REU TECHNICAL SERIES 67

BUFFALO PRODUCTION AND RESEARCH

FAO REGIONAL OFFICE FOR EUROPE

FAO REGIONAL OFFICE FOR EUROPE
INTER-REGIONAL COOPERATIVE RESEARCH
NETWORK ON BUFFALO (ESCORENA)

BUFFALO PRODUCTION AND RESEARCH

Edited by

Antonio Borghese

Professor Antonio Borghese
Istituto Sperimentale per la Zootecnia
Via Salaria, 31 - 00016 Monterotondo, Roma (Italy)
Tel. 0039 06900901 - 0690090232 - 3383172740
Fax 069061541
e-mail: antonio.borghese@isz.it
antonio.borghese@entecra.it

C.R.A.

Consiglio per la ricerca e la sperimentazione in agricoltura

ISTITUTO SPERIMENTALE PER LA ZOOTECNIA

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2005

PREFACE

For many years I have had the idea of writing a book about the buffalo: the species that I love and have studied for thirty years. The buffalo is a very quiet and intelligent animal, domestic but rustic, faithful and friendly, rich in history, and can now be found in many countries worldwide. It is employed as a draught animal, but also produces meat, horns, skin and particularly the rich and precious milk that gives creams, butter, yoghurt and many cheeses, including the delicious mozzarella.

The opportunity to write this book was facilitated by the FAO Regional Office for Europe aiming at promoting the diffusion of expertise and technologies among the regions within the framework of the European System of Cooperative Research Networks in Agriculture (ESCORENA). The FAO Regional Office for Europe supported me, as Coordinator of the FAO Inter-Regional Cooperative Research Network on Buffalo for Europe and the Near East, to produce this reference book on buffaloes all over the world with contributions from various buffalo experts and based on the results of my own research and work experience.

This experience was gained thanks to senior researchers devoted to the development and promotion of buffaloes. The first of these was Dr Augusto Romita, my first supervisor at the Istituto Sperimentale per la Zootecnia (the Animal Production Research Institute of the Italian Ministry of Agriculture in Monterotondo, Rome) with whom I conducted many experiments at Tormancina, the farm of the Institute, on buffalo calves and young bulls during the period from 1974 to 1980. He later prepared the first important research project on the buffalo species, financed by the Italian Ministry of Agriculture. This project examined the main aspects of buffalo production: nutrition and requirements, reproduction and physiology, and rumen microbiology, in collaboration with other Italian universities: Naples, Bologna, Perugia, Piacenza.

The second was Professor Giovanni De Franciscis, who shared with me his concept of working towards the realization of the good prospects for buffalo development in Italy and in the world. He promoted the first (1974) and the second (1982) International Buffalo Congress, and organized, in collaboration with the IBF (International Buffalo Federation, founded in 1985) and myself, the Fourth World Buffalo Congress (1997) in the Royal Palace of Caserta.

The third was Mr A. Qureshi, who raised my awareness of the essential role of buffaloes for the livelihood of many rural families in developing countries and encouraged me to establish the FAO Inter-Regional Cooperative Research Network on Buffalo.

I would also like to mention Professor Beniamino Ferrara, the famous university teacher in Animal Production of students of my generation in Naples, and a dear colleague and friend of mine, Tullio Di Lella, Professor of Animal Feeding and Nutrition at the Veterinary Faculty, Federico II University, Naples, Italy, who supported my dedication to buffalo research.

All these five colleagues have unfortunately now passed away and I feel somewhat sad and lonely without them. I would therefore like to dedicate this book to their memory.

Antonio Borghese
Monterotondo (Rome), Italy, 2005

INDEX

Chapter I Page
Antonio Borghese and Marco Mazzi
Buffalo Population and Strategies in the World.....1

Chapter II
Bianca Moioli
Breeding and Selection of Dairy Buffaloes.....41

Chapter III
Bianca Moioli and Antonio Borghese
Buffalo Breeds and Management Systems.....51

Chapter IV
Vittoria Lucia Barile
Reproductive Efficiency in Female Buffaloes.....77

Chapter V
Giuseppina Maria Terzano
Follicular Dynamics and Reproductive Technologies in Buffalo.....109

Chapter VI
Giuseppina Maria Terzano
Reproductive Application of Ultrasound in Buffalo.....137

Chapter VII
Stefano Terramoccia, Settimio Bartocci and Antonio Borghese
Nutritional Requirements in Buffalo Cows and Heifers.....145

Chapter VIII
Settimio Bartocci, Stefano Terramoccia and Simonetta Puppo
New Acquisitions on the Digestive Physiology of the Mediterranean Buffalo.....161

Chapter IX
Carmela Tripaldi
Buffalo Milk Quality.....173

Chapter X
Antonio Borghese
Buffalo Cheese and Milk Industry.....185

Chapter XI
Antonio Borghese
Buffalo Meat and Meat Industry.....197

Chapter XII
Giuseppina Maria Terzano, Simona Allegrini, Antonio Borghese, Cristina Roncoroni and Lavinia Alfieri
Metabolic and Hormonal Parameters in Buffaloes.....219

Chapter XIII
Antonio Fagiolo, Cristina Roncoroni, Olga Lai and Antonio Borghese
Buffalo Pathologies.....249

Chapter XIV
Antonio Borghese
Buffalo International Organizations.....297

List of Acronyms.....311

Chapter I

BUFFALO POPULATION AND STRATEGIES IN THE WORLD

Antonio Borghese and Marco Mazzi

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

The buffalo (*Bubalus bubalis*) population in the world is actually about 168 million head: 161 million can be found in Asia (95.83 percent); 3 717 million are in Africa, almost entirely in Egypt (2.24 percent); 3.3 million (1.96 percent) in South America, 40 000 in Australia (0.02 percent); 500 000 in Europe (0.30 percent).

ASIA

Asian buffalo or Water buffalo is classified under the genus Bubalus, species bubalis.
The *Bubalus bubalis* belongs to the class Mammalia, subclass Ungulata, order Artiodactyla, suborder Ruminantia, family Bovidae, subfamily Bovinae, tribe Bovini, which includes the following three groups: Bovina (cattle), Bubalina and Syncerina. Syncerina includes only the species *Syncerus caffer* (the African buffalo). Bubalina (the Asian buffalo) includes three species: *Bubalus depressicornis* or Anoa which lives in Indonesia, *Bubalus mindorensis* which lives in the Philippines and *Bubalus bubalis* deriving from the domestication of the *Bubalus arnee*, the Indian wild buffalo. The domestication of this species occurred relatively recently (5 000 years ago) compared to the domestication of *Bos taurus* and *Bos indicus* (10 000 years ago). Asian buffalo includes two subspecies known as the River and Swamp types, the morphology and purposes of which are different as are the genetics. The River buffalo has 50 chromosomes of which five pairs are submetacentric, while 20 are acrocentric: the Swamp buffalo has 48 chromosomes, of which 19 pairs are metacentric. The difference in the diploid number is only apparent. In fact, the large Swamp buffalo chromosome 1 originated from tandem fusion translocation between the River buffalo chromosome 4 (telomeres of p-arm) and 9 (centromere) (Di Berardino and Iannuzzi, 1981). During this phenomenon, the nucleolus organizer regions (NORs) present in the River buffalo chromosome 4p were lost and the centromere of chromosome 9 inactivated (Di Berardino and Iannuzzi, 1981). The two subspecies are inter-fertile and produce progeny with 49 chromosomes. Male crossbred progeny have sometimes displayed fertility problems while female progeny have manifested longer calving intervals only in the case of further backcross. Morphology of the two types differs considerably. Swamp buffaloes are less heavy, the adult male weight ranging between 325 and 450 kg, while the River type weighs between 450 and 1 000 kg. While the Swamp buffalo is reared mainly for draught purposes, although it also produces a valuable milk yield of up to 600 kg milk per year, the importance of the River buffalo depends on the high quality and quantity of the milk that it produces. River buffaloes are generally large in size, with curled horns and are mainly found in India, Pakistan and in some countries of western Asia. They prefer to enter clear water, and are primarily used for milk production, but are also used for meat production and for draught purposes. Swamp buffaloes are stocky animals with marshy land habitats. They are primarily used for draught power in paddy fields and haulage but are also used for meat and milk production. Swamp buffaloes are mostly found in south east Asian countries. A few animals can also be found in the north eastern states of India (Sethi, 2003). Each subspecies includes many breeds.

The production of milk and meat from buffaloes in Asian countries over the last decades has shown a varying pattern: in countries such as India, Sri Lanka, Pakistan and China, the milk yield per animal has increased by 2.44 percent, 1 percent, 1.45 percent and 1.55 percent,

respectively, while there has been either no change or only a negligible change in milk production in Bangladesh, Myanmar, Nepal and Vietnam. In some regions of east and south-east Asia, there has been a negative growth. Meat production from buffaloes has shown a growth of 1.43 percent only in Pakistan, while in other countries there was no change or a decline. At the Asia level, although buffalo milk production increased by 2.26 percent, meat production marginally declined (Dhanda, 2004).

Buffaloes are known to be better at converting poor-quality roughage into milk and meat. They are reported to have a 5 percent higher digestibility of crude fibre than high-yielding cows; and a 4-5 percent higher efficiency of utilization of metabolic energy for milk production (Mudgal, 1988).

1. INDIA

India has about 95 million animals which represents 56.5 percent of the world buffalo population. India is the first country in the world for number of buffaloes and milk production (about 134 million tons).

India is also the first country in Asia for scientific and technological development in buffalo nutrition, production, reproduction, biotechnologies and genetic improvement. Moreover India has implemented national programmes such as the "green revolution" (to increase crop production for animals), the "white revolution" (to increase milk productivity and satisfy human needs for proteins) and finally the "red revolution" (to increase meat production and strengthen the meat industry), particularly with regard to buffalo.

India possesses the best River milk breeds in Asia e.g. Murrah, Nili-Ravi, Surti and Jaffarabadi, which originated from the north-western states of India and have a high potential for milk and fat production apart from their use as a work animal and as a supplementary stock for use as meat production (Sethi, 2003). Indian Murrah is the most diffuse breed in the world: from Bulgaria to South America and all over Asia (Fig. 1). The production traits of the Indian breeds are reported in Table 1, with more details and figures given in Chapter III.

Table 1. Buffalo Breeds of India and their Production Traits (ICAR, 1997)

Name of the Breed	Average Lactation length (days)	Average Lactation yield (kg)	Milk Composition
Badhawari	272	780	Av.Fat 8.6% Total solid 17%
Jaffarabadi	319	2151	Av.Fat 7.86%
Marathwada	302	900	-
Mehsana	305	1893	Av.Fat 7%
Murrah	305	1675	Av.Fat 7.3%
Nagpuri	286	1055	Av.Fat 7.7%
Nili-Ravi	294	1820	Av.Fat 6.8%
Pandharpuri	305	1142	Av.Fat 7.0% SNF 9.28%
Surti	305	1289	Av. Fat 7.9%
Toda	200	500	Protein 4.45%

Recent statistics concerning buffalo demography show that the buffalo population in some countries like India, Pakistan, China, Vietnam is increasing at a rate of 1.5 percent/per year. Buffaloes are well adapted to a hot and hot humid climate and play a distinct role in the economy of farmers, which is primarily based on agricultural production systems. They provide high quality milk and meat and are a source of draught power for smallholders in countries of this region. In fact these animals are considered a financial asset since they serve as an insurance against the risk of crop failure due to natural calamities (Dhanda, 2004).

Less information is available regarding the production systems of Swamp buffaloes.

However, according to Faruque (2003) the Indian Swamp buffaloes have been mostly evolved for milk production and generally males are used for work in the paddy fields and performing

other agricultural operations.

Figure 1. Murrah buffaloes in the Central Institute for Research on Buffalo, Hisar, India

2. CHINA

China has a huge variety of buffalo genetic resources, unknown to most buffalo experts other than the Chinese. They are all of the Swamp type, with a long history of domestic livestock, and provide many products to the farmers.

In China each region has different types of buffaloes, to the extent that it is possible to say that buffaloes have adapted themselves to a range of climates, altitudes and temperatures as have the different cattle breeds that inhabit the various continents and countries. Therefore, in China there are buffalo breeds that can be found only in the lowlands, and other breeds that live only in the mountains.
The breeds of the lowlands are raised on fertile soils and paddy fields where intensive agricultural activities are carried out. This is the case of the Binhu breed (461 000 head; Fig. 2) in the Hunan province, the Xinyang breed (290 000 head) in the Henan province, the Enshi breed (77 000 head; Fig. 3) in Hubei, the Fuan breed (70 000 head) in the Fujian province, the Yanjin breed (45 000 head) in Yunnan, the Xinglong breed (24 000 head) in Hainan and the Wenzhou breed (10 000 head) in Zhejiang (Zhang Chunxi and Li Zhongquan, 2001).

Figure 2. Binhu buffalo

Figure 3. Enshi buffalo

Two further breeds inhabit the lowlands and can also be found along the saline seaside shores of the east sea: these are the Haizi breed (65 000 head) in Jiangshu and the Shanghai breed (36 000 head; Fig. 4) around the city of Shanghai. A similar adaptability to saline sandy terrain was already mentioned for another buffalo population in Bangladesh, in the bay of Bengal. The

most numerous breed in China is the Guizhou (1.46 million), a mountain breed of the Guizhou province: raised on natural pasture and of varying body size according to environmental conditions.

With regard to the other mountain breeds, there are the Fuling (415 000 head; Fig. 5) in Sichuan, the Dehong (390 000 head) and the Diandong (220 000 head) in Yunnan, the Dechang (190 000 head) in Sichuan, the Xilin (59 000 head) and the Fuzhong (57 000 head; Fig. 6) in Guanxi and the Dongliu (27 000 head; Fig. 7) in the Anhui province.

Figure 4. Shanghai buffalo

Figure 5. Fuling buffalo

Figure 6. Fuzhong buffalo

Figure 7. Dongliu buffalo

Most buffalo breeds tolerate all ranges of temperature, from 0°C in the winter to 30°C and over in the summer.

All buffaloes have long horns, a typical trait of the Swamp buffalo. The colour of the coat is grey, with varying intensities: from deep grey and blackish grey to brown, hoar and light grey. The majority of the breeds also have white spots either in the form of stripes on the breast or in the form of rings on the neck.

As for all Swamp buffaloes, Chinese buffaloes are used for draught, often as their only task. Exceptions are the Wenzhou breed, which is regularly milked and produces 1 020 kg milk in 278 days and the Jianghan (800 kg milk in 8-12 months). The Fuan breed is also sometimes milked, producing on average 2.6 kg milk/day, in a lactation of extremely variable length: 150 to 300 days (Zhang Chunxi and Li Zhongquan, 2001).

According to statistical data (FAO, 2003), the total number of buffaloes in China in 2003 was 22.759 million, the second largest population of buffalo in the world, representing 17.37 percent of the total bovine population in China. Scientific research on buffaloes began with some delay compared with other breeds.

China imported Murrah buffalo from India in the late 1950s and Nili-Ravi buffalo from Pakistan in the late 1970s. Since China imported two breeds of exotic dairy type buffaloes, experiments such as feeding observation, freezing of buffalo semen, artificial insemination and crossbreeding etc., had already been conducted, and good results were obtained. Over the past

20 years, important developments and breakthroughs in scientific and technological research on buffaloes have been achieved. In the area of breeding improvement, genetics and breeding, physiology and biochemistry, feeding and nutrition, reproductive technology, embryo biotechnology, dairy processing and disease prevention and treatment, many scientific and technological achievements have been acquired, and abundant scientific data has been accumulated (Liang Xian-wei et al., 2004). According to various studies milk performance has been markedly improved in crossbreds through the crossbreeding system applied in upgrading the two breeds such as crossbred Murrah F1, F2 and crossbred Nili-Ravi F1, F2. The average milk yields per lactation in Murrah crossbred F1, F2 are 1 240.5 kg and 1 423.3 kg respectively, which is higher than that of the local buffalo of 1 092.8 kg (this data comes from a selected herd, normally the local Swamp buffalo produces 500 - 800 kg per lactation) by 13.5 percent and 30.2 percent ($p<0.01$). In Nili-Ravi crossbred F1 and F2 milk yields per lactation are 2 041.2 kg and 2 325.6 kg, which are an improvement of 88.6 percent and 115.2 percent respectively ($p<0.01$). Triple-crossbred and their inter se crossing offspring are 2 294.6 kg and 1 994.9 kg respectively, which is an increase of 109.98 percent and 82.55 percent, compared with local buffalo ($p<0.01$) (Yang Bingzhuang et al., 2003), as reported in Table 2.

Table 2. Comparison of milk performance in different buffalo breeds (kg/day) (Yang Bingzhuang et al., 2003).

Breed	Lactations (n)	Lactation length (days)	Milk yield (kg)	Average milk yield per day (kg)	Highest daily milk yield (kg)
L	70	280.4±20.2	1092.8±207.4	3.79	6.6
M	237	324.7±73.9	2132.9±78.3	6.57	17.40
N	164	316.8±83.6	2262±663.9	7.14	18.40
MLF1	157	313.7±96.7	1240.5±479.8	3.95	7.57
MLF2	118	313.9±90.1	1423.3±534.5	4.53	8.30
NLF1	45	326.7±96.4	2041.2±540.9	6.25	16.65
NLF2	55	321.4±118	2325.6±994.4	7.22	19.35
N.MLF2	168	317.6±78.4	2294.6±772.1	7.22	18.80
N.MLG1	70	329.1±89.8	1994.9±635.0	6.06	18,50

L=local, M=Murrah, N=Nili-Ravi, G=Santa Gertrudis

The body size and body weight in crossbreds are greater than in local buffaloes, therefore meat yield performance in crossbreds is better than in local buffalo.

According to the data supplied by the Guangxi Buffalo Research Institute, a fattening experiment was conducted on triple-crossbreds and half Santa Gertrudis under the same conditions. The results showed that dressing percentage at 18 months was 53.0 percent and 59.9 percent respectively, net meat weight was 43.2 percent and 42.1 percent respectively, bone meat ratio was 1:4.5 and 1:4.4 respectively, and the quality of the buffalo meat was equal to that of bovine meat. The results showed that Murrah F2, had the greatest drawing ability (198.3 kg), next was the Murrah F2 (166.3 kg) and last was the local buffalo (111.8 kg). Meanwhile swamp buffalo can be crossed with River buffalo with high milk yields to create high milk yield cows (Yang Bingzhuang et al., 2003).

3. PAKISTAN

In Pakistan, the buffalo is the main dairy animal in the country. Out of the 22 million head of buffalo in Pakistan, 76 percent are found in the Punjab (24 percent in other provinces of the country: Sind, North West Frontier Provinces (NWFP), and Baluchistan). The Punjab supplies 73 percent of the total national milk production and 71 percent comes from buffaloes which are part of the traditional small mixed farming system which is integrated with crop production. Herd size is very small; 85 percent of buffaloes are raised in herds of one to five animals. There are 0.5 million landless farmers keeping dairy animals and contributing a significant (70 percent) share of the total milk production (Raza et al., 2000).

Table 3. Production performances of buffalo in Pakistan (Mudgal, 1999).

Characteristics	Nili-Ravi		Kundhi	
	Mean	Range	Mean	Range
Body weight at birth (kg)	-	-	-	-
Male	39.8	32-58	35.1	33.4-37
Female	37.7	27-45	32.3	31-34.5
Age at first calving (months)	47	30-54	52.7	48-57
Weight at first calving (kg)	625	544-695	495	407-585
Lactation length (days)	312	200-450	277	244-300
Lactation yield (litres)	2070	1700-2700	1825	1580-2018
Dry period (days)	160	95-240	176	134-214

Figure 8. Nili-Ravi buffaloes at the Livestock Research Institute, Bahadurnagar, Okara (Pakistan) (Borghese photo, 1992)

Recording of buffaloes is mainly undertaken in the seven institutional herds and on a few military farms. Apart from this, buffaloes at farm level are recorded under the progeny testing programme which has been operative since 1980. Dairying is still not undertaken on a commercial basis so the level of inputs is very low. Generally, animals are fed on crop residues with some additional forage/fodder grown for this purpose. Hay and silage making does not exist, except to some extent for institutional herds. Concentrates are fed to those animals that

are kept for the sale of milk. The government facilitates vaccination against contagious diseases at nominal costs. About 5-10 percent of breedable females are artificially inseminated while the rest are mated naturally with bulls of a good type. Credit facilities have also been made available to farmers for the purchase of milk yielding animals but on a limited scale (Khan et al., 1999; Khan, 2000).
The most common breeds present in Pakistan are the River Nili-Ravi (Fig. 8) and the Kundhi; their production performances are reported in Table 3.

4. PHILIPPINES

In the Philippines there are 3.2 million Carabao buffaloes, 99 percent belong to small farmers that have limited resources, low income and little access to other economic opportunities.
The Carabao Development Programme is a massive programme started in 1993 to improve the native Swamp buffalo locally known as the Carabao to develop their meat, milk and draught potential. An elite herd of Riverine buffalo has now been established at the Philippine Carabao Center, Science City of Muñoz, by importing about 3 000 Murrah buffaloes with pedigree performance records from Bulgaria. Each female crossbred when raised for milk can produce about 1 350 kg of milk per lactation (Cruz, 2003). The crossbreeding of Bulgarian Murrah (producing 1 800 kg per lactation) with a Swamp population (producing 400 kg per lactation, Fig. 9) obtained F1 (Fig. 10) with 1 100 kg and F2 with 1 350 kg mean production respectively. The Nueva Ecija Federation of Dairy Carabao Cooperatives (NEFDCCO) is a federation that includes 25 cooperatives in the Nueva Ecija area, and specializes in milk collection, in organization of the milk industry and product sales. Since the main purpose of the project is to elaborate a mechanism which will permit the Philippine group to select the parents of future generations of Carabao, it will be necessary for them to develop their livestock and design breeding programmes that will deliver rates of genetic improvement in the range 1.2 - 2.0 percent per year compounding. This appears to be a rather low rate of improvement. For example, if the Carabao population currently produces 700 litres per 305 days lactation then the expectation from genetic improvement alone (i.e. excluding increases due to improvements in nutrition, health, etc.) is an increase to 853 litres. If the milk is valued at 70 cents a litre then the value of the increased production would amount to about US$110 i.e. the income from one cow rises from US$490 in the first year to US$600 in the tenth year of the breeding programme. The difficulty in communicating the value of genetic improvement is that the increase is small per year and will not be attained with precision every year (Phillips, 2004).

Figure 9. Carabao buffalo near Muñoz, Philippines (Barile photo, 2004)

Figure 10. Murrah X Carabao near Muñoz, Philippines (Barile photo, 2004)

5. VIETNAM

Swamp buffaloes in Vietnam are mainly raised by smallholder farmers with small herds (four to eight head) partly used for draught power and partly for meat.

Table 4. Milk production of buffaloes in Vietnam (Nguyen van Thu, 2000a)

	Swamp	**(in the South) Swamp**	**(in the North) Murrah**	**F1 crossbred**
Lactation period (days)	-	210-360	236± 57.31	292±27.5
Milk yield (kg/day)	1.50	1.20-3.45	5.55	3.50

Traditional management dominates the buffalo production systems. Buffaloes play an important role in agriculture and in the life of Vietnamese farmers. They are the main source of draught power for land preparation and transportation in the rural areas, and supply a huge amount of fresh organic manure for cultivation. They are also well adapted to utilizing local feed resources, are economic to maintain, and a source of credit for the farmers. The main crop of Vietnam is rice, and sub-crops are maize, sweet potato, cassava, groundnut, soybean, sugarcane and vegetables. In the highland provinces, cassava is especially popular. Buffaloes are freely grazed on natural grassland, forests, at roadsides, canal banks, rice fields after harvesting, dikes, etc. The local buffaloes are of the Swamp type with a total population of nearly three million. In general, Vietnamese Swamp buffaloes have a small body size, a slow growth rate, late maturity, a long calving interval and a low milk yield, but are very well adapted to local ecological conditions and have good disease resistance. In the 1970s dairy Murrah buffaloes were imported from China, Bulgaria and India to improve the productivity of local buffaloes. These Murrah buffaloes adapted very well to the local conditions and were raised in many parts of the country. The male Murrah buffaloes were used to cross with female Swamp buffaloes. The crossbred F1 have improved body size, growth rate, draught power, milk yield and also reproductive performance, but at present the numbers of crossbred buffaloes are still small (Mai Van Sanh, 2004). Murrah buffaloes and crossbreds (Murrah x Swamp) are mainly reared at the research station in small numbers, consequently their performances are recorded and documented accurately. They are distributed in the northern, central and southern provinces of Vietnam. There are some good examples of breeding Murrah buffaloes for work and milk in village conditions in the northern provinces. Diluted semen and frozen semen from Murrah bulls were successfully produced for artificial inseminations (AI) through financial and technical support obtained from the Indian Government, but this programme was poorly developed (Nguyen van Thu, 2000b). Buffalo milk production in Vietnam is reported in Table 4.

6. SRI LANKA

The estimated number of buffalo owners in Sri Lanka is around 100 000. However, hardly any of them are full time buffalo farmers. About 87 percent are crop producers, who rear buffaloes as an additional source of income. About 64 percent use buffaloes for draught purpose, 34 percent for milk and draught, while only 2 percent keep buffaloes purely for milk. Buffaloes are spread throughout the country, with high concentrations in certain areas due to particular farming systems and market and socio-cultural reasns. The average herd size is around 22.5 animals. However, this figure is heavily dependent on the agro-ecological zone. Larger herds with an average of 40 to 50 animals are found in rice-growing areas of the dry intermediate zone. Smaller herds with an average of six to eight animals are found in mid and low zones (Bandara, 2000).

The present population is unevenly distributed across the major agro-ecological zones of the island which has an area of 65 000 square kilometres. The buffalo population has decreased

from 0.89 million in 1981 to 0.75 million (-15.45 percent) in 1997 with a large reduction in the wet (-20.42 percent) and wet-intermediate zones (-33.26 percent). The reasons for this are the increase in population pressure creating a high demand for land for residential and commercial purposes, especially in the wet zone, urban and peri-urban areas and increased mechanization of paddy cultivation practices and colonization of vast tracts of dry zone resulting in the dwindling of communal grazing grounds for free-ranging cattle and buffaloes. While there has been a decline in the total population, there has been a steady increase in the number of exotic River crosses in the population.

Figure 11. Crossbreds of Murrah and local Sri Lanka (Borghese photo, 2000)

Figure 12. Sri Lanka buffalo bull (Borghese photo, 2000)

The indigenous buffaloes which require many years to reach sexual maturity and are capable of producing only about one to two litres of milk over a very short lactation period of three to five months, have crossbred with the exotic riverine dairy buffaloes since 1950s. As a result a significant shift in the genetic composition of the buffalo population has been recorded. According to more recent reports on a national scale the percentage of herds carrying crossbred buffaloes has increased from 26.5 percent in 1985 to 30.4 percent in 1999 (Figs. 11 and 12).

In the case of Sri Lanka, buffalo milk with high fat and solid non-fat content fetches a higher price at the farm gate and offers the advantage of converting into value-added products, which helps to increase the profit margin several fold. The buffalo has been reported to be a ruminant with a higher potential to utilize marginal resources, a more rugged animal than its counterpart since it possesses the capability to perform well on marginal lands and withstand harsh environmental conditions. In the paddy field the buffalo offers a definite advantage since it possesses more stamina and broader hooves. Lastly, data is emerging in Sri Lanka as well as in other countries favouring the buffalo as a better meat animal than cattle. The above-mentioned advantages certainly highlight the great opportunities for harnessing the potential of water buffalo in order to meet the national needs in many developing Asian countries (Abeygunawarardena and Abeyratne, 2001).

7. BANGLADESH

In 2003 Bangladesh had 772 764 buffalo head owned by 270 228 holdings representing 1.52 percent of the total holdings in the country. The average buffalo head per holding was 2.67 (Faruque, 2003).
Bangladesh now has about 400 000 adult female buffaloes that are being used for draught or dairy purposes. These buffalo are found in the Bramhaputra-Jamuna flood plain of central Bangladesh, the Ganges-Meghna flood plain of southern Bangladesh and in institutional herds. Bangladesh has milk/dairy buffaloes of the Swamp crossbred and River types such as the Murrah and Nili-Ravi. The occurrence of crossbred dairy buffaloes indicates that the genetic improvement programme has been operative and is still running. A brief description of past and present breeding programmes (with relative successes and constraints) is given below (Faruque, 2000).

Recent studies indicate that Bangladesh possesses the following types/breeds of buffaloes (Table 5).

Husbandry and production systems for buffaloes vary depending on the topography and vegetation patterns of the country. Buffaloes are raised under an extensive system in the coastal and hilly areas where large-scale pasture land and enough green forage are available. Buffaloes are raised under a semi-intensive system on plain land and marshy land where there is limited pasture land. An intensive system for buffalo production is not practiced anywhere in Bangladesh even for institutional herds. The husbandry and care of the animals differs somewhat in the two systems. Nevertheless, there are some common practices. These common practices are: no housing system, no artificial insemination system, no routine vaccination programme and no animal identification and record-keeping system. One of the most important characteristics of buffalo production in Bangladesh is that they are raised by medium or large farmers who are generally considered rich in the locality. The staple food for buffaloes in Bangladesh is rice straw, which is an inadequate source of energy and protein. Sugarcane leaves, micro silage of sugarcane leaves, cassava leaves, roadside grass, elephant grass, maize with corn cob and pineapple bran are also used as feeding stuffs (Faruque, 2003).

Table 5. Types/breeds of buffaloes found in Bangladesh (Farouque, 2003)

Type/breed	Location	Population size, Phenotypes and Genotypes
Indigenous River type	Western and Central Part of the country	433 000 head Coat colour- Jet-black to black. Chromosome number = 50. Medium in size
Bangladeshi	Central and South West	4 500 head Light black coat colour, chevron and white stocking present. Chromosome number = 50. Medium in size
Indigenous Swamp type	Eastern part of the country	37 500 head Grey coat colour; chevron, white stocking and crescent horn are present. Chromosome number = 48. Small in size
Crossbred type (Indigenous X Nili Ravi)	Southern part of the country	40 000 head Phenotypes combination of Swamp type and Nili -Ravi. Medium in size
Non-descriptive type - Central part	South west and southern part of the country	207 569 head
Nili Ravi	Buffalo Breeding Farm	60 head

8. THAILAND

In the past Thailand had the second largest number of Swamp buffalo in the world. However this buffalo population drastically declined from 4.7 million in 1990 to 1.9 million in 1998. The number of buffaloes has decreased yearly and the present number is about 1.7 million and is tending to decrease gradually. In addition in some areas people prefer to consume the buffalo foetuses when the pregnant dames are slaughtered and in this way the buffalo population decreases even more dramatically. As reproductive efficiency is low due to the longer production cycle, the period for reproduction of two calves could be as long as four years. The 1.7 million head of buffaloes belong to 517 941 households. If the situation forces the buffalo population to decrease any further, the national buffalo population would risk disappearing completely (Suthikrai, 2002).

Approximately 83 percent of Thailand's buffaloes live in the northeast where most agricultural production is under rainfed conditions. Thai buffaloes are genetically of the Swamp type. The majority (90-95 percent) are grey to black in colour, while the rest are white. Most buffaloes are raised by small farmers in the rural areas.

Sixty percent of the Thai population belongs to small-scale farmers who raise buffalo in the backyard. It was an integral part of the crop production system. The breeding units of buffalo per family possess on average five to ten head from which no economic profit is made. There are very few farms that possess up to 50 head of buffalo and manage the herd as a commercial undertaking where animals are fed good quality feed and are well supervised. Buffalo breeding under village conditions is generally done by random mating. In fact, during the plantation season the buffaloes are tied up and fed with rice straw for almost four months resulting in a lack of opportunity to be bred during the plantation period. The animals, males and females, are grazed together in the paddy fields after the harvesting season. Consequently, unplanned

breeding occurs during the harvesting time when the villagers allow the buffaloes to graze together. It is obvious that in general, there is no recording system approach at the farmer level as on the government farms (Ancharlie Na-Chiangmai, 2000).

A programme on genetic improvement of Swamp buffaloes for use as a dual purpose animal (meat and draught) is in place. This programme is aimed at solving two basic problems, the decrease in number and the reduction in mature body weight and size of buffaloes under small farm production. Reproduction and growth performance of buffaloes in the matured herd were evaluated as the result of genetic improvement programmes over a 11-year period (1983-1993). With regard to reproductive performance, the age at first calving has been reduced to 3.5 years, the calving interval to 487 days, the conception rate has been increased to 80.5 percent, the calving rate to 76.9 percent and the calf crop to 70 percent (Pakapun Skunmun, 2000).

9. INDONESIA

The number of buffalo holdings in Indonesia in 1993 was 489 000 households; however in 1983 the number had been more than 593 000 households. However, the total population of buffalo in Indonesia during that period did not decline in line with the decline in the number of households with buffalo. In 1985 the total population of buffalo was 3 245 thousand, whereas in 1993, the total population was 3 238 thousand. Therefore according to these statistics in the period 1985 to 1993 the number of households was declining, but there was no significant change in the number of buffaloes. Thus it indicates that the rural buffalo maintained by small farmers in Indonesia can still make a potential contribution to the development of the dairy industry in Indonesia. The most populous province for buffalo in the year 1993 was Jawa Barat with 487 000, followed by DI Aceh with 454 000. The other provinces with relatively large populations of buffalo were Sulawesi Selatan with 342 000 and Sumatra Utara with 265 000. The population of buffalo in Jawa Tengah was also fairly high totalling 232 000, and in Sumatra Barat with 228 000. Moreover, the populations in Nusa Tenggara Barat, Nusa Tenggara Timur, and Sumatra Selatan were 227 000, 167 000, and 152 000, respectively, and the remaining population in each province was less than 100 000.

The buffalo breeds have been classified as Swamp and River subspecies, and most of the Indonesian buffalo are included in the Swamp one that consists of many types and varieties of breeds. There are varieties of the Swamp breeds in many different localities with divergences in size, weight, colour, marking and horn dimension. The Swamp buffalo is generally considered to be a working animal, but it also has a considerable capacity for milk production. Swamp buffalo are used for draught power in most areas and for beef in the Java lowland areas (Figs. 13 and 14) and the Sumatra uplands (Fig. 15).

Spotted buffaloes are highly prized (and therefore they command high prices) to be sacrificed and consumed on special occasions such as marriage ceremonies.

Most of the rural buffaloes maintained by small farmers in Indonesia produce less than 1 000 kg of milk per lactation. However, the production of fresh milk in Indonesia has not increased greatly over recent years and the level of production in the latter part of the 1990s was insufficient to satisfy the fast growing demand for this commodity. Around 90 percent of Indonesia's fresh milk production comes from smallholder dairy farms. Some of the problems these smallholders face are lack of capital, low technology, deficiencies in management of animal health, and insufficient human resources. In addition, in the case of beef cattle and buffalo, it is common practice to tether them by the roadside, and in such cases, feed is cut and carried to them. Alternatively, they may be herded to "waste" areas where they graze on crop residues, and feed supplements are rarely given in sufficient quantities, and during the non-productive period, it is thought that farmers do not give the animals supplementary feed. The age of first calving is late in comparison to temperate animals and the calving interval ranges from 18 to 24 months. Therefore, on the basis of its performance to date, the dairy (cow and buffalo) industry will be unable to meet the growing demand for milk and beef which

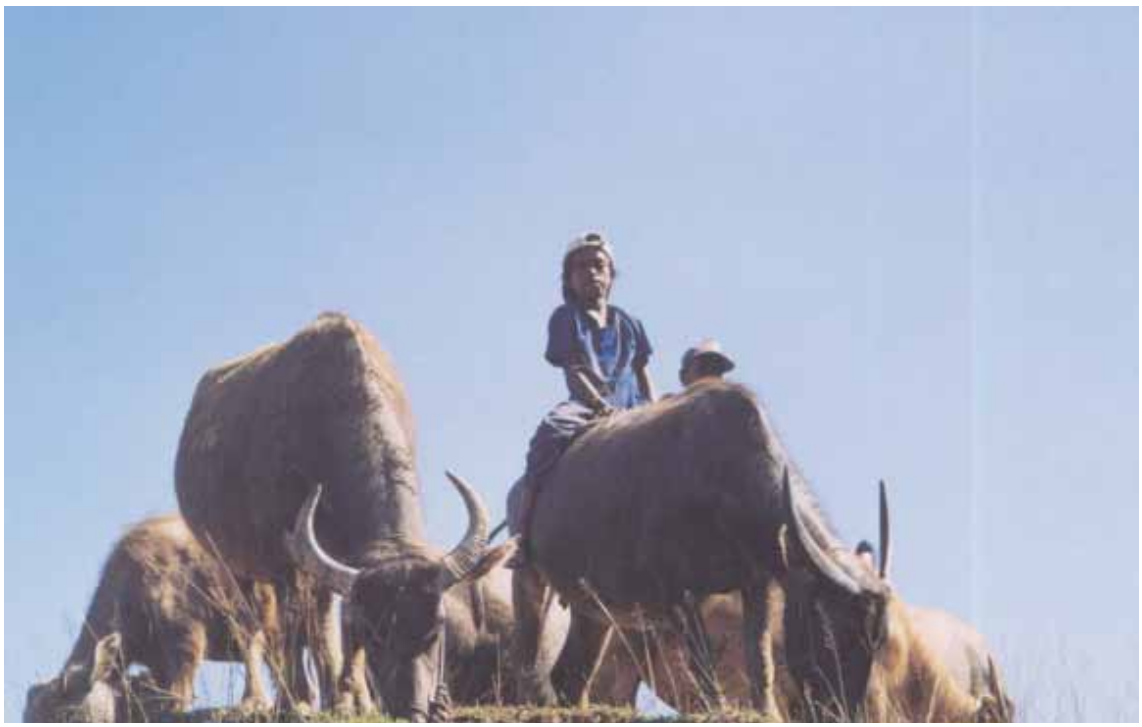


Figure 13, 14. Swamp buffaloes in the villages of Banten Province, West Java (Borghese photo, 2004)

Indonesia foresees for the future.
The primary objective of a new pilot programme will be to draw the attention of stakeholders to this situation and increase the availability of animal protein for improving human nutrition. These goals could be achieved by increasing the production of buffalo milk and meat through the improvement of the genetic capacity of buffalo, producing F1 and backcross buffaloes from Swamp and Mediterranean Italian River buffalo, to be used to increase buffalo milk production in Indonesia while maintaining and improving a nucleus of purebred Swamp buffaloes.

Indonesia is also the only country where the *Bubalus depressicornis* (Anoa) still exists (Fig. 16).

This is the smallest bovid in the world with a height at the withers of about 80 cm, with a live weight of 200 kg and with 30 cm long horns. Its colour is black and it is considered to be a wild buffalo.

Figure 15. Swamp buffaloes in Sumatra

Figure 16. *Bubalus depressicornis* (Anoa) typical species of Indonesia (Borghese photo, 2004)

10. MALAYSIA

The domestic water buffalo, commonly found in Malaysia, has been classified into the River and Swamp types. In 1998, the total population of buffaloes in Malaysia was about 170 000. They were mostly concentrated in the rice growing states of Kelantan, Terengganu, Kedah and Pahang in West Malaysia (60 percent). The Swamp buffalo is used for ploughing, harrowing and working in the rice fields. At the end of its working life, the Swamp buffalo is slaughtered and in this way accounts for about 16 percent of the current meat supply in Malaysia. The population of River buffaloes is less than 2 000 head of Murrah buffaloes brought by Indian immigrants at the beginning of the 20th Century.

Over the past two decades, there has been an alarming decline in the buffalo population in Malaysia with an average rate of population decline of 1.2 percent per year. This decline has been attributed to the displacement of buffalo by machinery for draught power in the rice fields, a low reproduction rate and a high extraction rate. Most farmers in rice-growing areas discontinued the rearing of buffaloes due not only to labour shortages, but also to the limited availability of grazing land. Most buffaloes, particularly the Swamp type, produce two calves every three years. Under field conditions, due to biological limitations as well as the seasonality of the feed supply, it is not possible for a buffalo to calve and then conceive immediately during the next few months when feed is still abundant. Thus breeding is delayed until the following year. Nearly all male buffaloes are castrated for draught purposes restricting the opportunities for mating.

Artificial insemination (AI) in the buffalo has not been practised in Malaysia due to poor oestrus detection techniques. Despite the availability of frozen semen and the fixed time insemination technique, there has been no progress in AI.

Formula for the fattening of buffalo calves using local feedstuffs, such as oil palm and rice by-products, have been devised in order to obtain a high average daily weight gain. Swamp buffaloes raised on feedlots using oil palm by-products as the major feed ingredient can reach a normal growth rate of about 0.59 kg per day and age at puberty was significantly lower in bulls on intensive grazing (21.5 months) than in bulls on free grazing (28 months) systems (Jainudeen and Wan Zahari, 2000).

MEDITERRANEAN AREA

The buffalo population in the Mediterranean area, typical for the climatic and cultural conditions, which includes Europe and the countries of the Near East, where the FAO Inter-Regional Cooperative Research Network on buffalo is operating, is about 5.5 million head, 3.4 percent of the world buffalo population, that is now about 168 million head.

A decrease in the number of buffaloes is occurring in some countries in the world and in Europe and the Near East (Bulgaria, Romania, Turkey) associated with three factors: holsteinization i.e. the substitution of low production cows and buffaloes with high production Holstein Friesian cows; mechanization, i.e. the substitution of draught animals with tractors and the poor market demand for buffalo products. On the contrary in Egypt, Iran and particularly in Italy buffalo numbers have increased due to the demand for particular products obtained only from buffalo milk and because the buffalo has changed from a rustic triple-purpose animal to become a dairy purpose animal.

In Italy particularly the increasing demand for buffalo mozzarella cheese both on the national and international markets, the Denomination of Controlled Origin (DOP) as "Mozzarella di Bufala Campana" for this cheese registered in Italy and in Brussels for the European Union (EU), and the milk quotas on surplus bovine milk imposed by the EU, led to an increase in the buffalo population of about 142 percent from 1993 to 2001 (compared with a 7.8 percent increase in the world population in the same period) and to an increase of 1600 percent (16 times) from 1957 to 2002. In Italy this increase in the number of buffaloes is not only remarkable for this percentage increase but also when compared with the trends in other species, which have all decreased over the last 50 years particularly for cattle, dairy cows and horses. The Italian dairy cow crisis was provoked by the milk quotas imposed by the EU, by farm structure inadequacy and lower dimensions in comparison with northern Europe, by BSE and Blue Tongue pathologies and by a reduction in reproductive life that is now no more than 2.5-3 calvings. Ovine livestock is also suffering due to new health regulations in the EU, insufficient pasture availability, Blue Tongue pathology, milk industry problems, and changes in consumer demand that now favours fresh and soft cheeses (such as mozzarella) rather than dry cheese (such as pecorino).

In eastern Europe and Turkey buffaloes were also used for draught. However, with the advent of more and more tractors, buffalo numbers have decreased. In countries of the Near East, where dairy cows give an average milk yield lower or similar to buffaloes, buffalo decline has not been registered and in Egypt they are still useful animals for draught. In Egypt, Iran and Azerbaijan there is a consumer preference for buffalo dairy products rather than for those derived from cow's milk. In Iran and Egypt the increase in buffalo numbers seems to be associated with a global improvement of animal production since the increase affected cattle to the same extent, whereas in Bulgaria and Turkey, alongside the consistent reduction in buffaloes, there has also been a drastic reduction in cattle.

In Table 6, buffalo population, productivity, number and percentage of recorded buffaloes are reported. The importance of animal recording for the process of selection is well recognized all over the world and is demonstrated by the fact that in most countries such activity is at least partially financed by governments, which consider it an important means for improving animal production.

An international non-governmental organization, the International Committee for Animal Recording (ICAR) has been active for over thirty years in the field of promotion and standardization of animal recording. A specific seminar, jointly organized by FAO and ICAR in the year 2000 (Workshop on animal recording for improved breeding and management strategies for buffaloes, Moioli et al., 2000), clearly evidenced the major constraints that have to be faced in order to implement a milk recording activity. These can be briefly summarized as follows: (i) lack of finance; (ii) farmers are reluctant to let other people know the production of

their animals; (iii) identification of the animals is expensive; (iv) recording costs increase proportionally with the distance between herds, and buffaloes are mainly raised by smallholders (two to five animals) scattered over wide country areas. These constraints explain why the percentage of recorded buffaloes in countries where buffalo seems to be more important than cattle is so low. The highest proportion of milk recorded buffaloes, in fact, is found in Italy (27.8 percent), in Iran it is 4.5 percent, while in other countries the recorded buffaloes are about 1 percent of total dairy females (Turkey, Romania) or less. We have high percentages of recorded buffaloes in Bulgaria, Syria and Greece, where the buffalo population is disappearing. All the cows (5 880) are recorded in Bulgaria since they are a small number: 5 640 on private farms and 240 on State and cooperative farms. A small nucleus is recorded in Romania, (0.7 percent total cows), in Egypt (0.2 percent) and in Azerbaijan (0.06 percent).
Recently milk recording began in Greece on 41 cows, in Syria on 640 cows and in Turkey on 1 000 cows at the Afion Buffalo Research Institute and in Ilikpinar Village (Hatay). No recording activity has been undertaken in Iraq and Albania.
Lactation length varies from 180 to 312 days, while the ideal period was 270 days with an average lactation milk yield of 1 600 kg.

Table 6. Buffalo population and productivity in European and Near East countries (Borghese, 2004)

Countries	Total Number	Adult Female	Lactation Milk Yield (kg)	Days Lactation	Milk Recorded Buffaloes	Recorded %
Italy	265,000	133,000	2,175	270	36,966	27.8
Egypt	3,717,000	1,487,000	1,600	312	3,040	0.2
Iran	400,000	208,200	1,600	220	9,300	4.5
Turkey	110,000	58,806	1,247	230	1,000	1.7
Azerbaijan	290,000	150,000	1,000	266	100	0.06
Romania	100,000	42,300	1,200	270	300	0.7
Iraq	98,000	40,000	1,320	270	NO	0
Bulgaria	9,200	5,880	1,870	278	5,880	100
Syria	4,500	1,800	1,191	254	640	35.5
Greece	1,000	500	1,020	240	41	8.2
Albania	100	70	400	180	NO	0

In Italy the milk production in 36 966 recorded buffaloes (ANASB 2003) was 2 175 kg in 270 days of lactation with 8.10 percent fat and 4.65 percent protein (Table 7) Recorded buffaloes are raised in 287 herds with an average of 128.8 head per farm. The productivity in other countries is lower, due to the fact that only Italy has undertaken a great deal of work on recording, selection and genetic improvement, health, and on improving feeding and livestock systems.

Milk composition improved in Italy in just a few years, with the average protein content moving from 4.4 to 4.73 percent in 2002 and to 4.65 percent in 2003, while the fat content moved from 7.3 to 8.3 percent in 2002 and to 8.1 percent in 2003 without operating any selection for the character of protein and fat content. Moreover the possibilities for genetic improvement for milk quantity and quality will be higher, if the selection pressure is increased reducing the number of bred females. At present there are many females in Italy producing more than

5 000 kg milk for lactation (270 days). Therefore the selection will be directed at the improvement of the yield of mozzarella cheese, not simply for milk production, since the farm income is based firstly on mozzarella cheese, secondly on the sale of pregnant heifers, and lastly on beef sales.

Table 7. Italian Buffaloes (ANASB 2003)

N° Head	265 000
N° Dairy buffaloes	133 000
N° Recorded buffaloes	36 966
% Recorded Buffaloes	27.8
N° Recorded farms	287
N° Head/farm	128.8
Kg Milk production (in 270 d)	2 175
% Fat	8.10
% Protein	4.65

In Europe and countries of the Near East, buffaloes are all of the River type, with similar phenotype but variable size, ranging between a minimum of 280 and 300 kg live weight for the adult female and male respectively in Egypt to a maximum of 900 and 1 000 kg in Iraq, the most frequent size being 600 and 800 kg (Table 8). There has been little exchange of breeding buffaloes among countries, therefore each population has its own phenotypic traits and performances. European buffaloes are all considered to be of the same breed, named the Mediterranean: in Italy the Mediterranean type was particularly selected and it is called Mediterranean Italian breed (figs. 28, 29, 30); in Turkey there is the Anatolian; in Egypt it is called the Egyptian; in Iraq there is the Khuzestani or Iraqi breed; in Azerbaijan it is called the Azeri or Caucasian; in Iran there are Azeri and Khuzestani breeds.

In Bulgaria, crossbreeding with the Murrah breed was undertaken, by importing in 1962 a considerable number of animals from India, and to a lesser extent in Azerbaijan by importing Murrah buffaloes from Bulgaria.

Age at first calving is, on average, 36 months (Table 8), in Italy a good proportion of buffaloes calve at 28 months of age whereas in Egypt and Syria a high number have the first calving after 40 months.

The main factor influencing the age at puberty (after genetics) is the nutrition level. In fact, experimental trials have demonstrated that heifers fed from the age of nine months with a high energy level diet (5.7 MFU/day) had a growth rate of 678 g/d while those fed a low energy level diet (4.4 MFU/day) had a daily gain of only 530 g. Furthermore, buffaloes of the first group reached puberty at 20.5 months while the others only at 23 - 24 months. The possibility of using grazing systems for buffalo heifers, which are less expensive than unifeed mixing or silage, was demonstrated by Borghese et al., (1997); in fact, no delay in the age at puberty was noted even in the grazing buffaloes provided that the average daily gain was not below 600 g/day.

Moreover in most European and Near East countries the puberty age is delayed because the requirements are not satisfied.

Average herd size (considering the number of adult breedable females) is below eight in the whole region, except Italy (90), Syria (35) and Iran (34). The proportion of breedable females to total buffaloes is about 50 percent in all countries. In the countries where the majority of buffaloes are reared in very small herds there are also a few bigger private, cooperative or state herds (Bulgaria, Egypt and Turkey).

Table 8. Type and feeding of buffaloes in Europe and the Near East (Borghese, 2003)

Country	Herd size (n° breedable buffaloes)	adult live weight (kg)		Age at first calving (months)	Housing	Feeding
		female	male			
Italy	90	650	800	28-32	loose in paddock	Common ration: 28% maize silage, 34% concentrates, 25% hay and straw 13% by-products
Romania	2.4	545	665	38-42	tied	Spring: leguminous, gramineous, hay - Winter: concentrates, wheat bran and silo - Sum./Aut.: graze, conc. or wheat bran
Bulgaria	2.5	600	800	34-37	tied	Alfalfa hay, straw, maize silage, green fodder or grazing, concentrates
Egypt	3.5	280	300	34-41	tied and paddock	Grazing + indoor feeding + cut and carry
Syria	35	490	580	36-42	paddock	Grazing, straw and concentrates
Turkey	8	410	510	36	tied and paddock	Summer: grazing and wheat bran - Winter: stems of maize concentrates
Iraq	10	900	1,000	36	paddock	In towns: concentrates, green forage, straw - In marshes: grazing on papyrus, and other plants
Iran	34	550	650	36-39	pasture	Green fodder, concentrates, straw, apple pulp, cotton seeds
Azerbaijan		530	780	36-39	tied	Maize silage, hay straw, hay silage, oilcake, concentrates
Greece				40		Graze all the year, concentrates only in winter
Albania						Grazing, roughage

The most common housing system is the one referred to as traditional, consisting of keeping buffaloes indoors at night and confined in fenced areas during the day (Egypt, Turkey, Iraq, Syria); in the favourable season they are allowed to graze during the day (Romania, Turkey and in some farms in Italy). In the marshes of south-west Iran, buffaloes are kept outdoors on pasture all year long, whereas in the northern areas around the Caspian Sea they are kept in barns only in winter. Lactating buffaloes are kept tied all year long in Bulgaria, Romania and Azerbaijan. In Italy they are housed loose in paddocks all year long, utilizing the same modern systems used for dairy cows, and the buffalo cows normally receive unifeed composed of maize silage, concentrates, hay, straw and sometimes by-products (Table 8). For example, a 600 kg live weight buffalo cow producing 10 kg milk, would be fed 15.3 kg dry matter (33 percent maize silage, 42 percent alfalfa hay, 17 percent concentrate with 38 percent proteins, 8 percent maize grain) with 12.7 Milk Feed Units, 2.1 kg crude proteins and 3.5 kg crude fibre. The same happens in Bulgaria, where the buffaloes receive unifeed composed of maize silage, concentrates, hay, straw, green fodder, and by-products. In other countries where the herds are small and domestic, the buffaloes are fed green forages indoors and outside are left grazing

(Egypt, Iran, Turkey, Syria, Greece). Concentrates are used more in Romania and Azerbaijan. One third of Iraq's buffaloes wallow in marshes all year round, the water reaching up to the middle of their body. They swim far and wide for feeding and when the water is high, they stand on platforms made of papyrus, reeds and mud. Sometimes the farmers build huts on these platforms to house the buffaloes and the platforms can be pushed to different parts of the marshes. Grazing in the favourable seasons is practiced everywhere at least for some of the buffaloes, except in Azerbaijan and Iraq. In any case, green forage "cut-and-carry" in the favourable season composed of legumes, varying from country to country, concentrates and by-products are the basic foodstuff. Green forage and hay are made mainly of alfalfa in Italy, Bulgaria, Romania and Turkey and *Trifolium alexandrinum* in Egypt. The most common by-products given to buffaloes are brewer grain residuals in Italy and Bulgaria, sugar beet-pulp in Italy, Bulgaria and Iran, cotton residuals in Egypt and Azerbaijan, tomato peel in Italy, apple juice residuals in Iran, sugar cane residuals in Egypt and Iran, stalk and cobs in Iran, Egypt and Romania and straw everywhere. In the Iraqi marshes, buffaloes are fed during the night with green forage cut by the farmer during the day. This forage is composed of reeds, papyrus, various water plants, and rice hulls when available.

In the countries examined, all herds have their own bull except in some areas of Romania, Bulgaria, Egypt and Turkey with very small herd sizes (two or three breedable buffaloes), where a group of bulls exists for breeding at the village level.

Artificial insemination is practiced very little: in Italy on 5 percent of buffalo cows, but it is rapidly increasing; in Azerbaijan on 0.7 percent; in Egypt on 0.3 percent and in Romania on 0.1 percent. In Bulgaria, in the large cooperative state farms, it is used on 80 percent of the buffaloes. In Turkey it began in 2002 near Ilikpinar village (Hatay) (Figs. 17 and 18) with Italian semen provided by the FAO Network project. In the other countries it is not used at all. The diffusion of artificial insemination in buffaloes is difficult because of seasonality.

The buffalo cow shows stages of partial anoestrous or even deep anoestrous during the spring and summer. Obviously seasonality depends on many factors, both genetic and environmental, mainly nutritional. In buffalo, as well as in other livestock, melatonin plays a fundamental role in initiating the hypothalamus-pituitary-ovarian axis activity with variations in level between three and ten pg/ml in daytime and between 20 and 90 at night, while levels depend upon daylight hours, and therefore season, as well as upon the age of the buffalo. In Italy the goal of having as many buffaloes as possible to calve before spring is of primary importance because the demand for buffalo milk is especially high in the spring and summer, with variations in the milk price between winter and summer of 50 percent.

For this reason, in Italy many research trials have been carried out to induce buffaloes to calve in the favourable season, in particular regarding oestrus induction which is also helpful for performing artificial insemination. Therefore, methods to induce oestrus are essential to remove anoestrus, to improve artificial insemination, and to increase out of season milk production. The hormonal treatments used to stimulate the hypothalamus-hypophysis axis are varied: 1. hormonal release factors (i.e. GnRh), 2. progesterone (i.e. PRID Progesterone Releasing Intravaginal Device) plus gonadotropine plus prostaglandine (see Chapter IV: "Reproductive efficiency in Female Buffaloes").

Figure 17, 18. Anatolian buffaloes submitted to oestrus induction and artificial insemination techniques (Borghese photo, 2002)

11. TURKEY

The water buffalo is called by different names such as Dombay, Camiz, Camis, and Komus in Turkish. It is recorded that it was introduced into Europe by the crusaders, and Mogul recounted that many buffaloes were raised in Trakya, in BC 3 000. Buffalo figures can be seen on signets made in Mesopotamia.

According to 1974 FAO statistics, at that time there were one million buffalo head in Turkey. From 1984 to 1997, there has been a decrease in the buffalo breeding population of 65 percent and the reason for this decrease in water buffaloes has been the preference for cattle over buffalo in the Ege and Marmara regions, where a large number of buffaloes were found. In Turkey, all the improvement efforts for genotypes were only practiced on cattle.

The buffalo population is about 110 000 head (Borghese, 2004), and only of the Anatolian breed. In 2002 Italian semen was introduced in Ilikpinar village (Hatay), for the local population of buffaloes in order to improve genetic and milk productivity (Sekerden et al, 2003).

In 1988 it was found that the average milk yield of buffaloes raised in controlled herds at first lactation period was 813.12±36.21 kg; it was also reported that at the first, second and third lactation period, the average milk yield was 983.4±58.45 (442-1715) kg respectively. In addition it was noted that the average milk yield of farmers under village conditions was 1,009.89±21.13 kg, and the average lactation period was 224.80±6.42 (121-368) days. The dry period was 188.04±11.17 (64 - 552) days. Milking was generally undertaken by hand. Milking by machine was carried out only around Istanbul. The average dairy yield of the buffalo cow was 5.08±1.71 kg.

Composition of the buffaloes' milk was: protein: 4.18±0.07 percent, total solids: 17.71±0.35 percent, and fat: 8.11±0.20 percent. The fat-free solid content of buffalo milk was 11.91±0.17 percent. The water content of the milk was 82.29±0.35 percent.

The milk production of the water buffalo is renowned and favoured particularly for the production of the famous unique Turkish desserts. This was one of the highest motivations for farmers to keep and raise water buffaloes near big cities (Soysal and Kok, 2004).

12. AZERBAIJAN

The most valuable buffalo gene fund of the USSR was in Azerbaijan. During the transition period following the break up of the Soviet Union no research facilities or farm management activities existed to assist buffalo breeders. As a result, the number of buffaloes in many regions of Azerbaijan fell drastically. Valuable breeds of buffaloes were slaughtered for meat. In order to counteract this shortage, the President of Azerbaijan, Heydar Aliyev, issued a decree for the Preservation of the Local Livestock Gene Pools in Azerbaijan. The Azerbaijan Association of Buffalo Breeders, founded in December 2001, played a leading role in passing this law.

There are approximately 300 000 buffaloes in Azerbaijan, including 140 000 female buffaloes with an average milking rate of around 1 200-1 600 kg (eight to ten percent fat content) with 305 days per lactation. On the state-supported buffalo breeding farm, there are 920 buffaloes, including 250 female buffaloes (Farajev and Bashirov, 2002).

The main problem hampering the development of buffalo breeding in Azerbaijan is the absence of high quality reproductive buffaloes and a lack of artificial insemination facilities. Financial assistance, such as that provided by Italy, was useful for the introduction of oestrus induction and artificial insemination techniques (Figs. 19 and 20), to develop milking and cheese industry management (Borghese, 2005) and to improve the local Azeri breed.

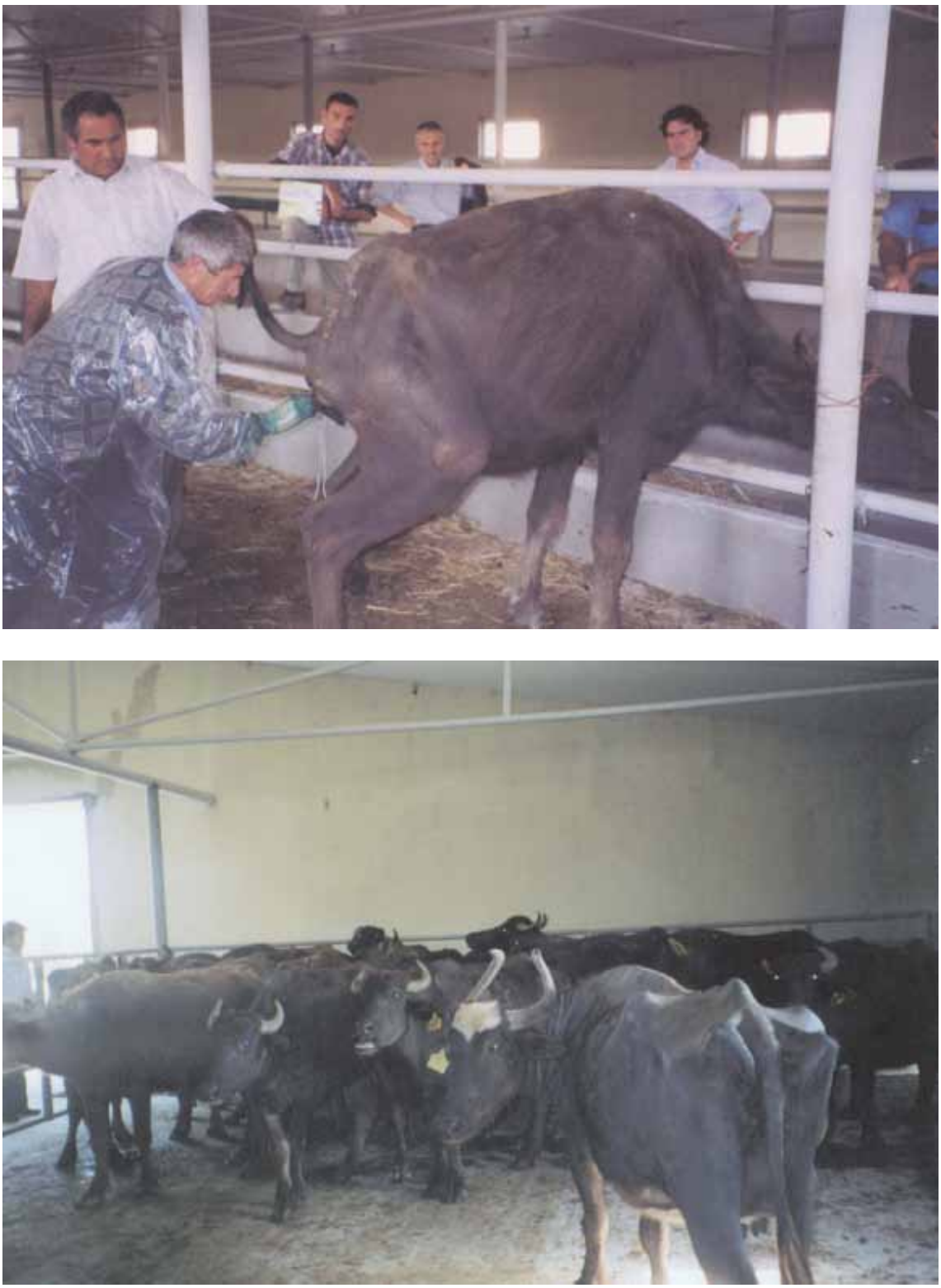


Figure 19, 20. Azeri buffaloes submitted to oestrus induction and artificial insemination techniques (Borghese photo, 2003)

13. ARMENIA

Due to the absence of animal recording, it is difficult today to obtain detailed information regarding the potential of buffaloes in Armenia. Scientific research has been conducted in the past in the Republic of Armenia. However, it is important to note that no research or selection has been carried out in Armenia recently. Since 1991, following the collapse of the former USSR, all livestock in Armenia, including buffaloes, became totally privatized. After privatization no precise livestock recording has been undertaken and the data presently available is merely the statistical data which was collected in the past. There are now about 1 000 buffaloes in Armenia (Marmarian, 2000).

14. IRAN

In the 1930s, there were 1 500 000 buffaloes in Iran. By 1995 this number had decreased to 500 000. The buffalo is a native animal of Iran, with over 80 percent of its population concentrated in the north and north-west (Azerbaijan province) and 18 percent in the south of the country. The overall buffalo population is increasing at about 1.3 percent annually, while on the contrary in some countries such as Iraq and Bulgaria, the numbers are dropping. Some of the main reasons for this decline may be industrialization, the increasing demand for buffalo meat but a lack of replacement of the slaughtered animals and farming diversification and income. Official neglect and pro-Holstein propaganda have caused a significant decrease in buffalo numbers in Iran in recent decades (Mohsen pour Azary et al., 2004).

The buffalo farming system in Iran is based on smallholders (99 percent); most of the herds have an average of five animals; a few herds have between 20 and 50 buffaloes and some of them have 300 buffaloes. Smallholders manage their animals according to the opportunities offered by the environment: on pasture, stubble, shrubs and grass. Most of them obtain their feeding by grazing along water sources: streams, rivers; ponds, lakes, integrated with the following products: citrus peels and pulp, sugar cane wastage, etc. In Khuzestan, buffaloes are raised outdoors throughout the year; but in the north-west they are housed in the autumn and winter. Buffalo farming in Iran can be considered to be at a good level since the owned or rented properties are of a large size and the land available for buffalo farming is also extensive. Buffalo farming has been a traditional activity for many decades (Kianzad, 2000).
Milk production in Iran is reported in Table 9 according to data published by Abdulwahid Ghanemi (1998).

Table 9. Milk production of buffaloes in Iran

	Khuzestani	**Azeri**
Lactation period (days)	210	210
Milk yield (kg/year)	1 865	1 200

15. IRAQ

In Iraq, according to data provided by Borghese (2004), there where 98 000 total River Khuzestani or Iraqi buffaloes, 40 000 adult females with kg 1 320 as medium lactation milk yield, in a 270 day lactation period. Presently it is impossible to estimate the real situation in Iraq because of the war. According to data provided by Magid (1996), buffaloes are bred in the marshes and swim far and wide for feeding on papyrus, reeds, common ash and other plants. When the flood water is high their owners have to go out and collect these plants in order to feed the buffaloes on platforms. Rice hulls are also given when available. Buffaloes in towns rarely graze on natural pasture; they are fed mostly on concentrates, green forage, straw and agricultural by-products.

16. EGYPT

The total number of buffaloes in Egypt reached about 3.717million in 2003, of which 42 percent were dairy cows, 6 percent buffalo bulls, 32 percent heifers less than two years old and 20 percent male calves less than two years old. While the annual growth rate for the buffalo population approached 3 percent over the last two decades, it still only accounts for 1 percent of the cattle population. The aggregate share of buffalo milk, from all types of production systems is about 81 percent of total milk production in Egypt.

The cost of milk production from buffaloes is also less than the cost of reconstituted imported powdered milk at the international market price. The return on one ton of concentrate feed mix generated by milk production confirmed the comparative advantage of buffaloes in Egypt. Recently, in association with the economic reform era and market liberalization, the commercial buffalo system has significantly expanded (Soliman and Sadek, 2004).

Figure 21, 22. Egyptian buffalo cows and calves reared in the Delta Region (Borghese photo, 2000)

There are different research institutes at the Ministry of Agriculture and at the University in Giza (Cairo) involved in developing projects concerning buffaloes and buffalo products. The breed is the River Egyptian (Figs. 21 and 22). The buffaloes are spread along the river Nile, in the Delta Region and at the Fayum Oasis.
Buffalo productivity in Egypt is about 210-280 days/lactation, an average of seven lactations and a milk yielding of 1 600 kg. The age at the first calving is 34-41 months (Fikri El-Kirabi, 1995).

17. ITALY

Buffalo livestock in Italy is a small reality in comparison with the large population numbers of many east Asian countries, but it is an important reality in economic terms, both for workers' occupation and as an example of typical Italian produce in the world. In addition the Italian Buffalo is the first in the world with regard to genetics, applied technologies, the monitoring of pathologies and the hygiene and quality of products.

The selection and genetic improvement is controlled by the ANASB (Italian Buffalo Breeders Association) and at the present time 27.8 percent of the total population of dairy buffaloes (Table 7) is recorded, both in the morning and in the evening, each month. Therefore many buffalo cows, producing more than 5 000 kg/lactation 270 days, have been identified. Many bulls are submitted to performance and progeny tests and many millions of semen doses from bulls of proven high genetic value are available for artificial insemination in Italy and in the world. There are many centres of semen production in the south of Italy, in Campania, where most buffaloes are reared, one (Cooperativa Fecondazione Artificiale, CoFA) is in Cremona, in the north of Italy. The breed is named the Mediterranean Italian to distinguish it from other European breeds which are not at the same genetic level. All data are collected by ANASB which decides on the selection goals, which are presently to increase not only the milk quantity but specifically the mozzarella cheese production according to the mozzarella index:

Mozzarella (kg) = Milk (kg) x (3.5 x % proteins + 1.23 x %fat - 0.88) / 100

The "Mozzarella di Bufala Campana D.O.P." is the primary product of buffalo livestock: it is sold for a minimum € 10.00 per kg in the cheese industries, much more in shops and is exported not only within Europe, but all over the world, from the USA to Australia. The demand exceeds the production and mozzarella is particularly sought by restaurants.

Italian buffalo management is exclusively intensive: dairy buffaloes are kept loose in paddocks close to the milking room, where the cows are submitted to udder control and mechanically milked twice a day. Milk production is sustained by diets with a high energy concentration (from 0.85 to 0.95 MFU/kg DM) and a high protein concentration (14-16 percent crude protein on DM), based on maize and other silages, cereal grains, soya, alfalfa or "graminaceae" hay and by-products.

The feeding stuffs movement and distribution is effected by mixing trucks; the movement and stocking of dung is also mechanized; therefore there are no smallholders in Italy, but only farmers with an average herd size of 90 head per herd. Heifers are also fed intensively in order to achieve puberty before 20 months.

The largest proportion of the buffalo population can be found in the Provinces of Caserta and Salerno (Campania region), and the next localities for size of population are the Provinces of Frosinone and Latina (Lazio region), which are in the Denomination of Protected Origin (D.O.P.) area.

The control and monitoring of pathologies is effected by the local veterinary services and by the "Istituto Zooprofilattico Sperimentale" (Animal Prophylaxis Research Institute), one for the Lazio region and another for the Campania region. The hygienic control of the milk production

and of the milk products in the industry is of a particularly high standard.
Research on the buffalo species is carried out by the "Istituto Sperimentale per la Zootecnia" (Animal Production Research Institute, Monterotondo, Rome) and by the Federico II University, Naples.

18. ROMANIA

The buffalo population in Romania was more than 200 000 head in 1996 (Popovici, 1996). Actually it is about 100 000 of Mediterranean breed (Table 6), sometimes crossbred with Bulgarian Murrah.
The average milk production is 1 200 kg per lactation (270 days). Buffaloes are still used today on small private farms for draught and the goal of the selection process is to create a dual-purpose type of animal (milk and meat), realizing good daily gains (600-800 g), in order to slaughter the males at 22 months with 460 kg of live weight. At present the calves are also fattened to be slaughtered at four months (100 kg of live weight).
The animals are housed and tied during the winter due to the unfavourable weather conditions and fed with hay, bran, concentrates, silage (Table 8), grazing on pasture in the warm season.

19. BULGARIA

In Bulgaria a new buffalo population, named Bulgarian Murrah, has been created through the importation of Indian Murrah in 1962 and later in 1975 by crossing them with indigenous Mediterranean. This activity was effected systematically under the scientific management of the Buffalo Research Institute in Shumen and the National Animal Selection Center (Alexiev, 1998). Buffaloes (Figs. 23 and 24) were raised on the State farms, kept tied in closed sheds, machine milked and fed maize silage, alfalfa or grass hay, straw and concentrates.
The animals were managed in separate groups according to physiological conditions: suckling calves, females four to twelve months, heifers, pregnant heifers and dry cows, milking cows.
Milk recording, selection, artificial insemination and progeny testing were coordinated by the Buffalo Research Institute.
After the changes in the political and social-economic system in 1989, buffaloes were transferred to the new private farms, where scientific and genetic activities were limited and the animal numbers have drastically declined.
Actually, there are only 9 200 head, of which 5 880 are cows (Table 6) of Bulgarian Murrah in Bulgaria. All these animals are submitted for milk recording and to artificial insemination.

Figure 23, 24. Bulgarian Murrah herds (Alexiev, 1998)

AMERICA

Today there is great enthusiasm about buffalo in America, particularly among buffalo breeders and livestock associations. Buffalo is considered to be the animal of the future, and there is justification for this. Buffalo numbers have significantly increased and it is felt that breeding policies have led to an all-round improvement in quality, as can be seen from Table 10.

Table 10. The buffalo population in America (Rocha Loures, 2001)

Country	Population
Argentina	50 000
Bolivia	5 000
Brazil	3 000 000
Colombia	30 000
Cuba	30 000
Ecuador	5 000
Paraguay	10 000
Peru	25 000
Venezuela	150 000
Trinidad and Tobago	10 000
Other countries (Belize, USA, Costa Rica, Guatemala, Mexico, Panama, Guyana)	30 000
Total	**3 345 000**

One of the characteristics that makes buffalo so widely used in these countries is their extraordinary ability to convert fibre into energy. Research trials indicate the superiority of the buffalo in food conversion and in the use of tropical forage and agricultural by-products. Therefore, it is emphasized that the buffalo does not compete with humans, for it does not necessarily use the main production from the crops. It is also an efficient tool in the recycling of nutrients in integrated production.

Other important characteristics of the buffalo are their rusticity, their ability to adapt to different climates and their high fertility rates, always superior to those of bovines. Buffalo breeding is a synonym for low production costs and high levels of productivity (Rocha Loures, 2001). According to recent data (Borghese, 2004) the buffalo population in Venezuela is 200 000 and 70 000 in Argentina. The present population all over America is about 3 415 000.

20. CUBA

In Cuba, buffalo introduction is relatively recent dating from 1983 to 1989. The River buffaloes now present in the country were originally imported from Trinidad, Tobago and Panama. They are the breeds used for upgrading the larger population composed of Swamp buffaloes imported from Australia. As has been well established, the Buffalypso or Trinidadian Buffalo is the result of crossbreeding between the Carabao and other River breeds such as the Murrah, Nili-Ravi, Jaffarabadi, Surti, Nagpauri and Bhadawari, which was undertaken in the sugar cane factories of the Sugar Carone between 1920 and 1930.

In an earlier publication, detailed information was given regarding the comparative

performance during five lactations of both buffalo types in relation to milk and fat production, specifying that the River (Buffalypso) buffalo was superior in milk yield (260 kg), with a 60 day longer lactation period than the Swamp buffalo, without any difference in fat content. In another work, it was indicated that the Buffalypso, when well managed, has a productive potentiality to produce 1 620 kg of milk (802 kg from milking and 817 kg to feed the buffalo calf) (Fraga et al., 2004).

21. TRINIDAD-TOBAGO

Water buffaloes are not indigenous to Trinidad and the River type milk buffaloes were imported into Trinidad from India at the beginning of the last century. Indian contract labourers used the males to haul sugar cane and the cows to provide milk for their families and neighbours. During the mid-19th century, selection and crossbreeding among the original imported milk breeds started with a view to developing a specialized beef animal now commonly referred to as the Buffalypso (Fig. 25). Consequently the milk production potential of these animals was ignored.

Figure 25. Buffalypso from Trinidad

However, since the late 1970s the use of Trinidad water buffaloes for meat as well as for milk production has been encouraged, not only in Trinidad but also throughout the Caribbean. In order to achieve this objective, the Ministry of Agriculture established a small milking herd at the Aripo Livestock Station during the early 1990s.

The average lactation duration was 191.6 days, which is lower than most values reported in literature. The mean total lactation yield, averaged over all lactations, and based on once a day hand milking with the calf suckling the mother was 611.3 kg (range: 767.4-444.2 kg). The average milk yield/day/cow was 3.09 kg (range: 0.50 15.42 kg). The average percentages for fat, protein, lactose, non fat solids, total solids, ash and Ca were 7.15, 4.03, 5.60, 8.84, 16.97, 0.85 and 0.23 respectively (Rastogi and Rastogi, 2004).

22. BRAZIL

At the same time as zebus were imported into Brazil from India, mainly between the 1940s and 1960s, some Murrah and Jaffarabadi buffaloes also arrived. In Brazil, these animals have found ideal conditions, such as thriving pastures, water, grazing space and suitable temperatures. These effects have been enhanced by the buffaloes' hardiness and adaptability.

Today there are approximately three million buffaloes in Brazil, which are found in all States, notably Parà, Maranhào, Cearà, Pernambuco, Rio Grande do Northe, Minas Gerais, Bahia, Rio de Janeiro, Sào Paulo, Paranà, Santa Catarina, Rio Grande do Sul and Mato Grosso do Sul. In these States, the buffalo population is growing at the rate of ten percent every year, because

breeders slaughter only culled males and females, and all other animals remain for reproduction.

In the 1970s, Brazilian buffalo breeders began using buffaloes professionally for dairy and meat production. At the same time, research on production, reproduction and nutrition began, and several regional associations of buffalo breeders were formed. At the end of the last decade, the associations initiated a programme to evaluate these animals, based on the data that had been gathered over the previous thirty years (Ramos et al., 2004).
In some Brazilian States, buffaloes have become an economic option, mostly for their milk yield and, consequently, for the elaboration of mozzarella cheese, originally produced in Italy. This product is well accepted on the consumer market, and secures high prices due to the substantial demand. For this reason buffaloes have conquered a space in the national cattle husbandry sector and are no longer seen as marginal contributors to the meat and milk yield cycle.
Under Brazilian conditions, the following results were recorded in a study with 659 pure or crossbreds of Murrah buffaloes, from 1979 to 1987, in the county of Pitangueiras (SP): an average milk yield of 725.49 ± 228.91 kg. The data reported, for 1 586 lactation records of the breeds Jafarabadi, Mediterranean, Murrah and their crossbreds (Fig. 26), in several regions of the country was: an average of 1 517.16 ± 407.62 kg milk, in an average lactation period of 248.81 ± 3.86 days. Tonhati and Cerón-Muñoz, after analysing 1 020 lactations, found an average value of 1 496.20 ± 605.72 kg milk, for a 270 day lactation. After taking into account 1 744 lactations of 1 268 females of different genetic groups, observed in six herds from 1996 to 1998, the estimated milk production was found to be 1 259.47 ± 523.09 kg, in 270 days of lactation (Tonhati and Cerón-Muñoz, 2002).

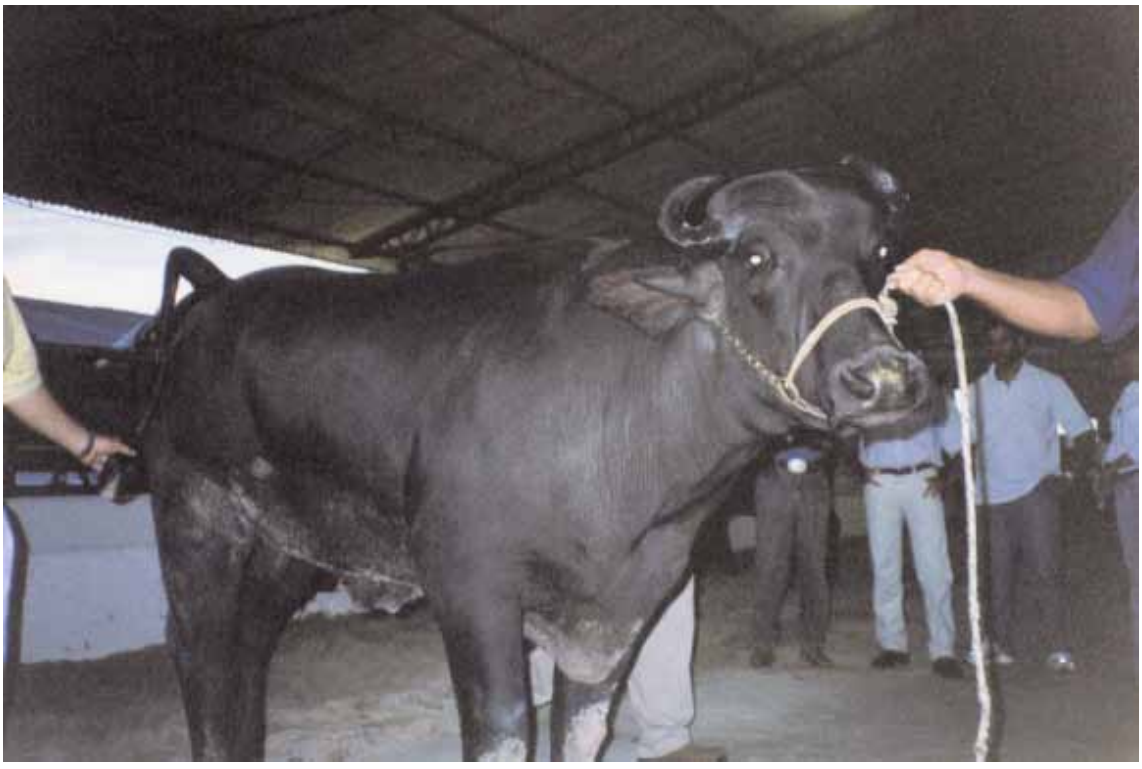


Figure 26. Murrah crossbred in Brazil (Borghese photo, 2002)

This data was based on 5 014 lactations, originating from 1 656 cows, who calved from 1973 to 2003, were sired by 234 bulls and were raised in twelve herds located in seven different Brazilian states (Parà, Rio Grande do Norte, Cearà, Bahia, Minas Gerais, Sao Paulo and Paranà). Only lactations yielding at least 3.0 kg/day and lasting a minimum of 150 days were taken into consideration. The objective was to evaluate the productive and reproductive performances of dairy buffalo cows, whose production was experimentally controlled.
The overall average for monitored milk yield, lactation length and age at calving was

1,589.57±605.14 kg, 265.63±49.00 days and 79.23±47.5 months respectively, which confirmed the data presented by Ramos et al (2001). The maximum yield per lactation was 5 796 kg whereas the minimum yield was only 351 kg of milk. The average milk production in this population was higher than the Brazilian average for cattle (1 265 kg/lactation), which is based on 19 million cows producing approximately 24 billion kilograms of milk (Ramos et al., 2004).

23. VENEZUELA

The buffalo population in Venezuela is about 200 000 heads (Borghese, 2004). There are different breeds: Mediterranean Italian, Bulgarian Murrah, Indian Murrah and others imported from other American countries. The management and feeding systems are almost entirely based on pasture and the primary purpose is for meat production. Therefore generally buffalo cows are not milked but give milk to their calves and the calves, in turn, are sold to the meat market. Each farmer often owns large properties (from 1 000 to 10 000 hectares) with numerous animals. Today there is a development of the milk production potential with the introduction of better genetic lines (Montiel-Urdaneta et al., 1997). Likewise, the technologies for milking cows, storing milk and for cheese production (together with technologies related to other milk products) are also developing. Although the promotion and expansion of buffalo production could solve the problem of the meat and milk deficit, there are many limiting factors such as government inertia regarding the existing sanitary problems and the absence of national development programmes (Reggeti, 2004).

24. ARGENTINA

The buffalo population in Argentina is about 70 000 head, notably Mediterranean, Murrah and Jafarabadi imported from Brazil, (Zava, 2004) and it is rapidly increasing. There are similar conditions to those in Venezuela, but the milk and cheese industry is better developed than in Brazil. In Argentina there is an active Buffalo Breeders Association (Asociacion Argentina de Criadores de Bufalos (AACB)) that works actively towards genetic and production improvement in the buffalo sector, particularly towards increasing meat production for export. The largest concentration of farmers in this regard is in the Corrientes and Formosa Provinces. Buffaloes are reared on an extensive system, on poor pasture on low fertile land, together with bovine herds, on farms with an area of between 750 and 2 000 hectares with about one head/two hectares. The problems in Argentina are: use of low-level technologies, inadequate sanitary conditions, low quality products and insufficient productivity (Vargas, 2004).

AUSTRALIA

Buffaloes were not native to Australia, they arrived with the first British settlements in the Northern Territory. Buffalo numbers are now estimated to be less than 40 000 - 50 000 head, with 20 000 in managed herds confined by fences and the remainder ranging over uncontrolled areas (monitored negative for TB) in southern and south eastern Arnhemland (an Aboriginal reserve), east of Katherine and along the south coast of Darwin. This feral population acts as a source of existent stock or replacement breeders for the controlled herd, while at the same time supplying some of the stock required for the current live export markets. Swamp buffalo are farmed on 30 to 40 properties in the Top End. Over the last 12 years there has been a movement of buffaloes between states with small herds (up to 100 head) scattered over all other states except Queensland (which currently prohibits their farming) (Lemcke, 2001).

From 1994 to 1997, government and private owners imported several River buffaloes (four bulls and four heifers) from the USA. Despite the loss of the two original bulls and one young calf, the purebred herd numbered 21 head in February 2001. Crossbred calves were first produced in 1995 and were involved in performance comparisons with purebred Swamp cows. A grading-up programme has also been carried out with the 3/4 and 7/8 progeny now available. The plan is to keep upgrading in this way in order to increase the number of purebreds available for distribution in Australia. Semen has been imported from milking herds in Italy and the first crossbred calves have been produced *in vitro* (Lemcke, 2001).

The general feed mix used was 2.0-2.5 kg/head/day of sorghum or maize with 0.5 0.75 kg of meat meal plus ad libitum roughage, generally a legume/grass hay plus sodium bicarbonate. During the trials it was found that buffaloes failed to increase their efficiency of utilization of grain above 30 percent of the total diet. Meat meal is no longer used in cattle feeding so an alternative is required; probably cottonseed meal is the most readily adaptable alternative. The most productive properties are those with a mixture of upland and floodplain terrain, and are capable of producing 400 kg Swamp buffalo at 2.0-2.5 years of age.

Significant improvements in these parameters have been achieved, firstly by using improved pastures and fertilizers, and secondly by the use of crossbreeding with the River blood (Lemcke, 2001).

AFRICA

Since Egypt has been considered as part of the Mediterranean area, due to its history, culture and geographical position, there is no tradition of buffalo farming in the African continent. Even if people commonly refer to the African buffalo, the indigenous wild African buffalo, which is very little known to most animal production scientists, this buffalo is a member of another species (*Syncerus caffer*). In the classification of the "Bovini" tribe, three groups have been distinguished: Cattle, Asian buffalo and African buffalo.

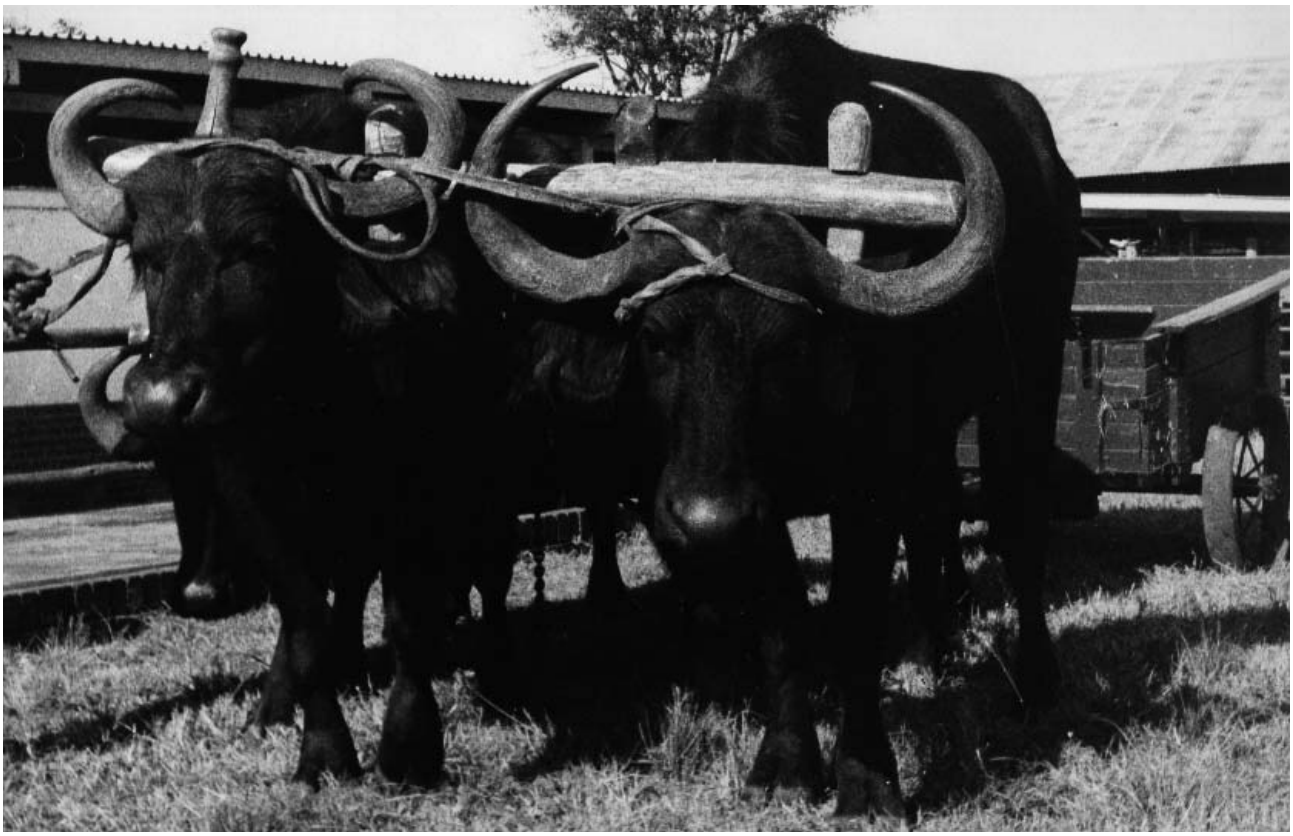


Figure 27. Bufalo cafro (Syncerus caffer), Zimbabwe (Antinori photo, 1984)

African buffalo therefore belongs not only to a different species with respect to Asian buffalo, but also to a different genus (genus Syncerus) with respect to the genus Bubalus of Asia and Europe, with a chromosome number of 52 in Syncerus in contrast to the 50 chromosomes of *Bubalus bubalis* River and 48 of *Bubalus bubalis* Swamp.

Very few studies have been undertaken on the African buffalo, which is found in the forest and savannah regions of Africa, South of the Sahara: Ethiopia, Sudan, Zaire, Congo, Chad and South Africa. The total population is about two to three million. In view of its tolerance to the tsetse fly and trypanosomiasis and its sustainability to the environment, the possibility to produce fertile hybrids with the Asian River buffalo appear attractive and in fact experiments of crossbreeding with the Indian buffalo have been carried out. Unfortunately these experiments were unsuccessful and therefore interbreeding between Syncerus and Bubalus appears impossible (Borghese and Moioli, 2000).

Further trials to domesticate the African buffalo have been undertaken in a few countries, with particular success in Zimbabwe (Fig 27), where it was proved that this animal, considered in the past to be wild and ferocious, could be used for draught.

Many trials have been undertaken in the past to introduce River buffaloes into Madagascar, Mozambique, South Africa, Tanzania, Zaire and the Congo (Alexiev, 1998), but these small buffalo colonies died out owing to disease (particularly trypanosoma), nutritional deficiencies, climate, etc., in fact buffaloes are more sensitive than cattle to direct solar radiation and temperature. In hot climates water availability is of high importance for buffaloes, which need

wallows, rivers or splashing water in order to reduce the heat load and thermal stress. Therefore the diffusion of the buffalo population in Africa and in the world depends on the availability of water, hence the fact that the *Bubalus bubalis* is commonly called the "Water buffalo" (Borghese and Moioli, 2000).

Figure 28, 29, 30 Mediterranean Italian Buffaloes at the Animal Production Research Institute (Barile photo, 2005).

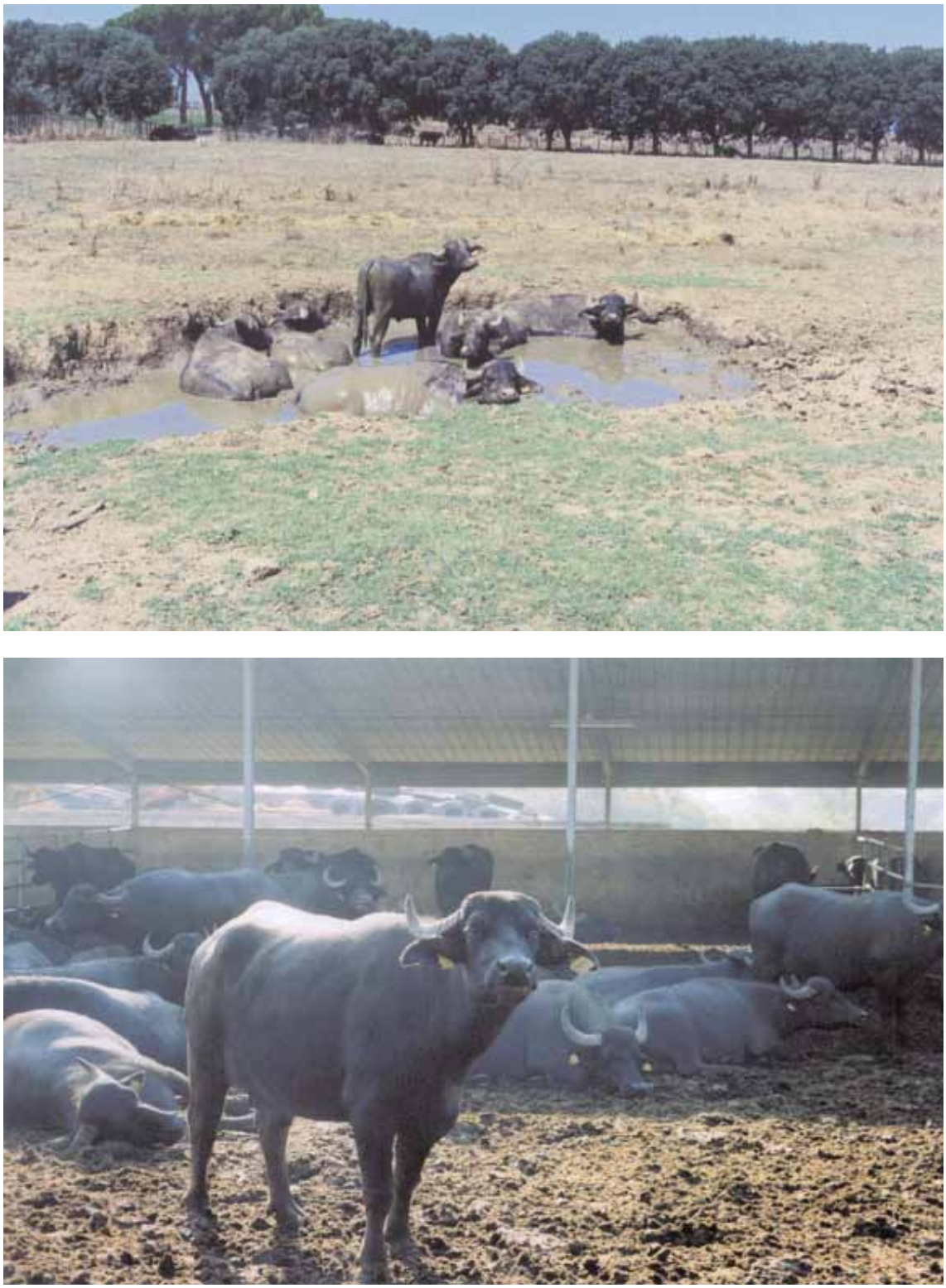


Figure 31, 32 Mediterranean Italian Buffaloes at the Animal Production Research Institute (Barile photo, 2005).

References

Abdulwahid Ghanemi, 1998. Buffalo population and production in Iran. Buffalo Newsletter, 10: 12-14.

Abeygunawarardena, H. and Abeyratne, A.S. 2001. Social-economics and future prospects of buffalo farming in developing Asia: evidence from Sri Lanka. Proc. of the Sixth World Buffalo Congress, Maracaibo, Zulia, Venezuela, 20 to 23 May: 25 48.

Alexiev, A. 1998. The Water buffalo. St. Kliment Ohridski University Press.

ANASB (Italian Buffalo Breeders' Association), 2003. Statistical data.

Ancharlie Na-Chiangmai, 2000. Development of a buffalo breeding scheme in Thailand. ICAR Technical Series, 4: 61-68.

Bandara, B.H.W.M.U.S. 2000. The current status of buffalo in Sri Lanka. Proc. of the Third Asian Buffalo Congress, Kandy, Sri Lanka, 27 to 31 Mar.: 93-97.

Borghese, A., Barile, V.L., Ficco, G., Galasso, A., Marchiori, E. and Terzano, G.M. 1997. Feeding system effect on reproduction performances in buffalo heifers. Proc. of the Fifth World Buffalo Congress. Caserta, Italy, 13 to 16 Oct.: 697-701.

Borghese, A. and Moioli, B. 2000. Feasibility of introducing buffaloes into some African countries. Third All African Conf. Anim. Agric., Alexandria, Egypt, 6 to 9 Nov.

Borghese, A. 2003. Buffalo production systems in Europe and the Near East. Proc. of the Fourth Asian Buffalo Congress, New Delhi, India, 25 to 28 Feb.: 13-23.

Borghese, A., 2004. Recent developments of buffaloes in Europe and Near East. Proc. of the Seventh World Buffalo Congress, Manila, Philippines 20 to 23 Oct.: 10 16.

Borghese, A. 2005. Personal information.

Cruz, L.C. 2003. Philippine Carabao Center. Tenth Anniversary, 27 Mar.

Dhanda, O.P. 2004. Developments in water buffalo in Asia and Oceania. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 17-28.

Di Berardino, D. and Iannuzzi, L. 1981. Chromosome banding homologies in Swamp and Murrah buffalo. J. Hered., 72: 183-188.

FAO. 2003. www.fao.org/dad.it.

Farajev, A.F. and Bashirov, E. B. 2002. The development of buffalo breeding in Azerbaijan. Journal of the Azerbaijan Dairy Industry, (2) Apr.-May: 9-10.

Faruque, M.O. 2000. Identification of the best genotype of buffalo for dairy purpose in Bangladesh and to improve their productivity. Research report: 1-80.

Faruque, M.O. 2003. Buffalo production system in Bangladesh. Proc. of the Fourth Asian Buffalo Congress, New Delhi, India, 25 to 28 Feb.: 31-35.

Fikri, El-Kirabi, 1995. Buffalo population and production in Egypt. FAO/RNE, Buffalo Newsletter, 3: 8.

Fraga, L.M., Fundora, O., Gutiérrez, M., Mora, M. and Gonzàles, M.E. 2004. Milk production characterization in a Water buffalo (Buffalypso) unit during a 4-year productive period under tropical conditions. Proc. of the Seventh World Buffalo Congress, Manila, Philippines 20 to 23 Oct.: 240-243.

ICAR (Indian Council of Agriculture Research), 1997. New Delhi, India.

Jainudeen, M.R. and Wan Zahari, M. 2000. Buffalo production in Malaysia. Proc. of the Third Asian Buffalo Congress, Kandy, Sri Lanka, 27 to 31 Mar.: 81-84.

Khan, M.S., Chandary, M.A. and Bhatti, N.M. 1999. Progeny testing Nili-Ravi buffaloes. Ranking of bulls for first six batches. Buffalo Newsletter, 13: 1-5.

Khan, S. 2000. Animal recording for improved breeding and management strategies of buffalo in Pakistan. ICAR Technical Series, 4: 21-23.

Kianzad, D. 2000. A case study on buffalo recording and breeding in Iran. ICAR Technical Series, 4: 37-44.

Lemcke, B. 2001. Buffalo production systems in Australia. Proc. of the Sixth World Buffalo Congress, Maracaibo, Zulia, Venezuela, 20 to 23 May: 104-118.

Liang Xian-wei, Yang Bing-zhuang, Zhang Xiu-fang, Zou Cai-xia and Huang You-jun, 2004. Progress of scientific research on buffaloes in China. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 29-35.

Magid, S.A. 1996. Buffalo population and production in Iraq. Buffalo Newsletter, 6: 6-8.

Mai Van Sanh, 2004. Buffalo production for beef in Vietnam. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 519-523.

Marmarian, Y. 2000. Buffalo breeding in Armenia. ICAR Technical Series, 4: 73 76.

Mohsen pour Azary A., Pirmohammadi, R. and Manafi Azar, Q. 2004. Breeding of buffaloes in West Azerbaijan of Iran. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 535-537.

Moioli, B., Maki-Hokkonen, J., Galal, S. and Zjalic, M. 2000. Workshop on animal recording for improved breeding and management strategies for buffaloes. ICAR Technical Series, 4.

Montiel-Urdaneta, Nestor S., Rojas, N., Angulo, F., Peroro, F., Hernandez, A., Zuleta, A., Cahuao, N. and Torres, I. 1997. Lactation length in buffaloes in a very dry tropical area of Venezuela. Proc. of the Fifth World Buffalo Congress. Caserta, Italy, 13 to 16 Oct.: 208-212.

Mudgal, V.D. 1988. Proc. of the Second World Buffalo Congress, New Delhi, India, 12 to 17 Dec.: 454.

Mudgal, V.D. 1999. Milking buffalo. In "Smallholder Dairying in the Tropics", International Livestock Research Institute, Chapter Six.

Nguyen van Thu, 2000a. Buffalo production research and development in Vietnam. Proc. of the Third Asian Buffalo Congress, Kandy, Sri Lanka, 27 to 31 Mar.: 105 115.

Nguyen van Thu, 2000b. Buffalo recording system in Vietnam. ICAR Technical Series, 4: 69-71.

Pakapun Skunmun, 2000. The current status of buffalo production in Thailand. Proc. of the

Third Asian Buffalo Congress, Kandy, Sri Lanka, 27 to 31 Mar.: 99-104.

Phillips, K.R. 2004. The Philippines-Aciar project designed to improve the production potential of local livestock. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 78-81.

Popovici, I. 1996. Buffalo Newsletter, 5: 9-10.

Ramos, A.A., Van Onselen, V.J. and Gonçalves, H.C., 2001. Promebul: "Sumàrio de Touros Bubalinos". Botucatu: 20p. tabs., PROMEBUL-Boletim Tecnico: n° 1.

Ramos A.A., Wechslew F.S., Gongalves H.C. and Mendes Malhado C.H., 2004. Milk yield from Brazilian dairy buffalo cows from 1973 to 2003. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 578-579.

Rastogi, L. and Rastogi, R.K. 2004. Milk production, milk composition and meat production from buffaloes (Buffalypso) in Trinidad, West Indies. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 524-528.

Raza, S.H., Ullah, A., Khan, S. and Teufel, N. 2000. Buffalo milk production by small, medium and large farmers and their response to different innovations in the Punjab (Pakistan). Proc. of the Third Asian Buffalo Congress, Kandy, Sri Lanka, 27 to 31 Mar.: 191-196.

Reggeti, J.G. 2004. Recent developments of buffalo system in South and North America. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 8-9.

Rocha Loures, 2001. Buffalo production systems in Americas. Proc. of the Sixth World Buffalo Congress, Maracaibo, Zulia, Venezuela, 20 to 23 May: 74-86.

Sekerden, Ö., Borghese, A., Köroslu, M., Uyar, A., Uras, H. and Ziya Güzey, Y. 2003. First oestrus synchronization and artificial insemination studies and effect of PRID treatment on conception rate in Anatolian Buffaloes. Proc. 2° Congr. Naz. Allev. Bufalo, Monterotondo, Roma, 28 to 30 Aug.

Sethi, R.K, 2003. Improving riverine and swamp buffaloes through breeding. Proc. of the Fourth Asian Buffalo Congress, New Delhi, India, 25 to 28 Feb.: 51-60.

Soliman, I. and Sadek, H. 2004. Impacts of productive and reproductive performances on investment efficiency of buffalo enterprises in Egypt. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 212-217.

Soysal, I. and Kok, S. 2004. Buffalo breeding in Turkey. Proc. of the Seventh World Buffalo Congress, Manila, Philippines, 20 to 23 Oct.: 547-548.

Suthikrai, W. 2002. Buffalo production in Thailand. Proc. of the First Buffalo Symposium of the Americas, Estacao das Docas-Belém-Parà-Brazil, 01 to 08 Sept.: 528-531.

Tonhati, H. and Cerón-Muñoz, M. F. 2002. Milk production and quality and buffalo genetic breeding in the state of São Paulo, Brazil. Proc. of the First Buffalo Symposium of the Americas, Estacao das Docas-Belém-Parà-Brazil, 01 to 08 Sept.: 267-280.

Vargas, P.M. 2004. Modelos ganaderos y produccion de bufalos de carne. In: "Bufalos en Argentina" Ed. Crudeli et al., Moglia SRL Corrientes, Argentina: 115 148.

Yang Bingzhuang, Li Zhongquan, Liang Xianwei and Zou Caixia, 2003. The advance of genetic improvement and the development of the dairy industry in the Chinese Water buffalo. Proc. of

the Fourth Asian Buffalo Congress, New Delhi, India, 25 to 28 Feb.: 27-30.

Zava, M. 2004: El Bufalo domestico. In: "Bufalos en Argentina" Ed. Crudeli et al., Moglia SRL Corrientes, Argentina: 17-54.

Zhang Chunxi and Li Zhongquan, 2001. Buffalo genetic resources in China. Buffalo Newsletter, 16: 1-7.

Chapter II

BREEDING AND SELECTION OF DAIRY BUFFALOES

Bianca Moioli

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

Breeding and official selection activity in any country depends on the economic importance of the considered livestock, therefore of the products that can be obtained from it. From Table 1, Column 6, it is evident that in four countries the numbers of buffaloes exceed the numbers of dairy cows, i.e. in Pakistan, Egypt, India and Nepal. Azerbaijan, Italy, Iran and Romania follow with values ranging from 15 to 2.6 percent.

The first step in the breeding and selection activity with regard to any dairy livestock is milk recording of the productivity of each yielding animal, the results of which, when appropriately merged with the genealogy data, allow a definition of the milk genetic merit of each individual, in particular of the bull for which we have no other milk record except the production of his related animals.

The importance of animal recording for the activity of selection is well recognized all over the world and is demonstrated by the fact that in most countries such activity is at least partially financed by governments, which consider it an important means for the improvement of animal production. An international non-governmental organization, the International Committee for Animal Recording (ICAR) has been active for over thirty years in the field of promotion and standardization of animal recording. The ICAR comprises 20 member countries and has obtained excellent results particularly in the dairy cattle sector, where one of its groups, entitled Interbull, regularly produces milk genetic merits of bulls that are comparable among different countries representing the most important dairy breeds: Holstein Friesian and Brown Swiss. What keeps buffaloes far behind the results obtained by dairy cows is the cost of the whole organization of milk recording, genealogy data registration and the selection activity. A specific seminar, jointly organized by FAO and the ICAR in the year 2000 (Workshop on animal recording for improved breeding and management strategies for buffaloes), made clearly evident the major constraints affecting the implementation of the milk recording activity, which can be briefly summarized as follows: 1. Lack of finance; 2. Farmers are reluctant to reveal to other people the levels of production of their animals; 3. Identification of the animals is expensive; 4. Recording costs increase proportionally to the distance between herds, and buffaloes are mostly raised by smallholders (two to five animals) scattered over wide country areas. These constraints illustrate why the percentage of recorded buffaloes (Table 1, Column 5) in countries where buffalo seem to be more important than cattle are so low.

The highest proportion of milk recorded buffaloes, in fact, is found in Italy (28.6 percent), Bulgaria (8.5 percent) and Iran (4.5 percent), countries where the numbers of buffaloes represent below ten percent of dairy animals.

A consistent number of recorded buffaloes (Column 4) is obviously found in countries with the highest presence of buffaloes (India and Egypt) which signifies that these countries have also taken steps towards implementing an organized milk improvement activity, on a solid scientific and technical basis.

The information regarding strategies for buffalo improvement and other associated activities, which are referred to in this document, were obtained through specific questionnaires that the coordination centre of the Buffalo Network, in collaboration with the ICAR Working Group on

Buffalo, regularly sends to reference centres in each country. The activities of each country will be separately examined below, beginning with those countries having the highest number of recorded buffaloes (Column 4).

India

The National Dairy Development Board (NDDB) was the promoter and is the executor of the whole recording and selection activity. The NDDB (organization covering the whole of India) was created to promote, finance and support producer-owned and controlled organizations. The NDDB's programmes and activities seek to strengthen farmer cooperatives and support national policies that are favourable to the growth of such institutions. Fundamental to the NDDB's efforts are cooperative principles; dairy cooperatives account for the major share of processed liquid milk marketed in the whole of India. Milk is processed and marketed by 170 Milk Producers' Cooperative Unions, which are merged into 15 State Cooperative Milk Marketing Federations. NDDB's programmes and activities seek to strengthen the operation of dairy cooperatives, as producer owned and controlled organizations. The NDDB supports the development of dairy cooperatives by providing them with financial assistance and technical expertise.

Table 1. Dairy cows, dairy buffaloes (females), total milk recorded buffaloes, percentage recorded out of total buffalo population, percentage of dairy buffaloes out of total dairy females (cattle + buffaloes). The countries are listed on the basis of total number of recorded cows. Years 2000 to 2003.

1	2	3	4	5	6
Country	Dairy cows	Dairy buffalo (females)	Total milk recorded buffaloes	Percentage recorded out of total buffalo population	Percentage of dairy buffalo out of total dairy females (cattle + buffalo)
India	35 500 000	46 000 000	-	-	56.40
- Gujarat		2 500 000	57 500	2.30	-
Pakistan	4 366 000	10 650 000	-	-	71.00
- Punjab	-	7 900 000	6 000	0.08	-
- North West Frontier Provinces	-	990 000	26 000	2.50	-
Italy	2 169 000	125 000	35 755	28.60	5.40
Egypt	1 253 000	1 487 000	3 034	0.20	54.00
Iran	3 543 000	208 200	13 236	6.30	5.00
Brazil	27 800 000	52 000	500	1.00	0.19
Bulgaria	430 000	4 980	425	8.50	1.10
Nepal	828 000	836 500	400	0.05	50.20
Turkey	5 700 000	58 806	200	0.34	1.00
Azerbaijan	820 000	150 000	100	0.06	15.00
China	53 000 000 (all)	8 500 000	-	-	-
China (only dairy)	4 633 000	2 900	-	-	0.06
Romania	1 600 000	42 300	-	-	2.60

The Dairy Cooperative Network operates in over 285 districts, covers nearly 1 031 281 village-level societies and is collectively owned by nearly 11 million farmer members. The most concrete results of the NDDB activity in buffalo improvement have been obtained in Gujarat and are outlined below.

Gujarat (India)

Five percent of all Indian buffaloes are raised in the State of Gujarat, in the west of India. In this state, buffalo recording has been carried out since 1987 under the programme entitled "Dairy herd Improvement Programme Actions " (DIPA). The recording systems were introduced with the objective of genetic improvement of buffaloes through a well-planned, field-based progeny testing programme.

In the year 2000, 2.5 million buffaloes were documented by milk recorders employed by the village cooperative society. These recorded buffaloes are all raised on smallholdings of one to five animals, which means over 800 000 recorded herds, and from these herds every year a total of forty young bulls are selected for progeny testing and sent to the artificial insemination (AI) station.

Average lactation production of milk recorded buffaloes is 1 071 kg (300 days) for the Meshana breed and 1 694 kg (292 days) for the Murrah crossbred. Fat content is 7.01 percent in the Meshana and 6.68 percent in the Murrah cross. Protein content is not recorded.

In the region, there are three natural breeding stations controlled by the cooperative and three AI stations. Forty percent of the 2.5 million buffaloes are given AI, while the remaining sixty percent are taken to the breeding station.

The three breeding stations belong to the Dairy Farmers' Cooperative Union, a non-governmental organization; yearly they keep about 260 bulls, of an average age of six years. The milk genetic merit of each bull is estimated on the basis of the milk production of 30 to 50 daughters per bull. An animal model is used for the calculation.

Two of the three AI stations are also owned by the Dairy Farmers' Cooperative Union; they keep 105 bulls which produce 730 000 semen doses a year. Ninety percent of them are progeny tested bulls or bulls born from progeny-tested bulls, while the remaining are new on-test bulls. There is an additional AI station owned by an international non-governmental organization: it keeps 155 bulls, producing altogether 410 000 semen doses a year. Ninety-five of them are progeny-tested bulls or bulls born from progeny-tested bulls, while the remaining are new on-test bulls.

The size of individual smallholdings does not permit the upkeep of their own breeding bull; this is the reason why the AI stations are frequently employed. The majority of farmers is given only one semen dose per buffalo; few of them request two doses. Conception at the first oestrus is 41 percent and the conception rate per year is 2.46 inseminations per conception.

Italy

In the past fifty years, buffalo numbers in Italy have increased 17 fold; it is therefore the livestock that has registered the highest increase in numbers. The reason for this increase lies in the fact that from a rustic triple-purpose animal, buffalo has become a dairy purpose animal. All produced milk is in fact processed into mozzarella cheese, and the increased demand for this cheese, both on the national and international market, together with the milk quotas (i.e. taxes on surplus cow milk production) imposed by the European Union, have favoured the increase of buffalo production. The Italian Ministry of Agriculture is responsible for the milk recording and selection activity through two specific organizations, the Italian Breeders' Association (AIA) and the Buffalo Breeders' Association (ANASB) which provide the technical staff for performing

these activities. The numbers for milk recorded buffaloes (2002) was 35 755, i.e. 28.6 percent of the buffalo population; average lactation milk production was 2 168 kg (270 days); fat content 8.28 percent and protein content 4.73 percent. Fat and protein content analysis is compulsory in the Italian milk recording system because the two results are included in the estimation of the genetic merit of milk, giving the highest importance to protein content, due to the conversion of the milk into mozzarella cheese. Recorded buffaloes are raised in 292 herds in 36 Italian districts (the average herd size is 122).

Selection activity started in the 1980s; five progeny testing cycles were performed from 1987 to 1994, testing comprehensively 43 bulls, and providing 17 positive bulls. Two more progeny testing cycles were performed from 1998 to 2002. In these two last cycles eight bulls were put on-test. 14 477 semen doses were produced from these bulls and 3 718 buffaloes were inseminated. The remaining available doses are 6 350. In 2003-2004 a further cycle was initiated during which four new bulls will be progeny tested.

The keeping of bulls and semen collection is performed in two different AI stations. Bull and cow genetic merit for milk and mozzarella production are published in special catalogues that are produced by ANASB biannually. In the catalogue, the top one percent of Italian buffalo cows with the highest genetic merit for mozzarella and milk production are listed.

Punjab (Pakistan)

The milk recording system and selection activity is implemented by the Livestock and Dairy Development Department of Punjab, through the Livestock Production Research Institute of Bahadurnagar. Six thousand buffaloes are milk recorded in seven very large herds (over 500 buffalo) belonging either to the research institute or to the army, as well as in 27 field recording centres, so including buffaloes in smallholdings of 5 to 20 animals. Average milk production (year 2000) was 1 823 kg (257 days). Fat and protein content are recorded only for the research herds. Breeding of buffalo is mainly through natural mating bulls, however, at the government livestock farms, 100 percent of females are inseminated by frozen semen from proven or on-test bulls. For bull selection a progeny-testing programme began in 1980, where high pedigreed bulls are selected on the basis of the milk production performance of their daughters. Government livestock farms are the principal centres for bull production. In Punjab, semen is produced and stored at Semen Production Units in Qadirabad (Sahiwal), Kalurkot (District Bakhar) and Karaniwala (District Bahawalpur) under the Directorate of Breed Improvement of Punjab, which controls the production and distribution of semen throughout the country.

North West Frontier Provinces - NWFP (Pakistan)

Twenty-five thousand buffaloes are milk recorded in the NWFP; the majority of them (76 percent) are raised in small herds of one to five animals, while 23 percent are raised in medium-size herds (6 to 20 animals). In these herds the owner himself records buffalo milk yield. There are also three public herds, owned by the Animal Husbandry Training Institute of Peshawar, by the Agricultural University and by the Pakistan Army, where the milk recording is performed by technical staff.

Five hundred breeding stations exist in the NWFP, each of them usually keeping one bull of five years of age. The stations are owned by private farmers and the milk genetic merit of the bull is judged according to the performance of his dam. Seventy-three percent of small owners (two to five animals) take their buffaloes to a breeding station, while only thirty percent of medium-size herds (up to 20 animals) take their buffaloes there.

There are three AI stations in the NWFP, two private ones and one run by the Government, keeping a total of 18 on-test bulls. Fifty thousand semen doses are produced annually. One percent of small herds (one to five animals) uses AI while five percent of bigger herds use it. The majority of farmers is given only one semen dose per buffalo; few of them request two doses. Conception at the first oestrus is 50 percent and the conception rate per year is two

inseminations per conception.

Ten percent of small owners raise their own breeding bull, while 20 percent of medium-size herds (up to 20 animals) does the same; 10 percent of medium-size owners buy an adult bull from a different owner. In the bigger herds, which number about 10 000 in the NWFP, breeding bulls are raised from the calves born in the same herd.

The milk genetic value of the bull is judged from the dam's performance. Buffaloes with an average milk yield of above ten litres per day are considered "Elite" buffaloes. Information on buffalo productivity is provided by the extension, research or university veterinarians, NGOs agents, other farmers, postgraduate students, farmers from the province of Punjab or animal dealers, when the breeding bull is purchased from outside the province.
It is estimated that 12 percent of buffaloes are kept grazing all day in village fields and therefore bred there by the grazing bulls.

Egypt

The Cattle Information System/Egypt (CISE) of the Cairo University records about 290 small (one to five animals), 27 medium (six to 20) and six large herds. Due to a lack of financing, fat and protein content cannot be recorded.

The Ministry of Agriculture and Land Reclamation (MALR) through the Animal Production Research Institute (APRI) records four State herds, belonging to APRI, the sizes of which are respectively 50, 70, 75 and 80 and 500 breedable females. The Breeders' Service Unit of APRI provides free complete milk analysis and Somatic Cell Count for the enrolled herds.
CISE is the only institution in Egypt performing data analysis centrally, producing monthly herd summaries and individual milk yield information. Calculation of the genetic merit of recorded buffaloes and breeding bulls is in progress.

The average milk production of milk recorded buffaloes (year 2002) is 2 030 kg (312 days) and the fat content is 8.2 percent. There are six breeding stations with a total of 60 bulls with an average age of five years. These stations belong either to APRI or MALR. All smallholders (one to five animals) take their buffaloes to the breeding stations, as well as 20 percent of the medium size (6 to 20) owners. In bigger herds, breeding bulls are mainly raised from their own male calves although 20 percent of them buy adult buffaloes (two to three years) from different owners. In all cases, breeding bulls are chosen on the basis of pedigree and performance results of the dams, when provided by CISE.

Artificial insemination is used in one percent of the medium to large herds. There are six AI stations owned by the Government and one by the University, possessing a total of 70 bulls.
Artificial insemination is still performed at research level; usually only one semen dose is offered at each oestrus, conception at the first oestrus being 30 percent.

Iran

In Iran milk recording and the selection activity is implemented by the central government through the Animal Breeding Centre of Karaj. The number of recorded buffaloes was 13 236 in 2003. Besides the official recording system provided by government technical staff, there is a semi-private system of recording performed by the farmer himself and by the staff of the local cooperative of farmers. In both cases, executive operations are supervised by the Animal Breeding Centre.

The semi-private system is more popular in the small herds (one to five animals) where 7 100 buffaloes are milk recorded.

There are no breeding stations in Iran, but two performance testing/AI stations, one in West

Azerbaijan (Jabal station), keeping ten bulls and one in Kuhzestan which will be inaugurated in 2004 with a capacity of 50 bulls. Bulls are preselected by provincial experts based on maternal performance and body type and then taken to the station at the age of between 6 and 18 months. Genetic merit of these bulls is estimated against an animal model which includes milk and fat yield, as well as body type parameters. Twenty-thousand semen doses are produced yearly by the Jabal AI station. Artificial insemination is still performed at a low level, since the activity only started in the year 2000; it is estimated that about 200 recorded buffaloes are offered AI yearly; two insemination at each oestrus are always offered, the conception at the first oestrus being 50 percent.

Smallholders (one to five animals) that rear 72 percent of buffaloes in Iran, grow their own calf to become a breeding bull in 50 percent of cases, they borrow a bull from a neighbour during the breeding season in 10 percent of cases, but leave their buffaloes to be bred in village fields by unknown bulls in 40 percent of cases. Medium-size farmers (6 to 20 animals) that rear 23 percent of buffaloes in Iran, grow their own calf to become a breeding bull in 40 percent of cases, they borrow a bull from a neighbour during the breeding season in 10 percent of cases, buy a bull from another farm with proven milk genetic merit provided by the Breeding Centre in 5 percent of cases, but leave their buffaloes to be bred in village fields by unknown bulls in 45 percent of cases. Bigger farmers (over 20 animals), that rear five percent of buffaloes, in 60 percent of cases they borrow a bull from a neighbour during the breeding season, or buy a bull from another farm with proven milk genetic merit provided by the Breeding Centre in 15 percent of cases, but never leave their buffaloes to be bred in village fields.

The milk recorded herds are provided with a wide set of information on the productivity of their buffaloes and breeding values of males that are centrally calculated from the productions of their daughters and related females.

Three breeds of buffalo are reared in the different regions of Iran: Azeri (70 percent); Kuhzestani (22 percent) and Mazandarani (eight percent). Average lactation milk production and lactation duration of the three breeds are as follows (2003): Azeri: 1 500 kg milk in 210-220 days; Kuhzestani: 1 950 kg milk in 210-240 days; Mazandarani: 1 300 kg milk in 220-230 days.

Brazil

Milk recording activity in buffalo of the various imported breeds (Murrah, Mediterranean and crossbred) is performed only in the research herds and in a few private herds for research purposes. About 500 buffaloes are recorded on an annual basis. Average milk production is 1 290 kg (241 days); fat and protein percent are respectively 7.04 and 4.25.

Bulgaria

Milk recording and selection activity in Bulgaria is promoted and executed by the Regional Agency for Selection and Reproduction with scientific and technical support from the Agricultural Institute, Department of Buffalo Breeding, Shumen.

The average milk production of the recorded buffaloes is 1 874 kg (278 days lactation); fat and protein percent are respectively 7.56 and 4.51.

The majority of buffaloes (3 976 i.e. 80 percent of the total population) are reared in small herds (one to five animals); in this herd-size class only 300 buffaloes are milk recorded. Consequently, no information is available to these farmers for improving buffalo productivity: 90 percent of them leave their buffaloes to be bred in village fields; however, five percent of them make use of the governmental breeding station, two percent buy a bull from another owner and three percent take the buffaloes to other herds for mating. Medium-size owners (6 to 20 animals) that rear 8.5 percent of the total population) use AI in five percent of cases, go to the breeding

station in another five percent of cases, and grow their own bull, or buy a different bull in 50 percent of cases; 40 percent of them leave their buffaloes to be bred in village fields. In addition there are ten larger herds (20 to 500 animals). In these herds AI is employed on over 70 percent of buffaloes and a proven genetic merit bull is purchased from other owners in 25 percent of cases.

There is one breeding station in Bulgaria, owned by the government, possessing three to four bulls on an annual basis. The genetic merit of these bulls is estimated using a BLUP Animal Model developed from the records of daughters and related animals. These calculations are performed by the Agricultural Institute, Department of Buffalo Breeding, Shumen.

In addition, there are two AI stations possessing four bulls, which provide 1 320 semen doses every year, of which 1 050 semen doses are from proven bulls. Buffalo are offered two or more inseminations at each oestrus; the conception rate at first oestrus is 45 to 55 percent.

Nepal

There are three institutional herds currently being milk recorded in Nepal. The Department of Livestock Services maintains a breeding herd of about one hundred Murrah cows and five to seven Murrah bulls which provide young bulls for dispersal under the crossbreeding programme throughout the country. The Agricultural Research Stations of the Nepal Agricultural Research Council (NARC) maintain two herds (in Lumle and Tarahara) of hill buffaloes (Lime, Parkote and Tarai breed) with the purpose of assessing the performance of indigenous stock in station production environments. In addition, the Agricultural Research Station, Lumle, has been carrying out milk recording activity in farmer buffaloes for the past 13 years in the western hill area.

Crossbreeding of indigenous buffaloes with Murrah has been the national policy of the genetic improvement programme. Both natural and AI methods are used. In the past, unrestricted grading up with Murrah blood has been the policy throughout the country, but recently, particularly in the central hill area, a limit of 62.5 percent Murrah blood was considered.

Average milk production (305 days lactation) is 1 372 kg; 1 048 kg and 1 031 kg respectively for the Murrah, Lime and Parkote breeds.

There are five breeding stations in Nepal, possessing in total 35 bulls, aged six years.
There are a total of 179 AI service centres in 43 accessible districts. The central Animal Breeding and AI Section in Kathmandu valley and the Regional Semen Banks supply frozen semen to these AI centres. Eleven thousand semen doses are produced annually. Each buffalo is offered 1.2 semen doses at each oestrus and the conception rate per year is 35 percent.

Turkey

No milk recording systems for buffalo at the national level are established in Turkey. However, good examples of recording activity are found in two research herds, the first owned by the Mustafa Kemal University of Antakia (Hatay province) and the second at Kocatepe Research Institute. The Department of Animal Production of the Mustafa Kemal University of Antakia performs milk recording activity in buffalo herds of the Hatay province. In total the milk performance of about 200 buffaloes is recorded.

No breeding station for buffalo or AI station exists in the country.

AI was provided to buffalo herds in the Hatay province as part of an FAO development project using semen from proven Italian bulls in the year 2002.

83 percent of buffaloes in Turkey are raised by smallholders (one to five animals); the

remaining 17 percent are raised by medium-size farmers (eight animals on average). All these farmers, except the mentioned recorded ones, leave their buffaloes to be bred in village fields by unknown bulls.

Azerbaijan

Milk recording in Azerbaijan is performed only for buffaloes of the four nationally controlled herds, that raise altogether 240 adult buffalo females.

Fifty-five percent of all buffaloes are raised in small herds (one to five animals); 12 percent in medium-size ones (6 to 20 animals); 21 percent in large herds (21 to 100 animals); there are moreover 101 herds comprising more than 100 buffaloes. All farmers grow their own bull replacements from their calves; small farmers often buy bulls from other owners at the age of three to five years. The practice of leaving buffaloes to be bred in village fields is not used.

China

Although the Chinese buffaloes are Swamp type, therefore not milking buffaloes, the perspectives of the dairy buffalo sector in China cannot be ignored in this context. Contrary to popular opinion, the Chinese are content to consume dairy products. The market for dairy products is growing by 18 percent per annum, the second fastest growth in the world after Brazil. In this context the Ministry of Agriculture, through its Dairy Project Office, has drafted a publication entitled "Dairy and beef buffalo sector investment promotion guide book for domestic and foreign investors (1999)".

The first dairy buffalo herd composed of Indian Murrah was introduced in Guandong in 1948. Further batches of Murrah were introduced in Guanxi and Hubei in 1957, and in Huizhan City and Zhanjiang in 1960. In 1962 and 1974 further Nili Ravi and Murrah breeding animals were imported. In 1987 the Ministry of Agriculture established a National Buffalo Development Project, and in 1996 a financing agreement for the water buffalo development project was signed with the European Union.

In 1997, 100 paillettes (straws) of Murrah semen were made available to the Buffalo Institute of Nanning. The defined target dairy buffalo provinces are: Guangdong, Guanxi, Yunnan, Jiangxi and Sichuan. The EU-China Water Buffalo Development Project is based on the following assumptions: investment in dairy processing plants; import of 100 000 to 200 000 semen doses from foreign countries for crossbreeding and improvement of milk production of indigenous buffaloes.

Buffalo genome research and applications

DNA technology in buffalo has been principally applied for parentage verification. DNA profiling has become a major tool in paternity verification and forensic medicine in humans, and among the various types of markers, that have been used for creating the profile, microsatellite-based markers overcome many of the difficulties associated with the previously used ones (RFLPs and genetic fingerprints produced by minisatellite probes) (Botstein et al., 1980; Jeffreys et al., 1991), which either suffer from low polymorphic information content or are difficult to interpret. Microsatellite sequences are stretches of tandemly repeated short sequence motifs, one to six nucleotides in length; polymorphisms arise from the difference in number of times the motif is repeated, and they have the advantage that, being 100-300 nucleotide long, they can be amplified by polymerase chain reaction (PCR) (Usha et al., 1995). A set of polymorphic DNA microsatellites useful in Swamp and River buffalo was produced by Moore et al. (1995) and the protocols for two microsatellite multiplex were referred by Blasi et al. (2003). The first multiplex includes: microsatellites INRA006, CSSM42, CSSM47, CSSM19, D5S2, MAF65, RM4, CYP21 and BM1013 and the second multiplex includes: microsatellites CSSM70, CSSM60, INRA026, BM0922, and BM1706. The two multiplex are routinely used for buffalo parentage verification in Italy and give a probability of exclusion of 0.99999.

DNA microsatellites, furthermore, have found widespread application in population genetics. FAO recommended the use of high polymorphic microsatellite markers in the programme strategy for the measurement of domestic animal diversity (FAO, 1998).
Barker et al. (1997) estimated the genetic diversity between and within eight Swamp and three River buffalo breeds based on the variation of 21 microsatellite loci. They found a significant differentiation between the Swamp and the River types, and among population within each buffalo type.
Moioli et al. (2001) analysed the genetic diversity between Italian, Greek and Egyptian buffalo populations, based on 13 microsatellite loci, showing that the differentiation between the Italian and Greek buffaloes was irrelevant, while the differentiation between the Egyptian and the other two is higher than the one found between the river populations in Asia examined by Barker et al. (1997).
The paper by Moioli et al. (2001) indicates also that the genetic analysis undertaken using the 13 microsatellites reflects effectively the isolation by distance of different populations, and that the genetic distances between groups of sampled animals of the same population describe the geography of the country.

References

Barker, J.S.F., Moore, S.S., Hetzel, D.J.D., Evans, D., Tan, S.G. and Byrne, K. 1997. Genetic diversity of Asian water buffalo: microsatellite variation and a comparison with protein-coding loci.

Blasi, M., Lanza, A., Rosati, A., Varlotta, C., Loffi, C., Di Gregorio, P., Coletta, A. and Gubitosi, L. 2003. A new protocol for genotyping microsatellites in buffalo. 54th Annual Meeting of the European Association for Animal Production, Rome, Italy, 31 Aug. to 3 Sep.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics, 32: 314-331.

FAO, 1998. Secondary Guidelines for the Development of National Farm Animal Genetic Resources Management Plans: Measurement of Domestic Animal Diversity (MoDAD). Rome.

Jeffreys, A.J., Macleod, A., Tamaki, K., Neil, D.L. and Mossekton, D.J. 1991. Minisatellite repeat coding as a digital approach to DNA typing. Nature, 354: 204 209.

Moioli, B., Georgoudis, A., Napolitano, F., Catillo, G., Giubilei, E., Ligda, Ch. and Hassanane, M. 2001. Genetic diversity between Italian, Greek and Egyptian buffalo populations. Liv. Prod. Sci., 70: 203-211.

Moore, S.S., Evans, D., Byrne, K., Barker, J.S.F., Tan, S.G., Vankan, D. and Hetzel, D.J.D. 1995. A set of polymorphic DNA microsatellites useful in swamp and river buffalo. Animal genetics, 26: 355-359.

Usha, A.P., Simpson, S.P. and Williams, J.L. 1995. Probability of random sire exclusion using microsatellite markers for parentage verification.

Chapter III

BUFFALO BREEDS AND MANAGEMENT SYSTEMS

Bianca Moioli and Antonio Borghese

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

BUFFALO BREEDS

Breeds (only for the River subspecies) are listed in alphabetical order. Breed names are those given in Mason (1996). A World Dictionary of Livestock Breeds, Types and Varieties, Fourth Edition. Wallington, UK. Population size is given for years 1999-2004, according to the different sources.

1. Anatolian

The Anatolian buffalo has been raised in Turkey for centuries, originating from Indian migration (7th Century), together with the expansion of Islam.
Population size: 110 000
Description: Black in colour, long hair, with variation in tail length and frequent white switch. Height at withers of adult male is 138 cm, body weight is 200-500 kg.
Height at withers of adult female is 138 cm, body weight is 200-500 kg.
Distribution: Concentrated in the Black Sea region, North of Middle Anatolia, Thrace, Hatay, Mus, Kars, Dyarbakir, Afyon, Sivas.
Husbandry: In dairy farms, housing differs from region to region. If grazing is available, the three to five buffaloes owned by the family are taken to graze together with the other buffaloes from the village. Mating and calving occur at the pasture. Generally on the ground floor of each house there are barns to keep the buffaloes in winter. The barns have no windows and the doors are tightly closed. Young animals are never taken outdoors in winter in the cold climates. Buffaloes are slaughtered together with cattle. Milking is done by hand except at the two existing research stations. Average slaughter weight is 300-350 kg, at the age of 18-20 months. Carcass yield is 53-55 percent. Overall growth rate is 400 g/day.

Dairy performance:

Lactation duration	220-270 days
Milk yield	700-1 000 kg
Milk fat	6.6-8.1 percent
Milk protein	4.2-4.6 percent

Products: a semi-hard cheese called "peyaz peyneri" is made from buffalo milk.
Ayran is a drink with water and buffalo yoghurt. Buffaloes are raised for milk production only as source of income that does not require any expenditure, i.e. in the areas that have natural feeding conditions. The price of buffalo milk is only slightly higher than the price for cows' milk. Meat production is all converted into sausages. The price of buffalo meat is 10 percent less than the price for beef.
Sources: Sekerden et al., 1996a,b; Sekerden et al., 2000, Borghese, 2005.

2. Azeri or Caucasian

This breed originates from the Indo valley (Indian buffalo). There is some evidence that buffalo were raised in Lorestan (Iran) in the 9th Century B.C. since six engraved buffalo heads have been found on a bronze stick from this period.
Population size: 600 000
Description: Black in colour, short horns growing backwards.
Height at withers of adult male is 137 cm, body weight is 400-600 kg.
Height at withers of adult female is 133 cm, body weight is 400-600 kg.
Distribution: In Iran, they are found in West Azerbaijan, East Azerbaijan and the Caspian Sea. In Azerbaijan, everywhere. In Georgia and Armenia, they were widespread until 1940, but then declined.
Husbandry: Housing differs from region to region. They are generally untethered in summer and tied up in winter. In some areas, milking females are tethered all year round.
Average slaughter weight is 300 kg, at the age of 15 months. Carcass yield is 50 percent. Overall growth rate is 420 g/day.

Dairy performance:

Lactation duration	200-220 days
Milk yield	1 200-1 300 kg
Milk fat	6.6 percent

Products: Milk, yoghurt, fresh cream, fresh cheese, butter, ice-cream, rice pudding, churned yoghurt, dried whey, ghee.
In Iran, the price of buffalo milk is twice that of cows' milk. Buffalo skin is used in the leather industry. Buffalo manure is used for fuel in rural areas.
Sources: Latifova, 2000; Turabov, 1991; Turabov, 1997a,b; Naderfard and Qanemi, 1997; Marmarian, 2000; Qanemi 1998; Borghese, 2005.

3. Bangladeshi

Population size: 5 000
Description: Black in colour, white spot on the forehead and tail-switch in some cases. Curled and short horns.
Indigenous Bangladeshi buffaloes of the River type are found in the South-West. In the remaining parts of the country they are either Swamp or crosses of exotic breeds: Nili-Ravi and Murrah type.
Sources: Faruque, 2000.

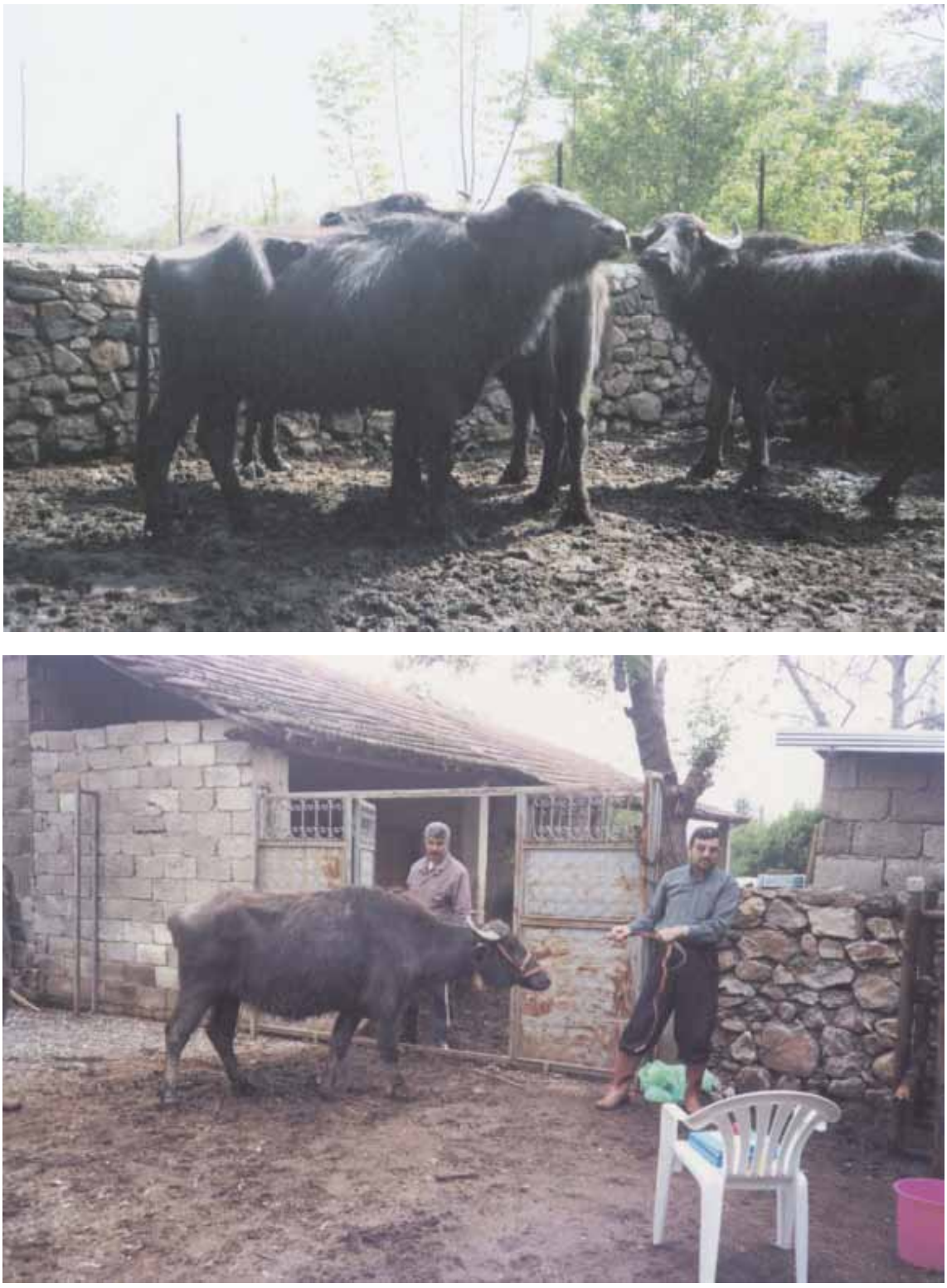


Figure 1, 2. Anatolian buffaloes in Ilikpinar village (Hatay, Borghese photo, 2002)

Figure 3, 4. Azeri buffaloes (Borghese photo, 2003)

Figure 5. Azeri buffalo, Iran, Mazandaran (Naderfard H. photo, Iran)

Figure 6. Bangladeshi buffaloes in coastal area (Faruque O. photo)

4. Bhadawari

This is an improved local breed. It is the result of selection of Indian breeds of buffalo. It is considered the best breed of buffalo in Uttar Pradesh.

Population size: 30 000.
Description: Copper coloured coat, scanty hair which is black at the roots and reddish brown at the tip. Sometimes it is completely brown. The neck presents the typical white colour ring. Tail switch is white or black and white. Horns are short and grow backwards.
Height at withers of adult male is 128 cm, body weight is 475 kg.
Height at withers of adult female is 124 cm, body weight is 425 kg.
Distribution: It is raised in the Agra and Etawa districts of Uttar Pradesh and in Bhind and Morena districts of Madhya Pradesh.
Husbandry: Buffaloes are traditionally managed under domestic conditions together with the calf. They are hand-milked twice a day. They are fed different kinds of roughages (barley and wheat straw, cornstalks, sugar cane residuals). In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are naturally mated. Some villages also provide artificial insemination.

The performance characteristics of the Bhadawari breed maintained at the Indian Grassland and Fodder Research Institute (IGFRI), Jhansi Centre (India) of the Network Project on Buffalo are presented below (Sethi, 2003):

Average body weight (kg)	385.5
Age at first calving (months)	48.6±0.58
First lactation 305 days or less yield (kg)	711±25
All lactation 305 days or less yield (kg)	812±23
All lactation total yield (kg)	781±29
All lactation length (days)	272±4
Average fat (percent)	7.2±0.4 to 13
Average dry period (days)	297±24
Service period (days)	179±10
Calving interval (days)	478±11
Average calf mortality (0-3 months)	12.15 percent

Sources: Alexiev, 1998; FAO, 2003; Sethi, 2003.

Figure 7. Bhadawari cow (Sethi, 2003)

Figure 8. Bhadawari bull with the typical ring on lower side of neck

5. Bulgarian Murrah

From 1962 to 1990, Murrah buffaloes from India were imported into Bulgaria and a new population of buffalo was created by upgrading the local buffalo.

Population size: 14 000
The buffalo population in Bulgaria has dramatically declined since the Second World War, with the advent of Holstein and mechanization. Furthermore, after 1989, privatization forced the cooperative buffalo farms to close down. The private sector is composed of small units which has made selection and recording more difficult.
Description: Black or black and brown or dark grey in colour.
Body weight of adult male is 700 kg.
Body weight of adult female is 600 kg.
Distribution: All over Bulgaria, Romania and South America.
Husbandry: Buffaloes are traditionally managed under domestic conditions together with the calf. They are hand-milked twice a day. Some milking machines are now available. During winter, they are kept in sheds and are fed different kinds of roughages: barley and wheat straw, cornstalks. In addition, they are given concentrate mixtures, sometimes mixed with beet pulp. During the summer, they graze all day long in the marshy areas and in the evening they return to their sheds. They are mated mainly through natural mating. Some villages provide artificial insemination. In state buffalo farms (200-400 buffaloes) they are managed according to their condition: heifers, lactating, pregnant, dry. Milking buffaloes are kept in closed sheds and tied up. During winter, they are allowed outside in paddocks for part of the day, in summer they are allowed to graze. They are always given concentrate mixture in addition to roughage. AI is used on all buffaloes.
Average slaughter weight is 400 kg, at the age of 16 months. Carcass yield is 50.4 percent. Overall growth rate is 750 g/day.

Dairy performance:

Lactation duration	270-305 days
Milk yield	1 800 kg
Milk fat	7.04 percent

Products: Yoghurt and milk by-products. Processed meat products are very important: all kinds of salami and sausages, Pastarma, lukanska and flat sausages.
Sources: Peeva et al, 1991; Peeva, 1996; Alexiev, 1998.

Figure 9, 10. Bulgarian Murrah bull and herd (Alexiev, 1998)

6. Egyptian

Buffaloes were introduced into Egypt from India, Iran and Iraq approximately during the middle of the 7th Century.

The distinction between the different types of Egyptian buffaloes is only environmental. It is the most important and popular livestock for milk production in Egypt.

Population size: 3 717 000
Description: Blackish grey in colour, horn form varies from lyre to sword-shaped. The head is long and narrow, the jaws are long and strong. Ears are long and dropping. The neck is rather long, thin and straight. The forelegs are rather short and heavy boned. Ribs are wide, deep and well sprung. The rump is sloping and the tail setting is low.
Height at withers of adult male is 178 cm, body weight is 600 kg.
Height at withers of adult female is 144 cm, body weight is 500 kg.
Distribution: All over the country, mainly in peri-urban areas and the Nile delta.
Husbandry: The farmer keeps manure in a solid state inside the animal enclosure. The solid manure is taken twice a year and spread in the fields before planting. The animals are slaughtered only in slaughterhouses, following the Islamic practice of cutting the jugular vein. Milking is done by hand, twice a day, mainly by women.
Average slaughter weight is 500 kg, at the age of 18-24 months. Carcass yield is 51 percent. Overall growth rate is 700 g/day.

Dairy performance:

Lactation duration	210-280 days
Milk yield	1 200-2 100 kg
Milk fat	6.5-7.0 percent

Products: The following cheeses are produced with the addition of cow milk: Domiati, Karish, Mish, Rahss.
Sources: El Kirabi, 1995; Nigm, 1996; Ragab and Abdel Salam, 1963; Mokhtar, 1971; Askar et al., 1973; Borghese, 2005.

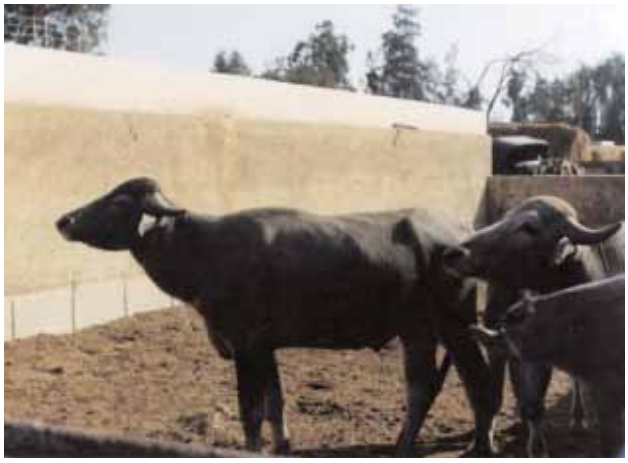


Figure 11, 12. Egyptian buffaloes from Fayum oasis (Borghese photo, 1996)

7. Jafarabadi

The existence of the Jafarabadi breed in Gujarat (India) is referred to in 1938.
Population size: 600 000
Description: Black coloured coat. Massive and long-barreled conformation. Horns are long, heavy and broad and sometimes they cover the eyes.
Height at withers of adult male is 142 cm, body weight varies from 600 to 1 500 kg.
Height at withers of adult female is 140 cm, body weight is about 550 kg, some individuals may weigh as much as 700-800 kg.
Distribution: It is one of the most important breeds in Gujarat. This breed is located principally between the Mahi and Sabarmati rivers in north Gujarat. Some breeding stock has been exported to Brazil.
Husbandry: Buffaloes are traditionally managed in domestic conditions together with the calf. They are hand-milked twice a day. They are fed different kinds of roughages: barley and wheat straw, cornstalks, sugar cane residuals. In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are naturally mated. Some villages also provide artificial insemination.

Dairy performance:

Lactation duration	350 days
Milk yield	1 800-2 700 kg
Milk fat	8.5 percent

The performance characteristics of the Jafarabadi breed maintained at the Junagarh Centre (India) of the Network Project on Buffalo are presented below (Sethi, 2003):

Average body weight (kg)	529±13
Age at first calving (days)	1 925±196
First lactation 305 days or less yield (kg)	1 642±283
First lactation total yield (kg)	1 642±283
All lactation 305 days or less yield (kg)	1 950±79
All lactation total yield (kg)	2 097±110
All lactation length (days)	320.1±11.6
Average fat (percent)	7.7±1.0
Average dry period (days)	159.8±10.9
Service period (days)	161.5±14.0
Calving interval (days)	509.8±20.1
Number of services per conception	1.4±0.1
Average calf mortality (0-3 months)	10.75 percent

Sources: Alexiev, 1998; Trivedi, 2000; Sethi, 2003.

Figure 13. Buffalo cows of Jafarabadi breed in Brazil (Alexiev, 1998)

8. Jerangi

Description: Black in colour, with small horns running backwards. It is a small animal.
Distribution: It is localized along the border of Orissa with Andhra Pradesh.
Husbandry: Buffaloes are traditionally managed in domestic conditions together with the calf. If grazing is available, they graze all day long. They are naturally mated.
It is a draught animal with a rapid pace.
Sources: Cockrill, 1974; FAO, 2003.

9. Kuhzestani or Iraqi buffalo

Population size: 200 000
Description: Horns are short and grow upward forming a ring at the end. In size, it is very likely the biggest buffalo breed in the world.
Height at withers of adult male is 148 cm, body weight is 800 kg.
Height at withers of adult female is 141 cm, body weight is 600 kg.
Distribution: In Iran, they are located in Kuhzestan and Lorestan. In Iraq, mainly in the South, in the peri-urban areas of Baghdad and Mosul.
Husbandry: Buffaloes are raised outdoors all through the year. They are housed in paddocks made of local plants (reeds, brushes, palm leaves) with a wall on one side, and three open sides. They are hand fed at the time of milking, morning and evening, with available green forage. They are also fed any type of by-products: waste of sugar cane, reeds from marshy land, home baked wastes. Those that swim in ponds and rivers are also fed aquatic plants.
Milking is done by hand in 95 percent of cases and in a few cases with movable milking machines, there are no milking establishments. Male buffaloes are very hazardous, strong and difficult to handle and always aggressive to humans. In a few cases, for tilling operations, they are castrated. Females are very sensitive to non-familiar persons and reduce milk yield with non-familiar milkers. Generally females are also not docile. Average slaughter weight is 400 kg, at the age of 12 months. Carcass yield is 50 percent. Overall growth rate is 580 g/day.

Dairy performance:

Lactation duration	200-270 days
Milk yield	1 300-1 400 kg
Milk fat	6.6 percent

Products: Milk, yoghurt, fresh cream, fresh cheese, butter.
Sources: National Buffalo Project, 1988; Magid, 1996; Saadat, 1997; Borghese, 2005.

Figure 14, 15. Iraqi buffalo near Mosul (Iraq) on the Tigris river (Al-Jamass R. photo)

10. Kundi

Domestication of draught animals in the Indus valley civilization is referred to about 4 500 years ago.
It is the second most important breed in Pakistan.
Population size: 5 500 000.
Description: Black in colour, short horns.
Height at withers of adult male is 135 cm, body weight is 700 kg.
Height at withers of adult female is 125 cm, body weight is 600 kg.
Distribution: Widespread in South Pakistan Sindh region.
Husbandry: Buffaloes are traditionally managed under domestic conditions together with the calf. They are hand milked twice a day. They are fed different kinds of roughages: barley and wheat straw, cornstalk, sugar cane residuals. In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are mated mainly through natural mating. Some villages also provide artificial insemination.
There are a few state buffalo farms with 500 to 1 000 milking buffaloes.

Dairy performance:

Lactation duration	320 days
Milk yield	2 000 kg
Milk fat	7.0 percent
Milk protein	6.0 percent

Sources: Alexiev, 1998; Tunio A.N., 1999.

11. Lime

The pure Lime breed is believed to have originated from the wild Arna and has been domesticated throughout the known history of Nepal.
The Lime buffalo is estimated to constitute 35 percent of the total indigenous buffalo population in the hills and mountains of the country.
Population size: 700 000
Description: Light brown colour, small body size, characteristic chevrons of grey or white hair below the jaws and around the brisket, small sickle-shaped horns, curved towards the neck.
Height at withers of adult female is 115 cm, body weight is 399 kg.
Distribution: The breed is found in the mountains, high hills and hill river valleys in Nepal. It is not found in the Terai plane.
Husbandry: Mainly raised under migratory conditions or semi-stall systems. The breed is a voracious eater and is fed only low quality feedstuff such as rice, wheat and millet straw. Small farmers exchange breeding animals within and between villages. Among the migratory herds, male and females are grazed together and mate freely during the breeding season from June to November.
Females are legally banned from slaughter; only culled animals are slaughtered for meat.

Dairy performance:

Lactation duration	351 days
Milk yield	875 kg
Milk fat	7.0 percent

Products: milk, ghee, meat, swiss-cheese, yoghurt, leather.
Sources: Rasali, 1997; Rasali, 1998a,b.

Figure 16, 17. Typical Lime buffalo (Rasali D. Photo, Nepal)

12. Manda

This is an improved local breed, resulting from the selection of Indian breeds of buffaloes.
Population size: 100 000
Description: Uni colour: grey, brown.
Distribution: It is raised along the border of Orissa with Andhra Pradesh.
It is a hardy breed, able to work under the hot sun. It is not very demanding in terms of feeding and acclimatizes very easily to various conditions.

Dairy performance:
Milk yield 4 kg/day
Products: Milk, ghee, cream, meat.
Sources: Cockrill, 1974; FAO, 2003.

13. Mediterranean or European

The Mediterranean buffalo originates from the Indian buffalo. It was introduced into Europe with the advent of Islam and the Arab occupation as well as through other central European conquerors in the 6th and 7th Centuries.
The buffalo population in Europe has been dramatically declining since the Second World War, with the advent of Holstein and mechanization.
Population size: 400 000
Description: Black, black and brown, dark grey coat. Horns are flat at the bottom, backwards and slightly outwards pointed, and backwards straightened; the top is pointed inwards. They have a compact conformation with a deep and wide chest as well as a developed pectoral. The back is short. The rump is short. The udder is medium size with squarely placed quarters and halves; the teats are cylindrical. Where machine milking is popular (only in Italy) udders are more regular and better shaped. Size, weight and productivity vary a lot according to the environment and management. Average herd size is below five breedable buffaloes in most countries, except in Italy where it is 90. The proportion of breedable females to total buffaloes is about 45 percent except in Italy where it is 62 percent, since males have little market potential.
The body weight of the adult female is 450-650 kg.
Distribution: Italy: 265 000 (Mediterranean Italian breed); Romania: 100 000; Brazil: 10 000; a few thousand in Greece, Albania and TFYR Macedonia; a few hundred in the United Kingdom, Germany, The Netherlands, Switzerland and Hungary.

Husbandry: The most common housing system is the one referred to as traditional, consisting of keeping buffaloes indoors at night and confined in fenced areas during the day. In the favourable season they are allowed to graze during the day. In Italy, they are housed loose in paddocks all year long, with the same modern systems used for dairy cows. One third of Italian buffaloes are also put out on pasture in the favourable seasons, or green forage "cut-and-carry" such as alfalfa can also be used. Maize silage, concentrates and by-products are the basic foodstuffs in Italy.

Performance varies very much depending on the area. There is no common practice to wean buffalo calves. When milking is done by hand, both male and female calves suckle from the dam. In some cases they suckle from a dairy cow. This results in a wide difference in daily gain up to weaning, as well as weaning weight and age. Males are now in greater demand as meat producers, therefore increased attention is being paid to their feeding and health.

Average daily milk yield reveals a huge variability, mainly depending on the feeding system. It can range from 3 to 4 kg milk/day for poorly fed animals to 15 kg/day in intensive management systems. In Bulgaria, Romania, TFYR Macedonia, Greece and Albania, extensive management systems are employed.
Average slaughter weight is 250-400 kg, at the age of 12-15 months.

Dairy performance:

Lactation duration	270 days
Milk yield	900-4 000 kg
Milk fat	8.0 percent
Milk protein	4.2-4.6 percent

Products: Mozzarella, treccia, scamorza and other cheeses, ricotta (Italy); Vladaesa cheese, Braila cheese (Romania); White brine cheese (Bulgaria, Romania); yoghurt, meat and meat industry products: bresaola, salami, sausages, cacciatorini (little salami), etc.,
Sources: Borghese and Moioli, 1999; Borghese et al., 2000; Borghese, 2005; Stravaridou, 1998; Bikocu, 1995; Popovici, 1996; Bunewski, 2000.

Figure 18, 19. Mediterranean Italian buffalo cow and herd in intensive system, Tor Mancina (Rome). (Borghese photo, 2004)

14. Meshana

The existence of the Meshana breed in north Gujarat, India, is referred to in 1940. This breed is the result of selection of Indian breeds of buffalo.
Population size: 400 000
Description: Characteristics are intermediate between Surti and Murrah. Jet black skin and hair are preferred. Horns are sickle-shaped but with more curve than the Surti. The udder is well developed and well set. Milk veins are prominent.
Body weight of adult male is 570 kg.
Body weight of adult female is 430 kg.
Distribution: Concentrated between the Mahi and Sabarmati rivers in Gujarat (India).
Husbandry: Buffaloes are traditionally managed under domestic conditions together with the calf. They are hand-milked twice a day. They are fed different kinds of roughages: barley and wheat straw, cornstalks, sugar cane residuals. In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are naturally mated. Some villages also provide artificial insemination.

Dairy performance:

Lactation duration	305 days
Milk yield	1 800-2 700 kg
Milk fat	6.6-8.1 percent
Milk protein	4.2-4.6 percent

Products: milk, ghee, cream, meat.

According to Sethi (2003), the average milk yield per animal per day in Mehsana buffaloes ranges from 4.37 to 4.81 kg. However, a systematic Mehsana breed improvement programme through field progeny testing was launched in 1985 in the milk shed area of the Mehsana district. 107 bulls were tested in eight batches. Average 305 day first lactation milk yield of 50 daughters of the top proven bulls of the first four batches in these buffaloes ranged from 2 085 to 2 312 kg.
Sources: Trivedi, 2000; Sethi, 2003.

Figure 20. Meshana heifers (Gujarat, India)
(Early Bulletin of National Dairy Development Board)

15. Murrah

In the north-west of the sub-Indian continent, buffaloes have long been selected for milk yield and curled horn. It is the most important and well-known buffalo breed in the world.
Population size: 2 000 000
Description: Black in colour. Massive and stocky animals, heavy bones, horns are short and tightly curled. Placid.
Height at withers of adult male is 142 cm, body weight is 750 kg.
Height at withers of adult female is 133 cm, body weight is 650 kg.
Distribution: From its origins in the centre of Haryana, it has spread to the Punjab, Ravi and Sutley valleys, north Sind and Uttar Pradesh. It has been exported to Brazil, Bulgaria and many countries of eastern Asia.
Husbandry: Buffaloes are traditionally managed in domestic conditions together with the calf. They are hand-milked twice a day. They are fed different kinds of roughages (barley and wheat straw, cornstalks, sugar cane residuals). In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are naturally mated. Some villages also provide artificial insemination.

Dairy performance:

Lactation duration	305 days
Milk yield	1 800 kg
Milk fat	7.2 percent

Products: Milk, ghee, cream, meat.

Sethi (2003) reported the performance characteristics at the Haryana Agricultural University (HAU) Centre, India.

Average body weight (kg)	495
Age at first calving (months)	50.6±2.0
First lactation 305 days or less yield (kg)	1 894±44
All lactation 305 days or less yield (kg)	2 183±136
All lactation total yield (kg)	2 226±152
All lactation length (days)	305±16
Average fat (percent)	6.70
Average dry period (days)	144±26
Service period (days)	146±27
Calving interval (days)	479±33

Sources: Reddy and Taneja, 1982; Pal et al., 1971; Cockrill, 1974; FAO, 2003, Sethi, 2003.

16. Nagpuri

It is an improved local breed, the result of a selection of Indian breeds of buffaloes.
Population size: 360 000
Description: Black in colour, sometimes there are white markings on the face, legs and switch. Horns are 50-65 cm long, flat-curved and carried back near to the shoulders. Nasal flap is mostly absent and even if present is very short.
Height at withers of adult male is 140 cm, body weight is 522 kg.
Height at withers of adult female is 130 cm, body weight is 408 kg.
Distribution: This breed is raised in the Nagpur, Wardha and Berar districts of Madhya Pradesh.

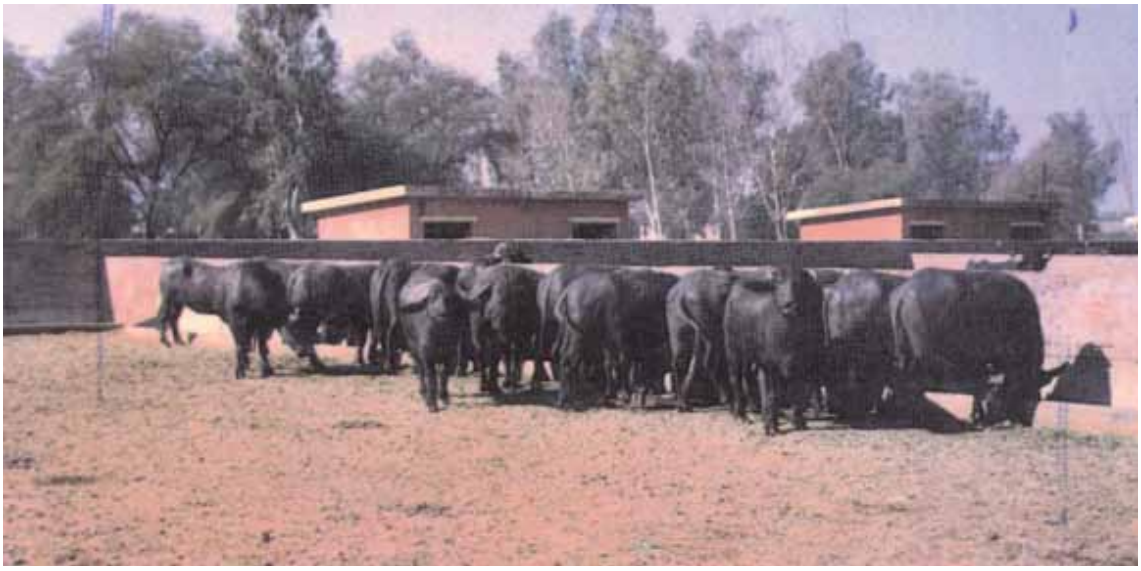
Husbandry: Buffaloes are traditionally managed under domestic conditions together with the calf. They are hand-milked twice a day. They are fed different kinds of roughages: barley and wheat straw, cornstalks, sugar cane residuals. In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are naturally mated.

Dairy performance:

Lactation duration	243 days
Milk yield	825 kg
Milk fat	7.0 percent

Products: Milk, ghee, cream, meat.

Sources: Cockrill, 1974; FAO, 2003; Sethi, 2003.



Figures 21, 22. Murrah bull and herd at the Buffalo Research Institute (Hisar, Haryana, India)

17. Nili-Ravi

Domestication of draught animals in the Indus valley civilization is referred to about 4 500 years ago. Nili and Ravi were two different breeds until 1950, but after this period it was difficult to distinguish between the two breeds probably due to an overlapping selection criteria of breeders. Thus, the common name Nili Ravi became popular. It is the most important livestock in Pakistan. It is also present in India and in the Punjab. This breed is similar to the Murrah in almost all characteristics except for the white markings on extremities and walled eyes; horns are less curled than in the Murrah; the udder is well shaped and extends well forward up to the naval flaps.
Population size: 6 500 000
Description: Black in colour, short horns.
Height at withers of adult male is 135 cm, body weight is 700 kg.
Height at withers of adult female is 125 cm, body weight is 600 kg.
Distribution: All over Pakistan but mainly in the Punjab.
Husbandry: Buffaloes are traditionally managed under domestic conditions together with the calf. They are hand-milked twice a day. They are fed different kinds of roughages: barley and wheat straw, cornstalks, sugar cane residuals. In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are naturally mated. Artificial insemination is available at the state farms and in some villages.

Dairy performance:

Lactation duration	305 days
Milk yield	2 000 kg
Milk fat	6.5 percent

Products: Milk, ghee, cream, meat.

The highest milk yielder at the Institute at Bahadurnagar produced over 4 000 kg while the breed champion produced as high as 6 535 kg in 378 days (Alexiev, 1988).
The performance characteristics of the Nili Ravi breed maintained at the Central Institute for Research on Buffaloes (CIRB) Sub-campus Centre (India) of the Network Project on Buffalo are presented below (Sethi, 2003):

Average body weight (kg)	546
Age at first calving (months)	39.97
First lactation 305 days or less yield (kg)	1 565
First lactation total yield (kg)	1 571
All lactation 305 days or less yield (kg)	1 946
All lactation total yield (kg)	1 969
All lactation length (days)	299
Average fat (percent)	7.1
Average dry period (days)	131
Service period (days)	151
Calving interval (days)	443
Number of services per conception	1.6
Average calf mortality (0-3 months)	7

Sources: Alexiev, 1998; Cockrill, 1974; FAO, 2003; Sethi, 2003.

Figure 23. Nili-Ravi bull at Livestock Research Institute, Bahadurnagar, Okara, Pakistan (Borghese photo, 1992)

Figure 24. Nili-Ravi bull cow at Livestock Research Institute, Bahadurnagar, Okara, Pakistan (Borghese photo, 1992)

18. Parkote

The hill buffalo of Nepal, named Parkote buffaloes, are the typical buffalo of the mid-hills and river valleys. However, due to the traditional practice of crossbreeding with Lime buffalo as well as recent crossbreeding with Indian Murrah, their population in pure form is now declining. At present the pure bred population is estimated at only 25 percent of the indigenous population in the hills and mountains of Nepal.

Population size: 500 000

Description: The Parkote are dark in coat colour and of medium-built body size, with sword-shaped horns directed laterally or towards the back. Black skin, black muzzle, black eyebrows. Usually they have no markings on the legs.

Distribution: The breed is raised in the mountains, high hills and hill river valleys of Nepal.

Height at withers of adult female is 114 cm, body weight is 410 kg.

Husbandry: Mainly raised under migratory conditions or semi-stall systems. The breed is a voracious eater and is fed only low quality feedstuff such as rice, wheat and millet straw. Small farmers exchange breeding animals within and between villages. Among the migratory herds, male and females are grazed together and mated freely during the breeding season from June to November.

Females are legally banned from slaughter; only culled animals are slaughtered for meat.

Figure 25. Typical Parkote buffalo (Rasali D. photo, Nepal)

Dairy performance:

Lactation duration	351 days
Milk yield	875 kg
Milk fat	7.0 percent

Products: milk, ghee, meat, swiss-cheese, yoghurt, leather.
Sources: Rasali, 1997; Rasali, 1998a,b.

19. Sambalpuri

Description: Black in colour, with white switch on tail, with narrow and short horns, curved in a semi-circle, running backward, then forward at the tip.
Distribution: This breed is raised around Bilaspur in Madhya Pradesh (India).
Husbandry: Buffaloes are traditionally managed under domestic conditions together with their calf. They are hand-milked twice a day. They are fed different kinds of roughages: barley and wheat straw, cornstalks, sugar cane residuals. In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are naturally mated. It is a good healthy draught animal with a rapid pace and it is comparatively the most productive breed of the region. Some exceptional buffaloes may yield as high as 2 300 to 2 700 kg in about 340 days.

Dairy performance:

Lactation duration	350 days
Milk yield	2 400 kg

Products: Milk, ghee, cream, meat.
Sources: Sethi, 2003

20. Surti

The existence of the Surti breed in north Gujarat (India) is referred to in 1940. It is the result of a selection of Indian breeds of buffalo. It is one of the most important breeds in Gujarat and in Rajasthan.

Population size: 500 000
Description: Black colour coat, skin is black or reddish. They have two white chevrons on the chest. Animals with white markings on forehead, legs and tail tips are preferred. Horns are flat, of medium length, sickle shaped and are directed downward and backward, and then turn upward at the tip to form a hook. The udder is well developed, finely shaped and squarely placed between the hind legs. The tail is fairly long, thin and flexible ending in a white tuft.
Height at withers of adult male is 131 cm; body weight is 700 kg.
Height at withers of adult female is 124 cm; body weight is 550-650 kg.
Distribution: Concentrated between the Mahi and Sabarmati rivers in Gujarat (India).

Husbandry: Buffaloes are traditionally managed under domestic conditions together with the calf. They are hand-milked twice a day. They are fed different kinds of roughages (barley and wheat straw, cornstalks, sugar cane residuals). In addition, they are given concentrate mixtures. If grazing is available, they graze all day long. They are naturally mated. Some villages also provide artificial insemination.

Dairy performance:

Lactation duration	350 days
Milk yield	2 090 kg
Milk fat	6.6-8.1 percent
Milk protein	4.2-4.6 percent

Products: Milk, ghee, cream, meat.

Sethi (2003) reported the following performance characteristics of the Surti breed maintained at the Maharana Pratap University of Agriculture and Technology (MPUAT) (India) Vallabhnagar Centre of the Network Project on Buffalo:

Average body weight (kg)	462±7.0
Age at first calving (months)	53.2±1.7
First lactation 305 days or less yield (kg)	1 295±57
All lactation 305 days or less yield (kg)	1 477±42
All lactation total yield (kg)	1 547±50
All lactation length (days)	311±7
Average fat (percent)	8.10
Average dry period (days)	234±21
Service period (days)	207±17
Calving interval (days)	510±16
Number of services per conception	2.55
Average percentage calf mortality (0-3 months)	7.0

21. Tarai

Population size: 940 000

Description: Black to brown colour coat; sometimes there is a white blaze on the forehead, tail switch is white. Horns are long and flat with coils bending backwards and upwards.

Height at withers of adult male is 127 cm; body weight is 375 kg.

Height at withers of adult female is 120 cm; body weight is 325 kg.

Distribution: This breed is raised in the Agra and Etawa districts of Uttar Pradesh and in the Bhind and Morena districts of Madhya Pradesh (India).

Husbandry: Buffaloes are traditionally managed under domestic conditions together with the calf. They are hand-milked twice a day. They are fed different kinds of roughages: barley and wheat straw, cornstalks, sugarcane residuals. If grazing is available, they graze all day long. The breed is well adapted to the difficult climatic and feeding conditions of the Tarai region. Sometimes it is crossbred with the Murrah.

Dairy performance:

Lactation duration	250 days
Milk yield	450 kg
Milk fat	6.6-8.1 percent
Milk protein	4.2-4.6 percent

Products: Milk, ghee, cream, meat.

Sources: Cockrill, 1974; FAO, 2003.

22. Toda

Population size: 6 000
Description: Unicolour, light or dark grey. Horns are set wide apart with recurved tip inwards, outward and forward. They are large and powerful animals.
Height at withers of adult male is 160 cm; body weight is 380 kg.
Height at withers of adult female is 150 cm; body weight is 380 kg.
Distribution: This breed is raised in the Nilgiris hills of Madras.
Husbandry: The breed is semi-wild and raised under semi-nomadic conditions, with total grazing.

Dairy performance:
Lactation duration: 200 days
Milk yield 500 kg
Products: Milk, ghee, cream, meat.

Sethi (2003) reported the following performance:

Average birth weight	27.9±0.43 kg
Average 305 days lactation yield	501±10.6 kg
Average lactation length	198.6±2.8 days
Average daily milk yield	2.53±0.44 kg
Average fat (percent)	8.22±0.08
Average carcass weight in adults	142.1±10.1 kg
Average calving interval	15.74±0.4 months

Sources: Cockrill, 1974; FAO, 2003; Sethi, 2003.

Figure 26. Surti cow (Sethi, 2003)
Sources: Trivedi, 2000; Sethi, 2003.

Figure 27. Toda buffalo in its natural habitat (ICAR Ad hoc Scheme, Breeding Research Station, Sandynallah)

References

Alexiev, A. 1998. The Water Buffalo. St. Kliment Ohridski University Press, Sofia.

Askar, A.A., Ragab, M.T. and Ghazy, M.S. 1973. Repeatability and heredity of some charcteristics in Egyptian buffalo. Ind. J. Dairy Sci., 6: 61-65.

Bikocu, Y. 1995. Buffalo population and production in Albania. Buffalo Newsletter, 4: 8.

Borghese, A. and Moioli, B. 1999. Buffalo population and products in Europe and the Middle Eastern countries. International Conference on Sustainable Animal Production. Hisar, India, 24 to 30 Nov.: 245-257.

Borghese, A., Moioli, B. and Tripaldi, C. 2000. Buffalo milk: processing and product development in the Mediterranean countries. Third Asian Buffalo Congress, Kandy, Sri Lanka, 27 to 31 Mar.: 37-46.

Borghese, A. 2005. Personal information.

Bunewski, G. 2000. Buffalo breeding in TFYR of Macedonia. Proc. Workshop on Animal Recording and Management Strategies for Buffaloes. ICAR Technical Series, 4: 77-78.

Cockrill, W.R. 1974. The husbandry and health of the domestic buffalo, FAO, Rome.

El-Kirabi, F. 1995. Buffalo population and production in Egypt. Buffalo Newsletter, 3: 8-9.

FAO. 2003. www.FAO.org/DAD-IS.

Faruque, O. 2000. Buffalo breeding in Bangladesh. Proc. Workshop on Animal Recording and Management Strategies for Buffaloes. ICAR Technical Series, 4: 51-54.

Latifova, E. 2000. Case study on buffalo recording. Breeding and management strategies of buffalo in Azerbaijan. Proc. Workshop on Animal Recording and Management Strategies for Buffaloes. ICAR Technical Series, 4: 45-50.

Magid, S. 1996. Buffalo population and production in Iraq. Buffalo Newsletter, 6: 6-7.

Marmarian, Y. 2000. Buffalo breeding in Armenia. Proc. Workshop on Animal Recording and Management Strategies for Buffalo. ICAR Technical Series, 4: 73 76.

Mason, I.L. 1996. A World Dictionary of Livestock Breeds, Types and Varieties, 4th Edition, Wallington, UK.

Mokhtar, S.A. 1971. Study on some economic traits of Holstein Friesian cattle in U.A.R. M.Sc.Thesis, Fac. Agriculture, Ain Shams Univ., Cairo, Egypt.

Naderfard, H.R. and Qanami, A. 1997. Buffalo breeding in Iran. Proc. Fifth Buffalo Congress, Caserta, 13 to 16 Oct.: 942-943.

National Buffalo Project, 1988. Ministry of Jihad, Teheran, Iran.

Nigm, A.A. 1996. Characterization of Egyptian buffalo. Proceedings of International Symposium on Buffalo Resources and Production Systems. Giza, Egypt, 17 Oct.

Pal, S., Basu, S.B. and Sengar, O.P.S. 1971. Heredity and genetic correlations for milk constituents in Murrah buffaloes. Ind. J. Animal Sci., 41: 1019-1021.

Peeva, T., Vankov, K. and Vasilev, M. 1991. Persistency index of lactation milk production in buffalo cows. Proc. of the Third World Buffalo Congress, Agricultural Academy, Varna, Sofia, 13 to 18 May: 61.

Peeva, T. 1996. Buffalo population and production in Bulgaria. Buffalo Newsletter, 4: 6-7.

Popovici, I. 1996. Buffalo population and production in Romania. Buffalo Newsletter, 5: 9-10.

Qanemi, A. 1998. Buffalo population and production in Iran. Buffalo Newsletter, 10: 12-14.

Ragab, M.T. and Abdel-Salam, M.F. 1963. Heredity and repeatability of bodyweight and growth rate in Egyptian buffalo and cattle. Egypt. J. Anim. Prod., 3: 15-26.

Rasali, D.P. 1997. Present status of indigenous buffalo genetic resources in the western hills of Nepal. Proceedings of the Fourth Global Conference on Conservation of Domestic Animal Genetic Resources. Rare Breeds International: 168-170.

Rasali, D.P., Joshi, H.D., Shrestha, H. and Gautam, D.C. 1998a. Assessment of the infertility situation in cows and buffaloes in the western hills of Nepal. Lumle Agricultural Research Centre. Working Paper, 98/40: 16.

Rasali, D.P., Joshi, H.D., Patel, R.K and Harding, A.H. 1998b. Phenotipic clusters and karyotypes of indigenous buffaloes in the western hills of Nepal. Lumle Agricultural Research Centre. Technical Paper, 98/2: 24.

Reddy, C.E and Taneja, V.K. 1982. Genetic trends for 300 day first lactation milk yield in Murrah buffaloes. Second World Congress on Genetics Applied to Livestock Production, Madrid, 4 to 8 October 1982: 152-156.

Saadat, M.N. 1997. In "Dairy goats and buffalo production". University of Teheran.

Sekerden, O., Kebapci, M. and Kopar, A. 1996a. Buffalo population and production in Turkey. Buffalo Newsletter, 5: 7-8.

Sekerden, O., Kebapci, M. and Kopar, A. 1996b. Blood transferrin types and genetic structure for transferrin types of buffalo population in Samsun province. Buffalo Newsletter, 5: 11-12.

Sekerden, O., Dogrul, F. and Erdem, H. 2000. Blood serumtransferrin types and genetic structure for transferrin types and growth performance of Anatolian buffalo. Kocatepe Enstitutu manda surusu sut ve dol verim ozellikleri. Ataturk University.

Sethi, R.K. 2003. Buffalo Breeds of India. Proc. of Fourth Asian Buffalo Congress, New Delhi, India, 25 to 28 Feb.

Stravaridou, S. 1998. Buffalo population and production in Greece. Buffalo Newsletter, 9: 7.

Trivedi, K. 2000. Buffalo recording systems in India. Proc. Workshop on Animal Recording and Management Strategies for Buffalo. ICAR Technical Series, 4: 5 12.

Tunio, A.N. 1999. Personal communication.

Turabov, T. 1991. Selection of Caucasian breed of buffalo. PhD Thesis. Academy of Agriculture, Baku, Azerbaijan.

Turabov, T. 1997a. The modern condition of buffalo production in Azerbaijan and the ways for further improvement. Proc. of the Fifth Buffalo Congress, Caserta, 13 to 16 Oct.: 884-888.

Turabov, T. 1997b. Buffalo population and production in Azerbaijan. Buffalo Newsletter, 8: 9-10.

Chapter IV

REPRODUCTIVE EFFICIENCY IN FEMALE BUFFALOES

Vittoria Lucia Barile

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

Introduction

The world buffalo population is continuously increasing and was estimated to be more than 170 million in 2003 as reported by FAO (2004). More than 95 percent of the world population is found in Asia where buffalo play a leading role in rural livestock production. Over the last decades buffalo farming has widely expanded in Mediterranean areas and in Latin American countries and several herds have also been introduced in Central and Northern Europe.

Buffalo farming is increasing in Italy too due to the growing market demand for buffalo milk that is utilized exclusively for the production of "mozzarella cheese". Another economic benefit deriving from buffalo milk production is that buffalo milk is not restricted by the specific European Union (EU) directive called "milk quotas", introduced to stop the increase of cow milk production. In fact, this regulation induced some farmers, in areas where Friesian cattle are traditionally reared, to consider the option of breeding milking buffaloes for the production of "mozzarella" cheese. This led to an expansion of buffalo breeding in the north of Italy too, away from the customary area, traditionally situated in southern Italy (Campania, Lazio and Puglia regions) where 95 percent of the Italian buffalo population is reared.

In spite of this expansion in buffalo breeding, there was no improvement in milk and meat production due to slow genetic progress. The productivity increase obtained over the last years is due mainly to an improvement in management techniques rather than to genetic selection. Reproductive efficiency is the primary factor affecting productivity and is hampered in the female buffalo by a delayed attainment of puberty, seasonality, long post-partum anoestrus and subsequent calving interval, and poor oestrus expression. Regarding the latter, artificial insemination (AI) which is the normal practice in cattle breeding, is limited in buffaloes due to the weakness of oestrus symptoms and variability of oestrus length that makes oestrus detection very difficult.

Studies have been undertaken in order to better understand the reproductive physiology of the buffalo and the factors affecting its behaviour. Considerable attention has been paid to reproductive endocrinology over the last two decades, with the aim of developing models to improve reproductive efficiency, particularly when controlled breeding techniques are utilized.

Puberty

Puberty in buffalo is delayed compared with cattle (Jainudeen and Hafez, 1993). The age at puberty is difficult to establish because of difficulties in oestrus detection in this species and most estimations appear to have been extrapolated from the age at first calving. According to Jainudeen and Hafez (1993), the River type exhibit first oestrus earlier (15 to 18 months) than the Swamp type (21 to 24 months). First conception occurs at an average body weight of 250 to 275 kg, which is usually attained at 24 to 36 months of age.
There is a large variation in age at puberty in different countries (Table 1).

In one of the first investigations to study the phenomenon of puberty and first conception in buffalo heifers Hafez (1955) reports that in Egyptian buffalo, on the basis of mating behaviour

Table 1. Puberty in buffalo heifers as reported by various authors.

Author	Type or Breed	Age at puberty	BW (kg)	Age at first conception	BW (kg)	Age at first calving
Hafez (1955)	Egyptian	406 d (13.5 m)	198	647d (21.5 m)	319	
Mohamed et al. (1980)	Egyptian	9.9 m				
Barkawi et al. (1989)	Egyptian	24.7 m	310			
Salama et al. (1994)	Egyptian	15.4 m	271			
Madan (1988)	Indian	16-40 m				
Saini et al. (1998)	Murrah	36.5 m 33.1 m	355.8 322.3			
Pathodiya et al. (1999)	Surti					1683 d (56.1 m)
Sule et al. (2001)	Surti	1365 d (45.5 m)		1418 d (47.3 m)		
Gogoi et al. (2002)	Murrah Surti					53.8 m 51.5 m
Ishaq (1972)	Nili-Ravi	30-33 m	450-519			
Naqvi and Shami (1999)	Nili-Ravi	976 d (32.5 m)				
Le Xuan Cuong (1983)	Vietnamese Swamp	30-36 m				
Kamonpatana et al. (1987)	Asian Swamp	24-25 m	300			
Tulloc and Grassia (1981)	Australian Swamp	14-19 m				
McCool et al. (1988)	Australian Swamp	30.3 m	318			
Okuda et al. (1999)	Brasilian	540 d (18 m)				850 d (28 m)
Ferrara (1964)	Italian					36 m
Salerno (1974)	Italian			27 m		39 m
Zicarelli et al. (1977)	Italian			26-35 m		44.7 m
Borghese et al. (1994a)	Italian	575.4 d (19.1 m) 623.1 d (20.7 m)	359.1 390.1			

d: days; m: months.

and rectal examination of uterus and ovaries, the average age at first oestrus and at first conception was 406 and 647 days respectively, with a body weight at first oestrus and first conception of 198 and 319 kg respectively. The period elapsing from first oestrus to first conception ranged from 52 to 438 days. More recently, Mohamed et al. (1980) kept calves with good feeding levels and sprayed them with water during the hot months and reported the youngest age at puberty for the Egyptian buffalo (9.9 months); while Barkawi et al. (1989), under the common practices in the state farms, reported the oldest age (24.7 months at 310 kg body weight). Salama et al. (1994) with an improved feeding system and managerial practices, and taking into account progesterone value to define more accurately the onset of puberty, obtained the average age at puberty of 15.4 months with an average body weight of 271 kg.

For the Indian buffalo, Madan (1988) reports a large variation in age at puberty ranging from 16 to 40 months depending on the breed, with an earlier age in the Surti and a later age in the Nagpuri. In contrast, Sule et al. (2001) reports in Surti buffalo an average first heat and first conception of 1365.06±12.85 and 1418.6±13.16 days (about 45.5 and 47.3 months) respectively. According to Saini et al. (1998), Murrah buffalo kept under normal management at the University farm, reach puberty at 36.5 months and 355.8 kg body weight, while improving management and splashing buffaloes with water during the hot period, shorten the age at first oestrus to 33.1 months and to 322.3 kg body weight. Gogoi et al. (2002) studying the age at first calving of Murrah and Surti buffaloes from some government farms, found that Murrah buffaloes had a higher age than Surti buffaloes (53.88±0.48 *vs* 51.51±1.18 months), while Pathodiya et al. (1999) found an average age at first calving for the Surti buffaloes of 1683.48±34.86 days (about 56.1 months).

In Pakistan, Naqvi and Shami (1999) studied the age at sexual maturity in the Nili-Ravi buffaloes and report a mean age of 976.49±9.2 days (about 32.5 months) ranging from 957.93±10.68 to 1015.26±17.39 days depending on farms. Similarly, Ishaq (1972) found for the Nili-Ravi an age at puberty of 30 - 33 months and at 450-519 kg body weight.

Kamonpatana et al. (1987) in the Swamp buffalo report a mean age of 24 to 25 months and a body weight of 300 kg at sexual maturity. In the Vietnamese Swamp buffalo, Le Xuan Cuong (1983) states that puberty is achieved between 30 and 36 months.

According to Tulloch and Grassia (1981), puberty in the Australian Swamp buffalo occurs between 14 and 19 months of age, while McCool et al. (1988), on the basis of progesterone profiles, reports a mean age at puberty of 30.3±6.1 months at a body weight of 318±54 kg.
For buffalo bred in Brazil, Okuda et al. (1999) report that the age at sexual maturity and the age at first calving averaged 540.9±146.88 days (about 18 months) and 850.0±169.13 days (about 28 months) respectively.

For the Italian buffalo data refer more to the age at first calving rather than the age at puberty. Ferrara (1964) reported an age at first calving of 36±4.7 months; likewise Salerno (1974) reported 27 months as the age at first conception and 39 months as the age at first calving. According to Zicarelli et al. (1977) Italian buffalo heifers on average have first conception at 26-35 months and first calving at 44.7 months. Later De Franciscis (1988) reported 32-33 months as the age at first calving. Borghese et al.(1994a), on the basis of progesterone levels and rectal examination of uterus and ovaries, stated that Italian buffalo heifers on average showed the first high value of progesterone at 575.4±84.5 days (about 19.1 months) and 359.1±51.8 kg of body weight, while they showed ovarian cyclic activity at 623.1±81.2 days (about 20.7 months) and 390.1±50.9 kg. Age and weight at puberty such as ovarian cyclic activity were affected by different farm conditions especially by feeding levels that improved growth and sexual maturity.

The delay in puberty and the consequent delay in conception is one of the problems that lead to the low reproductive efficiency of the buffalo species, thus lengthening the non-productive life. Many factors influence age at puberty, such as breed, season, climate, nutrition and growth

rate, and several experiments have been carried out at our institute aimed at advancing the age at first calving (Borghese et al., 1993a, 1994a, 1994b, 1996; Terzano et al., 1996; Borghese et al., 1997; Terzano et al.,1997). The pre- weaning and weaning systems are important in promoting growth and achieving puberty, therefore attention must be given to heifer management needs beginning from birth to ensure a correct weight increase. In fact, the animals that showed a higher daily gain before the trials reached puberty in a shorter time. The age at puberty is affected by the dietary energy level. The heifers fed with a high level diet (5.56 MFU/d) had a daily gain of 562 g *vs* 465 g of the heifers fed with a low level diet (4.42 MFU/d), and reached puberty 30 days earlier. However, it is possible to rear heifers on pasture obtaining the same performance of those reared with intensive feeding on condition that the daily gain is nearly 600 g/day. Heifers on pasture realized their reproductive performances with less energy consumption, the best feed efficiency and the lowest cost in terms of feeding stuff and management. Campanile et al. (2001), in a study on the effects of long-term and short-term nutritional management on growth and conception in buffalo heifers, concluded that nutritional management and growth from the time of weaning and during the pre-pubertal period has a considerable influence on age and body weight at first conception in buffalo heifers. In fact heifers bred with a good early management system conceived at a younger age compared with the others (543±16 and 844±11 days respectively). They also observed that the negative effects of early nutritional deficiency on reproductive function are not surmounted by a relatively short-term period of dietary supplementation.

Simulating the hormonal changes occurring around puberty may induce sexual maturity in heifers. Trials to induce and synchronize oestrus in buffalo heifers have been undertaken, although to a lesser extent than in cattle (Saini et al., 1988; Honnapagol and Patil, 1991; Andurkar and Kadu, 1995; Zicarelli et al., 1997a). Saini et al. (1988), using PRID (progesterone-releasing intravaginal device) plus PMSG (Pregnant Mare Serum Gonadotrophin) to induce oestrus in non-cycling buffalo heifers, reported that all animals in the treated group expressed oestrus while none expressed oestrus in the control group. Those authors reported that more intense oestrus symptoms and a better conception rate were obtained when PMSG was used with PRID, as PRID treatment alone failed to induce a fertile oestrus. Andurkar and Kadu (1995), using a progesterone intravaginal pessary (CIDR) with prostaglandin F2α and PMSG, induced oestrus in non-cycling buffalo (either cows or heifers) and found better fertility with a long-term (12 days) than a short-term (8 days) treatment.

Our work has also shown that the use of PRID together with PMSG treatment is able to induce fertile oestrus in non-cycling heifers (Barile et al., 2001a; Pacelli et al., 2001). This has an economic impact on buffalo production as a greater proportion of heifers can be bred early. In fact, the PRID treatment increased ($P \leq 0.01$) the proportion of heifers that became cyclic within 60 days from the start of the trial (Table 2). Moreover, treated animals had a higher conception rate (CR) compared with controls (65,0 *vs* 28,2 percent, Barile et al., 2001a; 66.6 *vs* 33.3 percent, Pacelli et al., 2001). A marked difference in conception rate (CR) was

Table 2. Rate of heifers becoming cyclic within 60 day after PRID + PMSG treatment.

Trial 1: PRID + 1000 IU ≤PMSG.
Trial 2: PRID + 1000 IU PMSG (Group A), PRID + 750 IU PMSG (Group B)

Trial	Treated (%)		Control (%)
1	Farm TM	70.0a	11.1b
	Farm IM	71.4a	18.6b
2	Group A	66.6a	33.3b
	Group B	73.3a	

a,b $P \leq 0.01$ within row
TM: Tormancina; IM: Iemma
Trial 1 (Barile et al. 2001a); Trial 2 (Pacelli et al. 2001)

Table 3. Conception rate (CR; March-June) in treated (PRID+PMSG) and control heifers in relation to their cyclicity before the treatment in two farms (TM and IM).

Farm	Cyclicity	Treated		Control	
		No. of animals	CR No. (%)	No. of animals	CR No. (%)
TM	cycling	10	8 (80.0)	10	6 (60.0)
	non-cycling	10	5 (50.0)a	9	0 (0.0)b
IM	cycling	2	2 (100.0)	2	1 (50.0)
	non-cycling	18	11 (61.1)a	18	4 (22.2)b

a,b $P \leq 0.01$ within row.
TM: Tormancina; IM: Iemma
(Barile et al., 2001a).

Table 4. Conception rate (CR; March - August) in treated (Group A: PRID + 1000 IU PMSG; Group B: PRID + 750 IU PMSG) and control heifers (Group C)

Groups	Cyclicity	No. of animals	CR No. (%)
A	Non cycling	15	(66.6)A
B	Non cycling	15	10 (66.6)A
C	Non cycling	15	5 (33.3)B

A,B $P \leq 0.05$
(Pacelli et al., 2001)

found between treated and control heifers that were non cycling at the beginning of the trial (Tables 3 and 4). In relation to the dose of PMSG utilized, no difference was found either in the number of animals becoming cyclic or in the CR using 1 000 IU *vs* 750 IU of PMSG (Pacelli et al., 2001), although Khan et al. (1995) using 1 400 IU *vs* 700 IU of PMSG showed that the low dose of PMSG was better than the high dose for oestrus induction and subsequent CR in non-cycling buffalo heifers.

Using the PRID regime, it is possible to synchronize oestrus in cycling heifers, overcoming the problem of oestrus detection and increasing the effectiveness of AI programmes in buffalo heifers. The CR to AI obtained in our work, utilizing either cycling or non-cycling animals,

Table 5. Conception rate (CR) in buffalo heifers at artificial insemination as reported by various authors

Reference	Treatment	CR (%)
Honnappagol and Patil (1991)	PGFα	12.5-62.5
Zicarelli et al. (1997a)	PRID or norgestomet + PMSG	20.20
Neglia et al. (2001)	PGF2α PGF2α + GnRH	55.00 44.40
Kumaresan and Ansari (2001)	Spontaneous oestrus: 6-12h 12-18h 18-24h	 16.67 28.99 33.33
Barile et al. (2001a)	PRID + PMSG	37.50
Pacelli et al. (2001)	PRID + PMSG	36.50

(37.5 percent, Barile et al., 2001a; 36.7 percent, Pacelli et al., 2001) was a good result relative to the small amount of data reported in literature (Table 5). Honnappagol and Patil (1991), using an analogue of prostaglandin F2*a* to synchronize oestrus in cycling Surti buffalo heifers, had a CR to AI ranging from 12.5 to 62.5 percent. Zicarelli et al. (1997a), using PRID in Italian cycling buffalo heifers, reported a CR to AI of only 20.2 percent; the same group of researchers, using prostaglandin or prostaglandin +GnRH had a CR of 55.0 percent and 44.4 percent respectively (Neglia et al., 2001). Indian authors (Kumaresan and Ansari, 2001), utilizing AI on spontaneous oestrus, reported a CR ranging from 16.67 to 33.33 percent in relation to the stage of oestrus; the highest CR was obtained when the heifers were inseminated at 18 - 24 hours after oestrus.

Seasonality

Although buffaloes are polyoestrus, their reproductive efficiency shows wide variation throughout the year. As reported by different authors (Shah et al., 1989; Singh and Lal, 1994; Zicarelli, 1997; Srivastava and Sahni, 1999), buffalo cows exhibit a distinct seasonal change in displaying oestrus, conception rate and calving rate. This may be the cause of the prolonged intercalving period since buffalo calving during the unfavourable season may not resume their ovarian activity until the following favourable season, decreasing their reproductive efficiency.

In a study undertaken over five years on Indian Murrah buffaloes, the maximum percentage of heats were observed in November and the minimum in June; the conception rates ranged from 41.85 percent to 44.85 percent during August to November, with the highest rate in September, and from 29.45 percent to 32.92 percent during the months of February to April, with the lowest rate in April (Reddy et al.,1999). Data collected on Surti buffaloes reared in Rajasthan confirm a distinct seasonality in breeding behaviour. The monthly and seasonal calving pattern recorded at the research station and in the field indicated that buffaloes calved all the year round but have a tendency to calve more during the rainy season (July to September) followed by the winter season (October to January). The breeding season started in the rainy period and the winter appeared the most favourable season while the summer appeared the most unfavourable season for buffalo reproduction (Sule et al., 2001). Also for buffaloes bred in Pakistan, Shah (1988) reported that the breeding frequency was highest during the winter, decreased in autumn and spring, and was lowest in the summer. According to Shah et al. (1989), it may be possible that during the summer season, farmers are unable to fulfil the fodder requirements of buffalo because of less fodder availability at this period. High environmental stress together with under-nutrition might therefore be responsible for the long periods of seasonal anoestrus in buffaloes. Similar effects of these factors on oestrus activity in Australian Swamp buffaloes were described by McCool et al. (1987). These authors find in an area subject to a monsoon rainfall pattern, the highest number of cyclic buffaloes during the late wet and early dry seasons (wet season being December to March; dry season being April to November). During the late dry season (August to November) fodder availability is low, ambient temperatures are highest and body condition deteriorates, therefore the authors hypothesized that the combined effects of these factors could be the cause of depressed oestrus activity in this season. In fact, few buffalo cows conceived in this period, as reported previously by Tulloch and Grassia (1981). Similarly, Vale et al. (1990) in a study on buffalo reproduction in the Amazon Basin, were of the opinion that the seasonality in buffalo could be due more to management factors and unavailability of green fodder rather than to the inability of the species to reproduce throughout the year. However, in Italy, where buffaloes are fed with a constant balanced diet in place of free grazing, a distinct seasonal reproductive pattern is also found (Zicarelli, 1992). With regard to this aspect, Zicarelli (1997), in his review on buffalo seasonality, emphasizes how the need of a species, mainly in wildlife, to coincide calving and weaning with favourable environmental conditions represents one of the causes of reproductive seasonality; therefore the tendency of buffalo to seasonality depends upon the environmental characteristics of their place of origin which are the subtropical zones of North of the equator, which condition the forage availability and thus the state of animal nutrition throughout the year.

Therefore, the reproductive seasonality in the buffalo does not seem to depend on diet, food availability or metabolic status, while climate and particularly photoperiod, depending on melatonin secretion, play a pivotal role (Parmeggiani et al.,1993; Borghese et al.,1995; Di Palo et al., 1997; Zicarelli, 1997). Melatonin is a hormone secreted by the pineal gland during the night and represents the endocrinal signal of the light-dark rhythm in the environment. The role of melatonin in the regulation of the circadian and annual rhythm is well known in the control of ovarian cyclicity in seasonal species such as sheep, goats and mares, while few investigations have been made to clarify the role of this hormone in buffalo reproduction. Parmeggiani et al. (1993, 1994) investigated whether melatonin could act as a transductional signal of photoperiod in buffalo. The investigations were carried out on Mediterranean buffalo cows reared in Italy. In this country, calving occurs mainly between July and December with the highest calving frequency in August-September; the intercalving interval is longer for deliveries occurring between February and June, indicating a decrease in the conception rate during the spring - summer seasons (De Franciscis, 1988; Borghese et al., 1993b) (Figures 1 and 2).

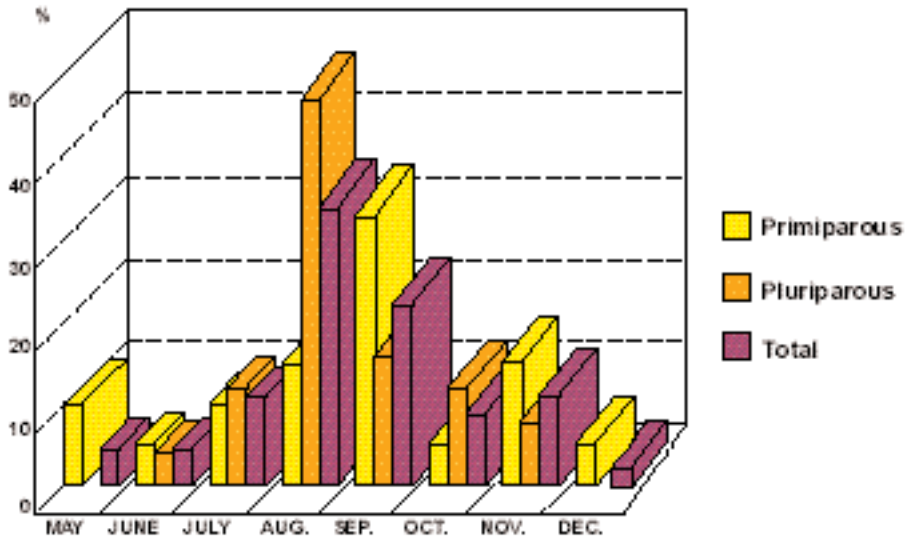


Figure 1. Trend of calving frequency in buffalo in Italy.
(data from Borghese et al., 1993b)

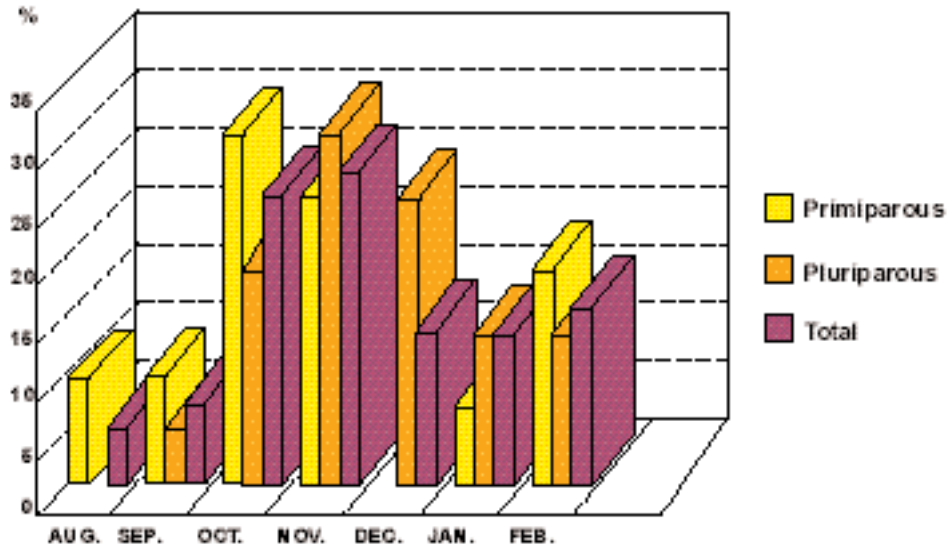


Figure 2. Trend of conception frequency in buffalo in Italy.
(data from Borghese et al., 1993b)

The proportion of buffaloes exhibiting oestrus during the period of short day length is significantly higher than during the period of long day length, indicating that decreasing daylight is a strong determinant of the resumption of ovarian activity. In fact Parmeggiani et al. (1993,1994), in a study of buffaloes reared in farms with a clear seasonal reproductive trend, found high levels of melatonin during the night and the persistence of these levels was clearly related to the photoperiod: they were the highest in December (35.22±2.07 pg/ml) and decreased progressively from March-April (35.0±2.07 pg/ml) to June (23.13±2.30 pg/ml). These secretory

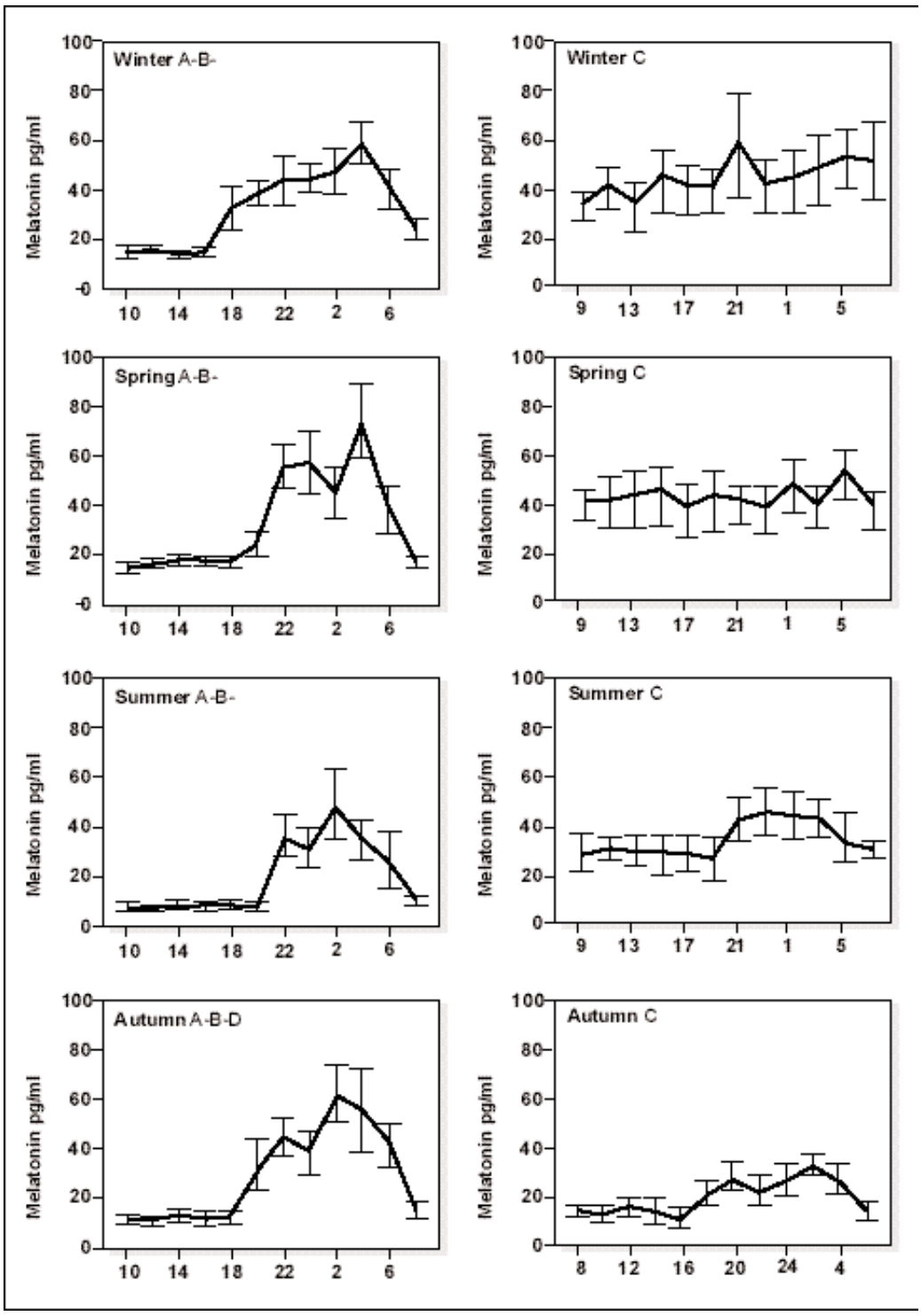


Figure 3. Circadian trend of melatonin in buffalo cows in different seasons.
On the left, farms A-B-D characterized by a higher trend of seasonal reproduction activity; on the right, farm C characterized by a lower trend of seasonal reproduction activity.
(Parmeggiani et al., 1994)

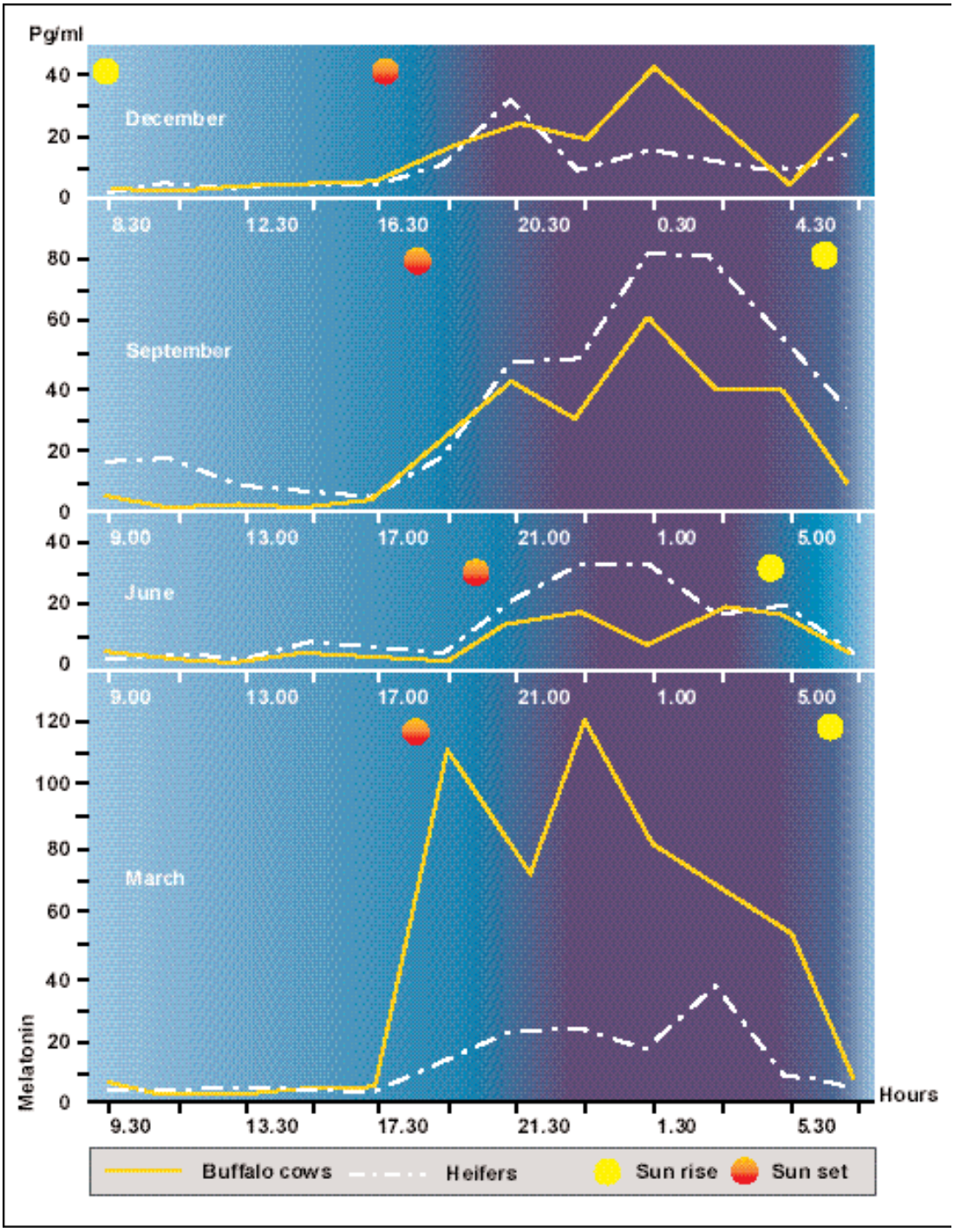


Figure 4. Circadian trend of melatonin in buffalo heifers and cows at equinoxes and solstices. (Borghese et al., 1995)

patterns were not observed in all the animals; in a farm where parturition frequency tended to be more uniformly distributed around the year, melatonin concentration was persistently high during the day (30-40 pg/ml), with a lack of evident melatonin increase during the night. According to the authors, the absence of strong seasonality in this particular farm was probably due to an extensive selection carried out with the aim of eliminating any seasonal breeder. However nutrition does not seem to be the cause of this difference since in all the farms a fairly similar diet was used. Borghese et al. (1995), also report, in a study carried out on buffalo heifers and cows in Italy, that the melatonin trend shows remarkable differences between seasons (Figure 4). In June at the summer solstice, the lowest values and less persistence of melatonin peak were found because of the shortest night, while the highest values were noted

at the equinoxes, particularly in September, the month corresponding to the start of hypothalamus-pituitary-ovarian axis activity.

The heifers showed significantly higher values during the day than in cows and in September also during the night, probably because they were close to the onset of puberty.

Therefore these data suggest a relationship between photosensitivity and the seasonal reproductive trend in this species.

The strong influence of photoperiod seems to be further demonstrated by the findings that the period of higher reproductive efficiency is reversed in the two opposite hemispheres (Zicarelli, 1997). In Brazilian buffaloes, Pires et al. (2002), in a study lasting 13 years, report that most of the births (86.73 percent) occurred during the first six months of the year, with 57.93 percent during February, March and April and only 0.65 percent during October and November. The reproductive period, moreover, is longer near the equator where the light/dark ratio is constant throughout the year. Da Silva and Grodzki (1991) have reported 95.4 percent of calving between December and May in the Parana State (Southern Brazil) that is characterized by a reverse light/dark ratio compared to the Northern hemisphere, and a calving concentration between April and September in the Para State (Northern Brazil) characterized by a constant length of the light/dark ratio during the year. In the zones near the equator, the satisfaction of nutritional requirements seems to prevail over light stimulus as a factor influencing reproductive activity.

In Italy, the reproductive seasonality of the species implies economic implications, since the milk production is totally utilized to produce fresh white "mozzarella" cheese. The demand for "mozzarella" cheese is mainly concentrated in the spring / summer period, while the higher milk production is during the autumn/winter months due to calving seasonality. In Italy under natural conditions, 70 to 80 percent of calving occurs in the last six months of the year (July to December) involving a higher production of milk in the period between the end of August and the middle of February, when the milk is not in great demand. In order to meet the market demands many Italian breeders (at present over 60 percent), over the last 20 years have managed to modify the natural calving calendar by keeping females without males from October to February, the period during which conception is undesired (out of breeding season mating) (Figures 5 and 6). The use of this reproductive strategy is spreading among Italian buffalo breeders also due to the higher price paid for buffalo milk produced during the spring and summer than that produced during the autumn and winter. This strategy is also attracting interest in other countries in order to cope with the need to guarantee a constant milk production for the market.

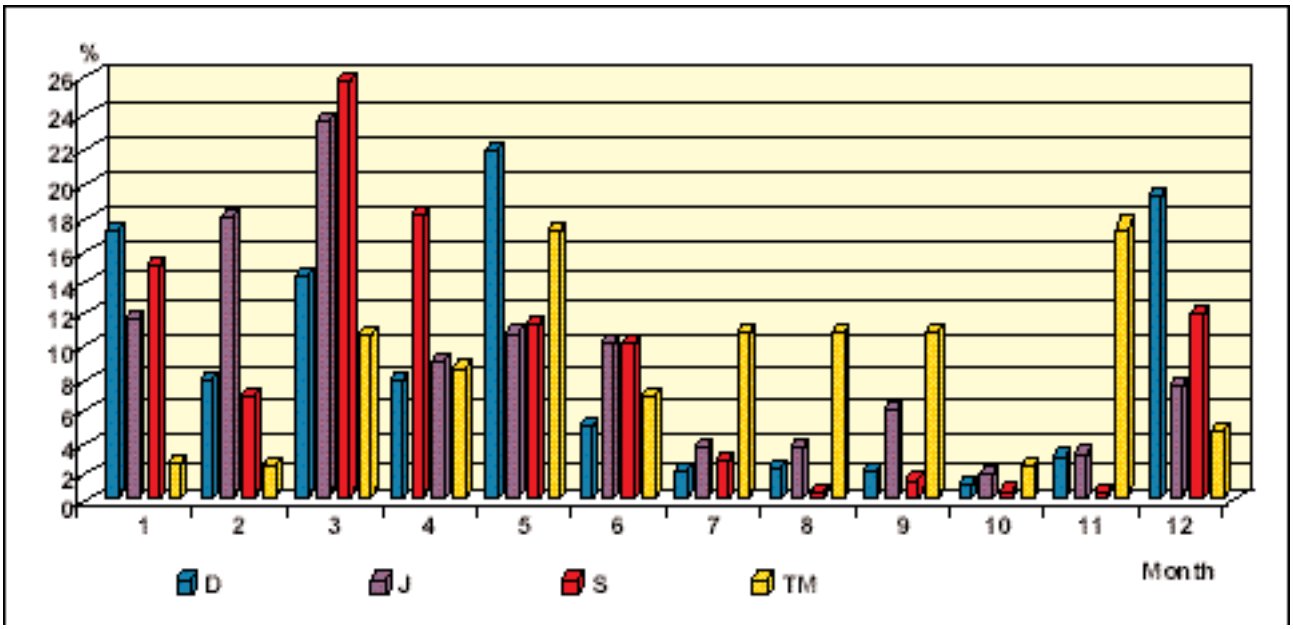


Figure 5. Calving frequency in pluriparous buffalo cows. Farms D-J-S utilized "out of breeding season mating"; in farm TM mating occurred during the natural breeding season period. (Zicarelli et al., 1994)

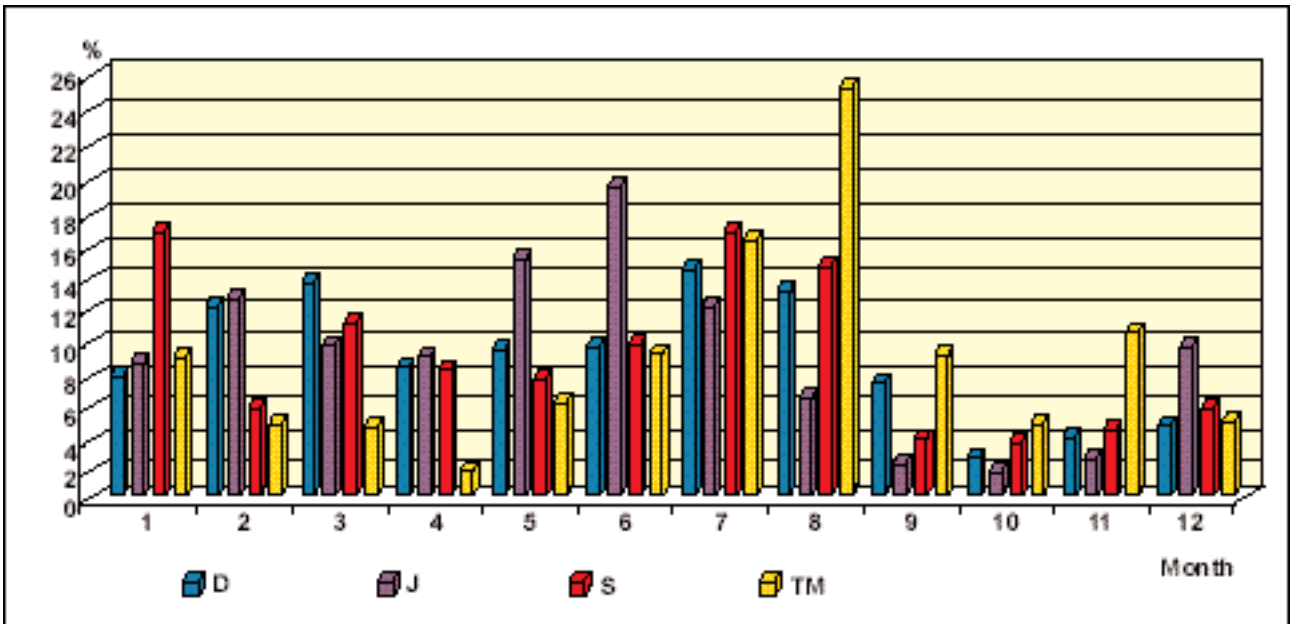


Figure 6. Calving frequency in primiparous buffalo cows. Farms D-J-S utilized "out of breeding season mating"; in farm TM mating occurred during the natural breeding season period.
(Zicarelli et al., 1994)

Anoestrus and Oestrus Induction

The long intercalving period is one of the major problems in buffalo breeding. The interval from calving to resumption of ovarian function is longer in buffalo when compared with cattle. Post-partum ovarian activity resumption, and subsequent conception, may be affected by several factors such as breed, nutrition plan, milk yield, suckling, uterine involution, season of calving (Ahmad et al.,1981; Jainudeen et al., 1983; McCool et al., 1987; Usmani et al., 1990; Borghese et al., 1993b; Qureshi et al., 1999b; Arya and Madan, 2001; Baruselli et al., 2001; Campo et al., 2002).

A large variability is reported in the literature for the intercalving period depending on the region where buffalo are raised and the calving season (Table 6). In India, Gill and Ruki (1985) referred to an intercalving period of 459 to 478 days for buffaloes calving from February to June and 369 to 391 days for those calving from July to January. In Pakistan, Ahmad et al. (1981) reported an average calving interval of 531.5 days with highly significant differences in length of calving interval due to the season of calving: 569.1 and 570.6 days in spring and winter *vs* 506.6 and 515.7 days in summer and autumn respectively. In Italy, Maymone and Pilla (1960) referred to an average intercalving interval of 447.5 days for the buffaloes kept mostly in stables and 411.7 days for those kept in semi-range conditions; they found that the intercalving period was shorter during the months in which parturitions were more numerous (August, September and October).

In Brazil, Pires et al. (2002) report that the mean interpartum interval was of 453.1± 127.26 days. In Cuba, Campo et al. (2002) have found an intercalving period of 384.0±2.3 days in buffaloes calved in the rainy season (June to September) and 361.0±2.5 days in those calved in the dry season (November to February). In Egypt, Barkawi et al. (1998) report an intercalving interval of 363.5±16.0 and 400.3±14.3 days in the cool season (November through April) and an interval of 387.0±15.3 and 441.5±14.3 days in the hot season (May through October), depending on different frequency in oestrus detection checking; thus buffaloes calving in the cool season had better reproductive performance than those calving in the hot season.

Body condition score (BCS) plays an important role in the reproductive performance of post-partum buffalo cows. Baruselli et al. (2001), in South-eastern Brazil, report that first post-partum oestrus was influenced by BCS at calving; cows with high BCS had an earlier first post-partum oestrus and a shorter service period than cows with lower BCS.

Suckling significantly increases the interval from parturition to first oestrus in buffalo. Jainudeen et al. (1983) found that in Malaysian Swamp buffaloes that suckled their calves the interval from parturition to first ovulation was 96±22 days in 32 percent of buffaloes, while over 68 percent were in anoestrus within 150 days post-partum. An earlier resumption of ovarian activity in milked rather than suckled buffaloes was found by El-Fouly et al. (1976). These authors report that only 38 percent of suckled buffaloes restored ovarian activity within 90 days from parturition in concurrence with the data of Janudeen et al.(1983). The extension of anoestrus period due to calf suckling is also reported by Usmani et al.(1990). They found a post-partum oestrus cyclicity resumption delayed by three to four weeks due to the practice of let buffaloes be suckled by their calves, before each milking, to stimulate milk let down. Arya and Madan (2001) also found a longer interval from parturition to first observed oestrus and a longer service period in suckled than weaned buffaloes (71.67±11.13 and 98.00±17.53 *vs* 44.17±8.58 and 70.33±9.56 days respectively). Therefore, suckling regulates the resumption of post-partum ovarian activity, but there is evidence to indicate that the season of calving may be more important than suckling.

The sensitivity of the species to the photoperiod, together with environmental factors, plays an important role in the regularity of oestrous cycle. Buffaloes calving in the autumn show shorter postpartum anoestrus than those calving in the spring and summer, since their ovarian activity

Table 6. Post-partum reproductive features in the buffalo cow as reported by various authors

Author	Country	Calving interval	Anoestrus length	Service period
Maymone and Pilla (1960)	Italy	411.7 - 447.5 d		
Ahmad et al. (1981)	Pakistan	506.6 - 570.6 d		
Gill and Ruki (1985)	India	369 - 478 d		
McCool et al. (1987)	Australia		5.8 m	
Borghese et al. (1993b)	Italy		25.2 d autumn 58.2 d summer	
Barkawi et al. (1998)	Egypt	400.3 - 441.5 d		
Qureshi et al. (1999a)	Pakistan		55.9 d breeding season 91.1 d low breeding season	
Qureshi et al. (1999b)	Pakistan		48.4 d autumn 185.9 d summer	67.2 d autumn 220.5 d summer
Naqvi (2000)	Pakistan			237.5 d
Arya and Madan (2001)	India		71.6 d suckled 44.1 d weaned	98.0 d suckled 70.3 d weaned
Pires et al. (2002)	Brazil	453.1 d		
Campo et al. (2002)	Cuba	361.0 - 384.0 d	39.0 d dry season 58.3 d rainy season	

d: days; m: months

resumption corresponds to the beginning of the short day-length period.
In the Australian Swamp buffalo cow, McCool et al.(1987), found a mean post partum anoestrus interval of 5.8±3.3 months with a variation depending on the season of calving: buffaloes calving in the late dry season (August to November) exhibited a longer interval than those calving earlier. Moreover, heavier cows had a shorter post-partum anoestrus. The late dry season is characterized by high temperatures and low fodder availability that lead to a deterioration in the buffalo's body condition and thus could be, according to the authors, the cause of delayed ovarian activity resumption. Qureshi et al. (1999a), in the Nili-ravi buffalo raised in Pakistan, found a shorter postpartum anoestrus interval in buffaloes calved during the normal breeding season (August to January) than those calved during the low breeding one (February to July) (55.95±4.90 *vs* 91.15±11.61 days). In another work, the same authors (Qureshi et al., 1999b) confirm the influence of season on the post-partum anoestrus length (185.95 days in summer *vs* 48.42 days in autumn) and on the service period (220.53 days in summer and 67.29 in autumn); the number of services per conception was also higher in the

summer with respect to that recorded in the winter. Likewise, Naqvi (2000) in a study in Pakistan, registered a service period of 237.57±4.5 days with a trend of reduction in the length of service period with an increase of parity (287.54±6.89 days in the 1st parity *vs* 107.95±19.72 days in the 8th parity); a shorter service period was recorded in buffaloes calving in the spring and winter compared with those calving in the summer and autumn. Campo et al. (2002) investigated the seasonal effect on uterine involution and post-partum ovarian activity in buffaloes raised in Cuba. These authors found no significant differences in the uterine involution between buffaloes calved in the rainy season (June to September) and those calved in the dry season (November to February), but seasonal influence was found in the resumption of ovarian activity: the first formation of corpus luteum after calving was found at 58.3±3.4 days in the rainy season and at 39.0±2.3 days in the dry one. In the Italian buffaloes, Borghese et al. (1993b) reported a post-partum anoestrus of 49.5±38.8 days in the primiparous and 51.3±26.0 days in the pluriparous. The early resumption of ovarian activity occurred mainly in October - November in the decreasing photoperiod. In fact, the buffaloes calved in the autumn showed a shorter post-partum anoestrus (19.9±10.9 days in primiparous and 25.2±12.7 days in pluriparous) than those calved in summer (50.8±24.3 days in primiparous and 58.2±24.3 in pluriparous). Sometimes the anoestrus is prolonged due to sudden climatic variation such as a fall in temperature, exposure to cold wind, heavy rain associated with low temperature or hot weather without any possibility of bathing or sheltering from the sun (Zicarelli, 1997).

In Italy another reason for prolonged anoestrus is the practice of "out of breeding season mating". In this case, buffaloes which calve in the first months of the year and do not become pregnant within 70 days from calving, will prolong anoestrus until the beginning of the short day-length period (autumn) (Zicarelli, 1997). The need to employ the strategy of "out of breeding season mating" with the aim of conciliating the production with the higher milk market demand in the spring / summer period, is in contrast with the condition of the greatest fertility of the herd that corresponds to the autumn season, period in which females are separated from males.
To increase fertility in the low breeding season and reduce the post-partum anoestrus and subsequent intercalving period, different hormonal treatments are utilized.

Prostaglandins have been used to induce oestrus in buffalo, but they work if a corpus luteum is present and therefore they can be useful in suboestrus animals, having a synchronizing more than an inducing effect (Dhalival et al., 1988; Sahasrabudhe and Pandit, 1997; Awasthi et al., 1998; Chohan, 1998; Kharche and Srivastava, 2001). The use of gonadorelin (GnRH), given by multiple injections or in microencapsulated form, did not appear efficacious and moreover their administration times are not of practical use (Minoia et al., 1984; Chantaraprateep et al.,1988; Shah et al., 1990; Fateh Mohammed et al., 1999; Takkar et al., 1999). More useful and efficacious have been the treatments using progesterone associated with gonadotrophin or gonadorelin (Zicarelli and Boiti, 1982; Rao and Sreemannarayana, 1983; Singh et al., 1983, 1984, 1988; Borghese et al., 1993c; Uma Shanker et al., 1999; Hattab and Osman, 2000).

Trials to remove anoestrus have been carried out on the Italian buffalo (Borghese et al., 1993c; Zicarelli et al., 1994). Buffalo cows found non-cycling at 150 days from calving, were submitted to the following treatments in the January-May period: a) subcutaneous implants of Norgestomet (synthetic Progesterone) + PMSG; b) Buserelin (GnRH-analogue) released by subcutaneous osmotic pump; c) Progesterone + Buserelin i.m. Buffaloes which began cycling were 81.3 percent, with no differences between treatments, while none of the control animals were found cycling in the same period (Table 7) (Borghese et al., 1993c). At the end of the breeding period, the fertility for all the treated animals was 73.8 percent *vs* 54 percent for the controls; nevertheless hormonal treatment was inefficacious on farms where buffaloes had serious infertility problems, and less efficient when utilized in the winter / spring period in respect to the summer period which is nearest to the reproductive season. In treated buffaloes the calving conception interval was reduced to about 40 days on average and the number of culled animals, because of infertility, was lower (18.1 percent *vs* 31.2 percent respectively in

treated and controls) (Zicarelli et al.,1994). Therefore these hormonal treatments have been able to reduce the intercalving interval and to increase the fertility of the herd out of the breeding season.

Better results have been obtained using a progesterone - releasing intravaginal device (PRID) associated with PMSG and prostaglandin. In a group of buffaloes on the experimental farm of our Institute which did not get pregnant in the autumn, PRID was used during the following spring and summer seasons in order to ascertain whether the treatment would improve the pregnancy rate in the low breeding season (Barile et al., 1996a; 1997).

Table 7. Rate of buffaloes becoming cyclic after different hormonal treatments (Borghese et al., 1993c)

Treatments	No. of animals	Non Cyclic No. (%)	Cyclic No. (%)
a) Norgestomet	14	2 (14.2)	12 (85.8)
b) Buserelin	17	5 (29.4)	12 (70.6)
c) Progesterone	33	5 (15.2)	28 (84.8)
Treated (a+b+c)	64	12 (18.7)	52 (81.3)
Control	52	52 (100)	0

The conception rate after PRID treatment (Table 8) was 21 percent at the induced oestrus while the total conception rate (up to the third oestrus) was 56.5 percent. This result is very satisfactory if compared to the conception rate ranging from 12.2 percent to 44.4 percent obtained on the same farm and during the same period (June-August) in the previous years without oestrus induction (Figure 7).

A significant difference was found between the lactating group and the non-lactating one (Table 8). The very low conception rate obtained for non-lactating buffaloes at first oestrus (4 percent) was probably due to the fact that they were animals which had fertility problems, as they had not become pregnant during the whole previous lactation period. For these buffaloes however PRID was helpful in improving cyclic activity so that a 50 percent conception rate was finally achieved.

Table 8. Conception rate in buffalo cows after PRID treatment

Group	No. of animals	Conception rate		
		1st oestrus No. (%)	Following oestrus (2nd+3rd) No. (%)	Total No. (%)
Lactating buffaloes (AI at first oestrus + natural mating at subsequent oestrus)	23	8 (34.8) a	8 (34.8)	16 (69.6)
Lactating buffaloes (natural mating)	17	4 (23.5) a	4 (23.5)	8 (47.1)
Non-lactating buffaloes (natural mating)	22	1 (4.0) b	10 (45.5)	11 (50.0)
Total	62	13 (21.0)	22 (35.5)	35 (56.5)

a,b $P \leq 0.001$
(Barile et al., 1997)

Similar results using PRID during the spring season were obtained by Zicarelli and Boiti (1982). They reported a conception rate of 50 percent for the cycling animals and 33 percent for the non cycling animals after the treatment.

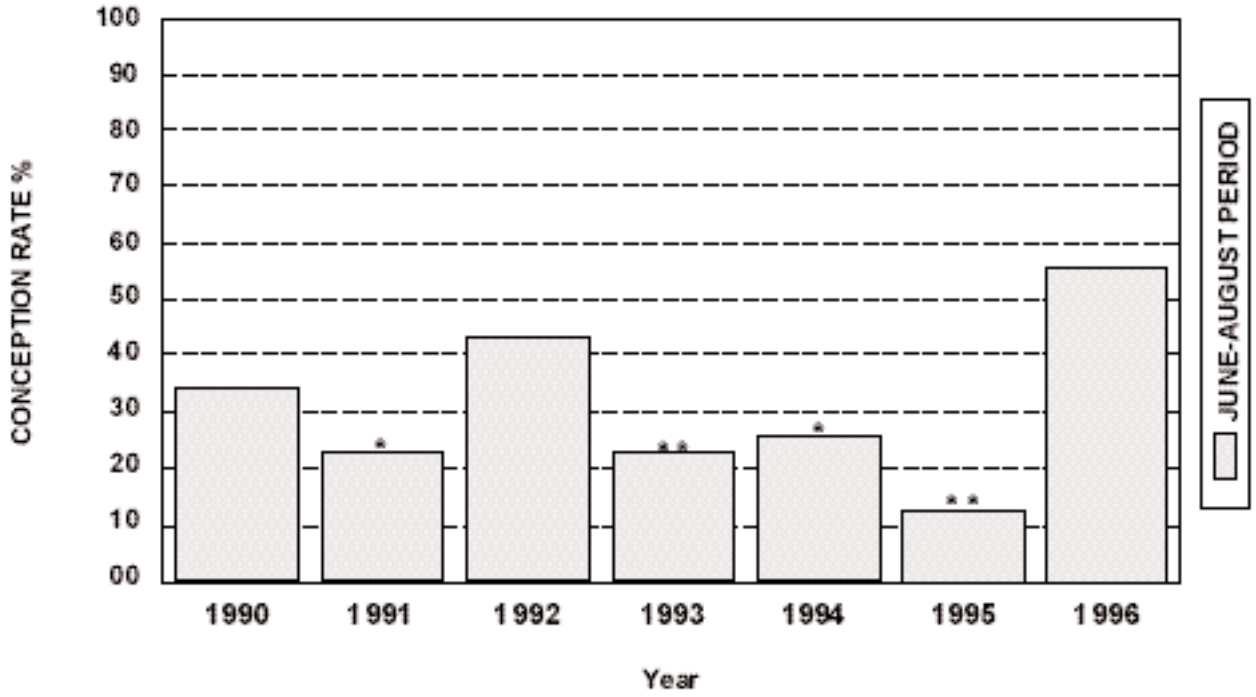


Figure 7. Conception rate in buffalo cows during the non breeding season (June August) using PRID (1996) or not (previous years).
(Barile et al., 1996a)

Oestrous Cycle

An accurate knowledge of the regulatory mechanisms associated with the oestrous cycle is necessary to increase the reproductive efficiency of the buffalo. Current knowledge of the basic pattern of changes in the hormone profile during the oestrous cycle and the basic pattern of follicle development, are important to develop models for improving reproductive efficiency, particularly when controlled breeding techniques using synchronization and superovulation protocols are utilized.

Up-to-date studies on the oestrous cycle, oestrous behaviour and the endocrinology of the oestrous cycle in the buffalo, have been recently reviewed by Beg and Totey (1999), Singh et al. (2000) and Malfatti (2003). Considerable variations in the reproductive traits of the different breeds have been observed. The average length of the oestrous cycle has been reported to be 21 days in the riverine type. Several factors such as climate, temperature, photoperiod, nutrition, have been shown to affect the length of oestrous cycle and the degree of heat expression. Oestrous behaviour in buffalo has a lower intensity than in cows and is therefore much more difficult to detect. Acceptance of the male is considered as the most reliable indication of oestrus in the buffalo. Salient signs of oestrus in River buffalo are reported to be frequent urination, bellowing, vulva swelling, mucous discharge, but they cannot be considered reliable indicators of oestrus because of their weak expression. The average duration of oestrus is 20 hours and appears to be slightly longer in the River buffalo than in the Swamp buffalo. Durations ranging from a short period of 9 hours to a long period of 56 hours have been reported.

In the Italian buffalo cow, a wide variability in the length of oestrous behaviour has been verified, depending on the month in which it was recorded and also according to some climatic factors (Campanile et al., 1988; Zicarelli et al.,1988). In relation to oestrous cycle length,

Zicarelli (1992) distinguishes these categories: short (<12 hours), medium (13-24 hours), long (24-48 hours) and very long (>48 hours) oestrus. In the short and medium oestrus the ovulation occurs after the end of oestrus (6-72 hours and 24-60 hours from oestrus beginning respectively). Depending on the ovulation time, the short oestrus often continues as silent oestrus. On the contrary, in the long and very long oestrus, ovulation can occur before the end of oestrous behaviour. Sometimes in these cases a second ovulation can be recorded after the end of oestrus and pregnancy occurs in the uterine horn on the same side as the last ovulation.

To better understand the endocrine factors involved in the control of ovarian activity in the buffalo, a research was elaborated to evaluate the secretory patterns of gonadotrophin (Luteinizing hormone (LH) and follicle stimulating hormone (FSH)), prolactin, ovarian steroids (progesterone and oestradiol-17β), and PGFM (a prostaglandin metabolite) through the oestrous cycle. The trials were carried out on buffalo cows of our Institute, in different seasons, and the results have been reported by Seren et al. (1994). Only 37.5 percent of the animals showed signs of heat. Oestrous behaviour lasted on average 32.7 hours, with a large individual variation (from 5 to 57 hours) as previously reported by Zicarelli (1992). In 16.6 percent of buffaloes showing oestrus, symptoms were present during the luteal phase and therefore recorded as false heats. The remaining 62.5 percent of buffaloes had a normal endocrine activity without external signs of oestrus (silent heat); the only sign in these animals was the mucous discharge. Regarding the interval between the beginning of oestrous behaviour and ovulation time, which is important for the application of artificial insemination, the value was 54.6 hours. Double ovulations were recorded in 33.3 percent of buffaloes; in this case the mean intervals between the beginning of oestrous behaviour and ovulation time was 40.4 hours for the first and 112 hours for the second ovulation. The perioestrous endocrine changes observed did not show clear difference between the seasons and are entirely similar to those recorded in cows (Figure 8). The progesterone concentration dropped two to four days before oestrus and ovulation. At the same time peak levels of PGFM were recorded; high pulses of PGFM were then found until luteolysis was completed. After the progesterone drop, oestradiol progressively increased triggering FSH and LH ovulatory peak. The mean interval between the LH peak and the ovulation time was 35.5 hours. Sometimes a second peak of LH occurred that preceded the following ovulation by 47.7 hours. No correlations were found between hormonal profile and oestrous behaviour in buffaloes with silent oestrus, anovulatory heat or double ovulations (Zicarelli et al.,1993).

In a recent study on buffalo oestrous behaviour in the presence of a teaser bull (Moioli et al., 1998), the average duration of interest shown by the bull towards a buffalo cow (from the very first to the last sign of interest) was 68 hours. Within this period, the phase of continuous courtship was longer and lasted on average 32 hours; this phase was considered the best variable to refer to for visual assessment of oestrus, because it is easy to detect even if the herd is observed only three or four times a day. In this study the interval between the LH peak and ovulation was found to be on average 25 hours for those animals which became pregnant after artificial insemination and 46 hours for those which did not become pregnant. It has been hypothesized that in this case an insufficient LH peak and a delayed luteinization are contributing causes to unsuccessful inseminations.

These studies indicate that the oestrus length and the ovulation time in the Italian buffalo manifest a wide variability with respect to buffalo raised in tropical conditions. Ovulation cannot be predicted from oestrous behaviour signs such as bellowing, frequent urination and mucous discharge, because they are not often displayed or when they are present they are very variable in relation to ovulation time and sometimes do not coincide with oestrus. For these reasons the application of artificial insemination is limited in the buffalo, taking into account the fact that a high conception rate depends mainly on insemination at a correct time relative to ovulation.

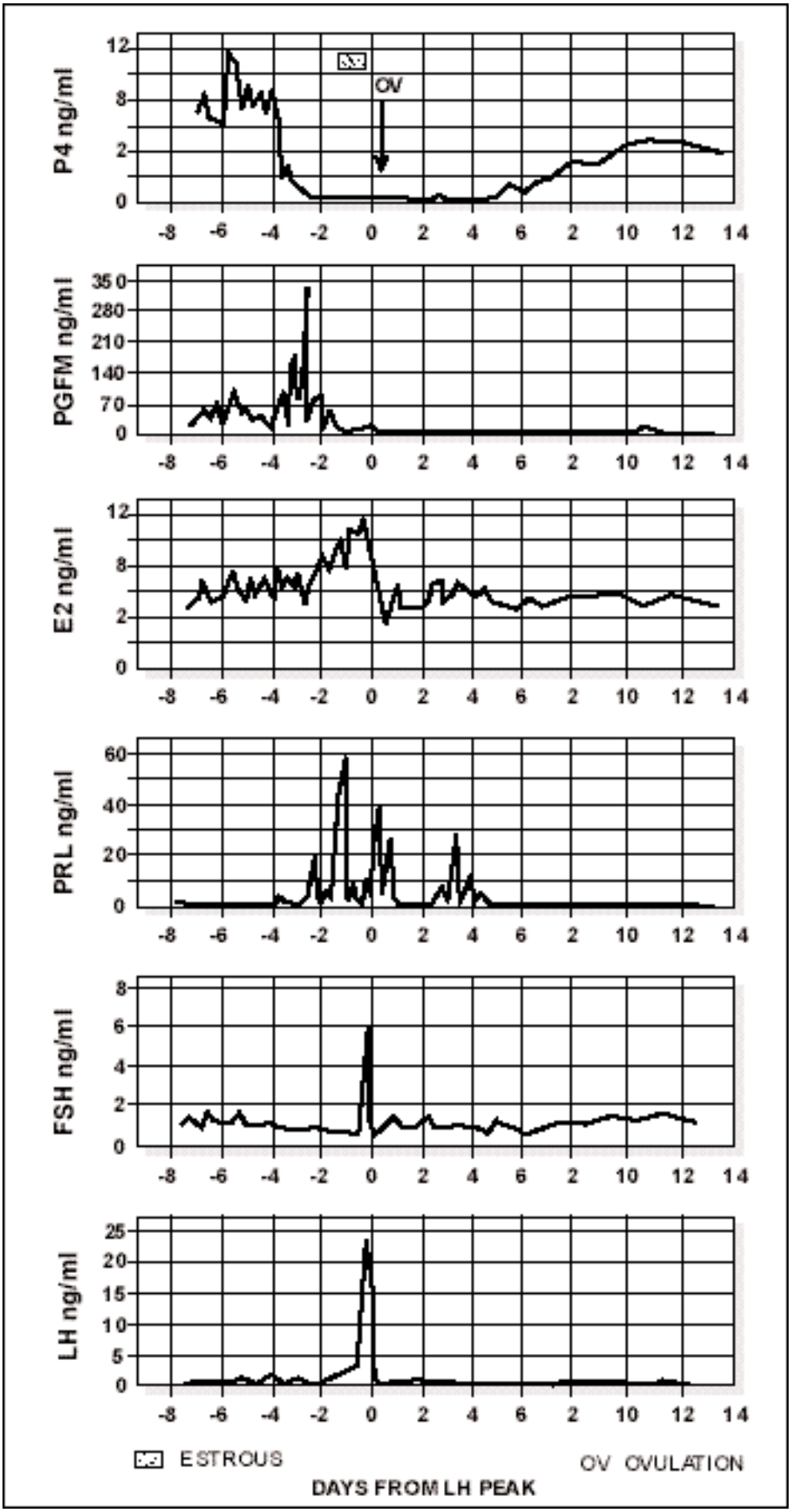


Figure 8. Perioestrus endocrine changes in the buffalo cow. (Seren et al., 1994)

Oestrus Control in order to apply Artificial Insemination

The presence of a teaser bull is helpful to identify buffaloes on heat (Figure 9); in this case the standing oestrus is the most reliable sign referable to a next ovulation, although a wide variability has been observed in the interval between the start of standing oestrus and LH peak (from 124 hours before the LH peak to 6 hours after the peak) (Barile et al., 1996b). The end of bull courtship and the end of bull acceptance by the female are reliable signs that indicate the end of heat and the occurrence of ovulation. Utilizing a teaser bull and inseminating the animals after the end of heat, Baruselli (1996) had a conception rate ranging from 40.44 percent to 60.68 percent depending on farms. Zicarelli et al. (1997b) have studied the effects of the presence or absence of a vasectomised buffalo bull on the reproductive efficiency of buffalo cows undergoing artificial insemination. They reported that exposure to a vasectomised bull increases the pregnancy rate in buffaloes inseminated at spontaneous (42.5 *vs* 18.9 percent) or induced oestrus (51.1 *vs* 33.3 percent). In the absence of the bull, pregnancy rate at AI was higher in cows inseminated at induced oestrus than at spontaneous oestrus (33.3 *vs* 18.9 percent). Similar results were found from our group in buffaloes inseminated at spontaneous oestrus in the presence of a vasectomised bull: pregnant cow rate was 56 percent in total and 40 percent at first oestrus (Moioli et al., 1998).

New approaches are being developed to provide automated systems of detection of oestrus using electronic technology in cattle such as pedometry and pressure sensing radiotelemetric HeatWatch® system (Nebel et al., 2000). Recently, studies on the efficiency of pedometers in buffalo oestrus detection have been carried out in Italy by Di Palo et al. (1999, 2001). They report that the pedometer has been found to be very useful for AI when visual observation of oestrus can be carried out only for a short time, providing a greater number of alerts for spontaneous oestrus to be inseminated; the conception rate at AI was 40 percent. A study on oestrous detection using radiotelemetry has been carried out in Brazil by Baruselli (2001). The author reports that the distribution of mountings during the day did not present significant differences showing that buffalo present a homogeneous distribution of oestrus during the 24 hours of the day. The use of a vaginal electrical resistance (VER) probe to predict oestrus and ovarian activity has been studied by Gupta and Purohit (2001) on Indian buffaloes. They proved that VER can be used successfully to predict the stage of oestrous cycle, ovarian status and ovulation; insemination at a low VER distinctly improves the conception rate in buffaloes (81.48 *vs* 16.66 percent with 26 and 40 ohms respectively).

The use of management schemes that do not require the identification of oestrus, contribute to the increase in the use of AI in buffalo herds, mainly because it is easy to perform. In order to apply a fixed time AI, thereby surmounting the problem of oestrus detection, different hormonal treatment schedules have been proposed (Tables 9 and 10). Various authors have recorded the use of PGF2*a* or one of its analogues in oestrus control in buffalo, often using an 11 day interval between two consecutive doses. The endocrine change after PGF2*a* induced luteolysis appears similar to that occurring at natural oestrus (Kamonpatana et al., 1979). Chohan et al., (1993) reported a fertility rate at AI of 22.8 percent in the low breeding season and 53.3 percent in the peak breeding season, in buffaloes synchronized with PGF2*a*, concluding that the use of PGF2*a* to synchronize oestrus should be undertaken in animals having a functional corpus luteum and preferably during the peak breeding season. Nevertheless, Sahasrabudhe and Pandit (1997) reported that a high percentage of suboestrus buffaloes expressed oestrus after PGF2*a* treatment during the hot season. The detection of oestrus after prostaglandin treatment, however, had posed problems because external signs of oestrus were found by some workers to be less apparent than at spontaneous oestrus. Baruselli (2001) detected a greater variation in the duration of oestrous manifestation after the administration of prostaglandin; moreover he found that the phase in which prostaglandin was administered interfered with the interval from administration and the beginning of oestrous manifestation and ovulation. Therefore protocols using fixed time insemination and only prostaglandin treatment have not produced good results. In order to decrease the variation in the ovulation time after prostaglandin treatment, the use of GnRH has been associated with

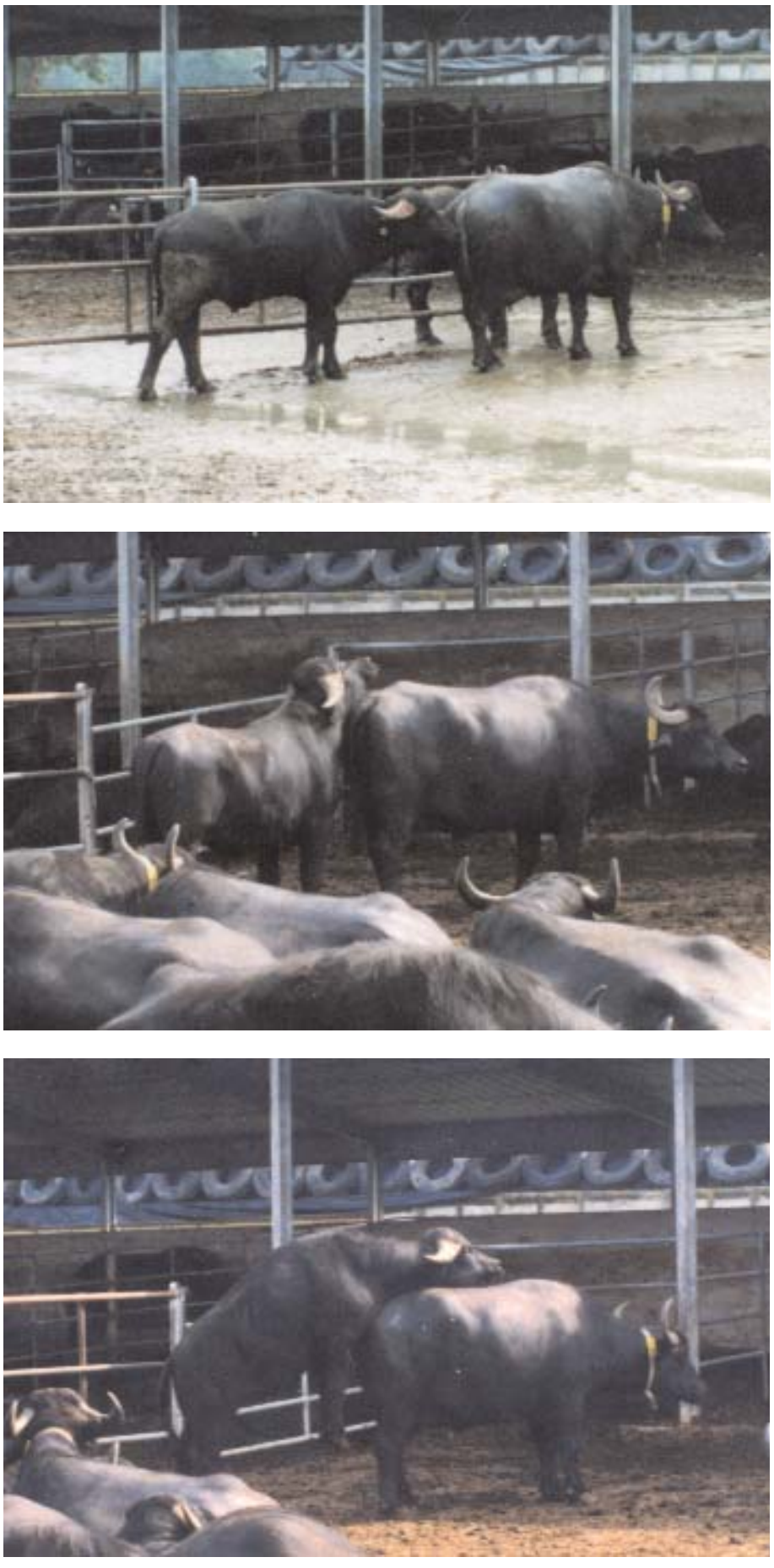


Figure 9. Use of teaser bull for oestrus detection: phase of courtship (a and b); standing oestrus (c). (Moioli photo, 1994)

that of prostaglandin. Some trials have demonstrated that oestrus synchronization and in particular ovulation synchronization can be obtained using GnRH + prostaglandin after seven days + GnRH after 36 to 48 hours (Ovsynch protocol). This second administration of GnRH improves the efficiency of fixed time insemination because it synchronizes the ovulation in a short period of time. Baruselli et al. (1999) using this protocol had a CR of 48.8 percent in buffaloes inseminated during the breeding season (autumn / winter) and 6.9 percent in those inseminated during the non-breeding season. Neglia et al. (2001) utilizing a synchronization treatment with prostaglandin have reported a CR of 43.4 percent; by adding an injection of GnRH to this treatment, at the time of the first insemination, they found a similar CR (45.8 percent).

Table 9. Hormonal treatments to control oestrus in order to apply AI in buffaloes. Use of PGFα and GnRH

Reference	Treatment	Period	Conception rate
Chohan et al. (1993)	PGF2α	Peak breeding season Low breeding season	53.3 22.8
Baruselli et al. (1999)	GnRH+PGF2α+GnRH	Breeding season Non-breeding season	48.8 6.9
Neglia et al. (2001)	PGF2α PGF2α +GnRH	Low breeding season	43.4 45.8
Baruselli et al. (2002)	GnRH+PGF2α+GnRH	Non-breeding season	28.2
de Araujo et al. (2002)	GnRH+PGF2α+GnRH GnRH+ PGF2α+LH	Breeding season	56.5 64.2
Neglia et al. (2003)	GnRH+PGF2α+GnRH	Low breeding season	36.0
Barile et al. (2004)	GnRH+PGF2α+GnRH	Low breeding season	42.5

Table 10. Hormonal treatments to control oestrus in order to apply AI in buffaloes. Use of progesterone intravaginal device.

Reference	Treatment	Period	Conception rate
Rao and Rao (1983)	PRID	Peak breeding season Rest of the year	40.7 25.3
Sing et al. (1988)	PRID PRID+PMSG	Summer	8-28 50.0
Barile et al. (2001c)	PRID PRID+500 IU PMSG	Low breeding season	17.5 26.0
Barile et al. (2001b)	PRID+1000 IU PMSG	Low breeding season	56.7
Baruselli et al. (2002)	CIDR+eCG+hCG	Low breeding season	53.5
Neglia et al. (2003)	PRID+1000 IU PMSG	Low breeding season	28.2
Barile et al. (2003)	PRID+ 1000 IU PMSG PRID+1000 IU PMSG+GnRH	Low breeding season	64.5 45.2
Barile et al., (2004)	PRID+1000 IU PMSG	Low breeding season	47.8

Other authors using the Ovsynch protocol reported a CR at AI ranging from 56.5 percent (de Araujo Berber et al., 2002), if used during the breeding season, to 36.0 percent (Neglia et al., 2003), and 42.5 percent (Barile et al., 2004) if used in the period of transition to seasonal anoestrus.

Natural or synthetic progesterone containing devices (injections, intravaginal pessary, ear implants along with estradiol, PMSG and prostaglandin) have been used successfully to improve synchrony of oestrus and conception in buffaloes. Baruselli (2001) used progesterone intravaginal pessary (CIDR-B) or progestagen ear implant (CRESTAR) along with estradiol to study the follicular dynamic during the retaining of implants in order to evaluate the appropriate moment for fixed time insemination in buffalo cows. The author found that the CRESTAR protocol was not efficient in synchronizing oestrus and ovulation, while animals treated with CIDR-B protocol ovulated, although the percentage of ovulated animals (66.6 percent) and synchronization of ovulation (varying from 32 to 96 hours) was not particularly efficient.

The synchronization protocols, however, are efficient if buffaloes are cyclic and therefore these protocols can be used during the breeding season (autumn). In the spring season there is a higher variability between the beginning of oestrus and the ovulation time and it is more difficult to establish the correct time for AI.

Our previous work showed that the use of a progesterone pessary (PRID) improves pregnancy rate in the low breeding season and, moreover, is able to induce synchronization so that insemination can be effected at fixed times, overcoming the problem of the difficult oestrus detection (Barile et al., 1996a, 1997). The protocol foresees the use of a progesterone releasing intravaginal device (PRID), containing 1.55 g natural progesterone and a gelatine capsule with 10 mg oestradiol benzoate, kept in place for ten days. On the seventh day after PRID insertion, an injection of 1000 IU PMSG and one of 0.15 mg cloprostenol, a prostaglandin F2*a* analogue, are given. At PRID removal buffaloes are artificially inseminated at fixed times from withdrawal. In the first trial, in order to deal with ovulation time variability animals were inseminated three times: at 48, 72 and 96 hours from withdrawal and, in addition the same synchronization treatment schedule was used in the peak breeding season (autumn) and the low breeding one (spring), to evaluate if there was any difference in the conception rate at AI (Barile et al., 1999). The fertility rate did not differ between the two seasons considered. In fact, in autumn and in spring, respectively the peak and the low breeding seasons for Italian buffalo, the CR was 46.2 percent and 44.3 percent. Rao and Rao (1983) investigating the PRID treatment both during the peak and the low breeding season found that fertility was much higher during October to January (peak breeding season) than during the rest of the year (40.7 percent *vs* 25.3 percent). Singh et al. (1988) found that the use of gonadotrophin in addition to the PRID treatment ensures a good ovulatory response in the low breeding season. In fact, during the summer months, they observed a pregnancy rate of 50 percent in Indian buffaloes synchronized with PRID + PMSG, which was higher than that observed in their previous work using treatment with PRID alone (8 percent to 28 percent; Singh et al., 1983; 1984). We also found that the use of PMSG increases the fertility that is related to the doses utilized; in fact CR was 26 percent in buffaloes in which PRID + 500 IU PMSG were used and 17.5 percent in buffaloes in which PRID was used without gonadotrophin (Barile et al. 2001c). In our treatment schedule the addition of PGF2*a* to PRID + PMSG was useful to avoid any presence of a functional corpus luteum at the device removal which could delay the synchronization as reported in cattle by Mialot et al. (1996) and in buffaloes by Subramanian and Devarajan (1991).

Therefore PRID associated with PMSG and prostaglandin can be successfully employed in the low breeding season to increase the effectiveness of AI programmes in improving the fertility rate. The lack of difference in the CR between autumn and spring allows the use of AI for application in breeding schemes during the low breeding season, thus resolving the need of Italian farmers to have conceptions between March and September in order to satisfy the higher market demand for buffalo milk in the summer.

To better define the proper time for AI following the PRID synchronization treatment we have evaluated the time of LH peak after pessary removal (Barile et al., 1998; Borghese et al., 1999). In buffaloes synchronized in spring (Barile et al., 1998) the interval from PRID removal to LH

peak was 54.7±12.3 hours ranging from 40 to 76 hours. Considering ovulation as the time at which a ruptured follicle was palpated, the interval from PRID removal to ovulation was 84±13.1 hours whilst the one from LH peak to ovulation was 31.0±8.9 hours, similar to the one found in previous work (Seren et al.,1994) in non treated buffaloes which was 36 hours on average. Evaluating the time of LH peak in oestrus synchronized buffaloes in two different seasons (Borghese et al.,1999), it was found that the interval from PRID removal to LH peak was 46.87±21.53 hours in November and 61.00±12.05 hours in March. The ovulation (checked daily by rectal palpation) occurred within 72 hours from PRID removal in November and within 96 hours in March. On the basis of these results it has been suggested that 72 and 96 hours after PRID removal are more appropriate times for AI in synchronized buffalo cows in the low breeding season while 48 and 72 hours could be better in the autumn. In fact, utilizing PRID+1000 IU PMSG and two AI schedules at 72 and 96 hours during the spring season we have obtained a CR ranging from 47.8 to 64.5 percent in different years (Barile et al, 2001b, 2003, 2004). These are good results considering that animals are treated in a period in which their reproductive efficiency is lower and that the treatment increases the fertility so that buffaloes that do not conceive at AI will become pregnant later during the natural breeding period.

In order to decrease the variation in ovulation time and increase the effectiveness of fixed time AI, GnRH was used in association with PRID treatment, but the conception rate did not improve with respect to that found using the PRID protocol alone (45.2 percent and 64.5 percent with PRID+GnRH or PRID respectively) (Barile et al., 2003). Satisfactory results (53.5 percent of CR) during the non-breeding season have been obtained by Baruselli et al. (2002) using a progesterone intravaginal device (CIDR) associated with eCG and hCG (equine and human Chorionic Gonadotrophin), since the animals received only one insemination (62 hours from CIDR withdrawal). Recently, we have compared the efficiency of PRID and Ovsynch protocols for the application of fixed time AI in buffalo cows in the Spring. The two different hormonal schedules utilized showed the same efficiency in obtaining oestrus synchronization and a good conception rate at AI, in the Spring. Although the fertility rate did not differ significantly between the PRID and Ovsynch protocols (47.8 percent and 42.5 percent respectively), a higher conception rate was found in buffaloes synchronized with PRID compared with Ovsynch, as PRID treatment was efficient in removing the anoestrus status in non-cycling animals (Barile et al., 2004). This conclusion is supported by the work of Baruselli et al. (1999) that using an Ovsynch protocol resulted in a CR of 48.8 percent in buffaloes inseminated during the breeding season (autumn-winter) and 6.9 percent in those inseminated during the non-breeding season. In fact, the same researchers, comparing CIDR+eCG+hCG treatment to GnRH+PGF2*a*+GnRH (Ovsynch protocol) in the non-breeding season, resulted in a higher CR at AI in animals treated with CIDR (53.5 percent *vs* 28.2 percent) (Baruselli et al., 2002).

Conclusion

Improvement of reproductive efficiency in the buffalo can be obtained by directing attention to management systems and utilizing controlled breeding techniques.

The application of oestrus induction techniques permits the possibility of inducing fertile oestrus in non cycling heifers, in order to increase fertility in the low breeding season and reduce the intercalving period. Different treatments are utilized to induce oestrus, such as prostaglandin, gonadorelin, progestagen, however improved results have been obtained using PRID plus PMSG and prostaglandin. To identify buffaloes in heat, in order to apply AI, the presence of a teaser bull can be helpful. New approaches are being developed to provide automated systems of detection of oestrus using electronic technology such as pedometry and radiotelemetry. To apply a fixed time AI, thereby overcoming the problem of oestrus detection, different hormonal treatment schedules have been proposed. Protocols using fixed time insemination and only prostaglandin treatment have not provided good results. The use of GnRH, in association with that of prostaglandin, improves the efficiency of fixed time

insemination because it synchronizes the ovulation in a short period of time but this treatment is efficient when buffaloes are cyclic. The use of PRID associated with PMSG and prostaglandin can be successfully employed in the low breeding season thereby proving to be the preferred treatment when oestrus synchronization and AI are programmed out of the breeding season.

References

Ahmad, N., Chaudhry, R.A. and Khan, B.B. 1981. Effect of months and season of calving on the length of subsequent calving interval on Nili Ravi buffaloes. Anim. Reprod. Sci., 3: 301-306.

Andurkar, S.B. and Kadu, M.S. 1995. Induction of oestrus and fertility with CIDR device and combination in non cycling buffaloes. Indian J. Anim. Reprod., 16 (2): 81-84.

Arya, J.S. and Madan, M.L. 2001. Post partum reproductive cyclicity based on ovarian steroids in suckled and weaned buffaloes. Buffalo J., 17 (3): 361-369.

Awasthi, M.K., Tiwari, R.P. and Pangaonkar, G.R. 1998. Induction of oestrus and fertility with low dose of prostaglandin F2 alpha in suboestrus buffaloes. Indian J. Anim. Sci., 68 (10): 1049-1050.

Barile, V.L., Galasso, A. and Carretta, A. 1999. Preliminary results on conception rates in Italian buffaloes after the use of intravaginal progesterone device and artificial insemination. Buffalo Newsletter (FAO Bulletin, Rome), 6: 3-4.

Barile, V.L., Moioli, B., Terzano, G.M., Napolitano, F., Puppo, S., Debenedetti, A., Malfatti, A., Catalano, A. and Borghese, A. 1996b. Ovulation prediction to improve artificial insemination in buffalo cows. Bulgarian J. Agric. Sci., 2: 103 107.

Barile V.L., Galasso A., Marchiori E. and Borghese A., 1997. Effect of PRID treatment on oestrus synchronization and progesterone levels in Italian buffaloes. In: Proc. Fifth World Buffalo Congress, Caserta, Italy, 13-16 October: 738-743.

Barile, V.L., Galasso, A., Terzano, G.M., Malfatti, A. e Barbato, O. 1998. Valutazione del picco di LH in bufale (Bubalus bubalis) sottoposte a sincronizzazione dell'estro ai fini della inseminazione artificiale [Evaluation of LH peak in oestrus synchronized buffaloes (Bubalus bubalis) to perform artificial insemination]. In: Atti LII Convegno Nazionale S.I.S.Vet., Silvi Marina, Italy; 17 - 19 Settembre: 85-86.

Barile, V.L., Galasso, A., Pacelli, C., Francillo, M., Cigliano, A., Penna, L., Panfili, M., Fiorini, M. and Borghese, A. 1999. Conception rate in synchronized and artificially inseminated buffalo cows in two different seasons under field conditions. In: Recent Progress in Animal Production Science.1. Proc. XIII A.S.P.A. Congress, Piacenza, Italy. 21-24 June: 262-264.

Barile, V.L., Galasso, A., Marchiori, E., Pacelli, C., Montemurro, N. and Borghese, A. 2001a. Effect of PRID treatment on conception rate in Mediterranean buffalo heifers. Livest. Prod. Sci., 68: 283-287.

Barile, V.L., Galasso, A., Carretta, A., Marchiori, E. and Borghese, A. 2001b. Evaluation of different timed inseminations on conception rate in synchronized Italian buffaloes. In: Proc. Sixth World Buffalo Congress, Maracaibo, Venezuela, 20-30 May : 172-178.

Barile, V.L., Pacelli, C., Galasso, A., De Mauro, G.J., Francillo, M., Cigliano, A., Penna, L., Panfili, M., Terzano, G.M. e Borghese, A. 2001c. Inseminazione artificiale nella bufala. - Risultati di prove condotte nel Lazio. [Artificial insemination in buffaloes. - Results of trials carried out in the Lazio region]. In: Atti 1° Congresso Nazionale sull'Allevamento del Bufalo, Eboli, Italy, 3-5 Ottobre: 354-358.

Barile, V.L., De Santis, G., Malfatti, A., Barbato, O. and Borghese, A. 2003. Use of GnRH in association with PRID treatment in oestrus synchronization protocol for fixed timed insemination in buffalo cow. In: Atti 2° Congresso Nazionale sull'Allevamento del Bufalo, Monterotondo, Italy, 28-30 Agosto: 285-289.

Barile, V.L., Pacelli, C., De Santis, G., Penna, L., Veloccia, C., Allegrini, S., Lomolino, R., Barbato, O. and Borghese, A. 2004. Fixed time artificial insemination in buffalo using two different hormonal schedule for oestrus synchronization. Preliminary results. In: Proc. 7th

World Buffalo Congress, Manila, Philippine, October 20-23, vol.II: 585-587.

Barkawi, A.H., Mokhless, E.M. and Bedeir, L.H. 1989. Environmental factors affecting age at puberty in Egyptian buffaloes. Buffalo J., 5 (1): 71-78.

Barkawi, A.H., Khattab, R.M. and El-Wardani, M.A. 1998. Reproductive efficiency of Egyptian buffaloes in relation to oestrus detection systems. Anim. Reprod. Sci., 51: 225-231.

Baruselli, P.S. 1996. Reprodução de bubalinos. In: I Simposio Brasileiro de Bubalinocultura, Cruz das Almas, Brasil: 29.

Baruselli, P.S., Madureira, E.H., Visintin, J.A., Barnabe, V.H, Barnabe, R.C. and Amaral, R. 1999. Timed insemination using synchronization of ovulation in buffalo. Rev. Bras. Reprod. Anim., 23: 360-362.

Baruselli, P.S. 2001. Control of follicular development applied to reproduction biotechnologies in buffalo. Atti 1° Congresso Nazionale sull'Allevamento del Bufalo, Eboli, Italy, 3-5 Ottobre: 128-146.

Baruselli, P.S., Barnabe, V.H., Barnabe, R.C., Visintin, J.A., Molero-Filho, J.R. and Porto R. 2001. Effect of body condition score at calving on postpartum reproductive performance in buffalo. Buffalo J., 17 (1): 53-65.

Baruselli, P.S., Carvalho, N.A.T., Henriquez, C.E.P., Amaral, R., and Nichi, M. 2002. Synchronization of ovulation for timed artificial insemination during the off breeding season in the buffalo. In: Proc. First Buffalo Symposium of Americas, Belém, Brasil: 418-420.

Beg, M.A. and Totey, S.M. 1999. The oestrous cycle, oestrous behaviour and the endocrinology of the oestrous cycle in the buffalo (Bubalus bubalis). Anim. Breed. Abs., 67 (5): 329-337.

Borghese, A., Terzano, G.M., Barile, V.L., Annicchiarico, G. and Parmeggiani, A. 1993a. Onset of puberty in Italian buffalo heifers: Note II-Influence of bull exposure on age at puberty. In: Proc. International Symposium "Prospects of Buffalo Production in the Mediterranean and the Middle East", Cairo, Egypt, 9-12 November 1992, EAAP Publication, Pudoc, Wageningen, n. 62: 370-373.

Borghese, A., Barile, V.L., Terzano, M.G., Annichiarico, G., De Benedetti, A. and Malfatti, A. 1993b. Anoestrous length in Italian buffalo cows. Note I. In: Proc. International Symposium "Prospect of Buffalo Production in the Mediterranean and in the Middle East", Cairo, Egypt, 9-12 November 1992. EAAP Publication, Pudoc, Wageningen, n. 62: 389-392.

Borghese, A., Barile, V.L., Campanile, G., Esposito, L. e Pacelli, C. 1993c. Induzione dell'estro in bufale acicliche. Nota I. Percentuale di ciclicità. [Oestrus induction in acyclic water buffaloes. Note I. Cyclicity rate]. In: Atti 5° Meeting Nazionale "Studio della Efficienza Riproduttiva degli Animali di Interesse Zootecnico", Bergamo, Italy, 30 Aprile:125-129.

Borghese, A., Terzano, G.M., Barile, V.L., Annicchiarico, G., Allegrini, S., Zicarelli, L., Montemurro, N., Pacelli, C., Campanile, G., Esposito, L., Di Palo, R., Boni, R., Seren, E. e Parmeggiani, A. 1994a. Pubertà e mantenimento dell'attività ciclica ovarica nella bufala. [Puberty and maintenance of ovarian cyclic activity in buffalo] Agricoltura Ricerca, 153: 5-16.

Borghese, A., Terzano, G.M., Barile, V.L. and Parmeggiani, A. 1994b. Season and feeding level effects on onset of puberty in buffalo heifers. In: Proc. Fourth World Buffalo Congress, Sao Paulo, Brazil, 27-30 June: 525-527.

Borghese, A., Barile, V.L., Terzano, G.M., Pilla, A.M. and Parmeggiani, A. 1995. Melatonin trend during season in heifers and buffalo cows. Bubalus bubalis, 1: 61 64.

Borghese, A., Terzano, M.G., Barile, V.L., Catalano, A. and Malfatti, A. 1996. Onset of puberty in buffalo heifers in different feeding and management systems. In: Proc. International

Symposium on Buffalo Resources and Production Systems, Cairo, Egypt, 14 -17 October: 41-46.

Borghese, A., Barile, V.L., Ficco, G., Galasso, A., Marchiori, E. and Terzano, G.M. 1997. Feeding system effect on reproduction performances in buffalo heifers. In: Proc. Fifth World Buffalo Congress, Caserta, Italy, 13 - 16 October: 697-701.

Borghese, A., Barile, V.L., Terzano, G.M., Galasso, A., Malfatti, A. and Barbato, O. 1999. Time of LH peak in oestrus synchronized buffaloes in two different seasons. In: Book of Abstracts of the 50th EAAP Annual Meeting, Zurich, Switzerland, 22-26 August: 166.

Campanile, G., Di Palo, R., Ferrari, G., Intrieri, F. and Zicarelli, L. 1988. Influence of farm and climatic elements on the alfaprostol-induced heats in Mediterranean buffalo cows. In: Proc. Second World Buffalo Congress, New Delhi, India, 12 - 16 December, vol. III: 40-48.

Campanile, G., Di Palo, R., Gasparrini, B., D'Occhio, M.J. and Zicarelli, L. 2001. Effects of early management system and subsequent diet on growth and conception in buffalo heifers. Livest. Prod. Sci., 71 (2/3): 183-191.

Campo, E., Alons,o J.C., Hincapie, J.J., García, L., Faure, O. and Fernández, O. 2002. Seasonal influence on uterine involution and postpartum ovarian activity in river buffaloes. Bubalus bubalis, 8 (3): 59-63.

Chantaraprateep, P., Loachit, C., Kobayashi, G., Techakumphu, M., Virakul, P., Kunavongkrit, A. and Prateep, P. 1988. Ovarian responses to gonadotrophin stimulation in swamp buffalo. Buffalo Bull., 7 (4): 82-86.

Chohan, K.R., Iqbal, J. and Asghar, A.A. 1993. Influence of season on fertility of oestrus synchronized buffalo. Buffalo J., 9 (1): 65-67.

Chohan, K.R. 1998. Oestrus synchronization with lower doses of PGF2*a* and subsequent fertility in suboestrus buffalo. Theriogenology, 50 (7): 1101-1108.

Da Silva, M.E.T. and Grodzki, L. 1991. Study of correlations between climatic factors and seasonal fertility of female buffaloes in the Northeast of the State of Parana - Brazil. In: Proc. Third World Buffalo Congress, Varna, Bulgaria, 13 - 18 May: 689-700.

de Araujo Berber, R.C., Madureira, F.H. and Baruselli, P.S. 2002. Comparison of two Ovsynch protocols (GnRH versus LH) for fixed timed insemination in buffalo (Bubalus bubalis). Theriogenology, 57 (5): 1421-1430.

De Franciscis, G. 1988. Buffalo production system in Europe. In: Proc. Second World Buffalo Congress, New Delhi, India, 12-16 December, vol.II: 21-42.

Dhalival, G.S., Sharma, R.D. and Singh, G. 1988. Efficacy of prostaglandin F2 alpha administration for inducing oestrus in buffalo. Theriogenology, 29 (6): 1401-1406.

Di Palo, R., Parmeggiani, A., Spadetta, M., Campanile, G., Esposito, L., Seren, E. and Zicarelli, L. 1997. Influence of changing farm on repeatability of melatonin plasma level in Italian Mediterranean buffalo. In: Proc. Fifth World Buffalo Congress, Caserta, Italy, 13-16 October: 758-761.

Di Palo, R., Campanile, G., Prandi, A., Ciaramella, R. and Zicarelli, L. 1999. Preliminary results on the use of pedometers to detect oestrus in dairy buffaloes. In: Recent Progress in Animal Production Science.1. Proc. XIII A.S.P.A. Congress, Piacenza, Italy 21-24 June: 265-267.

Di Palo, R., Campanile, G. e Zicarelli, L. 2001. Tecnologie utilizzate per la rilevazione dei calori e inseminazione strumentale nella specie bufalina. [Use of new technologies for heat detection and artificial insemination in the buffalo] In: Atti 1° Congresso Nazionale sull'Allevamento del Bufalo, Eboli, Italy, 3 - 5 Ottobre: 100-113.

El-Fouly, M.A., Kotby, E.A. and El-Soubhy, H.E. 1976. Post partum ovarian activity in suckled and milked buffaloes. Theriogenology, 5: 69-79.

FAO (Food and Agriculture Organization of the United Nations), FAOSTAT Agriculture Data, 2004. http:\\apps.fao.org/default.htm.

Fateh, Mohammed, Dhaliwal, G.S. and Sharma, R.K. 1999. Clinical efficacy of GnRH analogue (buserelin) and oestradiol benzoate treatments in anoestrus buffaloes. Indian J. Anim. Sci., 69 (5): 310-312.

Ferrara, B. 1964. Su alcuni aspetti dell'allevamento del bufalo in Italia. [About some aspects of buffalo breeding in Italy]. Rivista di Zootecnia, 37: 304 319.

Gill, S.S. and Rurki, G.S. 1985. Effect of season of calving on the reproductive behaviour of water buffalo (Bubalus bubalis). In: Proc. First World Buffalo Congress, Cairo, Egypt: 604-612.

Gogoi, P.K., Das, D., Goswami, R.N., Nahardeka, N. and Das, G.C. 2002. Studies on age at first calving in Murrah and Surti buffaloes maintained in Assam. Indian Vet. J., 79 (8): 854-855.

Gupta, K.A. and Purohit, G.N. 2001. Use of vaginal electrical resistance (VER) to predict oestrus and ovarian activity, its relationship with plasma progesterone and its use for insemination in buffaloes. Theriogenology, 56 (2): 235-245.

Hafez, E.S.E. 1955. Puberty in the buffalo cow. J. Agr. Sci., 46: 137-142.

Hattab, S.A. and Osman, R.A. 2000. Use of Norgestomet and oestradiol valerate injection with norgestomet implant and/or PMSG for induction of oestrus in anoestrous buffaloes. Vet. Med. J., Giza, 48 (1): 135-143.

Honnappagol, S.S. and Patil, R.V. 1991. Oestrus synchronization and fertility in buffalo heifers using Carboprost Tromethanine. Indian J. Anim. Reprod., 12 (2): 177-179.

Ishaq, S.M. 1972. Ninth Annual Report. Directorate of Livestock Farms, Punjab, Lahore.

Jainudeen, M.R., Bongso, T.A. and Tan, H.S. 1983. Postpartum ovarian activity and uterine involution in the suckled swamp buffalo (Bubalus bubalis). Anim. Reprod. Sci., 5: 181-190.

Jainudeen, M.R. and Hafez, E.S.E. 1993. Cattle and Buffalo. In: Hafez E.S.E. (Ed.), Reproduction in Farm Animals, 6th ed., Lea and Febiger, Philadelphia: 315-329.

Kamonpatana, M., Kunawongkrit, A., Bodhipaksha, P. and Luvira, Y. 1979. Effect of PGF2*a* on serum progesterone levels in the Swamp buffalo (Bubalus bubalis). J. Reprod. Fertil., 56: 445-449.

Kamonpatana, M., Pansin, C., Timsard, V., Limsakul, A., Vechabusakorn, A., Savasri, S., Parnpai, R. and Pikultong, P. 1987. Indication of puberty as related to fertility based on body weight, age and profiles of reproductive hormones in swamp buffaloes. Buffalo J., 3 (2): 181-194.

Khan, A.H., Chohan, K.R., Chaudhry, R.A. and Naz, N.A. 1995. Comparison of two different doses of PMSG for oestrus induction in non cycling buffalo heifers. Pakistan Vet. J., 15 (1): 34-37.

Kharche, S.D. and Srivastava, S.K. 2001. Fertility following treatment of suboestrus buffaloes with prostaglandin F2*a*. Indian J. Anim. Reprod., 22 (2): 170 - 171.

Kumaresan, A. and Ansari, M.R. 2001. Evaluation of conception rate in buffaloes (Bubalus bubalis) with reference to semen quality, stage of oestrus and inseminator. Indian J. Anim. Sci.,71 (2): 144-145.

Le Xuan Cong, 1983. Performances in Vietnamese Swamp buffalo. Buffalo Bulletin, 2 (2): 12-13.

Madan, N.L. 1988. Status of Reproduction in female buffalo. In: Proc. Second World Buffalo Congress, New Delhi, India, 12-16 December, vol. III: 89-90.

Malfatti, A. 2003. Recent advances in buffalo endocrinology. In: Atti 2° Congresso Nazionale sull'Allevamento del Bufalo, Monterotondo, Italy, 28 - 30 Agosto: 161-176.

Maymone, B. e Pilla, A.M. 1961. L'intervallo interpartum della bufala in rapporto alla stagionalità dei parti ed all'effetto di altri fattori extragenetici. [Intercalving interval in the buffalo cow related to calving seasonality and other extragenetic factors]. Ann. Istituto Sperimentale Zootecnico, Roma, 15: 15-60.

McCool, C.J., Townsend, M.P., Wolfe, S.G. and Entwistle, K.W. 1987. Endocrinological studies on pregnancy, post partum anoestrus and seasonal variation of ovarian activity in the Australian Swamp buffalo cow. Buffalo J.,1: 67-72.

McCool, C.J., Townsend, M.P., Entwistle, K.W., Wolfe, S.G. and Ridd, C.A.J. 1988. Puberty in the Australian Swamp Buffalo Heifer. Buffalo J., 2: 237-241.

Mialot, J.P., Gipoulou, Ch., Bihoreau, J.L., Roux, M.E., Ponsart, C., Pobel, T. et Deletang, F. 1996. Synchronisation des chaleurs chez les vaches allaitantes lors de vêlage d'automne avec l'association PRID+Hormo P2*a*. [Oestrus synchronization after autumn calving period with PRID+Hormo P2? in suckled beef cows]. Renc. Rech. Ruminants, 3: 192.

Minoia, P., Zicarelli, L., Lacalandra, G. and Mastronardi, M. 1984. Follicular development in anoestrus water buffalo by means of pulsatile doses of GnRH. In: Proc. 10th International Congress on Animal Reproduction and Artificial Insemination, Urbana-Champaign, USA, 10-14 June, vol. II: 13-14.

Mohamed, A.A., El-Ashry, M.A. and El-Serafy, A.M. 1980. Reproductive performance of buffalo heifers bred at a young age. Indian J. Anim. Sci., 50: 8.

Moioli, B.M., Napolitano, F., Puppo, S., Barile, V.L., Terzano, G.M., Borghese, A., Malfatti, A., Catalano, A. and Pilla, A.M. 1998. Pattern of oestrus, time of LH release and ovulation and effects of time of artificial insemination in Mediterranean buffalo cows. Anim. Sci., 66: 87-91.

Naqvi, A.N. and Shami, S.A. 1999. Comparative performance of early and late maturing Nili Ravi buffalo heifers. Asian-Australasia J. Anim. Sci., 12 (3): 336 - 340.

Naqvi, A.N. 2000. Effect of parity and season of calving on service period in Nili-Ravi buffalo in Pakistan. Asian-Australasian J. Anim. Sci., 13 (3): 287-291.

Nebel, R.L., Dransfield, M.G., Jobst, S.M. and Bame, J.H. 2000. Automated electronic systems for the detection of oestrus and timing of AI in cattle. Anim. Reprod. Sci., 60-61: 713-723.

Neglia, G., Midea, D., Caracciolo di Brienza, V., Rossi, N. e Zicarelli, L. 2001. Associazione del GnRH alle prostaglandine nella inseminazione artificiale della bufala Mediterranea Italiana. [GnRH associated with prostaglandin in artificial insemination of the Mediterranean Italian buffalo cow]. In: Atti 1° Congresso Nazionale sull'Allevamento del Bufalo, Eboli, Italy, 3-5 Ottobre: 337-340.

Neglia, G., Gasparrini, B., Di Palo, R., De Rosa, C., Zicarelli, L. and Campanile, G. 2003. Comparison of pregnancy rates with two oestrus synchronization protocols in Italian Mediterranean Buffalo cows. Theriogenology, 60 (1): 125-133.

Okuda, H.T., Starke Buzetti, W.A., Fagliari, J.J., Passipiéri, M., Zocoller Seno, M.C. and Bergamaschine, A.F. 1999. Idade a pubertade, idade a primeira cria e intervalo entre partos de búfalos.[Observations of puberty, age at first calving and calving interval in buffalo cows].

Veterinária Notícias, 5 (1): 91-96.

Pacelli, C., Barile, V.L., Lenza, R., Terzano, M.G., Montemurro, N. and Borghese, A. 2001. Comparison of two different doses of PMSG on conception rate in Mediterranean buffalo heifers treated with PRID. In: Proc. Sixth World Buffalo Congress, Maracaibo, Venezuela, 20-30 May: 160-165.

Parmeggiani, A., Seren, E., Esposito, L., Borghese, A., Di Palo, R. and Terzano, M.G. 1993. Plasma levels of melatonin in buffalo cows. In: Proc. International Symposium "Prospect of Buffalo Production in the Mediterranean and in the Middle East", Cairo, Egypt, 9-12 November 1992. EAAP Publication, Pudoc, Wageningen, n. 62: 401-403.

Parmeggiani, A., Di Palo, R., Zicarelli, L., Campanile, G., Esposito, L., Seren, E., Accorsi, P.A. e Soflai, S.M. 1994. Melatonina e stagionalità riproduttiva nella bufala. [Melatonin and reproductive seasonality in the buffalo cow]. Agricoltura Ricerca, 153: 41-48.

Phathodiya, O.P., Jain, L.S. and Tailor, S.P. 1999. Age at first calving and its correlation with economic traits in Surti buffalo. Indian Vet. J., 76 (10): 902-905.

Pires, R.M.L., Reichert, R.H., Martinez, A.C. and Baruselli, P. 2002. Índices reprodutivos de búfalos da raça Murrah. [Some reproductive traits in a Murrah buffalo herd]. Revista Brasileira de Reprodução Animal, 26 (2): 119-120.

Qureshi, M.S., Habib, G., Samad, H.A., Lodhi, L.A. and Usmani, R.H. 1999a. Study of factors leading to seasonality of reproduction in dairy buffaloes. II. Non nutritional factors. Asian-Australasian J. Anim. Sci., 12 (7): 1025-1030.

Qureshi, M.S., Safi, G.M., Dhanani, J. and Kaka, I. 1999b. Reproductive performance of dairy buffaloes in the northern hilly areas of Pakistan. Buffalo J., 15 (3): 391-396.

Rao, A.R. and Rao, Ch.C. 1983. Synchronization of oestrus and fertility in buffaloes with a progesterone releasing intravaginal device. Vet. Rec., 113: 623 624.

Rao, A.V.N. and Sreemannarayana, O. 1983. Induction of ovulatory oestrus and fertility in non cycling buffaloes with norgestomet during low breeding season. Therigenology, 19 (3): 305-309.

Reddy, A.O., Ramesha, K.P. and Rao, M.K. 1999. Effect of climate on the incidence of oestrus, conception and cycle length in Murrah buffaloes. Indian J. Anim. Sci., 69 (7): 485-489.

Sahasrabudhe, S.A. and Pandit, R.K. 1997. PGF2*a* induced oestrus in suboestrus Murrah buffaloes during the summer. Indian J. Anim. Sci., 67: 513-514.

Saini, M.S., Galhotra, M.M., Sangwan, M.L. and Razdan, M.M. 1988. Use of PRID in inducing oestrus and its effect on the sexual behaviour of Murrah buffalo heifers. Indian J. Dairy Sci., 41 (1): 40-42.

Saini, M.S., Dhanda, O.P., Singh, N. and Georgie, G.C. 1998. The effect of improved management on the reproductive performance of pubertal buffalo heifers during the summer. Indian J. Dairy Sci., 51 (4): 250-253.

Salama, M.A.M., Mokhless, E.M. and Barkawi, A.H. 1994. Pubertal performance of Egyptian buffalo heifers. Buffalo J., 10 (1): 61-66.

Salerno, A. 1974. Statistiche vitali nei bufali. [Performances in buffalo]. In: Atti I Convegno Internazionale sull'Allevamento Bufalino nel Mondo, Caserta, Italia, 7-9 Novembre: 115-140.

Seren, E., Parmeggiani, A., Mongiorgi, S., Zicarelli, L., Montemurro, N., Pacelli, C., Campanile, G., Esposito, L., Di Palo, R., Borghese, A., Barile, V.L., Terzano, M.G., Annicchiarico, G. e Allegrini, S. 1994. Modificazioni endocrine durante il ciclo estrale nella bufala. [Periestrous endocrine changes in Italian buffaloes]. Agricoltura Ricerca, 153: 17-24.

Shah, S.N.H. 1988. Comparative studies of seasonal influence on breeding behaviour and conception rate of dairy buffaloes and zebu cattle. In:Proc. Eleventh Int. Congr. on Anim. Reprod. Dublin (Ireland), III: 538.

Shah, S.N.H., Willemse, A.H. and Van de Wiel, D.F.M. 1989. Influence of season and parity on several reproductive parameters of Nili-Ravi buffaloes in Pakistan. Anim. Reprod. Sci. 21, 177-190.

Shah, S.N.H., Willemse, A.H. and Van De Wiel, D.F.M. 1990. Reproductive performance of Nili-Ravi buffaloes after a single injection of GnRH early post partum. Tropic. Anim. Health and Prod., 22 (4): 239-246.

Singh, B. and Lal, K. 1994. Effect of seasons on the incidence of breeding and conception rate in buffaloes. Indian J. Anim. Sci., 64: 314-316.

Singh, G., Singh, G.B., Sharma, R.D. and Nanda, A.S. 1983. Experimental treatment of summer anoestrus buffaloes with norgestomet and PRID. Theriogenology, 19 (3): 323-329.

Singh, G., Singh, G.B., Sharma, R.D. and Nanda, A.S. 1984. Ovulation and fertility after PRID, PRID+GnRH and GnRH in anestrous buffaloes. Theriogenology, 21 (6): 859-867.

Singh, G., Dhaliwal, G.S., Sharma, R.D. and Biswas, R.K. 1988. Treatment of summer anestrous buffalo (Bubalus bubalis) with progesterone releasing intravaginal device plus pregnant mare serum gonadotropin. Theriogenology, 29 (5): 1201-1206.

Singh, J., Nanda, A.S. and Adams, G.P. 2000. The reproductive pattern and efficiency of female buffaloes. Anim. Reprod. Sci., 60-61: 593-604.

Srivastava, S.K. and Sahni, K.L. 1999. Effect of season on oestrus and conception in village cows and buffaloes. Indian Vet. J. 76 (5): 385-387.

Subramaniam, A. and Devarajan, K.P. 1991. Oestrus synchronization in nondescript Indian buffaloes with a new intravaginal progesterone pessary and PGF2*a*. Buffalo J., 7 (1): 101-105.

Sule, S.R., Taparia, A.L., Jain, L.S. and Tailor, S.P. 2001. Reproductive status of Surti buffaloes maintained under sub-humid conditions of Rajasthan. Indian Vet. J., 78 (11): 1049-1051.

Takkar, O.P., Narinder Singh, Jaght Kaur, and Chaudhary, K.C. 1999. Augmentation of post-partum reproductive efficiency by use of GnRH in buffaloes, Indian J. Anim. Reprod., 20 (1): 9-11.

Terzano, G.M., Barile, V.L., Francia, U., Malfatti, A., Todini, L. and Borghese, A. 1996. Onset of puberty in buffalo heifers bred on pasture or in intensive feeding systems. Bulgarian J. Agric. Sci., 2: 89-92.

Terzano, G.M., Galasso, A., Barile, V.L., Pacelli, C., Montemurro, N. and Borghese, A. 1997. Effect of feeding system and puberty on blood metabolites trends in buffalo heifers. In: Proc. Fifth World Buffalo Congress, Caserta, Italy, 13 - 16 October: 951-956.

Tulloch, D.G. and Grassia, A.A. 1981. A study of reproduction in the Water Buffalo in the Northern Territory of Australia. Australian Wildlife Res., 8: 335-348.

Uma Shanker, Agarwal, S.K., Satish Kumar and Greesh Mohan, 1999. Oestrus response and fertility using progestagen ear implant in noncyclic buffalo (Bubalus bubalis). Indian J.Vet. Res., 8 (1): 54-58.

Usmani, R.H., Dailey, R.A. and Inskeep, E.K. 1990. Effects of limited suckling and varying prepartum nutrition on postpartum reproductive traits of milked buffaloes. J. Dairy Sci.,73: 1564-1570.

Vale, W.G., Ohashi, O.M., Sousay, J.S. and Ribeiro, H.F.L. 1990. Studies on the reproduction of Water buffalo in the Amazon Basin. In: Livestock Reproduction in Latin America. Proceedings of the final research coordination meeting. Bogotà, Colombia, 19-23 September 1988. IAEA, Vienna: 201-210.

Zicarelli, L., Di Lella, T. e De Franciscis, G. 1977. Osservazione e rilievi sui parametri riproduttivi e produttivi di bufale in allevamento presso un'azienda della valle del Sele. [Observations on the reproductive and productive parameters of buffalo bred on a Sele valley farm]. Acta Medica Veterinaria, 23: 183-206.

Zicarelli, L. e Boiti, C. 1982. Risultati preliminari sull'impiego in primavera di spirali vaginali con progesterone nella bufala. [Preliminary results on the use of a progesterone intravaginal device in Italian buffalo cows during the spring season]. In: Atti II Convegno Internazionale sull'Allevamento Bufalino nel Mondo, Caserta, Italia, 29 Settembre - 2 Ottobre: 412-419.

Zicarelli, L., Infascelli, F., Esposito, L., Consalvo, F. and De Franciscis, G. 1988. Influence of climate on spontaneous and alfaprostol induced heats in Mediterranean buffalo cows bred in Italy. In: Proc. Second World Buffalo Congress, New Delhi, India, 12-16 December 1988, vol. III: 49-56.

Zicarelli, L. 1992. Recenti acquisizioni sull'attività riproduttiva nella bufala. [Advances in buffalo cow reproduction]. In: Atti 4° Meeting Nazionale "Studio della Efficienza Riproduttiva degli Animali di Interesse Zootecnico", Bergamo, Italy, 10 Aprile: 9-39.

Zicarelli, L., Campanile, G., Seren, E., Borghese, A., Parmeggiani, A. and Barile, V.L. 1993. Periestrus endocrine changes in Italian buffaloes with silent oestrus or ovarian disorders. In: Proc. International Symposium "Prospects of Buffalo Production in the Mediterranean and the Middle East", Cairo, Egypt, 9-12 November 1992, EAAP Publication, Pudoc, Wageningen, n. 62: 397-400.

Zicarelli, L., Campanile, G., Esposito, L., Di Palo, R., Boni, R., Spadetta, M., Montemurro, N., Pacelli, C., Borghese, A., Barile, V.L., Terzano, G.M., Annicchiarico, G., Allegrini, S., De Benedetti, A., Malfatti, A., Lucaroni, A. e Todini, L. 1994. Anaestro e induzione dell'estro in bufale acicliche. [Anoestrus and oestrus induction in acyclic buffaloes]. Agricoltura Ricerca, 153: 25-40.

Zicarelli, L. 1997. Reproductive seasonality in buffalo. Bubalus bubalis, 4, Suppl.: 29 - 52.

Zicarelli, L., De Filippo, C., Francillo, M., Pacelli, C. and Villa, E. 1997a. Influence of insemination technique and ovulation time on fertility percentage in synchronized buffaloes. Proc. Fifth World Buffalo Congress, Caserta, Italy, 13-16 October: 732-737.

Zicarelli, L., Esposito, L., Campanile, G., Di Palo, R. and Armstrong, D.T. 1997b. Effect of using vasectomized bulls in artificial insemination practice on the reproductive efficiency of Italian buffalo cows. Anim. Sci., 47: 171-180.

Chapter V

FOLLICULAR DYNAMICS AND REPRODUCTIVE TECHNOLOGIES IN BUFFALO

Giuseppina Maria Terzano

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

The general characteristics of reproduction like seasonality, cyclicity and ovulation differ widely in mammals for the following reasons:

a) reproductive activity may take place during the whole year or at defined seasons, according to the species and their adaptation to environmental conditions; thus, photoperiod plays a determinant role in seasonal breeders such as rodents, carnivores and ruminants (sheep, goats, buffaloes, deer, etc.,). An extreme situation is observed in foxes with only one ovulation per year, occurring in January or February;

b) mammals may be distinguished according to the absence or presence of spontaneous ovulations: in the first group of mammals (rabbits, hares, cats, mink, camels, Llama), the ovulation is induced by mating and cyclicity is not obvious; in the second group, ovulation occurs spontaneously in each cycle, separating the follicular phase from the luteal phase;

c) the length of cycles is quite different among species: small rodents have short cycles of four or five days, farm animals and primates have longer cycles (sheep: 17 days; cow, goat, buffalo, horse and pig: 21 days; primates: 28 days), and dogs are characterized by long cycles of six to seven months, including a two month luteal phase (Concannon, 1993);

d) ovulation rates differ widely among species and breeds within a given species: in sheep for example, Merinos d'Arles or Ile-de-France breeds have only one ovulation per cycle, whereas average rates of two to four ovulations per cycle are observed in prolific breeds like Romanov or Finn (Land et al., 1973). An extreme situation is observed in some insectivores such as Elephantulus, with ovulation rates as high as 120 (Dryden, 1969).

Despite these differences, it is now established that common mechanisms regulate the ovarian function (Monniaux et al., 1997).

1. Oogenesis and the beginning of follicular growth

Oogenesis begins in the early life of the females and results in the constitution of a pool of primordial follicles. At the time of sexual differentiation, the foetal female gonad is constituted of oogonia and somatic cells from the mesonephros. Oogonia develop from primordial germ cells that have migrated into the ovary early in embryogenesis. Schematically, oogenesis involves three phases: a prolific phase (the oogonia divide actively), a meiotic phase (primary oocytes are formed), and an intense germ cells degeneration phase. The oocytes that survive to degeneration are arrested at the diplotene stage of the first meiotic division and are surrounded by a single layer of granulosa cells, constituting structures called primordial follicles. The three phases of oogenesis clearly overlap, but important differences of chronology are observed among the species studied. The meiotic phase occurs during the prenatal life in most mammals: in the rabbit oocytes formation begins two days after birth, in the pig oocytes formation also begins early during foetal life but oogenesis is completed only during the first weeks after birth, in sheep and cattle the oogonia and oocytes are formed during the first half of foetal life and in water buffaloes the formation of primordial follicles is finished completely before birth (at

127.84 ± 11.55 days, when crown-rump length (CRL) is = 22.84 ± 4.74 cm), whereas in woman the oogenesis finishes at birth (Figure 1).

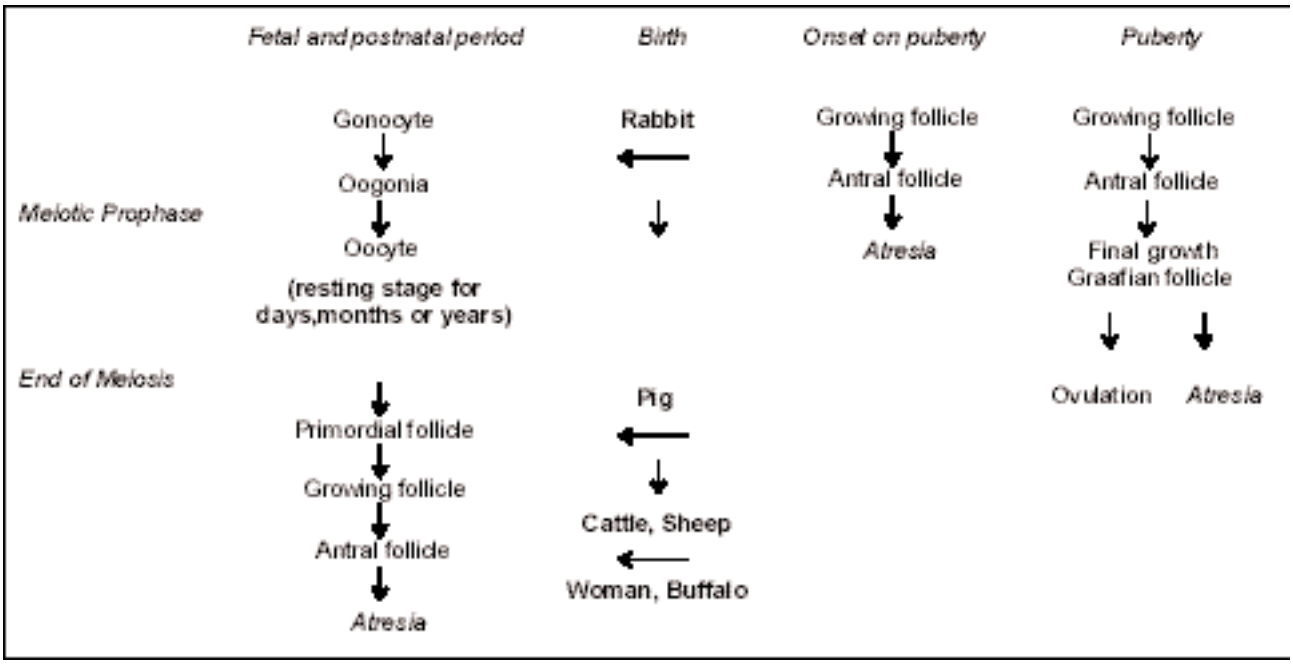


Figure 1. Gametogenesis in female mammals from fetal life to active sexual life

At the end of oogenesis, the ovary encloses millions of primordial follicles within a framework of interstitial tissue and is lined with ovarian epithelium erroneously called germinal epithelium: as oogonia completely disappear, the oocytes formed during the foetal and neonatal period are the only source of oocytes available during the entire sexual life. As soon as the primordial follicle reserve is constituted, it rapidly decreases by atresia and from the end of the period of oogenesis, some primordial follicles continuously begin growth, but up to puberty all disappear owing to atresia: for example a cow foetus that has 2 700 000 oocytes at day 110 of gestation has only 70 000 oocytes at birth; with continual follicular growth and maturation throughout her life, an old cow may have only 2 500 potential ova. In the human foetal ovaries the number of germ cells decreases from 7 000 000 to 1 000 000 between six months of gestation and birth.
It is now established that a number of oocytes, probably only one percent of the total, reach maturity and is released through ovulation.

Follicles are in a constant state of growth and maturation. A histological section of the cortex of a reproductively active female reveal these maturation stages. The primary follicle stage is followed by a proliferation of granulosa cells surrounding the potential ovum giving rise to a secondary follicle. Later in the development, an antrum will form by fluid collecting between the granulosa cells and separating them. At this stage the follicle is classified as a tertiary follicle, also called a Graafian follicle.

The ovary performs two major functions: a) the cyclic production of fertilizable ova and b) the production of a balanced ratio of steroid hormones that maintain the development of the genital tract, facilitate the migration of the early embryo and secure its successful implantation and development in the uterus. The follicle is the ovarian compartment that enables the ovary to fulfill its dual function of gametogenesis and steroidogenesis.

Macroscopically the features of the buffalo ovary (Figure 2) differ widely from those of cattle; the ovary of the buffalo was earlier described as roundish in shape, about 2.5 cm long and weighing 3.9 g (Lutkuke and Rao, 1962) whereas the latter is oval in shape, about 3.7 cm long and weighing 8.6 g (Sisson, 1953).

Physiologically, the buffalo ovary shows scarce reproductive potentiality because it contains less follicles (primary and antral) than those found in cattle and it seems that buffalo oocytes rapidly undergo atresic degeneration. The corpus luteum of the buffalo is deeply embedded in the ovary and is greyish in colour while in cattle it often juts out on the surface of the ovary and is yellowish. The size varies among buffaloes and active ovaries are larger than inactive ones.

Figure 2. Buffalo ovary

2. Advanced technology applied in buffalo

In buffalo important advances in artificial breeding and in the control of reproduction have been made over the past few years. The earliest development in reproduction technology was the use of artificial insemination (AI) which, through the widespread dissemination of genes carried by high quality males, has been considered, up until now, the most effective method for breeding.

Progress has also been made, but with less impact, with the development of oestrus synchronization, superovulation and the transfer of embryos derived "in vivo". Increased interest in the "in vitro" embryo-production (IVEP) technologies, for faster propagation of superior germoplasm, has led to the development of the transvaginal ultrasound-guided follicular puncture (Ovum pick-up or OPU): this latter technique, combined with the IVEP technology, has great potential for improving the genetic development of this species through the maternal lineage.

The recent application of ultrasonographic techniques in the study of buffalo follicles is elucidating the patterns of follicular growth, development and regression that can lead to the improvement of fertility in the female buffalo.

3. Follicular dynamics in buffalo

More recently, the development of ultrasonic probes used intrarectally to observe females' ovaries has clearly confirmed that ovarian follicular development during the oestrus cycle occurs in a wave-like pattern in cattle, thus providing the bases for improving fertility, synchronizing oestrus with more precision and enhancing superovulatory responses.

A good understanding of the processes involved in the growth and differentiation of vesicular follicles destined for ovulation is also essential in order to optimize buffalo reproduction. Diagnostic ultrasonography for the assessment of ovarian structures is a reliable and accurate method for identifying and measuring follicles, especially important since manual palpation through the rectum in buffalo is not completely accurate.

The studies and advancements that have led to our current understanding regarding patterns of follicular development, are listed below in chronological order:

1960 The two-waves concept for follicular growth during the bovine oestrus cycle was propounded. (Rajakoski,1960).

1972 and 1981 The lifespan and fate of individual follicles during the oestrus cycle of heifers

was directly examined. (Dufour et al., 1972; Matton et al., 1981).

1982-1983 Growth and differentiation of estrogen-active and estrogen-inactive follicles during the oestrus cycle were distinguished. (Ireland and Roche, 1982, 1983a,b).

1984 Ultrasound to monitor sizes of follicles during the oestrus cycle of heifers was used. (Pierson and Ginther, 1984).

1987 The concept of dominant follicles, as observed in primates, is applied to cattle and the three-wave hypothesis for development of dominant follicles during the oestrus cycle was proposed. (Ireland and Roche,1987).

1988 Ultrasound analysis and ovarian maps to track growth and atresia of individual follicles throughout the oestrus cycle of heifers were used. (Fortune et al., 1988, Savio et al., 1988, Sirois and Fortune, 1988).

1990-present The autocrine and paracrine role of intrafollicular factors in the regulation of follicular growth, differentiation and function is studied (Ireland et al., 2000).

1992 The radioimmunoassay (RIA) method for determining the transient peak in basal serum concentrations of FSH before each follicular wave was used. (Adams et al., 1992).

1993 The decreased episodic pattern of secretion of LH associated with termination of a follicular wave was studied. (Savio et al., 1993, Stock et al., 1993).

3a. Follicular population

In buffaloes, the follicular system has not been studied as much as in cattle. Singh et al. (1984) delineated the pattern of development and atresia of large follicles (8 mm) on the surface of the ovaries of buffalo heifers. In 65 percent of the postpubertal heifers they found larger follicles at mid-cycle than one to three days before oestrus concluding that these findings complied with the theory of Rajakoski (1960): the follicles at mid cycle become atretic and a new growth wave of follicles begins about mid-cycle and gives rise to the follicle ovulating after oestrus. The buffalo species is characterized by a reduced follicle reservoir compared to that of cattle: the number of primordial follicles has been reported to be approximately 12 000 - 19 000 in riverine buffalo heifers (Samad and Nasseri, 1979).

Furthermore, through ovarian histological evaluations, Danell (1987), studying the follicular system of cycling and non-cycling Surti buffalo heifers, reported 12 636 primordial follicles in cyclic buffalo heifers, (less than the 150 000 primordial follicles reported in cattle, Erickson, 1966) and 10 132 primordial follicles in the non-cycling animals, with a range of 1 222 - 40 327 in an ovary pair. He observed more atresia in buffalo follicles (66.7 percent) than in bovine follicles (50 percent) and detected the same pattern of follicular dynamics in buffalo as observed by Rajakoski (1960) in cattle. Le Van Ty et al., (1989) in a study of swamp buffalo reported a number of antral follicles equal to only 20 percent of those observed in cattle under similar conditions (47.5 *vs* 233.0) and the observed number of non atretic follicles (> 1.7 mm) was between one and five (average 2.9) for buffalo and 17 and 32 (average 22.1) for cattle.

The total number of surface follicles per ovary in abattoir buffalo ovaries at random stages of reproduction has been reported to range from 5.14 (2.5, 1.2, 0.82 and 0.62 follicles of 0-4, 5-8, 9-12 and > 12 mm diameter, respectively); Kumar et al., (1997) to 6.06 (5.30, 0.54 and 0.17 follicles of 0.4, 5-8 and 8 mm diameter, respectively); Madan et al., (1996).

In swamp buffaloes, Smith (1990) reported the number of ovarian follicles of various sizes including also those atretic at different age groups (Table 1).

Table 1. Number of primordial, growing, secondary, tertiary and atretic follicles in buffaloes at various ages (mean ± sd).

Follicle class	Age groups		
	2 years	**7-8 years**	**12-14 years**
Primordial	47.189 ± 39.23	5.996 ± 2.52	3.673 ± 1.97
Growing	4.233 ± 3.5	18.0 ± 13.95	17.0 ± 17.91
Secondary	324 ± 3.23	14.0 ± 11.4	8.0 ± 8.7
Tertiary	62.7 ± 37.78	9.0 ± 6.98	6.67 ± 5.7
Atretic	126.5 ± 22.5	139 ± 50.2	138 ± 37.2

The ovaries obtained from two-year old Swamp buffaloes showed a relatively high rate of transformation from the primordial developing to tertiary follicles; in seven to eight year old and 12 to 14 year old buffaloes this transformation was not noticeable.

The number of secondary follicles in the pubertal buffaloes was low, indicating a slower transitional rate of the growing follicles to secondary follicular stage (7.65 percent); a decline in the number of follicles, from growing (4.233 *vs* 18.0 *vs* 17.0) to tertiary stage (62.7 *vs* 9.0 *vs* 6.67) was observed with age. The rate at which the primordial follicles are stimulated to develop to pre-antral and, subsequently, to antral stage is, in part, dependent upon the size of the pool of primordial follicles (Krarup et al., 1969). A nearly ten-fold lower population of primordial follicles could be, in part, the major factor contributing to the lower number of antral follicles in buffalo compared with that in cattle. The transformation of primordial follicles through the growing stage to the tertiary stage appears to be very inefficient: this can also be seen in the high level of atretic follicles that in buffalo is higher than that reported in cattle (Settergren, 1964). In fact, in the earliest report on follicular atresia in buffalo, Danell (1987) and Ocampo et al., (1994) found a high level of follicles to be atretic (66.0 percent and 82.0 percent, respectively). Molar ratios of progesterone (P4) and oestradiol (E2) have been used to clarify cattle ovarian follicles into atretic and non atretic categories (Grimes et al., 1987). In recent studies, (Palta et al., 1998a, 1998b) classified buffalo ovarian follicles into oestrogen active (E2/P4 molar ratios > 100) and oestrogen inactive/atretic (E2/P4 molar ratios < 100) and observed 92 to 95 percent of follicles to be atretic in abattoir ovaries at random stages of reproduction. The percentage of atretic follicles was lower in large (74 percent) compared with medium (97 percent) and small (92 percent) follicles. In cattle, Danell (1987), using histological evaluation, reported a value of 50 percent; Grimes et al., (1987), using P4/E2 molar ratios, reported a value of 70 percent and Blondin et al., (1996), using flowcytometry in ovaries of cycling cattle reported a value of 16 to 38 percent.
The small number of follicles and the higher level of follicular atresia may explain in part the reported lower superovulatory response and embryo production in buffaloes compared to cattle (Table 2).

Table 2. Follicular and embryo-production efficiency in buffalo and cattle (Zicarelli, 1998).

	Buffalo	**Cow**
Primordial follicles (n)	20 000	100 000
Antral follicles (n)	47	233
Non atretic follicles (n)	39	207
Recovered oocytes (n)	10.6	14.7
Oocytes used for IVEP (%)	58	96
Cleavage rate (%)	30	86
Blastocysts/oocytes (%)	6.3	29.4
Blastocysts/head (n)	0.39	4.20

3b. Follicular dynamics

The use of ultrasound technology in animal reproduction has played an important role in the collection of data regarding ovarian follicular dynamics and related hormonal profiles in domestic animals (Fortune et al., 1991; Ginther et al., 1996; Pierson and Ginther, 1987) as well as other species (Figueiredo et al., 1997; Radcliffe et al., 2001) and has demonstrated that the follicular turnover during the oestrus cycle occurs in waves, each wave being characterized by the synchronous development of a group of follicles (Baruselli et al., 1997). Ovarian follicular growth in buffaloes is similar to that observed in cattle and is characterized by waves of follicular recruitment, growth and regression (Baruselli,1997; Baruselli et al., 1997); the same authors have shown that buffaloes typically show two follicular waves (63.3 percent) and three follicular waves (33.3 percent) during an oestrus cycle, with the first wave beginning around day 0 (day of ovulation). Cattle also commonly have three follicular waves (Sirois and Fortune, 1988; Savio et al., 1988) and two follicular waves (Ginther et al., 1989a, b) (Fig.1). Unlike cattle (Rhodes et al., 1995, Savio et al., 1988, Sirois and Fortune, 1988) buffalo do not display four wave cycles.

In each wave of follicular growth, one dominant follicle develops and suppresses the other follicles. Dominant follicles grow and reach maximum diameter in the middle of the oestrus cycle: when there are high levels of progesterone, there is no ovulation; regression starts allowing a new wave growth to occur. The dominant follicle that develops during the last wave of follicular growth in each oestrus cycle is the ovulatory follicle (Figure 3).

Based on ultrasound analysis, most animals have one *(First wave)* or two waves *(First wave, Second wave)* of follicular development during the luteal phase and a single wave of follicular development *(Ovulatory wave)* during the follicular phase. *Cohort* refers to a group of similar sized nearly synchronously growing follicles. *Emergence* marks the beginning of a wave and is the first day a 4 - 5 mm follicle is the largest in a new wave. The beginning of *selection* cannot be determined by ultrasonography, however, the end of selection occurs simultaneously with the onset of dominance. *Deviation* is when growth rates between the dominant and largest subordinate follicle begin to differ. *Dominance* occurs when the largest follicle in a wave is 1 to 2 mm larger than the next largest follicle and growth of all subordinate follicles ceases. *Subordinate follicles* are all nondominant follicles in a wave. Loss of dominance marks the end of a wave and is detected at emergence of the next wave. The *growing phase* for a follicle begins on the day of the oestrus cycle of its emergence and ends the day in which the diameter of the follicle ceases to increase. The *static phase* is from the day the follicle diameter ceases to increase (end of growing phase) until the day follicle diameter decreases minus one day. The *regressing phase* is the last day of the static phase until the follicle is no longer detectable, which is usually when it reaches four to five mm in diameter.

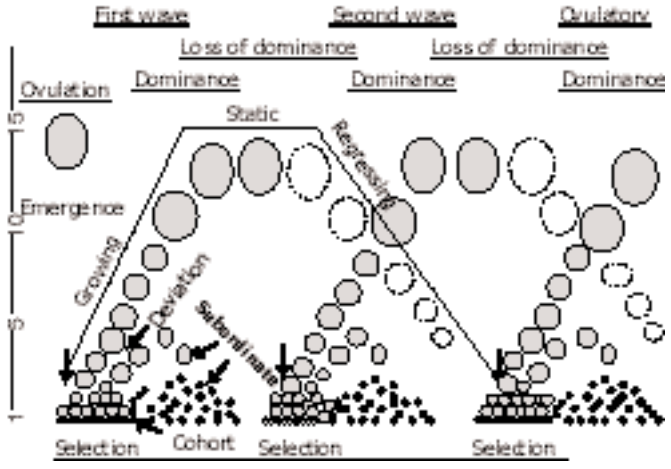


Figure 3. Description of the physiological terms associated with each wave of follicular dynamics during the oestrus cycle of animals. (Ireland J.J. et al., 2000).

3c. Follicular dynamics during an oestrus cycle

The dynamics of follicular turnover, including the length of the interovulatory interval (Knopf et al., 1989; Savio et al., 1990), emergence of waves, number of follicle 3 mm at emergence of waves (Knopf et al., 1989), persistence and maximum diameter of the dominant anovulatory and/or dominant ovulatory follicles (Table 3) (Fortune et al., 1988; Ginther et al., 1989a,b; Bo et al., 1995), have been expressed showing the similarity between buffalo and cattle.

In an earlier study Baruselli et al., (1997) described the follicular dynamics of an oestrus cycle in buffalo (Table 3).

Table 3. Characteristics of follicular turnover during an oestrus cycle in buffaloes having two or three-wave patterns (Baruselli et al.,1997).

	N° waves	
	2	**3**
Percent of Buffaloes	66.33	33.33
Length of interovulatory intervals (d)	22.27±0.89	24.50±1.88
Emergence of first wave (d)	1.16±0.50	1.10±0.32
Emergence of second wave (d)	10.83±1.09	9.30±1.25
Emergence of third wave (d)		16.80±1.22
N° of follicles 3 mm at emergence of wave		
First wave	7.72±4.64	7.50±2.75
Second wave	6.66±3.12	6.50±2.36
Third wave		5.11±1.37
Persistence of first dominant follicle (d)	20.67±1.18	17.9±3.47
Maximum diameter (cm)	1.51±0.24	1.33±0.18
Persistence of second dominant follicle (d)		13.30±2.96
Maximum diameter (cm)		1.11±0.21
Persistence of ovulatory follicle (d)	11.44±0.92	7.70±1.91
Maximum diameter (cm)	1.55±0.16	1.34±0.13
Growth rate of ovulatory follicle (mm/d)	0.131±0.01	0.172±0.02
Length of oestrus cycle (d)	21.84±1.01	24.00±2.24
Length of luteal phase (d)	10.40±2.11	12.66±2.91

In the above studies the growth and static phases of the dominant anovulatory follicles and the growth phase of the ovulatory follicle were also described (Table 4). Specifically, the beginning of a wave (also called emergence) is defined as the first day of the oestrus cycle when a growing follicle four to five mm in diameter in a new wave is detected by ultrasound. According to this definition, in an oestrus cycle with two waves, the first wave begins at approximately day one (day 0 = day of ovulation) and the second wave begins at approximately day 11, both in buffalo and cattle; for the first and second waves, the maximum size of each dominant follicle is 15 mm, reached on days 9 and 22 of the oestrus cycle. In a cycle with three waves, the waves emerge, on average, at days 1, 9 and 16 both in buffalo and in cattle (Baruselli et al., 1997; Manik et al., 1998a,b; Savio et al., 1988; Sirois and Fortune 1988; Ginther et al., 1989; Fortune et al., 1991). There were no differences between second- and third-wave cycles with regard to the day of emergence of the first wave; the second wave appeared earlier in the oestrus cycle with three waves than with two waves.

Two- and three-wave cycles were significantly different with regard to: the persistence of the

first dominant follicle (20.7 d *vs* 17.9 d), the length of the growing phase (7.39 d *vs* 5.50 d) and the static phase (6.88 d *vs* 5.30 d), the last day of the growing phase (8.55 d *vs* 6.60 d), the beginning of the regressing phase (15.4 d *vs* 11.9 d), the maximum diameter of both the first dominant follicle (1.51 cm *vs* 1.33 cm) and the ovulatory follicle (1.55 cm *vs* 1.34 cm); no difference was found with regard to the length of the regressing phases (6.40 *vs* 7.10). Two- and three-wave cycles were also significantly different with regard to the maximum diameter of both the first dominant follicles (1.51 cm *vs* 1.33 cm) and the ovulatory follicle (1.55 cm *vs* 1.34 cm). Terzano et al., (2001) also reported the diameter of ovulatory follicles ranging between 1.32 cm and 1.50 cm.

However, in two-wave cycles, no differences were observed between the maximum diameters of first dominant follicle and ovulatory follicle (1.51 cm *vs* 1.55 cm) and in three-wave cycles, the maximum diameter of the first dominant follicle was significantly larger than that of the second dominant follicle (1.33 cm *vs* 1.11 cm).

No correlation was observed between the diameter of ovulatory follicle (beginning of oestrus cycle), first dominant follicle (diestrus) and second ovulatory follicle (end of cycle).

Two- and three-wave cycles were significantly different with regard to the average length of intervals to oestrus and ovulation and the average length of luteal phase. The mean progesterone level during the luteal phase was lower in two-wave than in three-wave cycles.

Similar follicular waves can also be observed in prepubertal buffalo heifers (Baruselli et al., 1997a; Presicce et al., 2003).

Table 4. Characteristics of dominant follicles (DF) for each wave during two-and three-wave cycles in buffalo (Baruselli et al., 1997)

	N° waves	
	2	**3**
First dominant anovulatory follicle		
Growing phase		
Beginning day	1.16±0.50	1.10±0.32
End day	8.55±2.33	6.60±1.42
length (d)	7.39±1.55	5.50±1.22
growth rate (cm/d)	0.172±0.01	0.187±0.02
Static phase		
maximum diameter (cm)	1.51±0.24	1.33±0.18
beginning day	15.43±2.33	11.90±1.68
end day	21.83±1.20	19.00±3.12
length (d)	6.40±0.85	7.10±0.92
growth rate (cm/d)	0.148±0.02	0.113±0.02
Second dominant anovulatory follicle		
Growing phase		
Beginning day		9.30±1.25
End day		14.50±2.01
length (d)		5.20±0.61
growth rate (cm/d)		0.174±0.02
Static phase		
maximum diameter (cm)		1.11±0.21
beginning day		18.21±1.43
end day		22.60±3.06
length (d)		4.39±0.89
growth rate (cm/d)		0.136±0.02
Ovulatory follicle		
Growing phase		
Beginning day	10.83±1.09	16.80±1.22
End day	22.27±0.89	24.50±2.01
length (d)	11.44±0.91	7.70±0.55
growth rate (cm/d)	0.131±0.01	0.172±0.02

In all buffaloes studied, there was little variation between the numbers of follicles for the different waves. This finding agrees with the high repeatability observed in the number of follicles per wave in cattle (Boni et al.,1993) and suggests that the number of follicles recruited depends on the individual animal. No information was available on the heredity of this characteristic. However, the selection of animals based on the number of follicles per wave is encouraging because of the positive correlation found between the number of small follicles at the beginning of superovulatory treatment and superovulatory response (Romero et al., 1991).

To summarize, follicular dynamics in buffalo is similar to that observed in cattle; there may be marked individual variation in follicular dynamics among buffaloes, with as few as one to as many as three waves of follicular growth occurring within an oestrus cycle: the two waves pattern appears to be the most common; the number of follicular growth waves during an oestrus cycle is linked to the length of luteal phase and of the oestrus cycle.

The elucidation of patterns of follicular development in the buffalo ovary could provide new experimental models for studying the regulation of follicular development and dominance, and could generate information that could help to explain variability in response to oestrus synchronization and superovulatory protocols. Moreover it could also provide new ideas for future improvement of fertility in female buffalo.

3d. Endocrine basis of the wave pattern

Ovarian follicular dynamics in the buffalo species have been studied by several authors although these studies include little information on the hormonal aspects related to wave emergence and follicle development. However, because there are striking similarities between cattle and buffalo in terms of follicular dynamics, the basic endocrine mechanism leading to the occurrence of the wave pattern, could, presumably, be similar in the two species. It is now established also in buffalo that a transient rise in serum concentrations of FSH begins each follicular wave (Presicce et al., 2003), and a decreased episodic secretion of LH is associated with loss of dominance and with the end of a nonovulatory follicular wave.

Today it is clear that several intrafollicular growth factors identified in the follicular fluid of individual follicles, are also involved and some factors have been identified (Table 5). In vitro studies have shown that these growth factors could have endocrine, autocrine or paracrine actions that modify gonadotropin stimulated follicular growth and differentiation.

Table 5. Growth factors and animal reproduction

Acronym	Term
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
PDGF	Plateled-derived growth factor
IGF	Insulin-like growth factor (including IGF-binding protein)
TGF-β	Transforming growth factor-β (including inhibin and activin)
HGF	Haematopoietic growth factor (cytokines)

Their exact role in folliculogenesis is not yet clear but increasing evidence suggests that growth factors modulate follicle growth, acting in a paracrine or endocrine way and regulating proliferation, differentiation and survival of follicular cells (Monniaux et al., 1997).
Changes in insulin-like growth factor-1 (IGF-1) and IGF-binding proteins during follicular development have been reported by various authors in the rat (Zhou et al., 1991), pig (Hammond et al., 1993), human (El-Roeiy et al., 1993), sheep (Gordon I., 1999) and cattle (Kruip A.M., 1997). Changes in the profile of IGF binding proteins have suggested than these proteins may be an important regulator of IGF-1 action on cell proliferation and steroidogenesis within the ovary.

Other authors have reported evidence of paracrine and autocrine effects of epidermal growth factor (EGF) and fibroblast growth factor (FGF) on follicle and luteal function (Kaipia A. and Hsueh J.A., 1997).

4. Multiple ovulation and embryo-transfer (MOET) in buffalo - Limits and perspectives

In buffalo, multiple ovulation and embryo-transfer (MOET) technology is of relatively recent origin. The pioneering work of Drost et al., 1983 in the USA, resulted in the birth of the first buffalo calf through non surgical transfer. This work aroused considerable interest in buffalo-rearing countries leading to subsequent reports on this species (Table 6).

Table 6. Review of superovulation and embryo (E) collection in buffalo (Misra, 1997)

Country	Breed	Treat-ment	Donor super-ovulated	Donor flushed	Total CL (mean)	Total E (mean)	Viable E (mean)	Authors
USA	River	FSH	1	1	2	1	1	Drost et al., 1983
Malaysia	Swamp	PMSG PMSG GnRH	5 5		(5.2) (3.2)	0 0	0 0	Sharifuddin and Jainudeen, 1984
USA	River	FSH	26	19		18	1	Drost et al., 1985
Bulgaria	River	FSH PMSG	19 54			35 ↓		Vlahov et al., 1985
Bulgaria	Murrah & MurrahX	FSH PMSG	19 19		(4.3) (1.9)	24 11		Karaivanov, 1986
Bulgaria	Murrah & MurrahX	FSH PMSG		45 26		66 28		Karaivanov et al., 1987
Bulgaria	Murrah & Medit.	FSH	34	24	(4.0)	(1.2)	(1.7)	Drost et al., 1988
Bulgaria	Murrah & Medit.	FSH PMSG	126 75	123 66	(4.0) (3.4)	146 (1.2) 54 (0.7)	132 38	Alexiev et al., 1988
Thailand	Swamp FSH	PMSG	7 5	7 5	26 15	10 6		Chantaraprateep et al., 1988
Japan	Murrah & Swamp	PMSG	4	3	9	2	0	Ocampo et al., 1988
India	Surti	FSH	1	1	3	1	1	Misra et al., 1988
India	River	FSH	24	24	72	35	24	Yadav et al., 1988b
India	Murrah & Surti	FSH PMSG		145		165		Kurup, 1988
India	Murrah	FSH PMSG	12 12	12 11	57 47	22 8		Deshpande et al., 1988
India	Murrah	FSH PMSG	14	9	(4)	20	19	Sing et al., 1988c
India	Murrah	FSH PMSG	15 7	15 7	42 22	8 3	5 1	Madan et al., 1988
Pakistan	Nili-Ravi	FSH	12	7	(3.7)	(0.5)		Rail et al., 1988b

Country	Breed	Treat-ment	Donor super-ovulated	Donor flushed	Total CL (mean)	Total E (mean)	Viable E (mean)	Authors
Malaysia	Swamp	FSH PMSG	17 32		(3.6) (4.3)	1 1		Sharifuddin and Jainudeen, 1984
Bulgaria	Murrah	PMSG FSH	20 5	20 5	112 44	31 10		Karaivanov et al., 1990
India	Murrah	FSH	73	69	(3.7)	(2.7)	(1.5)	Misra et al., 1990
Italy	Medit	PMSG	10	6	27	16		Schallenberger et el., 1990
India	Murrah	FSH	14	14	31	11	5	Singla and Madan, 1990
India	Murrah	FSH	16	16	(6.8)	(4.4)	(3.1)	Misra et al., 1991
India	Murrah	FSH	22	22	82	52	30	Ambrose et el., 1991
Philippines	Murrah	FSH PMSG	7 4	7 4	720 6	14 0	4 0	Cruz et al., 1991
India	Murrah	FSH PMSG	115 10	70 10	249 31	60 0	33 0	Jain et al., 1992
Vietnam	Swamp	FSH	15	15	90			Uoc et el., 1992
Pakistan	Nili-Ravi	FSH	20	20	75	35	22	Ullah et al., 1992
India	Murrah	FSH PMSG	814	718		1452	804	NDDB, 1992
India	Murrah	FSH PMSG	217	175		353	174	NDRI, 1992
India	Murrah	FSH PMSG	35	29		45	28	NII, 1992
India	Murrah	FSH PMSG	556	497		1302	712	Misra et al., 1994
India	Murrah	FSH PMSG	41 158	41 154	(8.2) (6.3)	(5.1) (3.5)	(3.4) (2.2)	Rao, 1994
Italy	Medit	FSH	51		(5.6)	(1.8)	(1.4)	Zicarelli et al., 1994
China	Swamp	FSH	6	6	46	26	22	Wang et al., 1994
India	Murrah	FSH FSH	8 8		(3.38) (2.25)			Beg et al., 1997
India	Surti	FSH	13	29	(2.48)	(0.76)		Sarvaiya et al., 1997

Superovulation associated with embryo-recovery and embryo-transfer to synchronized recipient females is considered an effective means of increasing the contribution of high quality females to the gene pool of the population.The succesful application of multiple ovulation and embryo-transfer technology largely depends on superovulation for which the essential factor is the treatment with exogenous gonadotrophins. Superovulation, in fact, requires the stimulation of a significantly increased number of pre-ovulatory follicles by the administration of gonadotrophins simulating the effect of FSH. In cattle superovulatory response is much higher than in buffalo and ovulation rates of the order of 15 are frequently recorded: in general 60 to 70 percent of recovered embryos are suitable for transfer: in comparison with cattle embryo transfer (ET), the use of this technology in the buffalo is much more limited. Worldwide MOET technology in cattle has developed on a large scale and about 715 000 bovine transferable embryos, yielding an average of six transferable embryos (5.5 to 7.3), are collected from close to 120 000 donors; almost 50 percent were transferred as fresh embryos and 50 percent were transferred as frozen embryos (Table 7).

Table 7. Number of bovine in vivo-derived embryos transferred (AETE 2000)

Continents	Flushes	Transferable Embryos	Number of transferred embryos		
			Fresh	Frozen	Total
Africa	1 765	10 005	3 766	1 949	5 715
N.America	51 224	299 180	98 391	99 495	197 886
S.America	12 719	92 400	58 423	34 929	93 352
Asia	11 519	74 811	11 684	38 487	50 171
Europe	26 429	145 305	54 286	75 494	129 780
Oceania	15 508	92 655	29 182	14 626	43 808
Total	**119 164**	**714 356**	**255 732**	**264 980**	**520 712**

The procedure commonly used in buffalo for ovarian superstimulation was quite similar to that employed in cattle; however, MOET programmes in buffalo typically resulted in the recovery of small numbers of embryos (one to three) from donor females.

For many years the superovulatory effect of PMSG and FSH have been used to increase ovulation rates in buffaloes and have been applied in conjunction with progestagen and/or prostaglandin F2α treatments to regulate the oestrus cycle. Although the number of corpora lutea was often similar in FSH- and PMSG treated buffaloes, the recovery of embryos after flushing often favoured FSH (Table 6).

An endocrinological evaluation of superovulation by 3 000 IU of PMSG in buffaloes, attempted by Shallenberger et al., (1990) showed that PMSG treatment rapidly induced LH surges of low magnitude, causing unovulated follicles to become endocrinologically active; they further suggested that high oestrogen levels during the early luteal period may activate subclinical uterine infections, which may affect embryonic development.

Anti-serum to neutralize PMSG has resulted in a decreased number of large follicles with variable effects on the number of transferable embryos (Manik et al., 1999).

Palta et al., (1996a) examined the effect of 2 500 IU of PMSG on peripheral inhibin levels recording a sustained elevation in plasma inhibin which they speculated may result in the suppression of endogenous FSH secretion.

The effect of hCG and GnRH, given at oestrus, on the ovulation rate and embryo production in PMSG-treated buffaloes was reported by Ismail et al., (1993). They found that the embryo

recovery percentage was higher after hCG treatment (25 percent *vs* 9 percent in controls) but GnRH proved to be ineffective.

The response of Mediterranean buffaloes induced to superovulate with 2 500 IU of PMSG or 1 050 IU of human menopausal gonadotrophin (HMG) was reported by Alvarez et al., (1994). They recorded an average of 2.3 and 3.0 corpora lutea for PMSG and HMG, respectively. Progesterone levels in the donor animals at the start of superovulatory treatment were found to be extremely variable and this was considered to be a factor contributing to the poor ovarian response. The injection of a single i.m. injection of FSH in the post-scapular region has been reported as effective as the multiple dose regimen (Kasiraj et al., 1992) or to produce a lower superovulatory response compared to a multiple injection regimen (Misra, 1997).

Terzano et al. (2004a) evaluated the relationship of plasma inhibin A (analysed in duplicates by a human sandwich type of immunoassay) to ovarian follicular development in prepuberal Mediterranean Italian buffaloes subjected to two different ovarian stimulation protocols. The data suggested that the medium/large follicles are the most important source of hormone production and that serum inhibin A determined during FSH treatment may provide a useful marker in the control of ovarian hyperstimulation.

Despite these efforts, the variability in the ovulatory response and the low yield of transferable embryos have always been the most important factor affecting the economical use of embryo-transfer technology in this species. It was initially assumed that the low embryo recovery rate in buffalo was related to a poor follicular response to exogenous gonadotrophins. However, in recent studies undertaken by ultrasound evaluation, 9 to 14 ovulatory size follicles were consistently observed in buffaloes stimulated with FSH (Baruselli et al., 1999). This was associated, on average, with ovulation rates of 62.8 percent, a value similar to that found in cattle (Desaulniers et al., 1995; Shaw et al., 1995; Stock et al., 1996). In the same study, the number of ovulations presented a high correlation (0.86;$P < 0.01$) with the number of corpora lutea found on the day of embryo collection, but only one to three ova/embryos were recovered (average recovery rate/CL = 30 percent). In cattle, on the contrary, Shaw et al., (1995) reported a recovery rate proportional to the number of ovulations. In a subsequent study, evidence was obtained for a relatively low rate of transfer of oocytes to the oviduct in buffaloes (Baruselli et al., 2000). It was concluded that the recovery of a low number of embryos in MOET programmes was not necessarily a result of poor superstimulatory responses; rather, it would appear that the failure of oocytes to enter the fallopian tubes and/or impaired transport of ova/embryos in the reproductive tract are major contributing factors to low embryo recovery. This latter condition has implications for direct oocytes aspiration from follicles and the linking of this approach with in vitro fertilization. A negative correlation ($r = 0.31$;$P<0.07$) between the number of large (>0.8 mm) follicles present on the day of embryo collection and the number of embryos recovered was observed: follicles not ovulating in response to the endogenous LH surge continued to secrete large amounts of estradiol, adversely affecting the functionality of the infundibulum and passage of ova into the oviducts. It seems that the failure of some follicles to ovulate depends on incomplete follicular maturation and therefore on lack of sufficient LH receptors at the time of the preovulatory surge release of LH. A GnRH agonist-LH protocol, developed in cattle (D'Occhio et al., 1997, 1998, 1999) was used in buffaloes to verify whether it consistently induced ovulations and increased embryo recovery. In females treated with a GnRH agonist the endogenous pre-ovulatory surge release of LH is blocked and ovulation is induced by injection of exogenous LH. It would appear that the GnRH agonist-LH protocol provides full control on ovulations, including fixed-timed artificial insemination after follicular superstimulation and a reduced number of inseminations. Zicarelli et al., (2000) failed to find significant differences in ovarian follicular response in buffaloes treated with GnRH agonist LH protocol and in those treated with a conventional MOET protocol. Carvalho et al., (2002) reported the GnRH agonist LH protocol to be efficient in the control of follicular dynamics and in the time of ovulation in superovulated buffaloes but a relatively low embryo recovery rate remains a fundamental problem in buffaloes.

Several authors have attributed the poor superovulatory response of buffaloes to inherent endocrine patterns as well as to the characteristics of the follicular population and ovarian folliculogenesis. Recent interest and research activity in ovarian function have contributed greatly to our understanding of the ovary, particularly with respect to follicular dynamics and its control. Based on recent findings regarding endogenous mechanisms controlling follicular wave emergence, follicle selection and dominance, new ideas for artificial manipulation of ovarian function are being investigated. Up to now the most important trials on follicular dynamics were performed on bovines, using ultrasound examination of the ovaries. Attention has been given in recent investigations on buffalo to superstimulatory responsiveness with specific regard to the status of follicular wave development. An increase in the number of ovulations has been reported when superstimulatory treatments were initiated in the absence of a dominant follicle or when the dominant follicle was in a regressing or plateau phase (Taneja et al., 1995). The concept regarding the need to mobilize the small follicles to the stage considered to be responsive to superovulatory treatment has been the basis for trials on hormone pretreatment prior to main superovulatory regimen. Although in cattle some reports have shown that superovulatory response was improved by administering FSH at the start of the donor's oestrus cycle, in buffalo some studies failed to find evidence of any useful effect of such FSH priming (FSH on day three and four) (Joshi et al., 1992; Aggarwal et al., 1995). The folliculogenesis studies have shown a great variation regarding the day the second wave starts, showing the difficulty in standardizing the superovulatory schemes in the middle of the oestrus cycle (Barros et al., 1993; Beg et al., 1997). In monitoring follicular growth by ultrasound, authors reported a greater superstimulatory response when treatment started before (day one) rather than after (day five) manifest selection of the dominant follicle (Adams et al., 1992, 1994; Nasser et al., 1993). In a direct comparison of the superstimulatory response of first follicular wave versus the second one, the results revealed no differences in the number of ovulations induced or the number of ova/embryos recovered in heifers in which superstimulatory treatments were started on day of emergence of wave one or wave two. In cattle, several reports have confirmed that a superovulatory response could be elicited when begun at the time of wave emergence, near the expected time of the pre-wave FSH surge. Superstimulation of the first follicular wave after ovulation (wave one), rather than of the subsequent waves, was chosen because the day of ovulation (day 0) could be used as a convenient and consistent point of reference for the emergence of wave one. However this procedure is difficult to perform under field conditions. A way to perform this would be the synchronization of follicular waves by hormonal or mechanical methods and to perform superovulatory treatment at the onset of the second wave, as proposed for bovines (Bo et al., 1995, 1996). In buffalo a low individual variation was found for the number of follicles recruited for different waves of the same oestrus cycle. These results concur with the high repeatability found in the engagement of follicles in each wave of follicular growth in bovines (Boni et al., 1997b). This suggests that the number of follicles engaged depends on individual characteristics. Although no information exists on the heredity of this characteristic, the selection of female buffalo based on the number of follicles per wave is encouraging due to the positive correlations found between the number of small follicles at the beginning of superovulatory treatment and superovulatory response in cattle (Romero et al., 1991). This selection becomes more important in buffalo, showing a smaller number of follicles (Danell,1987; Le Van Ty et al., 1989).

In these first ventures, it is clear nowadays that the application of cattle ET technology to buffalo has met with limited success and much remains to be done in developing procedures specifically for this species.

5. Ovum Pick-up and in vitro embryo production

In buffalo the low efficiency of superovulation (SO) and embryo-transfer (ET) programmes had led to an increased interest in the in vitro embryo production (IVEP) technologies for faster propagation of superior germoplasm. One of the relatively recent breakthroughs in the practical world of animal reproduction is the combined application of the existing in vitro

fertilization technology and transvaginal ultrasound-guided follicular puncture (Ovum pick-up or OPU) to improve the genetic progress of this species through the maternal lineage. Within this framework, transvaginal oocyte recovery by puncture and aspiration of antral follicles has become a routine procedure in most laboratories where in vitro embryo production is part of the services offered to breeders.
Worldwide a considerable number of bovine oocytes have been collected (the number of approximately 160 000 is an underestimate) (Table 8). Japan, in particular, leads all other countries with around 8 000 in vitro-produced embryos. Europe, notably Italy and the Netherlands, is also actively involved in this in vitro production and transfer of embryos. Several hundred nuclear-transferred embryos have reportedly been transferred by Korean teams for experimental purposes.

Table 8. The number of bovine in vitro-produced embryos transferred (AETE 2000)

Continents	**Transferable embryos collected**	**Number of transferred embryos**		
		Fresh	**Frozen**	**Total**
Africa	421	31	17	48
N.America	1 384 (*)	2 182	117	2 299
S.America	92 (*)	27	42	69
Asia	136 751	4 089	6 114	10 203
Europe	24 146	6 074	7 314	13 388
Oceania	n.d	895 (*)	50	945
Total	**166 794**	**13 298**	**13 654**	**26 952**
(*) Only one country from this region has reported data				

The OPU technique is a non invasive and repeatable procedure for recovering immature oocytes from individual known donors. The possibility of collecting large numbers of meiotically competent oocytes, suitable for in vitro embryo production (IVEP), renders the OPU*IVEP technique competitive to SO for embryo production. Furthermore, the Ovum pick-up (OPU) technique can be performed in non cyclic females, in pregnant cows, in subjects with patent oviducts or genital tract infections, in animals not responsive to hormonal stimulation. It can also be employed as a means of obtaining embryos from clinically infertile but valuable animals. In buffalo the number of transferable embryos/donor/session is lower with OPU + IVEP *vs* MOET, but it is significantly higher over longer periods of time because the MOET programmes cannot be repeated before 100 days (Table 9).

Table 9. Embryo production efficiency in vivo (ET), in vitro (IVEP) and by OPU+IVEP in buffalo (Zicarelli, 1998).

	ET	**IVEP**	**OPU+IVEP**
Total embryos/session	1.8	0.4-0.8	0.17-0.37
Transfer embryos/session	1.7	0.3-0.6	0.15-0.33
Embryo production in 100 d	3.6	0.4-0.8	5.1-11.1
Transferable embryos in 100 d	3.4	0.3-0.6	4.5-9.9

The use of OPU+IVEP in the field could represent a valid approach to speed up genetic improvement by decreasing the generation interval. It has been estimated that a selection scheme based on this technique, applied in a closed nucleus of farms, will decrease the generation interval from 6.28 to 3.25 years and the genetic increase will be about 30 to 25 percent compared to progeny testing (Zicarelli 2003).
OPU has been successfully applied in the buffalo species since 1994 (Boni et al.) and subsequent studies dealing with this technique have been reported by several authors (Galli et al., 1998; Di Palo et al., 2001) showing a low yield of good quality oocytes per ovary, compared

to cattle (on average 2.4 *vs* 10.0, respectively) (Gordon, 1994; Gasparrini et al., 2000).

Boni et al., (1997b) recorded a high individual variability and a low repeatability of the follicular recruitment; the latter probably because the number of follicles recruited varied cyclically and follicular wave was observed. Although OPU resets the follicular population, a cyclic pattern is still observed, perhaps because of the autocrine mechanism. Nevertheless, it might be possible to predict the ability of animals to recruit follicles on the basis of the first four transvaginal ultrasound-guided follicular puncture sessions, as observed in cattle. In fact, the number of total and small follicles recruited within the first four puncture sessions were significantly correlated with the total production (r=0.72 and 0.83, respectively). The importance of this finding is highlighted by the correlation existing between the number of follicles and COCs (r=0.61), Grade A and B COCs (r=0.42) and blastocyst production (r=0.24). The possibility of undertaking a selection of the donors for OPU and embryo production programmes may further improve genetic progress. A limitation to this technique may be the functional exhaustion of the follicular pool (Zicarelli et al., 2003) after six months of OPU. In this trial a productive phase (first six months characterized by high blastocyst production) and an unproductive phase (lasting three months characterized by a low number of follicles and no embryo production) were observed. This finding highlights the need to further investigate this aspect and evaluate whether a resting period is required to better exploit the donor's potentials.

5a. In vitro maturation

Most attempts at producing buffalo embryos in vitro have been based on the methods employed in cattle (Gordon, 1994) and the majority of experimental work in this species utilized ovaries from slaughtered animals as a source of oocytes. Research in buffalo IVF technology has been mainly reported from the developing countries of Asia where the greatest number of buffaloes are found (of the 152 million buffaloes in the world, 96.6 percent are found in Asia), providing not only milk but also meat and consequently a high ovary availability from which to collect oocytes. In Italy, on the contrary, the estimated population of buffaloes is 250 000 *vs* 8.5 million cattle and the culling rate is also lower (12 percent *vs* 25 percent). In addition to this, buffaloes are bred mostly for milk production and are usually slaughtered at the end of their productive life span. Furthermore, the yield of good oocytes per ovary is low compared with cattle (2.4 *vs* 10, respectively) (Kumar et al., 1997; Gordon, 1994). Although the multi step process of IVEP has been successfully used for producing morulae/blastocyst (Madan et al., 1994a; Boni et al., 1999; Gasparrini et al., 2000; Caracciolo di Brienza et al., 2001) and pregnancy in buffalo (Madan et al., 1994b; Suzuki et al., 1992; Chauhan et al., 1997a) the efficiency, in terms of transferable embryos (TE) and development to term, has been very low (Madan et al., 1996). The IVEP technology involves several sequential steps, from the recovery of oocytes to the in vitro maturation (IVM) of the selected oocytes, in vitro fertilization (IVF) and in vitro culture (IVC) of zygotes up to the morula or blastocyst stage but so far many crucial questions still remain to be satisfactorily resolved.

A deeper knowledge of buffalo oocyte/embryo physiology, metabolism and culture requirements is necessary to optimize the efficiency of innovative reproductive strategies in this species.

The ultrastructure of buffalo oocytes during IVM is dealt with in a report by Boni et al., (1991). An ultrastructural study was carried out to assess whether oocyte maturation was accomplished also at a cytoplasmic level in a system that was previously shown to successfully support nuclear maturation (Boni et al., 1992); studies with confocal microscopy have shown that the highest proportion of MII oocytes occurs at a shorter time in buffalo compared to cattle (19 hours *vs* 24 hours, respectively) (Neglia et al., 2001).

During maturation, considered an important step for further development, the oocytes undergo a series of modifications necessary to acquire developmental competence. Therefore the development of a suitable IVM system is critical.

Authors are increasingly using defined rather than undefined media in evaluating the role of various factors in maturation rate and so several complex media (TCM-199 and Ham's F-10) (Totey et al., 1992, 1996; Ocampo et al., 1996), different sources of serum (foetal calf serum-FCS and buffaloes oestrus serum BES) (Totey et al., 1993; Chauhan et al., 1998; Samad et al., 1998) and hormones (Follicle stimulating hormone-FSH, Luteinizing hormone-LH and 17 estradiol) (Totey et al., 1992, 1993) have been evaluated. The role of granulosa cells in the maturation process has also been demonstrated (Bacci et al., 1991). Subsequent studies have been performed to evaluate the role of growth factors in oocyte maturation and post-fertilization development. In this regard the buffalo follicular fluid (BUFF), used as a supplement of IVM media and in replacement of hormones and serum additives, has yielded high maturation, fertilization and blastocysts rates (Chauhan et al., 1997). According to Palta et al., (1996, 1998) the beneficial effect of BUFF during IVM is related to the presence, in this supplement, of gonadotriphins, estradiol, progesterone and several growth factors. The latter play an important role in oocyte maturation and post-fertilization development, acting as a local modulator of gonadotrophin action on mammalian oocytes. IGF-1, IGF-2 and insulin enhance oocyte maturation in buffalo oocytes, as well as fertilization and development to the blastocyst stage (Pawshe et al., 1998), acting in synergy with FSH as autocrine and paracrine modulators of granulosa cells and therefore promoting mitosis, steroidogenesis and protein synthesis. EGF improves cumulus expansion, nuclear maturation and cleavage rate of cumulus-enclosed buffalo oocytes without affecting the post-fertilization embryonic development (Chauhan et al., 1999).

In buffalo Boni et al., (1992) have found that the oocytes and early embryos show extreme sensitivity to oxidative damage, due to their high lipid content. It is known that glutathione (GSH) plays a critical role in protecting mammalian cells from oxidative stress; the latter is thought to be a major factor affecting in vitro mammalian embryo development; GSH content increases during in vivo maturation in the ovary and this reservoir protects the oocytes in the later stages of development (Perreault et al., 1998). Based on these observations, De Matos et al., (1997, 2000) showed that cysteamine, a low molecular weight tiol compound, added to the maturation media, improves bovine and ovine embryo development and quality increasing GSH synthesis. Gasparrini et al., (2000), by supplementing the IVM medium with 50 M of cysteamine, obtained an increased proportion of tight morula and blastocyst-stage buffalo embryos (22.6 percent *vs* 14.9 percent) and, more interestingly, embryo quality was also improved. However, no beneficial effect was recorded on maturation and cleavage rates. The authors speculated that cysteamine-induced GSH synthesis may significantly enhance buffalo embryo development either by protecting the embryos from oxidative stress or by affecting the delicate process of cytoplasmic maturation, that in buffalo may be impaired by the fact that oocytes are often surrounded by only a few layers of cumulus cells. Eppig (1996) suggested that GSH production is critical for the acquisition of development competence of oocytes at a cytoplasmic level and de Matos et al., (1995, 1997) proposed the measurement of GSH at the end of IVM as a reliable indicator of cytoplasmic maturation.

5b. In vitro fertilization

Relative to cattle, buffalo sperm appears to have poor fertilizing capacity and low viability if the semen is frozen with liquid nitrogen. In fact, despite a similar maturation rate (87 percent *vs* 94 percent) a significantly lower cleavage rate (65 percent *vs* 84 percent) is observed in buffalo *vs* cattle (Gasparrini, 2003). In contrast with previous reports (Totey et al., 1992; 1993); Chuangsoongneon et Kamonpatana, 1991; Bacci et al., 1991), Wilding et al., (2003) reported non significant differences between frozen and fresh buffalo semen in penetration and cleavage rate (69.4 percent *vs* 79.6 percent and 60.3 percent *vs* 70.5 percent, respectively). In the same trial the mitochondrial activity of buffalo semen was also assessed showing it to be only slightly lower in cryopreserved *vs* fresh semen. These results suggest that other factors may contribute to the low efficiency rate in buffalo IVF.
As with cattle and other farm animals, considerable variability exists among buffalo bulls in the fertilizing capacity of sperm (Totey et al., 1993b): an accurate screening of the sperm of

several bulls is required in order to identify a suitable semen for IVF programmes.
Sperm needs to undergo capacitation to acquire fertilizing ability; this process can be induced in vitro either by pre-incubation with heparin (Chauhan et al., 1997; Boni et al., 1999) or by adding heparin to the IVF medium (Totey et al., 1996; Gasparrini et al., 2000). In a previous report Totey et al., (1993) showed heparin was able to capacitate buffalo sperm in a dose-dependent manner. High sperm motility is required to accomplish fertilization, although when frozen-thawed sperm is employed, this can be carried out by the swim-up method (Boni et al., 1994a,b; Chauhan et al., 1997a; Nandi et al., 1998) or by Percoll density gradient (Totey et al., 1993b; Boni et al., 1999; Gasparrini et al., 2000). Different motility-inducing substances are used during IVF such as caffeine (Bacci et al., 1991; Madan et al., 1994b; Chauhan et al., 1997a), theophilline (Jainudeen et al., 1993) or a mixture of penicellamine, hypotaurine and epinephrine (Totey et al., 1993b; Madan et al., 1994b), all enhancing the sperm motility and fertilization rate. Basic media such as Tyrode's modified medium (TALP) (Totey et al., 1996; Gasparrini et al., 2000) or Brackett and Oliphant (BO) (Madan et al., 1994a, b; Chauhan et al., 1997a; Nandi et al., 1998) have been found suitable for IVF in buffalo. In this regard, several studies have suggested that BO medium supported higher fertilization and cleavage rates than TALP medium (Totey et al., 1992; Madan et al., 1994a; Ocampo et al., 1996) with average fertilization and cleavage rates of 30 percent to 78 percent and 28 percent to 69 percent respectively. As described above, oocyte maturation in vitro, at least at nuclear level, occurs earlier than in cattle (Neglia et al., 2001), but results are not improved by anticipating the IVF; moreover a linear decrease in efficiency is observed starting from 27 hours of maturation (Gasparrini, 2003).

The efficiency of IVF is also affected by the sperm concentration and, consequently, by the length of sperm-oocyte incubation. Increasing sperm concentration from one through five to 10*106/ml increase polispermy from 24 percent to 43 percent and 64 percent, respectively (Ocampo, 1996), which can be reduced by shortening the co-incubation time. Authors suggested the use of a concentration of 2*106/ml, which yields a high fertilizing rate, avoiding the occurrence of polispermy (Totey et al., 1993b). The positive effect of cumulus cells at the time of IVF has been observed also in buffalo, similar to cattle (Zhang et al., 1995).

5c. In vitro culture

The development of a suitable system for supporting in vitro embryonic development is the most critical step to increase the buffalo IVEP efficiency. Although buffalo embryos have been successfully cultured in ligated rabbit (Chantarapatreep et al., 1989c) and sheep (Galli et al., 1998) oviducts, the use of intermediate hosts is unsuitable for large-scale embryo production. Following the development of co-culture systems in sheep (Gandolfi and Moore, 1987), several authors (Chuansoongneon et al., 1991; Jaunuden et al., 1993) developed a buffalo oviductal epithelial cell co-culture system, with or without a cumulus cell monolayer (Madan et al., 1994b), supporting embryonic development up to the blastocyst stage, but with very low efficiency (8.10 percent). In cattle, established cell lines in a pathogen free-form, such as Buffalo Rat Liver (BRL) cells (Reed et al., 1996) and Vero cells (Lay et al., 1992) have been successfully used for culturing buffalo embryos in vitro (Boni et al., 1994 a,b; 1999), avoiding any risk of transmitting infectious diseases by way of oviductal cells from slaughterhouse material. The use of chemically defined cell-free medium termed Synthetic Oviductal Fluid (SOF) or Potassium Simplex Optimized Medium (KSOM), earlier used in cattle (Tervit et al., 1992), has become necessary to acquire a better understanding of metabolic pathways and biochemical requirements of buffalo embryos in vitro which, in turn, would allow the formulation of an optimal species specific culture system.

A higher blastocyst rate and improved quality was obtained when embryos were cultured in SOF medium compared with the co-culture system with BRL cells (13.5 percent *vs* 7.0 percent, respectively) (Boni et al., 1999). Recently, Caracciolo di Brienza et al., (2001) have reported a higher blastocyst rate, evaluated on the total COCs, in SOF (22.6 percent) and in KSOM (23.8 percent) culture systems.

References

Adams, G.P., Matteri, R.I., Kastelic, J.P., Ko, J.C.H. and Ginther, O.J. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J.Reprod.Fertil., 94: 177-188.

Aggarwal, S.K., Shankar, V. and Yadav, M.C. 1995. Effect of priming with Folltropin in early cycle on superovulatory response in buffaloes. Proc. Natl. Symposium "Modern Trends in Reproductive Health for Enhancing Livestock Fertility and Productivity". 13 to 15 Jan., Akola 444 104. Abstr. 5, 12: C-55.

Alexiev, A., Vlahov, K., Kavaivanov, Ch., Kacheva, D., Polikronov O., Petrov, M., Nikolov, N., Drogoev, A. and Radev, P. 1988. Embryo transfer in buffaloes in Bulgaria. Proc. of the Second World Buffalo Congress, New Delhi, II: 591-595.

Alvarez, P.H., Nogueira, J.R., Meirelles, C.F. and Baruselli, P. 1994. Blood progesterone concentration and ovarian response of Mediterranean buffalo cows superovulated with PMSG or HMG. Buffalo Bulletin,13: 64-67.

Bacci, M.L., Galeati, G., Mattioli, M., Boni, R. and Seren, E. 1991. In vitro maturation and in vitro fertilization of buffalo oocytes. Proc. Third World Buffalo Congress, Varna, 3: 599-603.

Barros, C.M., Figueiredo, R.A., Papa, F.O. and Rocha, G. 1993. Follicular growth in Nelore cows after PGF2β administration. J.Anim.Sci., 71: 216.

Baruselli, P.S. 1997a. Folliculogenesis in buffalo. Bubalus bubalis, Supplement, 4: 79-92.

Baruselli, P.S., Mucciolo, R.G., Visintin, J.A., Viana, W.G., Arruda, R.P., Madureira, E.H., Oliveira, C.A. and Molero-Filho. 1997b. Ovarian follicular dynamics during the oestrus cycle in buffalo (Bubalus bubalis). Theriogenology, 47 (8): 1531 1547.

Baruselli, P.S., Mucciolo, R.G., Arruda, R., Madureira, E.H., Amaral, R. and Assumpcao, M.E.O.A. 1999. Theriogenology, 51: 401.

Baruselli, P.S., Madureira, E.H., Visintin, J.A., Porto-Filho, R., Carvalho, N.A.T., Campanile, G. and Zicarelli, L. 2000. Failure of oocytes entry into oviduct in superovulated buffalo. Theriogenology, 53: 491.

Beg, M.A., Sanwal, P.C. and Yadav, M.C. 1997. Ovarian response and endocrine changes in buffalo superovulated at midluteal and late luteal stage of the oestrus cycle: a preliminary report. Theriogenology, 47: 423-432.

Blondin, P., Dufour, M. and Sirard, M.A. 1996. Analysis of atresia in bovine follicles using different methods: flow cytometry, enzyme-linked immunosorbent assay and classic histology. Biol.Reprod., 54: 631-637.

Bo, G.A., Adams, G.P., Caccia, M., Martinez, M., Pierson, R.A. and Mapletoft, R.J. 1995. Ovarian follicular wave emergence after treatment with progestogen and estradiol in cattle. Anim. Reprod. Sci., 39: 193-204.

Bo, G.A., Adams, G.P., Pierson, R.A. and Mapletoft, R.J. 1996. Effect of progestogen plus estradiol-17β treatment on superovulatory response in beef cattle. Theriogenology, 45: 897-910.

Boni, R., Santella, L., Dale, B.V. and Zicarelli, L. 1991. An ultrastructural study of maturation in buffalo oocytes. In Proc. of the Seventh Meeting of the European Embryo Transfer Association (Cambridge): 128.

Boni, R., Santella, L., Dale, B., Rovello, S., Di Palo, R. and Barbieri, V.M. 1992. An ultrastructural study of maturation in buffalo oocytes. Acta Media Veterinaria, 38: 153-161.

Boni, R., Roelofsen, V.M., Pieterse, M., Wurth, Y. and Kruip, A.M. 1993. Follicular recruitment after repeated removal of all follicles >2 mm in bovine ovary. J.Reprod. Fertil., 12: 40.

Boni, R., Di Palo, R., Barbieri, V. and Zicarelli, L. 1994. Ovum pick-up in deep anestrus buffalo. Proc. Fourth World Buffalo Congress, 3: 480-482.

Boni, R., Rovello, S., Gasparrini, B and Zicarelli, L. 1997a. Pregnancies established after transferring embryos yielded by ovum pick-up and in vitro embryo production in Italian buffalo cows. Proc. Fifth World Buffalo Congress, Caserta, Italy: 787-792.

Boni, R., Roelofsen, V.M., Pieterse, M., Kogut, J. and Kruipth, A.M. 1997b. Follicular dynamics repeatability and predictability of follicular recruitment in cows undergoing repeated follicular puncture.Theriogenology, 48: 277-289.

Boni, R., Rovello, S., Gasparrini, B., Langella, M. and Zicarelli, L. 1999. In vitro production of buffalo embryos in chemically defined medium. Buffalo J., 1: 115 120.

Caracciolo di Brienza, G., Neglia, G., Masola, N., Gasparrini, B., Di Palo, R. and Campanile, G. 2001. Produzione embrionale in vitro in media chimicamente definiti. Atti Primo Congresso Nazionale sull'allevamento del bufalo. 3-5 Ott., Eboli (Sa): 341-344.

Carvalho, N.A.T., Baruselli, P.S., Zicarelli, L., Madureira, E.H., Visintin, J.A. and D'Occhio, M.J. 2002. Control of ovulation with a GnRH agonist subsequent to superstimulation of follicular growth in buffalo: fertilization and embryo recovery. Theriogenology (in press).

Chantarapatreep, P., Lohachit, C., Techakumphu, M., Kobayashi, G., Virakul, P., Kunavongkrit, A., Prateep, P. and Limskul, A. 1989a. Early embryonic development in the Thai Swamp buffalo (Bubalus bubalis). Theriogenology, 31 (6) : 1131-1138.

Chantarapatreep, P., Kobayashi, G., Virakul, P., Kunavongkrit, A., Techakumphu, M., Prateep, P. and Dusitsin, N. 1989b. Success in embryo transfer in the Thai Swamp buffalo (Bubalus bubalis). Buffalo bulletin 8(1): 4-5.

Chantarapatreep, P., Lohachit, C., Techakumphu, M., Kobayashi, G., Virakul, P., Kunayongkrit, A., Prateep, P. and Limskul, A. 1989c. Early embryonic development in the Thai swamp buffalo (Bubalus bubalis). Theriogenology, 3 1 (6): 1131-1139.

Chauhan, M.S., Katiyar, P.K., Singla, S.K., Manik, R.S. and Madan, M.L. 1997a. Production of buffalo calves through in vitro fertilization. Ind J Anim Sci, 67: 306-308.

Chauhan, M.S., Palta, P., Das, S.K., Katiyar, P. and Madan, M.L. 1997b. Replacement of serum and hormone additives with follicular fluid in the IVM medium: Effect on maturation, fertilization and subsequent development of buffalo oocytes in vitro. Theriogenology, 48: 461-469.

Chauhan, M.S., Singla, S.K., Palta, P., Manik, R.S. and Madan, M.L. 1998. In vitro maturation and fertilization, and subsequent development of buffalo (Bubalus bubalis) embryos: Effects of oocytes quality and type of serum. Reprod.Fertil.Dev. 10: 173-177.

Chauhan, M.S., Singla, S.K., Palta, P., Manik, R.S. and Madan, M.L. 1999. Effect of epidermal growth factor on the cumulus expansion, meiotic maturation and development of buffalo oocytes in vitro. Vet.Ret. 144: 266-267.

Chuangsoongneon, U. and Kamonpatana, M. 1991. Oocyte maturation, in vitro fertilization and culture system for developing pre-implantation swamp buffalo embryos using frozen thawed semen. Buffalo Journal, 2: 189-198.

Concannon, P.W. 1993. Biology of gonadotrophin secretion in adult and prepubertal female dogs. Journal of Reproduction and Fertility Supplement 1: 1-6.

Danell, B. 1987. Oestrus behaviour, Ovarian morphology and Cyclical variation in follicular system and endocrine pattern in Water Buffalo Heifers. Ph.D.Thesis, Uppsala. Swedish University of Agricultural Sciences.

De Matos, D.G., Furnus, C.C., Moses, D.F. and Baldassarre, H. 1995. Effect of cysteamine on glutathione level and developmental capacity of bovine oocytes matured in vitro. Molec.Reprod.Dev. 42: 432-436.

De Matos, D.G., Furnus, C.C. and Moses, D.F. 1997. Glutathione synthesis during in vitro maturation of bovine oocytes: role of cumulus cells. Biol.Reprod. 57: 1420-1425.

De Matos, D.G. and Furnus, C.C. 2000. The importance of having high glutathione level after bovine in vitro maturation on embryo development: Effect of B mercaptoethanol, cysteine and cystine. Theriogenology, 53 (3): 761-771.

Desaulniers, D.M., Lussier, J.G., Goff, A.K., Bousquet, D. and Guilbault, L.A. 1995. Follicular development and reproductive endocrinology during and after superovulation in heifers and mature cows displaying contrasting superovulatory responses. Theriogenology, 44: 479-497.

Diancourt, M.A. 2001. Regulation of ovarian follicular dynamics in farm animals. Implications for manipulation of reproduction. Theriogenology, 55: 1211-1239.

Di Palo, R., Neglia, G., Campanile, G., Presicce, G.A., Spadetta, M., Caracciolo di Brienza, V., Gasparrini, B. and Zicarelli, L. 2001. Seasonal effect on follicle production and oocyte recovery by ovum pick-up in the Mediterranean Italian buffalo (Bubalus bubalis). Proc. 27th Ann.Confer.Int.Embryo Transfer Soc.: 404.

D'Occhio, M.J., Sudha, G., Jillella, D., White, T., Maclellan, L.J., Walsh, J., Trigg, T.E. and Miller, D. 1997. Use of a GnRH agonist to prevent the endogenous LH surge and injection of exogenous LH to induce ovulation in heifers superstimulated with FSH: A new model for superovulation. Theriogenology, 47: 601-613.

D'Occhio, M.J., Sudha, G., Jillella, D., White, T., Maclellan, L.J., Walsh, J., Trigg, T.E. and Miller, D. 1998. Close synchrony of ovulation in superstimulated heifers that have a downregulated anterior pituitary gland and are induced to ovulate with exogenous LH. Theriogenology, 49: 637-644.

D'Occhio, M.J., Jillella, D. and Lindsey, B.R. 1999. Factors that influence follicle recruitment, growth and ovulation during ovarian superstimulation in heifers: Opportunities to increase ovulation rate and embryo recovery by dealing with the exposure of follicles to LH. Theriogenology, 51: 349-351.

Drost, M., Wricht, J.R., Cripe, W.S. and Richter, A.R. 1983. Embryo transfer in water buffalo (Bubalus bubalis).Theriogenology, 20: 579-584.

Dryden, G.L. 1969. Reproduction in Suncus murinos. Journal of Reproduction and Fertility Supplement, 6: 377-396.

Dufour, J., Whitmore, H.L., Ginther, O.G. and Casida, L.E. 1972. Identification of the ovulating follicle by its size on different days of the oestrus cycle in heifers. J.Anim.Sci., 34: 85-87.

El-Roeij, A., Chen, X., Roberts, V.J., LeRoith, D., Roberts, C.T. jr. and Yen, S.S.C. 1993. Expression of IGF-1 and IGF-2 and the IGF-1, IGF-2 and insulin receptor genes and localization of the gene products in the human ovary. J. of Clin. Endocr. and Metabolism. 77: 1411-1418.

Eppig, J. 1996. Coordination of nuclear and cytoplasmic oocyte maturation in eutherium mammals. Reprod.Fertil.Dev. 8: 485-489.

Erikson, B.H. 1966. Development and senescence of post-natal bovine ovary. J.Anim.Sci., 25: 800-805.

Figueiredo, R.A., Barros, C.M., Pinheiro, O.L. and Soler, J.M.P. 1997. Ovarian follicular dynamics in the Nelore breed (Bos indicus). Theriogenology, 47: 1489 1505.

Fortune, J.E., Sirois, J. and Quirk, S.M. 1988. The growth and differentiation of ovarian follicles during the bovine oestrus cycle.Theriogenology, 29: 95-109.

Fortune, J.E., Sirois, J., Turzillo, A.M. and Lavoir, M. 1991. Follicle selection in domestic ruminants. I.Reprod.Fertil Suppl, 43: 187-198.

Fortune, J.E. 1993. Follicular dynamics during the bovine oestrus cycle: A limiting factor in improvement of fertility? Anim. Reprod. Sci., 33: 111-125.

Galli, C., Duchi, R., Crotti, G. and Lazzari, G. 1998. Embryo production by ovum pick-up in the water buffalo.Theriogenology, 49: 400.

Gandolfi, F. and Moor, R.M. 1987. Stimulation of early embryonic development in sheep by co-culture with oviduct epithelial cells. J.Reprod.Fertil. 8: 23-28.

Gasparrini, B., Neglia, G., Di Palo, R., Campanile, G. and Zicarelli, L. 2000. Effect of cysteamine during in vitro maturation on buffalo embryo development. Theriogenology, 54: 1537-1542.

Gasparrini, B. 2003. Advances in biotechnologies in buffalo species: prospects and constraints. Atti II° Cong. Naz. sull'allevamento del buffalo: 193-218.

Ginther, O.J., Kastelic, J.P. and Knopf, L. 1989a. Composition and characteristics of follicular waves during the bovine oestrus cycle. Anim.Reprod. Sci., 20: 187 200.

Ginther, O.J., Knopf, L. and Kastelic, J.P. 1989b. Temporal association among ovarian events in cattle during oestrus cycle with two and three follicular waves. J.Reprod.Fertil., 87: 223-230.

Ginther, O.J., Wiltbank, M.C., Fricke, P.M., Gibbons, J.R. and Kot, K. 1996. Selection of the dominant follicle in cattle. Biol.Reprod., 55: 1187-1194.

Gordon, I. 1994. Aspiration techniques: Old and new. In Laboratory Production of Cattle Embryos. Wallingford,UK: Cab International: 71-72.

Gougeon, A. 1996. Regulation of ovarian follicular development in primates: facts and hypotheses. Endoc.Rev.17: 121:155.

Grimes, R.W., Matton, P. and Ireland, J.J. 1987. A comparison of histological and non histological indices of atresia and follicular function. Biol. Reprod., 37: 82 88.

Hammond, J.M., Samaras, S.E., Grimes, R., Leighton, J., Barber, J., Canning, S.F. and Guthrie, H.D. 1993. The role of IGFs and EGF related peptides in intraovarian regulation in the pig ovary. J. of Reprod. and Fertil. Suppl. 48: 117 125.

Ireland, J.J. and Roche, J.F. 1982. Development of antral follicles in cattle after prostaglandin-induced luteolysis: changes in serum hormones, steroids in follicular fluid and gonadotrophin receptors. Endocrinology, 111: 2077-2086.

Ireland, J.J. and Roche, J.F. 1983a. Development of nonovulatory antral follicles in heifers: changes in steroids in follicular fluid and receptors for gonadotrophins. Endocrinology,112: 150-156.

Ireland, J.J. and Roche, J.F. 1983b. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: changes in concentration of hormones in follicular fluid and specific binding of gonadotrophins to follicles. J. Anim. Sci, 57: 157-167.

Ireland, J.J. and Roche, J.F. 1987. Hypotheses regarding development of dominant follicles during a bovine oestrus cycle. Follicular growth and Ovulation rate in Farm Animals. J.F. Roche and D. O'Callaghan, eds. Martinus Nijhoff Publishers,The Netherlands.: 1-18.

Ireland, J.J., Mihm, M., Austin, E., Diskin, M.G. and Roche, J.F. 2000. Historical perspective of turnover of dominant follicles during the bovine oestrus cycle: key concepts, studies, advancement and terms. J. Dairy Sci., 83:1648-1658.

Ismail, S.T., Abboud, M.Y., Tawfik, M.S., Essawi, S. and Mohamed, K.M. 1993. Effects of HCG and GnRH on the ovulation rate and embryo production in buffalo cows superovulated with PMSG. Buffalo Journal, 9: 129-134.

Jainudeen, M.R. 1989. Embryo transfer technology in the buffalo: A review. A paper presented to the Joint FAO/IAEA/ACIAR Research Coordination and Planning Meeting on Buffalo Productivity, 20-24 Feb., CSIRO, Rockhampton, Queensland, Australia.

Jainudeen, M.R., Takahashi, Y., Nihayah, M. and Kanagawa, H. 1993. In vitro maturation and fertilization of swamp buffalo (Bubalus bubalis) oocytes. Anim.Reprod.Sci. 3: 205-212.

Joshi, B.V., Rajeshwaran, S. and Misra, A.K. 1992. Effect of FSH-P priming on superovulatory response in buffalo (Bubalus bubalis).Theriogenology, 37: 232.

Kaipia, A. and Hsueh, J.W. 1997. Regulation of ovarian follicular atresia. Annual review of Physiology, 59: 349.

Karaivanov, C., Kacheva, D., Petrov, M. and Sapundjiev, E. 1990. Superovulatory response of river buffalo (Bubalus bubalis). Theriogenology, 33: 453-464.

Kasiraj, R., Rao, M.M., Rangareddi, N.S. and Misra, A.K. 1992. Superovulatory response in buffalo following single subcutaneous or multiple intramuscular FSH administration. Theriogenology, 37: 234.

Knopf, L., Kastelic, J.P., Schallenberger, E. and Ginther, O.J. 1989. Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. Domest.Anim.Endocrinol., 6: 111-119.

Krarup, T., Pederson, T. and Faber, M. 1969. Regulation of oocyte growth in the mouse ovary. Nature, 224: 187-188.

Kruip, A.M. 1997. Follicle-oocyte relationship in cattle. Bubalus Bubalis, 4: 71 77.

Kumar, A., Solanki, V.S., Jindal, S.K., Tripathi, V.N. and Jain, G.C. 1997. Oocytes retrieval and histological studies of follicular population in buffalo ovaries. Anim.Reprod.Sci., 47: 189-195.

Kurup, M.P.G. 1988. Present status of embryo transfer in buffalo and future expectations. Proc. of the Second World Buffalo Congress, New Delhi, II: 587 590.

Land, R.B., Pelletier, J. and Mauleon, P. 1993. A quantitative study of genetic differences in the incidence of oestrus, ovulation and plasma luteinizing hormone concentration in sheep. Journal of Endocrinology. 58: 305-317.

Lay, Y.M., Stein, D.F., Soong, Y.K., Tang, Y.X., Grifo, J., Malter, H.E., Talansky, B.E., Cohen, J., Liu, H.C. and Rosenwaks, Z. 1992. Evaluation of Vero cell co culture system for mouse embryos in various media. Hum. Reprod., 7: 276 280.

Le Van Ty., Chupin, D. and Driancourt, D.A. 1989. Ovarian follicular population in buffaloes and cows. Anim. Reprod.Sci., 19: 171-178.

Lutkuke, S.N. and Rao, A.S.P. 1962. Studies on the biometry of the reproductive tract of the buffalo cow. Indian J. Veter.Sci., 32: 106-111.

Madan, M.L., Singla, S.K. and Jain, G.C. 1988. Ovulatory response to different superovulatory treatment amongst buffalo (Bubalus bubalis). XIth International Congress on Animal Reproduction and A.I., Dublin, Ireland 1: 172.

Madan, M.L., Singla, S.K., Chauhan, M.S. and Manik, R.S. 1994a. In vitro production and transfer of embryos in buffaloes. Theriogenology, 41: 139-143.

Madan, M.L., Chauhan, M.S., Singla, S.K. and Manik, R.S. 1994b. Pregnancies established from water buffalo (Bubalus bubalis) blastocyst derived from in vitro matured, in vitro fertilized oocytes and co-cultured with cumulus and oviductal cells. Theriogenology, 42: 591-600.

Madan, M.L., Das, S.K. and Palta, P. 1996. Application of reproductive technology to buffalo. Anim.Reprod.Sci., 42: 299-306.

Manik, R.S., Singla, S.K., Palta, P. and Madan, M.L. 1999. Changes in follicular population following treatment of buffaloes with eCG and Neutra-eCG for superovulation. Anim. Reprod. Sci., 56: 31-38.

Manik, R.S., Singla, S.K. and Madan, M.L. 1998. Comparative follicular dynamics in superovulated crossbred cows and water buffalo. Asian Aust. J. Anim. Sci. 11: 65-70.

Matton, P., Adelakoun, V., Couture, Y. and Dufour, J.J. 1981. Growth and replacement of the bovine ovarian follicles during the oestrus cycle. J.Anim.Sci., 52: 813-820.

Mehmood, A., Anwar, M. and Javed, M.H. 1989. Superovulation with PMSG beginning on three different days of the cycle in Nili Ravi buffalo (Bubalus bubalis). Buffalo Journal 5 (1): 79-84.

Misra, A.K., Joshi, B.V., Rajeshwaran, S., Motwani, K.T. and Yadav, M.C. 1988a. News item appeared in the national dailies, The Indian Express, 6 Oct., The Times of India.

Misra, A.K., Yadav, M.C. and Motwani, K.T. 1988b. Successful embryo transfer in a buffalo (Bubalus bubalis). Proc. of the Second World Buffalo Congress, New Delhi, India, I: 56.

Misra, A.K. 1997. Application of biotechnologies to buffalo breeding in India. Bubalus bubalis,

Suppl. 4: 141-166.

Monniaux, D., Huet, C., Besnard, F., Clèment, F., Bosc, M., Pisselet, C., Monget, P. and Mariana, J.C. 1997. Follicular growth and ovarian dynamics in mammals. Journal of Reproduction and Fertility. Supplement 51: 3-23.

Murphy, M.G., Boland, M.P. and Roche, J.F. 1990. Pattern of follicular growth and resumption of ovarian activity in postpartum beef suckler cows. J. Reprod. Fertil. 92: 333-338.

Nandi, S., Chauhan, M.S. and Palta, P. 1998. Effect of cumulus cells and sperm concentration on cleavage rate and subsequent embrionic development of buffalo (Bubalus bubalis) oocytes matured and fertilized in vitro. Theriogenology: 1251 1262.

Nasser, L.F., Adams, G.P., Bo, G.A. and Mapletoft, R.J. 1993. Ovarian superstimulatory response relative to follicular wave emergence in heifers.Theriogenology, 40: 713-724.

Neglia, G., Marino, M., Di Palo, R., Wilding, M., Caracciolo di Brienza, V., Dale, B., Gasparrini, B. and Zicarelli, L. 2001. A comparison of in vitro maturation in buffalo (Bubalus bubalis) and bovine oocytes using confocal microscopy. Theriogenology, 55: 488.

Ocampo, M.B., De Asis, A.T., Ocampo, L.C. and Kanagawa, H. 1994. Histological observation of follicular atresia in swamp buffalo. Buffalo Bull., 13: 51-55.

Ocampo, L., Ocampo, M.B., Aquino, F.P., de Vera, R. and Cruz, L. 1996. Blastocyst formation of Swamp buffalo embryos in co-culture system. Proc. Second ABA Congress, Manila, Phil.: 412-416.

Palta, P. 1988a. Fertility augmentation by inhibin based fecundity vaccines: potential application in buffaloes. Buffalo J., 14: 1-9.

Palta, P. and Chauhan, M.S. 1988b. Laboratory production of buffalo (Bubalus bubalis) embryos. Reprod.Fertil.Dev., 10: 379-391.

Palta, P. and Madan, M.L. 1995. Alterations in hypophysial responsiveness to synthetic GnRH at different postpartum intervals in Murrah buffalo (Bubalus bubalis). Theriogenology, 44: 803-811.

Palta, P., Prakash, B.S., Manik, R.S. and Madan, M.L. 1996a. Inhibin in individual buffalo ovarian follicles in relation to size. Ind. J. Exp. Biol. 34: 606-608.

Palta, P. and Madan, M.L. 1996b. Effect of gestation on the GnRH-induced LH and FSH release in buffalo (Bubalus bubalis). Theriogenology, 46: 993-998.

Palta, P., Bansal, N., Prakash, B.S., Manik, R.S. and Madan, M.L. 1998. Follicular fluid inhibin concentrations in relation to follicular diameter and estradiol-17β, progesterone and testosterone concentrations in individual buffalo ovarian follicles. Ind. J. Exp. Biol. 36: 768-774.

Palta, P., Bansal, N., Prakash, B.S., Manik, R.S. and Madan, M.L. 1998a. Interrelationship between follicular size and follicular fluid estradiol-17β, progesterone and testosterone concentrations in individual buffalo ovarian follicles. Asian Aust.J.Anim.Sci. 11: 292-299.

Palta, P., Bansal, N., Prakash, B.S., Manik, R.S. and Madan, M.L. 1998b. Endocrinological observation in individual buffalo ovarian follicles. Indian J. Anim. Sci. 68: 444-447.

Parnpai, R., Timsard, V., Kamonpatana, M., Pansin, C., Sophon, S., Jetana, T., Limsakul, A.

and Austin, C.R. 1985. Recovery of a swamp buffalo embryo using the nonsurgical technique. Buffalo Journal 1 (1): 77-82.

Pawshe, C.H., Appa Rao, K.B.C. and Totey, S.M. 1998. Effect of IGF-1 and its interaction with gonadotrophins on in vitro maturation and embrionic development, cell proliferation, and biosynthetic activity of cumulus-oocytes complex and granulosa cells in buffalo. Mol. Reprod. Dev. 49: 277-285.

Perreault, S.D., Barbee, R.R. and Slott, V.I. 1988. Importance of glutathione in the acquisition and maintenance of sperm nuclear decondensing activity in maturing hamster oocytes. Dev. Biol. 125: 181-186.

Pierson, R.A. and Ginther, O.J. 1984. Ultrasonography of the bovine ovary.Theriogenology, 21: 495-504.

Pierson, R.A. and Ginther, O.J. 1987. Follicular population dynamics during the oestrus cycle of the mare. Anim.Reprod.Sci., 14: 219-231.

Presicce, G.A., Parmeggiani, A., Senatore, E.M., Stecco, R., Barile, V.L., De Mauro, G.J., De Santis, G. and Terzano, G.M. 2003. Hormonal dynamics and follicular turnover in prepubertal Mediterranean Italian buffalo. Theriogenology, 8860: 1-9.

Radcliffe, R.W., Eyres, A.I., Patton, M.L., Czekala, N.M. and Emslie, R.H. 2001. Ultrasonographic characterization of ovarian events and fetal gestational parameters in two southern black rhinoceros (Diceros bicornis minor) and correlation to fecal progesterone. Theriogenology, 55: 1033-1049.

Rahil, T., Chaudhary, R.A., Khan, I.H., Ahmed, W. and Anwar, M. 1988. Superovulation in Nili Ravi buffalo using FSH at two different stages of the oestrus cycle. Proc. of the Second World Buffalo Congress, New Delhi, India, III: 115-118.

Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. Acta Endocrinol., 34: 7-68.

Reed, W.A., Suh, T., Bunch, T.D. and White, K.L. 1996. Culture of in vitro fertilized bovine embryos with oviductal epithelial cells, buffalo rat liver (BRL) cells, or BRL-cell-conditioned medium. Theriogenology, 45: 439-449.

Rhodes, F.M., De'Ath, G. and Entwistle, G. 1995. Animal and temporal effects on ovarian follicular dynamics in Braham heifers. Anim. Reprod. Sci. 38: 265-277.

Romero, A., Albert, J., Brink, Z. and Seidel jr, G.E. 1991. Numbers of small follicles in ovaries affecting superovulation response in cattle. Theriogenology, 35: 265.

Samad, H.A. and Nasseri, A.A. 1979. A quantitative study of primordial follicles in buffalo heifers ovaries. Compendium 13 FAO/SIDA Int. Course Anim. Reproduction.

Samad, H.A., Khan, I.Q., Rehman, N.L.J. and Ahamad, N. 1988. The recovery, in vitro maturation and fertilization of Nili-Ravi buffalo follicular oocytes. Asian Aust. J. Anim. Sci. 11: 491-497.

Savio, J.D., Keenan, L., Boland, M.P. and Roche, J.F. 1988. Pattern of growth of dominant follicles during the oestrus cycle in heifers. J.Reprod.Fertil., 83: 663 671.

Savio, J.D., Boland, M.P. and Roche, J.F. 1990. Development of dominant follicles and length of ovarian cycles in postpartum dairy cows. J. Reprod.Fertil., 88: 581 591.

Savio, J.D., Thatcher, W.W., Badinga, L., de la Sota, R.L. and Wolfenson, D. 1993. Regulation of dominant follicle turnover during the oestrus cycle in cows. J.Reprod.Fertil., 97: 197-203.

Settergren, I. 1964. The ovarian morphology in clinical bovine gonadal hypoplasia with some aspects of its endocrine relations. Acta Veterinaria Scandinavia 5, Suppl. 1.

Shallemberger, E., Wagner, H.G., Papa, R., Hartl, P. and Tenhumberg, H. 1990. Endocrinological evaluation of the induction of superovulation with PMSG water buffalo (Bubalus bubalis). Theriogenology, 34: 379-392.

Shaw, D.W., Farin, P.W., Washburn, S.P. and Britt, J.H. 1995. Effect of retinol palmitate on ovulation rate and embryo quality in superovulated cattle. Theriogenology, 44: 51-58.

Singh, G., Sharma, S.S., Singh, G.B. and Sharma, R.D. 1984. Studies on palpable changes of corpus luteum during various phases of oestrus cycle in buffalo heifers. Indian vet. J. 61: 660-663.

Singh, M., Matharoo, J.S., Sodhi, H.S., Sharma, R.D., Takkar, O.P., Hundal, R.S., Gill, S.S., Karaivanov, K.C., Alexiev, A. and Radev, P. 1988b. Embryo transfer in buffalo. Successful pregnancy through nonsurgical embryo transfer. Proc. of the Second World Buffalo Congress, New Delhi, India, I: 107.

Singh, M., Sodhi, H.S., Matharoo, J.S., Sharma, R.D., Takkar, O.P., Karaivanov, K.C. and Alexiev, A. 1988a. Embryo transfer in buffalo. Response of various hormone regimens on superovulation. Proc. of the Second World Buffalo Congress, New Delhi, India, I: 106.

Sirois, J. and Fortune, J.E. 1988. Ovarian follicular dynamics during the oestrus cycle in heifers monitored by realtime ultrasonography. Biol.Reprod., 39:308 317.

Sirois, J. and Fortune, J.E. 1990. Lengthening the bovine oestrus cycle with low levels of exogenous progesterone: a model for studyng ovarian follicular dominance. Endocrinology, 12: 916-925.

Sisson, S. 1953. The anatomy of domestic animals. Grossman J.D. ed.,4th ed., Saunders, Philadelphia.

Smith, O.F. 1990. Follicular dynamics in Philippine water buffalo (Bubalus bubalis). Unpub. Ph.D. Thesis, Central Luzon State University, Nueva Ecija, Philippines: 227.

Stock, A.E. and Fortune, J.E. 1993. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology, 132: 1108-1114.

Stock, A.E., Ellington, J.E. and Fortune, J.E. 1996. A dominant follicle does not affect follicular recruitment by superovulatory doses of FSH in cattle but can inhibit ovulation. Theriogenology, 45: 1091-1102.

Suzuki, T., Singla, S.K., Sujata, J. and Madan, M.L. 1992. In vitro fertilization of water buffalo follicular oocytes and their ability to clave in vitro. Theriogenology, 38: 1187-1194.

Taneja, V.K., Nanda, S.K., Datta, T.K. and Bhat, P.N. 1988. Embryo transfer in buffalo. Present status and future research needs. Proc. of the Second World Buffalo Congress, New Delhi, India, II: 603-609.

Taneja, M.G., Singh, S.M., Totey, S.M. and Ali, A. 1995. Follicular dynamics in water buffalo superovulated in presence or absence of a dominant follicle. Theriogenology, 44: 581-597.

Techakumphu, M., Lohachit, C., Chantarapatreep, P., Prateep, P. and Kobayashi, G. 1989. Preliminary report on cryopreservation of Thai swamp buffalo embryos: Manual and Automatic methods. Buffalo Bulletin 8 (2): 29-36.

Tervit, H.R., Whittingham, D.G. and Rowson, L.E. 1972. Successful culture in vitro of sheep and cattle ova. J. Repr. Fertil., 30: 493-497.

Terzano, G.M., Barile, V.L., De Santis, G., Senatore, E.M., Stecco, R., De Mauro, G.J., Parmeggiani, A. e Presicce, A. 2001. Monitoraggio ecografico in bufale italiane sottoposte a sincronizzazione follicolare ed inseminazione strumentale. Atti I Congresso Nazionale sull'allevamento del bufalo. Eboli (Sa): 359-362.

Terzano, G.M., Catone, G., Todini, L., Malfatti, A., Pacelli, C., D'Alessandro, A. and Borghese, A. 2004. Plasma inhibin A level and follicular development in prepuberal buffalo heifers superovulated with FSH or FSH/eCG: preliminary results. Proc. Seventh World Buffalo Congress, Manila, Philippines: 644-647.

Totey, S.M., Singh, G.P., Taneja, M., Pawshe, C.H. and Talwar, G.P. 1992. In vitro maturation, fertilization, and development of follicular oocytes from buffalo (Bubalus bubalis). J. Reprod. Fertil. 95: 597-607.

Totey, S.M., Pawshe, C.H. and Singh, G.P. 1993a. In vitro maturation and fertilization of buffalo oocytes (Bubalus bubalis): effects of media, hormones and sera. Theriogenology, 39: 1153-1171.

Totey, S.M., Pawshe, C.H. and Singh, G.P. 1993b. Effect of bull and heparin, and sperm concentrations on in vitro fertilization of buffalo (Bubalus bubalis) oocytes matured in vitro. Theriogenology, 39: 887-898.

Totey, S.M., Daliri, M., Appa Rae, K.B.C., Pawshe, C.H., Taneja,, M. and Chillar, R.S. 1996. Differential cleavage and developmental rates and their correlation with cell number and sex ratios in buffalo embryos generated in vitro. Theriogenology, 45: 521-533.

Wilding, M., Gasparrini, B., Neglia, G., Dale, B. and Zicarelli, L. 2003. Mithocondrial activity and fertilization potential of fresh and cryopreserved buffalo sperm. Proc. International Embryo Transfer society,. 11-14 January 11-14, Auckland, New Zealand, Theriogenology, 59: 466.

Zhang, L., Jiang, S., Wozniak, P.J., Yang, X. and Godk,e R.A. 1995. Cumulus cell function during bovine oocyte maturation, fertilization and embryo development in vitro. Mol. Reprod. Dev. 40: 338-344.

Zhou, J., Chin, E. and Bondy, C, 1991. Anatomy of the human IGF system. Biol. of Reprod. 48: 467-482.

Zicarelli, L. 1998. Esperienze di superovulazione ed Ovum pick-up nella bufala allevata in Italia. Convegno ARSIAL su "Biotecnologia e Zootecnia Regionale": 11-28.

Zicarelli, L., Baruselli, P.S., Campanile, G., Di Palo, R., Gasparrini, B., Neglia, G. and D'Occhio, M.J. 2000. Proc. of the 14th Intern. Congr. on Anim. Prod.,Vol.2: 0125.

Zicarelli, L. 2003. Advances in buffalo reproduction. Atti II° Congresso Nazionale sull'allevamento del buffalo. Monterotondo, Roma: 233-254.

Chapter VI

REPRODUCTIVE APPLICATION OF ULTRASOUND IN BUFFALO

Giuseppina Maria Terzano

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

The use of ultrasound as a diagnostic technique to evaluate reproduction has enhanced our understanding of the ovarian and uterine processes during the oestrus cycle and pregnancy and our ability to manipulate these processes in order to improve reproductive performance and increase genetic improvement of farm animals. Its use has also provided a "window" to examine the environment of the foetus in order to better understand the interaction between the foetus and its mother and to accurately predict foetal sex. The advent of ultrasound has changed the static glimpses that were achieved with palpation, laparoscopy or post-mortem examination into real-time images.

The earlier methods such as those based on the Doppler principle have now been superseded by real-time B-mode ultrasound and equipped with a linear-array 5 or 7.5 MHz intra-rectal probe. The method is non-invasive and interactive and a principal reason for the increased routine use of ultrasound in farm animals has been the development of inexpensive, portable equipment. Several companies now offer excellent ultrasound units for diagnostic examination of large or small animals (i.e Aloka, Universal Medical System, Classic Medical Supply Inc., E.I. Medical).

Today ultrasound is used for the following examinations:

- Ovarian status determination
- Onset of puberty determination
- Follicular monitoring for diagnosis or pharmacological treatments
- Ovulatory follicles and ovulation time determination
- Ovulation time or anovulatory condition determination
- Corpus luteum monitoring
- Stage of the oestrus cycle determination
- Luteal persistence and anovulatory conditions differentiation
- Establishment of optimal time for artificial insemination
- Oocytes recovery through ultrasound Ovum Pick-up
- Recipients testing for MOET programmes
- Early diagnosis of pregnancy
- Embryo growth characterization
- Foetal viability and age determination
- Foetal number and gender determination
- Post-partum uterine involution determination
- Embryonic death rate (by lack of heartbeat) determination

In this chapter some useful applications will be reported regarding the use of ultrasound for monitoring reproduction in buffalo.

1. Monitoring Ovarian Structures

Before the ultrasound, evaluation of ovarian follicles was limited to palpation, laparoscopy or visual examination of excised ovaries. With the advent of ultrasound, however, non-invasive, repeated monitoring of follicular and luteal development became possible (Figure 1).
Resolution and clarity of ovarian images depend on the quality of the ultrasound equipment

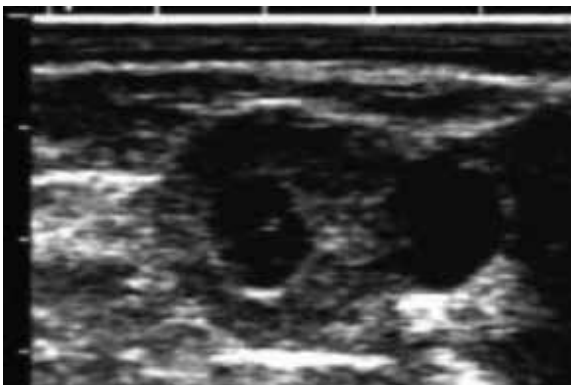


Figure 1. Ultrasound image of the buffalo ovary
with a corpus luteum (on the left) and follicle (on the right).

and the experience of the operator (Sirois and Fortune, 1988). However, ultrasound is a more sensitive method than palpation via the rectum for detecting and measuring ovarian follicles, especially those within the ovarian stroma (Pieterse M.C.et al., 1990). In heifers correlation coefficients between ultrasound measurements and counts obtained by slicing ovaries after slaughter ranged from 0.80 to 0.92 for follicles detected in various size categories and was 0.97 for diameter of the largest follicle (Pierson and Ginther, 1987).

Up to now the most important trials on follicular studies have been performed on bovines, using ultrasound examination of ovaries.

In buffalo, ultrasound monitoring of ovarian function has also been used to determine that follicular development occurs in two or three waves throughout the oestrus cycle.

Ovarian follicular growth in buffaloes is similar to that observed in cattle and is characterized by waves of follicular recruitment, growth and regression (Baruselli, 1997a; Baruselli et al., 1997b). By ultrasound the same authors have shown that buffaloes typically show two follicular waves (63.3 percent) and three follicular waves (33.3 percent) during an oestrus cycle, with the first wave beginning around day 0 (day of ovulation). Also cattle commonly have three follicular waves (Sirois and Fortune,1988; Savio et al.,1988) and two follicular waves (Ginther et al., 1989 a,b, Karaivanov, 1986) (Fig.1). Unlike in cattle (Rhodes et al., 1995, Savio et al., 1988, Sirois and Fortune, 1988), ultrasound monitoring proved that buffalo do not show four wave cycles.

Following each wave of follicular growth, one dominant follicle develops and suppresses the other follicles. Dominant follicles grow and reach maximum diameter in the middle of the oestrus cycle. When there are high levels of progesterone, there is no ovulation; regression starts allowing a new wave growth to occur. The dominant follicle that develops during the last wave of follicular growth in each oestrus cycle is the ovulatory follicle (Fig.2).

The echotexture characteristics of the dominant follicle may be correlated with the functional and endocrine status of the follicle. In cows, after the dominant follicle reaches its peak diameter, referred to as the static phase, granulosa cells are sloughed into the antrum and this debris increases the echogenic heterogeneity of the antral fluid. The changes in follicular echotexture measured by computer-assisted echotexture analysis coincided with the ovulatory potential of the follicle and steroid content of the follicular fluid (Singh et al., 1998; Tom et al., 1998). At present, however, there is no method to determine the physiological status of a large follicle without serial examinations and retrospective analysis. Future use of computer assisted image analysis may improve the diagnostic potential of ultrasound to determine the health of a large follicle in a single examination: in buffalo this will be of significant importance in detecting the health of ovulatory follicles after the application of oestrus synchronization protocols for fixed time artificial insemination. In addition, based on recent findings regarding

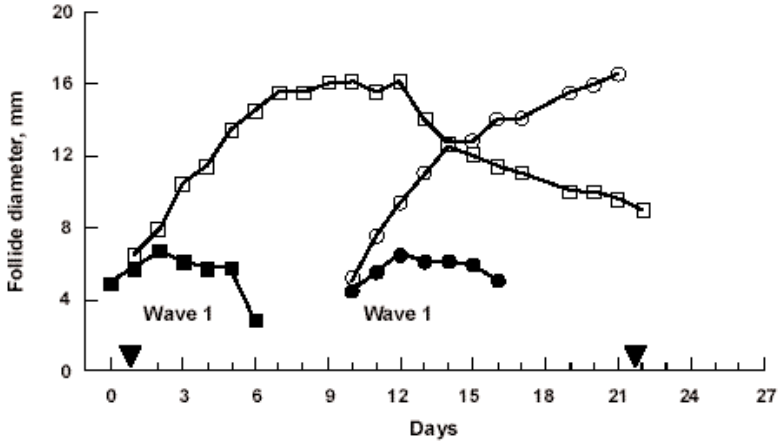


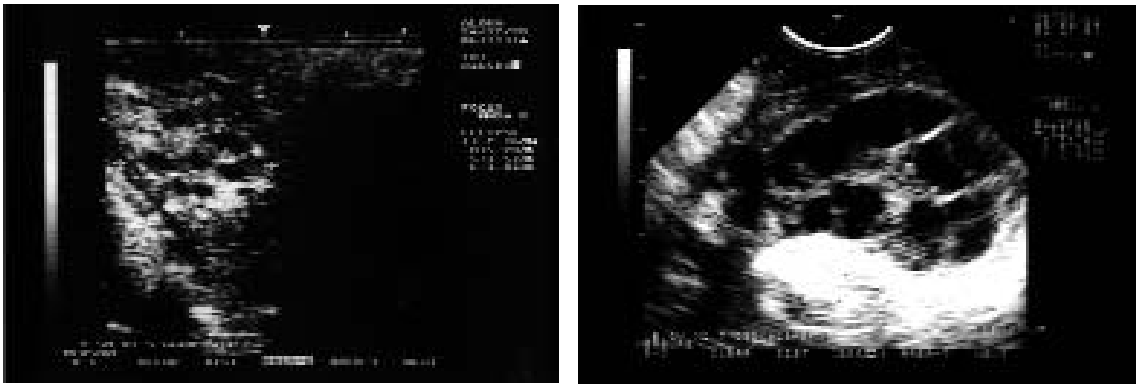
Figure 2. Buffalo oestrus cycle characterized by two follicular waves

endogenous mechanisms controlling follicular wave emergence, follicle selection and dominance, new ideas for artificial manipulation of ovarian function are being investigated.

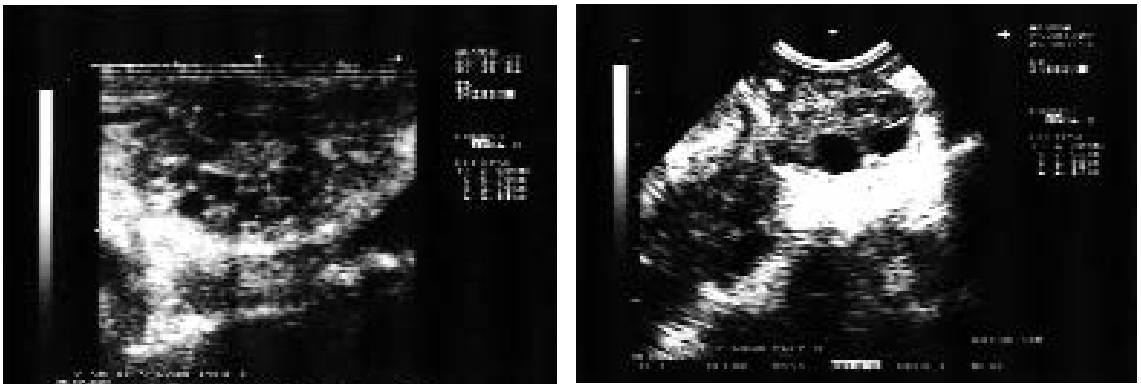
Recent ultrasound investigations in buffalo have paid particular attention to superstimulatory responsiveness with specific regard to the status of follicular wave development. An increase in the number of ovulations has been reported when superstimulatory treatments were started in the absence of a dominant follicle or when the dominant follicle was in a regressing or plateau phase (Taneja et al., 1995).The ultrasound folliculogenesis studies have moreover revealed a great variation on the day the second wave starts, demonstrating the difficulty in standardizing the superovulatory schemes in the middle of the oestrus cycle (Barros et al., 1993; Beg et al., 1997). When monitoring follicular growth by ultrasound, authors reported a greater superstimulatory response when treatment was started before (day 1) rather than after (day 5) manifest selection of the dominant follicle (Adams et al., 1992, Nasser et al., 1993). In a direct comparison of the superstimulatory response of the first follicular wave vs the second one, the results revealed no differences in the number of ovulations induced or the number of ova/embryos recovered in heifers in which superstimulatory treatments were started on the day of emergence of wave 1 or wave 2. In cattle several reports have confirmed that a superovulatory response could be elicited when begun at the time of wave emergence, near the expected time of the pre-wave FSH surge. Superstimulation of the first follicular wave after ovulation (wave 1), rather than of the subsequent waves, was chosen because the day of ovulation (day 0) could be used as a convenient and consistent point of reference for the emergence of wave 1. However this procedure is difficult to perform under field conditions. A way to perform this would be to synchronize the follicular waves by hormonal or mechanical methods and to perform superovulatory treatment at the onset of the second wave, as proposed for bovines (Bo et al., 1995, 1996). By using ultrasound in buffalo a low individual variation was found for the number of follicles recruited for different waves of the same oestrus cycle.

Ovulation is detected by ultrasound as the acute disappearance of a large follicle (≥10 mm) that was present at a previous examination. As in buffalo the corpus luteum (CL) is deeply embedded in the ovary, its ultrasonic detection may be more sensitive than detection by palpation, this being dependent on the experience of the individual performing rectal palpation (McDougall et al., 1999). Detection of a CL with ultrasound is based on the differences in echogenicity between the stroma and the luteal tissue. In buffalo a mature developing CL was recognizable within the first one to three days from ovulation by an increasingly distinct border separating it from the remaining ovarian stroma together with a darker grey granular echotexture (Senatore et al., 2002). The ability to discern CL from the stroma depends on the skill of the ultrasound technician. Occasionally it can be difficult to differentiate the CL from the stroma due to the size of the CL and the area of the ovary occupied by the CL. Usually the stroma can be differentiated from the CL by the presence of small follicles dispersed throughout

the stroma (Terzano, unpublished data, ISZ). Ultrasound machines with expanded gray scale capabilities enhance the ability to differentiate ovarian structures due to subtle differences in echogenicity.



Ovary before FSH-4d (a) Ovary after FSH 4d (b)



Ovary before PMSG+FSH (c) Ovary after PMSG+FSH (d)

Figure 3. Representative ultrasonographic images of ovaries of buffalo heifers before (a and c) and after (b and d) treatment with 4-d FSH and FSH + PMSG.

Ultrasonography may provide a good method of evaluating the follicular development after synchronization with different hormonal protocols for artificial insemination (Terzano et al., 2001; Barile et al., 2004) and after different superstimulatory treatments (Terzano et al., 2004a,b) (Figure 3) and of evaluating corpora lutea in embryo transfer programmes. In fact, embryo-transfer practitioners often reject recipients presented for transfer based on the absence of palpable luteal tissue or the presence of a small, irregular, fluid-filled or soft CL; ultrasound may provide a better method of evaluating CL's in embryo-transfer recipients (Beal W.E., unpublished).

2. Ultrasound evaluation of the uterus

The ultrasound appearance of the buffalo uterus, as in cattle, is dependent on the stage of the oestrus cycle. Variation in the appearance of the uterus involves changes in endometrial thickness, vascularity and the presence of intraluminal fluid. During oestrus the endometrium is very echogenic, the endometrial/miometrial border is evident and throughout the uterine lumen it is possible to see small fluid accumulation. The echogenicity and puffed up appearance of the uterine endometrium decreases by three or four days after ovulation. The uterine horns are extended during and immediately after oestrus (Bonafos et al., 1995).

Real-time, B-mode ultrasound has been reported to detect pregnancy in cattle as early as 9 (Boyd et al., 1988) or 12 days into gestation (Pierson and Ginther, 1984). The potential advantages of using ultrasound for pregnancy diagnosis are that the presence of an embryo can be detected earlier than by palpation per rectum and that direct physical manipulation of the gravid reproductive tract is unnecessary with ultrasound. The latter fact should reduce the risk

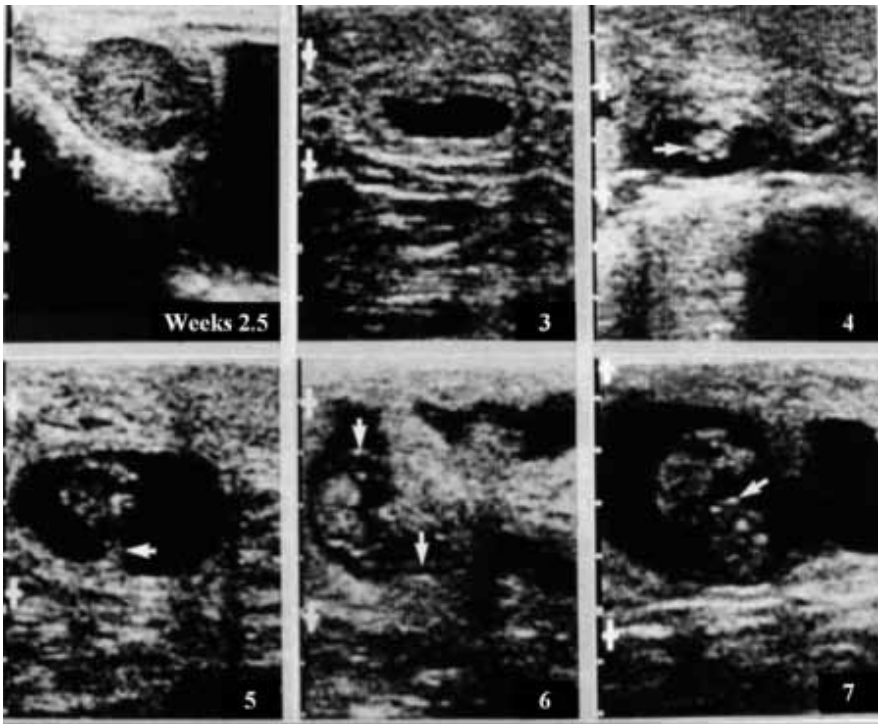


Figure 4. Ultrasound images of buffalo embryos and foetuses.

of inducing embryonic mortality. The use of ultrasound rather than palpation per rectum may also improve the consistency of early (< 40 days) pregnancy diagnosis by reducing the variation in accuracy among practitioners.

In buffalo, pregnancy was determined as early as 20 days with ventral view of the foetus (Presicce et al., 2001).

The embryo is defined as a distinct echogenic structure within the nonechogenic, fluid-filled vesicle. Presence and vitality of the embryo can be confirmed by the detection of a heartbeat at as early as three weeks of gestation: the embryo initially appears as a short, straight echoic line (three weeks), later becomes C shaped (four weeks) and by 4.5 weeks of gestation assumes an L shape (Figure 4).

3. Determination of foetal age

Various ultrasound methods for estimating animal foetal growth have been described in the literature (Kahn W., 1991; Noia et al., 2002). These techniques are based on serial measurements of specific somatic parameters in the foetus: measurements of crown rump length, head diameter and trunk diameter are actually the easiest predictive measurements to estimate gestational age (Figure 5).

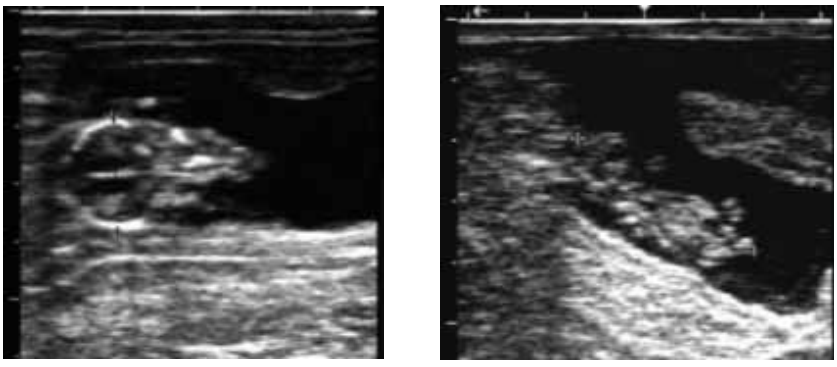


Figure5. Determination of buffalo foetal head (left panel) and crown rump length (right panel) by ultrasound.

Crown rump length (distance from the tail head to the greater curvature of the skull) is easily measured in embryos of foetuses presented in frontal or sagittal view. Head and trunk diameter measurements (recorded at their maximal diameters) require a cross-section or frontal presentation. Experience has revealed that crown rump length is best for estimating ages of embryos less than 50 days and head or trunk diameters are more easily obtained for foetuses over 50 days old.

The regressions and correlation coefficients between the development of the ovine (Noia et al., 2002) and bovine (Kahn W., 1991) foetus and age of gestation has been obtained for several different features. The use of these measurements in formulas to estimate age results in the least variation between the estimated and actual ages.

4. Foetal gender determination

The genital tubercle is embryonic tissue that gives rise to the clitoris in the female and to the glans penis in the male. It originates between the rear legs of the foetus and migrates just caudal to the umbilicus in the case of the male and ventral to the anus in the female. After day 50 of gestation, male and female foetuses can be differentiated by the relative location of the genital tubercle (presumptive penis or clitoris) and development of genital swellings into a scrotum in the male foetus. Diagnosis of sex should be made by visualization of either male or female sex organs and should be nearly 100 percent accurate. Determinations made on the basis of absence or inability to identify the organs either ventral to the tailhead or caudal to the umbilicus may result in lower accuracy.
Ultrasound imaging of 28 buffalo foetuses on day 50 to 65 (period considered critical for foetal gender determination) has been performed every day (Presicce et al., 2001).The position of the genital tubercle was considered to be diagnostically relevant for both males (n=16) and females (n=12) by day 57, with confirmation of the sex to occur by day 59. The hyperechogenic image of the buffalo genital tubercle did not show any appreciable differences from the bovine genital tubercle. A good flat ventral view of the foetus at day 57 was essential for gender determination and at this stage a good view was always reached within two minutes of ultrasound scanning for each animal. Echographic confirmation of gender was performed from day 65 to 67 and 100 percent efficiency was verified.

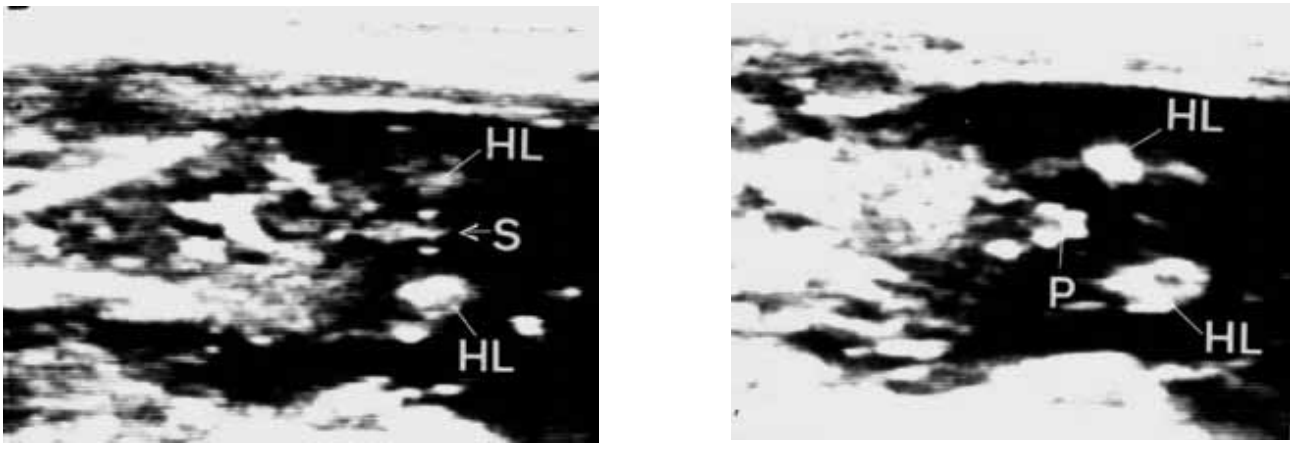


Figure 6. Ultrasound images of a male foetus (frontal view).
Right panel shows hind limbs (HL) and penis (P); left panel shows scrotum (S).

The ultrasound transducer must be manipulated within the rectum to provide a frontal, cross-sectional or sagittal image of the ventrum of the foetus. The umbilicus and tail serve as excellent landmarks when determining the location of the genital tubercle or the presence or absence of the scrotum.

References:

Adams, G.P., Matteri, R.I., Kastelic, J.P., Ko, J.C.H. and Ginther, O.J. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J.Reprod.Fertil., 94: 177-188.

Barile, V.L., Pacelli, C., De Santis, G., Maschio, M.F., Terzano, G.M. and Borghese, A. 2004. Follicular dynamics in buffalo cows treated with two different protocols of oestrus synchronization. Proc. Seventh World Buffalo Congress, Manila, Philippines, Oct.20-23: 588-591.

Barros, C.M., Figueiredo, R.A., Papa, F.O. and Rocha, G. 1993. Follicular growth in Nelore cows after PGF2α administration. J.Anim.Sci., 71: 216.

Baruselli, P.S. 1997a. Folliculogenesis in buffalo. Bubalus bubalis, Supplement, 4: 79-92.

Baruselli, P.S., Mucciolo, R.G., Visintin, J.A., Viana, W.G., Arruda, R.P., Madureira, E.H., Oliveira, C.A., and Molero-Filho. 1997b. Ovarian follicular dynamics during the oestrus cycle in buffalo (Bubalus bubalis). Theriogenology, 47 (8): 1531 - 1547.

Beg, M.A., Sanwal, P.C. and Yadav, M.C. 1997. Ovarian response and endocrine changes in buffalo superovulated at midluteal and late luteal stage of the oestrus cycle: a preliminary report. Theriogenology, 47: 423-432.

Bo, G.A., Adams, G.P., Caccia, M., Martinez, M., Pierson, R.A. and Mapletoft, R.J. 1995. Ovarian follicular wave emergence after treatment with progestogen and estradiol in cattle. Anim. Reprod. Sci., 39: 193-204.

Bo, G.A., Adams, G.P., Pierson, R.A. and Mapletoft, R.J. 1996. Effect of progestogen plus estradiol-17β treatment on superovulatory response in beef cattle. Theriogenology, 45: 897-910.

Bonafos, L.D., Kot, K., and Ginther, O.J. 1995. Physical characteristics of the uterus during the bovine oestrus cycle and early pregnancy. Theriogenology, 43: 713-721.

Boyd, J.S., Omran, S.N. and Ayliffe, T. R. 1988. Use of a high frequency transducer with real-time, B-mode ultrasound scanning to identify early pregnancy in cows. Vet. Rec., 123: 8-11.

Ginther, O.J., Kastelic, J.P. and Knopf, L. 1989a. Composition and characteristics of follicular waves during the bovine oestrus cycle. Anim.Reprod. Sci., 20: 187 - 200.

Ginther, O.J., Knopf, L. and Kastelic, J.P. 1989b. Temporal association among ovarian events in cattle during the oestrus cycle with two and three follicular waves. J.Reprod.Fertil., 87: 223-230.

Kahn, W. 1991. Atlas und lehrbuch der ultraschalldiagnostik: 11- 252.

Karaivanov, C. 1986.Comparative studies on the superovulatory effect of PMSG and FSH in Water buffalo (Bubalus Bubalis). Theriogenology, 26: 51-59.

McDougall, S. and Rhodes, F.M. 1999. Detection of a corpus luteum in apparently anoestrus cows by manual palpation, transrectal ultrasonography and plasma progesterone concentration. New Z. Vet. J., 47: 47-52.

Nasser, L.F., Adams, G.P., Bo, G.A. and Mapletoft, R.J. 1993. Ovarian superstimulatory

response relative to follicular wave emergence in heifers. Theriogenology, 40: 713-724.

Noia, G., Romano, D., Terzano, G.M., De Santis, M., Di Domenico, M., Cavaliere, A., Ligato, M.S., Petrone, A., Fortunato, G., Filippetti, F., Caruso, A. and Mancuso, S. 2002. Ovine foetal growth curves in twin pregnancy: ultrasonographic assessment. Clin.Exp. Obst. and Gyn., XXIX 4 : 251-256.

Pierson, R.A. and Ginther, O.J. 1984. Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology, 22: 225-233.

Pierson, R.A. and Ginther, O.J. 1987. Follicular population dynamics during the oestrus cycle of the mare. Anim. Reprod. Sci., 14: 219-231.

Pieterse, M.C., Taverne, M.A.M., Kruip, A.M. and Willemse, A.H. 1990. Detection of corpora lutea and follicles in cows: A comparison of transvaginal ultrasonography and rectal palpation. Vet. Rec., 126: 552-554.

Presicce, G.A., De Santis, G., Stecco, R., Senatore, E., De Mauro, G.J. and Terzano, G.M. 2001. Foetal gender determination by ultrasound in the Mediterranean Italian buffalo (Bubalus bubalis). Theriogenology, vol.55 (1): 532.

Rhodes, F.M., De'Ath,, G. and Entwistle, G. 1995. Animal and temporal effects on ovarian follicular dynamics in Braham heifers. Anim. Reprod. Sci. 38: 265-277.

Savio, J.D., Keenan, L., Boland, M.P. and Roche, J.F. 1988. Pattern of growth of dominant follicles during the oestrus cycle in heifers. J. Reprod. Fertil., 83: 663 - 671.

Senatore, E.M., De Santis, G., Barile, V.L., Stecco, R., De Mauro, G.J. and Terzano, G.M. 2002. Corpus luteum measurements, echotexture and plasma progesterone in adult and puberal Mediterranean Italian buffalo (Bubalus bubalis). Theriogenology, vol.57: 792.

Singh, J., Pierson, R.A. and Adams, G.P. 1998. Ultrasound image attributes of bovine ovarian follicles and endocrine and functional correlates. J. Reprod. Fertil., 112: 19-29.

Sirois, J. and Fortune, J.E. 1988. Ovarian follicular dynamics during the oestrus cycle in heifers monitored by realtime ultrasonography. Biol. Reprod., 39: 308 - 317.

Taneja, M.G., Singh, S.M., Totey, S.M. and Ali, A. 1995. Follicular dynamics in water buffalo superovulated in the presence or absence of a dominant follicle. Theriogenology, 44: 581-597

Terzano, G.M., Barile, V.L., De Santis, G., Senatore, E., Stecco, R., De Mauro, G.J., Parmeggiani, A. e Presicce, G.A. 2001. Monitoraggio ecografico in bufale italiane sottoposte a sincronizzazione follicolare ed inseminazione strumentale. Atti I° Congresso Nazionale sull'Allevamento del Bufalo, Eboli, (SA), 3-5 Ott: 359-362.

Terzano, G.M., Catone, G., Todini, L., Malfatti, A., Pacelli, C., D'Alessandro, A. and Borghese, A. 2004a. Plasma inhibin A level and follicular development in prepuberal buffalo heifers superovulated with FSH or FSH/eCG: preliminary results. Proc. Seventh World Buffalo Congress, Manila, Philippines. 20-23 Oct: 644-647.

Terzano G.M., Catone, G., Allegrini, S., Mazzi, M., Bracci, M., Lomolino, R. and Borghese, A. 2004b. Ovarian response in stimulated prepuberal Mediterranean Italian buffaloes (Bubalus bubalis). Proc. 7th World Buffalo Congress: 745-746.

Tom, J.W., Pierson, R.A. and Adams, G.P. 1998. Quantitative echotexture analysis of bovine ovarian follicles. Theriogenology, 50: 339-346.

Chapter VII

NUTRITIONAL REQUIREMENTS IN BUFFALO COWS AND HEIFERS

Stefano Terramoccia, Settimio Bartocci and Antonio Borghese

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

In order to provide the appropriate feeding when considering the varying physiological phases of animals, the evaluation of the nutritional requirements becomes a determining factor. There are two different periods in the life of the buffalo cow: the lactating period and the dry period. The dry phase is defined by the lapse of time between the end of lactation, the parturition and the onset of the next lactation, which in buffalo lasts for approximately 270 days. In this survey the nutritional requirements of heifers and the buffalo herd, lactating and non lactating will be analysed.

The evaluation of the nutritional requirements of the dry buffalo herd

As mentioned above the dry phase is the period which elapses between one lactation and another, in buffalo this dry phase lasts approximately four months, the gestation period is longer than in bovines, and considered as an unproductive phase by some breeders. Since the dry period in buffaloes is longer than that of dairy cows, possible rationing errors, even though slight, could have negative repercussions with significant consequences both for the subsequent lactation and for the wellbeing of the animal itself (Zicarelli, 2000). Proto (1993) carried out the first research to evaluate the nutritional requirements in buffaloes providing indicative values for rations of non-lactating buffaloes (Table 1). In the dry period the buffalo herd must procure its own maintenance requirements in addition to the gestation demands since the needs of the foetus increase compared to the previous months and additional food supplements are essential. Proto (1993) considered the nutritional requirements applied to dairy cows adequate also for non-lactating buffaloes and suggested an energy-protein level of 0.65 Milk FU/kg DM and 10.5 percent of crude protein. Similar data was reported by Bertoni et al. (1994), who recommended the following energy-protein trend for diets of the non-lactating buffalo herd: 0.63-0.65 Milk FU/kg DM and 10-11 percent of crude-protein (Table 2), confirming Proto's study. During the dry phase Bertoni et al. (1994) recommended that the amounts of protein in the rations should be higher than 10 percent because, with a lower quantity the rumen activity could be compromised. By providing these indications, for the non lactating buffalo diet, the issue arose (Bertoni et al., 1994) whether the high recycle of urea could allow for at least a 10 percent reduction of dry matter in the crude protein content of maintenance rations; during the dry phase the requirements are almost identical to maintenance. Di Lella (2000) provided the first response; an *ad libitum* diet for the non-lactating buffalo herd should be able to provide an energy content not inferior to 0.65 Milk FU/kg DM and above all a protein concentration lower than 10 percent, with a suggested value of 9 percent. During the dry phase the animals should be fed with fresh forage or hay of good nutritional value and it is advisable to provide 15 percent DM with a concentrate, therefore re-establishing the reserves of liposoluble vitamins, oligominerals and, by means of hydrosoluble vitamins, to normalize the rumen fermentation and hepatic functions. The diet that characterizes this phase has a low rumen fermentation rate, which conditions the production of volatile fatty acids and favours the proliferation of cellulolytic bacteria. While still in this phase a decrease of the absorbent process with a drop in the rumen papillae activity is noticed. The nutritional requirements increase following parturition and the diets show differing characteristics with a great increase of non structural carbohydrates (NSC) and in protein content. The sudden changes in the diet are not supported by variations within the rumen such as an increase of the amilolytic

population and development of the rumen papillae, which occur at a slower rate. Therefore it is important for the dry buffalo to be fed the same diet as when lactating; this new breeding technique could start at least three weeks before the presumed parturition by forming a group "near partum". This group should be administered with a suitable feed with an energy content of at least 0.90 Milk FU/kg DM, a reduction of structural carbohydrates in the diet and an increase of NSC. In addition to the energy content, the diet for buffaloes "near partum" must guarantee the appropriate amount of nitrogen compounds: according to Di Lella (2000) the optimal protein requirement in the last phase of gestation should be 13 percent.

In 1999 the Technical-scientific Committee of the consortium for the protection of Campania Buffalo Mozzarella Cheese was established. The Committee drafted standard guidelines for the regulation of both hygiene and nutrition in buffalo herds related to the Campania Buffalo Mozzarella Cheese DOP, published in 2002. In the cited work all the indications concerning the nutrition of buffaloes are presented. Furthermore the Technical-scientific Committee suggested, where possible, to subdivide the animals according to a breeding technique in which the Body Condition Score (BCS) is evaluated, the aim being, by means of the correct diet, to achieve the ideal weight within the ninth month of gestation. Table 3 reports the nutritional requirements for the non-lactating buffalo herd. The average energy level of the last three months is approximately the same as that previously suggested by various authors (Proto, 1993; Bertoni et al., 1994; Di Lella, 2000), while the optimal level for crude protein is indicated at 800 g/d, with a protein level of approximately 7.5 percent. Particular attention is given to mineral content, especially when comparing the calcium:phosphorus ratio. In fact from the ninth month the Ca:P ratio must be 1:1.1 to avoid the possibility of vaginal and/or uterine prolapse (Zicarelli et al., 1982). In the diet high Ca:P ratios cause an alteration of the normal Ca:Mg ratio in the blood, as a result the excitability of the uterine-vaginal muscle fibre undergoes alteration, causing atonicity of the organ leading to prolapse (Campanile et al., 1989). An excess of calcium during the dry phase can cause a minor parathyroid activity with consequent low values of calcemia at calving. Integration by means of hyper phosphorus salts tends to draw the ratio of the macro-elements closer together stimulating the parathyroid activity (Campanile et al., 1995). The mineral supplement, which is calculated bearing in mind the calcium, phosphorus and magnesium content in the diet, can be added to the concentrate or given separately. To provide a well-balanced supplement it is essential to assay the mineral composition of the foodstuff administered to the animals. Due to this the Technical-scientific Committee considers it unwise to administer poliphita hays, alphapha hay and Italian ryegrass hay, during the dry phase which accounts for 4.0-7.0 g/kg DM of calcium and 2.5-4.8 g/kg DM of phosphorus. From this point of view oat hay, wheat straw and maize silage (in quantities not higher than 5.0-7.0 kg/head/d) appear to be more suited being poor in minerals. The ample variations of these elements in the diet do not influence the final content.

The nutritional requirements of the non-lactating buffalo herd have also been evaluated by Bartocci et al. (2002). Twenty farms were assessed in the Lazio region (central Italy) and were subdivided according to the daily milk yield: high yield (> 9 kg), intermediate yield (8-9 kg) and low yield (< 8 kg). The diets for the non-lactating buffalo herd were characterized by an average energy content of 0.64 Milk FU/kg DM, no significant differences emerged between the farm categories. The protein content demonstrated an average of 7.5 percent, with a higher statistically significant difference between the low yield farms and the other two categories (6.3 vs 8.0, 8.1 percent; $P<0.05$). Bartocci et al., (2002) evaluated the nutritional requirements of the dry buffalo herd only on the farms with high and intermediate milk yields (Table 4). Such a low protein content (7.9 percent) could be justified by the nitrogen metabolism in buffaloes that differs from cattle (Abdullah et al., 1990; Kennedy et al., 1990). Moreover, studies carried out by Puppo et al., (2002) indicate a greater protein digestibility in buffaloes compared to cattle in diets with a high content of structural carbohydrates. This all leads to the conclusion that buffaloes have a greater capacity to utilize protein sources at least from those diets adapted for the dry phase, therefore giving the breeder the opportunity to formulate diets with a low protein content.
It is clear that great progress has been made with regard to the understanding of the

nutritional requirements of non-lactating buffaloes. Not so many years ago Proto (1993) and Bertoni et al., (1994) asserted that the non-lactating buffalo could be fed in the same way as the dairy cow, during the same physiological period. However, results from subsequent studies (Technical scientific Committee, 2002; Bartocci et al., 2002) demonstrate that the energy level in the non lactating buffalo herd can fluctuate from 0.60 to 0.65 Milk FU/kg DM, while the protein level can drop to 7.5 percent DM in the diet. In our opinion this protein level, which may appear low when compared to cattle, requires further research. In addition particular attention should be given to the Ca:P ratio which should be 1:1.1 from the ninth month.

The evaluation of nutritional requirements in the lactating buffalo herd.

The standard lactation phase in buffaloes is 270 days, the milk yield increases after calving and reaches a peak between the fourth and the sixth week. Besides quantity variations buffalo milk is also subject to a variation in the chemical composition during lactation, this phenomenon is much more evident in this species compared to cattle. This implicates greater attention when observing the lactation curve, bearing in mind the chemical variations when calculating the production requirements. According to Proto (1993), particular attention should be given to the milk fat percentage variation which ranges from 6.0 to 12.0 percent and influences the energy requirements. In the same way the protein level which varies between 3.5 to 5.5 percent influences the protein requirements. Table 5 records the protein and energy requirements for the production of 1 kg of buffalo milk relative to the fat and protein content (Proto, 1993). In addition to the energy and protein requirements, the mineral demands should be considered, with particular attention to the calcium, phosphorus and magnesium contents. The production requirements of these three elements, according to the same author, can be considered the same as for bovines bringing the levels to 6.7 g calcium, 2.2 g phosphorus and 0.9 g magnesium, per kg of milk yield. Other criterium suggested by Proto (1993) was to transform buffalo milk into milk standardized to 4.0 percent fat and 3.1 percent protein, by using Di Palo's equation (1992):

kg of standard milk = kg of milk produced * (((g fat - 40)+(g protein - 31))* 0.01155+1.0)

Once the conversion of buffalo milk was carried out Proto considered that the energy requirements of bovines were suitable when calculating buffalo requirements: 0.44 Milk UF/kg of milk normalized to 4 percent fat, and subsequently applying the production requirements for milk determined by the Institut National de la Recherche Agronomique, France (INRA, 1988).

Bertoni et al., (1994) proposed that one breeding technique could be to divide the lactating animals into two groups: one with a yield higher than 8-9 kg and the other with a lower yield. In the former group the suggested ration has an energy density of 0.80-0.85 Milk FU/kg DM and 13.5-14.5 percent CP; in the latter group the density drops to 0.76-0.80 Milk FU/kg DM and 12.5-13.5 percent CP (Table 6). The same authors recommended a diet containing mainly forage since the buffalo utilizes this much better than concentrates; furthermore, in order to avoid digestive problems, in the rumen or intestine, the crude lipid and starch + sugar content must not exceed respectively 4.0-4.5 percent DM and 16.0 17.0 percent DM (Bertoni et al., 1994).

Zicarelli (1999) likewise paid particular attention to buffalo diets during the lactation phase. When employing the equation of Di Palo (1992) and comparing buffalo milk to that of dairy cows, with the same energy produced per 1 kg of milk (Table 7), the fact emerges that buffalo milk is characterized by a lower protein and phosphorus value compared with that produced by dairy cows. According to the same author, analogous to variations of dairy cow milk, in the first 50 days circa of lactation buffaloes register a dry matter intake lower than their requirements, which leads to an inevitable weight loss. As a consequence the milk yield tends to decrease since the animals have the ability to accumulate reserves as a precautionary measure for periods of scarce forage availability, thus aiding their wellbeing while not favouring the galactopoiesis. Possible excesses of energy intake in buffaloes do not cause the "fat cow syndrome" typical in bovines, but modify the chemical composition of the milk, especially the lipid content. As the milk yield gradually augments during lactation, the requirements increase

according to the quantity of the milk yield: on average it can be considered that with a rise of 1 kg milk the requirements increase to 0.76 Milk FU, while the intake of dry matter rises to 0.475 kg. After 150 days from parturition the buffaloes tend to ingest more than their requirements, therefore accumulating excessive reserves. In order to prevent excessive weight gain in this phase the energy density should be lowered, the NDF increased and the starch reduced (not higher than 18 percent DM). A greater adipose reserve is most common in animals that exceed 270 days of lactation due to fertility reasons, or in animals with low yields. With the remaining animals this phenomenon appears less evident since the previous condition is easily re-established in the dry phase. As previously mentioned the buffalo milk protein quota, compared to energy produced is lower than that of dairy cows. One of the characteristics of the buffalo lies in the protein degradability in the rumen which is greater than that in cows (Terramoccia et al., 2000); furthermore the permanence time of foodstuff in the buffalo rumen is greater in comparison to cattle, while there is an inverse tendency in the intestinal tract (Bartocci et al. 1997). This characteristic favours the by pass proteins employed to a lesser degree than in cattle, therefore avoiding fertility or mastitis problems in the event of excessive protein. Zicarelli (1999) suggests a protein ration of 2.47 g CP for every gram of protein in the milk (similar values to those of dairy cows). At the onset of lactation as the intake is lower, it is advisable to increase the protein quota by 10 percent, bearing in mind that the requirements are not adequate if a diet containing less than 13.5 percent CP is used (Campanile et al., 1995). Each kg of buffalo milk contains 1.8-2.0 g calcium and 1.1-1.2 g phosphorus; as far as the maintenance requirements are concerned values provided by INRA (1988) for dairy cows apply. Zicarelli (1999) calculated that for milk production, the calcium requirements reach the value of 5.2-5.8 g/kg milk circa and the phosphorus requirements are 2.1-2.3 g/kg milk. Table 8 reports the conversion factors which consent the technician to calculate the milk yield normalized to 8.30 percent fat and 4.73 percent protein and subsequently to calculate the requirements and formulate the ration.

Another research which provides indications for the nutritional requirements of the lactating buffalo herd (Table 9) is that elaborated by the Technical-scientific Committee of the consortium for the protection of Campania Buffalo Mozzarella Cheese (2002). This work combines the experience gained in the various research centres (University of Naples - two faculties and the Animal Production Research Institute, Rome) that have studied this species to a greater extend. According to the authors the intake of dry matter depends on: the weight, the production level and the physiological phase of the animal, also on the forage: concentrate ratio and lastly on the quality of the feeds used to formulate the ration. The requirements reported in Table 9 have been evaluated considering 20 percent primiparous incidence within the lactating group. Moreover the possibility of weight gain recovery was considered which in buffaloes occurs between 100 and 170 days after calving, this period corresponds to the passage from the catabolism to the anabolism phase of the lactation curve. The considered Milk FU were calculated by evaluating the energy necessary to assure the milk production of the herd. As regards protein content the Technical-scientific Committee has decided to quote the values obtained by the research centres which are part of the working group. These values differ from the theoretic requirements because they not only consider the production of protein in the milk, the growth development of the primiparous and weight recovery of the animals, but also what endocrine - metabolic effects the feed proteins have on the buffalo milk yield. For example, the percentage of crude protein suggested by the Technical-scientific Committee for a group of buffaloes that produce 12 kg/d of normalized milk is 15.9 percent, compared to requirements calculated at 13.2 percent. Slight excesses of protein in the buffalo diet do not determine those negative effects that are usually detected in the dairy cow. Studies on lactation buffaloes demonstrate that protein concentrations greater than those arising from the calculation regarding only the requirements, show a rise of azotemia but also result in an increase of glycemia and a reduction of insulinemia. This particular metabolic condition guarantees a greater availability of glucose for the udder due to the synthesis of lactose, which in turn favours the galactopoiesis due to the osmotic effect. When formulating the rations for the lactating buffalo herd it must be considered that elevated levels of structural carbohydrates limit the ingestion capacity and that greater concentrations of highly fermentable starches and

sugar can lead to an excessive weight gain which results in a shorter lactation curve. The calcium and phosphorus contribution is correlated to the productive requirements of the herd; so in this case the Ca:P ratio must be 2:1, so that the quantity of these two minerals is in proportion to the amount of milk produced (Technical-scientific Committee, 2002).

Table 10 reports the indicative requirements of the lactating buffalo herd elaborated by Bartocci et al. (2002). These data were obtained by evaluating the amount of dry matter intake, the chemical composition, the nutritional value and the milk yield for an entire lactation phase of 258 buffaloes, on 20 buffalo farms. In order to estimate indicative requirements of lactating buffaloes regression equations were calculated ($P<0.01$) between the normalized milk quantity (8.30 percent fat, 4.73 percent protein) and the average daily net energy consumed, protein, structural and non-structural carbohydrates of the diets administered *ad libitum* on 20 monitored farms:

Milk FU/d = 7.16 + 0.66*kg of milk ($R^2 = 0.80$)
CP (g/d) = 314.72 + 187.35*kg of milk ($R^2 = 0.87$)
NDF (g/d) = 8864.30 - 198.92*kg of milk ($R^2 = 0.76$)
NSC (g/d) = 4762.92 + 150.36*kg of milk ($R^2 = 0.81$)

The data for net energy ingestion of proteins and structural and non-structural carbohydrates, resulting from the previous equations are considered an estimate of the nutritional requirements of the lactating buffalo herd, corresponding to a normalized milk yield varying from 7 to 12 kg/d. When dividing the above-mentioned daily requirements, calculated by means of the previous equations, per ingestion of dry matter, the concentrations of nutritional principles of the diet are obtained which are necessary to satisfy maintenance requirements and the milk yield (Table 10). The data refers to a buffalo herd with 20 percent circa primiparous, the average weight for the multiparous of 650 kg and for primiparous of 570 kg. The maintenance requirements were evaluated by employing the INRA method (1988) for dairy cows, as specific data are not available. In order to obtain a normalized milk yield of 10 kg/d, an average live weight increase of 18.8 kg was estimated, which takes into account a weight reduction in the first forty days and a consequent gain between 100 and 170 days of lactation. From the daily weight gain for primiparous, estimated at 300 g/d, it was possible to calculate the energy and crude protein needed to produce 1 kg of normalized milk in 0.72 Milk FU and 145 g. When confronting the data of the nutritional requirements (7-12 kg/d) reported in Tables 9 and 10 the following considerations emerge: the daily ingestion of dry matter to produce 7 kg of milk is 16.0 kg according to Bartocci et al., (2002) while the Technical-scientific Committee (2002) estimated an intake of 14.7 kg DM. This difference, 1.3 kg DM, with the increase of the milk yield almost tends to disappear, 0.65 kg DM per 10 kg of milk; the same intake of dry matter (17 kg) with 12 kg of milk produced. Therefore, for the highest milk yield, the difference between the two studies when evaluating the dry matter intake is minimal; conflicting values were reported for the lower milk yields. However the intake capacity of the buffalo species needs to be considered as it has yet to be defined due to the differing results obtained from the various research centres. The total energy intake required to produce 7-10-12 kg of milk according to Bartocci et al., (2002) is 11.84, 13.74, 15.13 Milk FU/d vs 12.05, 14.16, 15.64 Milk FU/d of the Technical-scientific Committee (2002); consequently there is substantial agreement between the two studies concerning the total energy to administer in order to obtain the same milk yield. The crude protein amounts calculated to produce 7-10-12 kg of milk/d are almost equal, and precisely 1 626, 2 188 and 2 565 g/d of crude protein for Bartocci et al. (2002); 1 617, 1 996 and 2 240 g/d of crude protein for the Technical-scientific Committee (unpublished data). The higher values of crude protein (2 102, 2 463, 2 705 g/d) reported in Table 9 refer to the recommended (not calculated) values which also take into account the endocrine metabolic effect of the proteins. When comparing the fibre contribution no substantial differences emerge in the values of NDF obtained by the two works. The evaluation of the non structural carbohydrate (NSC) contribution appears rather interesting; Bartocci et al., (2002) report higher values compared to those of the Technical-scientific Committee (2002) because in the latter work the protein level increased and also the fat content in the diet was considered.

In conclusion the optimal protein level is the one recommended by the work of the Technical-scientific Committee (2002). As this is the latest work which takes into account not only the calculated protein, parameters used by Bartocci et al. (2002), but also the endocrine metabolic effects that an addition of protein has on milk yield. The two studies agree on the total energy required for the various productions; the dry matter intake needs to be specified for the medium-low milk yields (7-9 kg of milk/d) of the buffalo herd.

The evaluation of nutritional requirements of buffalo heifers

In many countries the requirements of heifers are not a problem; the heifers stay on pasture, often on very poor pasture, or they are fed with straw or with bad hay. But this is not the correct and economic approach; in fact, as already stated in Chapter IV, the age of puberty and therefore the reproduction efficiency of the herd is affected by many factors: both genetic (breed, sire, etc.) and environmental factors (i.e. season, climate, management, feeding, etc.). The age of puberty is particularly influenced by the diet energy level that enhances growth and sexual maturity.

Therefore in some countries, such as Italy, farmers prefer to give the correct diet to heifers satisfying the necessary requirements, in order to obtain high daily gains, to anticipate sexual maturity, to realize early puberty, early conception and early calving, and thereby reducing the unproductive period in the herd.

In this connection, a series of experiments was performed at the Animal Production Research Institute in Rome in order to determine what daily gain is the optimum and with which feeding stuffs it is possible to realize such gains, and thereby ascertain, the most efficient system for the feeding and management of buffalo heifers in economic terms and in reproduction efficiency.

Experiment 1: Different farms
This first trial (Borghese et al.,1993; Esposito et al., 1993) was carried out at the Tormancina farm (TM), 18 km north east of Rome (42° latitude North) and in three other farms (D-J-S) situated in the Campania Region of southern Italy (40.5 41° latitude North). The heifers of TM farm were housed in open feed-lots and fed unifeed ad libitum (maize silage 55 percent, alfalfa hay 17 percent, wheat straw 12 percent, beet pulp 9 percent, soya bean meal 1 percent, brewer grain 6 percent, 0.76 Milk FU/kg DM), while in D-J-S farms the animals were fed unifeed (maize silage, hay, straw, concentrates) in restricted diets: 4.21 3.73 3.83 Milk FU/day between 400 and 500 days of age and 5.10 4.42 5.25 Milk FU/day between 500 and 650 days of age, respectively, on the three farms.

Experiment 2: Low and high feeding levels
The heifers were housed in open feed-lots, subdivided in two groups, and fed two different diets according to standard requirements in order to obtain 450 g (low level group) or 650 g (high level group) daily gains respectively (Terzano et al., 1993; Borghese et al., 1994). The forage/concentrate ratios were: 4.42:1 - 2.46:1 respectively in the low level and in the high level groups; the diet components were: hay (81.4-70.7 percent respectively), soya bean meal (10.1-8.7 percent respectively), maize meal (8.5-20.6 percent respectively).

Experiment 3: Intensive feeding versus grazing feeding
The heifers were housed in feed-lots and randomly assigned to intensive feeding or to the pasture system: in the intensive system they received maize silage ad libitum (DM 33 percent, crude protein 8 percent, crude fibre 21 percent, 0.85 Milk FU/kg DM), plus hay and protein-mineral-vitamin supplement; the natural pasture botanical composition was: 50 percent grass, 40 percent legume and 10 percent other species (DM 20-70 percent, crude protein 14 percent, crude fibre 30 percent, 0.50-0.85 Milk FU/kg DM) (Terzano et al., 1996). The trial was repeated for two consecutive years taking into account that the pasture could be subjected to variability due to the different seasons, so the feeding systems are reported as maize silage 1 - pasture 1

for the first year and maize silage 2 - pasture 2 for the following year. Experiments 2 and 3 were carried out at the same farm (Tormancina).
Protein requirements (100-150 g PDI/100 kg live weight) were satisfied in all the trials except in the grazing one during the dry season.

Experiment 4: Maize silage and unifeed versus grazing feeding
The trial was carried out on 27 Mediterranean buffalo heifers, housed in feed-lots, treated against helminthes and randomly assigned to three groups at the average initial age of 8.5-9.0 months (Borghese et al., 1997).
1. Maize silage - nine heifers were fed maize silage ad libitum (DM 33 percent, crude protein 8 percent, crude fibre 21 percent, 0.85 Milk FU/kg DM) plus hay (about 20 percent on fed maize silage) and protein-mineral-vitamin supplement.
2. Pasture - eight heifers were fed natural pasture (50 percent graminaceae, 40 percent leguminosae and 10 percent other species, DM 20-70 percent, crude protein 10-21 percent, crude fibre 18-35 percent, 0.50-0.85 Milk FU/kg DM).
3. Unifeed - ten heifers were fed unifeed (DM 43.7 percent, crude protein 15.3 percent, crude fibre 22.4 percent, 0.84 Milk FU/kg DM).

During each trial the animals were weighed monthly in order to evaluate their growth rate; starting from about the thirteenth month of age they were tested every ten days by rectal palpation in order to determine the presence of follicle and corpus luteum and to assess the development of ovaries, cervix and uterine horns. At the same time blood samples were collected and plasma progesterone (P4) was assayed by RIA. Heifers were considered to have achieved puberty and cyclic ovarian activity when plasma P4 levels exceeded 1.5 ng/ml for two consecutive samples with a low value interval. After two cycles, as confirmed by rectal palpation, the heifers were mated.

Results of the four trials

The puberty age of all the reported trials (Table 11) shows a large variability depending on several factors. The pre-weaning and weaning systems which had influenced the daily gain obtained before the trials started could be important in promoting growth and achieving puberty. In fact, considering a mean of 40 kg body weight at birth, the animals that had shown a higher daily gain before the trial reached puberty in a shorter time.

Most of the heifers required a body weight of 380-420 kg to achieve puberty. In this case the feeding level plays a pivotal role in order to promote weight gain, and body and sexual growth. In experiment 1 on the TM farm, where the heifers received 4.5-5.5 Milk FU/d, all the 30 animals achieved cyclicity before 20 months at a body weight of 421 kg (679 g/d), while on J farm, where the heifers received 3.7-4.4 Milk FU/d, the lowest daily gain (472 g/d) was registered and only seven animals (24 percent) achieved puberty before two years. On D farm (4.2-5.1 Milk FU/d), where a daily gain of 525 g was recorded, 28 heifers out of 30 became cyclic and 25 conceived. Contrary to the other farms, where a constant daily gain was recorded for the whole trial period, on S farm 300 g daily gains were recorded up until 500 days with 3.8 Milk FU/d, after 500 days a high compensative increase (740 g/d) was achieved with 5.2 Milk FU/d and all 30 heifers became cyclic and conceived, even if at a higher age (658 d) and at a lower weight (358 kg) than on the TM farm. On this farm the best feeding efficiency (7.36 Milk FU/kg gain) was executed. This trial showed how a proper feeding level may anticipate the onset of puberty and affect the incidence of pregnancies.

In experiment 2 (Table 11) significantly higher daily gains and more favourable ages and weights at puberty were achieved with a high feeding level (5.6 Milk FU/day) than with the low level (4.4 Milk FU/day). The feed efficiency in the low and high level diets was about the same. These results confirmed the feeding level effect on growth and on body and sexual development and on the onset of puberty, as noted by other authors in Swamp heifers in Malaysia (Dollah et al., 1989), in Nili-Ravi in Pakistan (Chaudhary et al., 1983; Asghar et al., 1983) and in Murrah

in India (Kaur and Arora, 1989). Most of the animals had cyclic ovarian activity when the first P4 >1.5 ng/ml appeared. Two buffalo heifers showed ovarian disorders; one persistent corpus luteum and one luteinic cyst. In this trial, as in experiment 1 at the same farm (TM), the start of cyclic ovarian activity was influenced by decreasing photoperiod with the highest concentration in the autumn. Nine animals, born between December and May, achieved puberty from the following October to February at about 22 months of age (614 d in the high level group, 686 d in the low level group), while 15 heifers, born after May were not able to achieve puberty within the favourable season of the following year and delayed ovarian activity until the next autumn, at an average age of 27 months (796 d in the high level group, 825 d in the low level group). Therefore it was also confirmed in this study that the age at puberty is affected by the season of birth.

In Experiment 3 during the course of the first year (maize silage 1 - pasture 1) significantly higher (+42 percent) gains were obtained with the intensive system (693 g/d) than with the grazing one (488 g/d). In the second year (maize silage 2 - pasture 2) the differences between the intensive and grazing groups were notably reduced: 679 (+6.6 percent) versus 637 g/d, certainly due to the better conditions of the pasture and the climate of the second year, which permitted constant daily gains similar to that obtained with ad libitum feeding. On the contrary the poor pasture of a very hot summer (the first year) halted the heifers' growth, determining even a diminution of their body weight which, however, was followed by a prompt recovery of growth in the autumn. In both trials, the puberty age was about the same in the intensive and pasture groups (Table 11), due to the balancing growths realized by the heifers on pasture, that were able to attain the same body and sexual development during the autumn, the season which normally promotes the onset of cyclic ovarian activity. Very early puberty was realized by the maize silage group (16 months, 23 days before the pasture group) at 402 kg body weight (22 kg more than the grazing one, in the first year), while in the following trial, puberty age was delayed until 20 months with the maize silage and until 19 months in the grazing one, achieving body weights comparable to those of the previous year. Feed efficiency was also about the same and more convenient in comparison to that of the previous trials characterized by more intensive feeding systems. The grazing system was the most convenient in economic terms. All the animals had cyclic ovarian activity, as detected by rectal palpation, when the first progesterone >1.5 ng/ml appeared and so the animals conceived at a very early age; less than 20 months (first year) and at about 22 months in the second year without variations between groups.

In Experiment 4, seven heifers from the maize silage group (77.8 percent), seven of the pasture (87.5 percent), and all ten animals of the unifeed group achieved puberty within two years of age (Table 12). Therefore data are reported on 24 animals. All the animals showed cyclic ovarian activity, as detected by rectal palpation and by progesterone assay, when the first P4>1.5 ng/ml appeared, without following anoestrous period. No persistent corpus luteum nor luteinic cyst were found.

The heifers in this trial achieved puberty between July and October, due to the favourable effect of decreasing photoperiod on cyclic ovarian activity by melatonine intermediate action (Borghese et al., 1995). Since these heifers were born in the winter (December-March), they showed a longer anoestrous period than heifers born in the spring-summer (May-August), which had been utilized in other trials; the latter also achieved puberty in the autumn (October-December) at a very early age (15-18 months), since these animals had been born near the autumn, while the heifers of this trial, born in the winter, achieved puberty at 18-20 months. Therefore, as in previous trials, the age at puberty is confirmed to be affected by the season of birth.

How the feeding system affected body weight during the trial (Fig.1). The unifeed group showed higher body weight particularly between 498-550 days of age ($P<0.05$). During this period the animals obtained the maximum average daily gain (Fig. 1), that was more than 1.0 kg/d, but this group demonstrated another period (366-466 days) with a 600-800 g/daily gain which is

similar to the values attained by the other feeding groups. The maize silage group was more uniform in the daily gain during the whole trial (600-800 g/d) and consequently for body weight trend. Heifers on pasture showed a minimum daily gain at 366 days (600 g/d) during winter when the pasture was poor, but later they realized balancing growths of more than 1.0 kg/d (Fig. 1) at 426 days during the spring when the pasture was rich. At the end of the trial all the groups demonstrated the lowest daily gain since body maturity was achieved at about 20 months of age and 420 kg of weight. The highest average daily gain obtained with unifeed (824 g, Table 12) significantly affected ($P<0.05$) the age of puberty, which was 17.7 months in comparison with 19 on pasture and 20 months with maize silage. The heifers on pasture achieved puberty with the lowest body weight (386 kg, Table 12), about 38 kg less than the other groups, one month later than the unifeed groups and one month prior to the maize silage group.
The heifers on pasture achieved these reproductive performances with the lowest cost in terms of feeding stuffs and management.

Six heifers on pasture (85.7 percent) conceived at 668.5 days of age, about 100 days after the onset of puberty, 47 days after being bull exposed. One heifer did not conceive within the two months with the bull. Seven heifers fed maize silage (100 percent) conceived at 697 days of age, about 61 days after the onset of puberty, 56 days after being bull exposed. No heifer with unifeed conceived in the same period, though they were bull exposed at 582 days of age for two months, but it was due to the bull's fertility. Therefore the pasture system promoted the best performances in buffalo heifers, due to the economy of feeding and management, with favourable daily gains and an early age at puberty and at conception.

The conclusion of these experiments is that the best results are obtained by using unifeed which guarantees the integration of different feeding stuffs, this means the optimum of crude protein (12 16 percent) and crude fibre (20-24 percent) concentration, good mineral and vitamin content, good energetic concentrations (0.76-0.84 Milk FU/kg DM), convenient daily gains (680-800 g), the best feed efficiency (5.8-7.0 Milk FU/kg daily gain), early puberty (530-600 days) at a correct body weight (400-420 kg) and early conception before two years.

These results are valid for the Mediterranean Italian Breed, but probably they could be extended with some variations to all River breeds.

The requirements average in heifers, commonly used to prepare diets on Italian farms, is reported in Table 13.

Table 1. Indicative characteristics of requirements of the dry buffalo herd, average live weight = 600 kg (Proto, 1993).

Dry matter (kg)	10.5
NE_L (Milk FU/kg DM)	0.65
CP (% DM)	10.5
Dig. Prot (% DM)	7.0
CF (% DM)	30.0
NDF (% DM)	60.0
Starch + Sugars (% DM)	9.0

Table 2. Indicative characteristics of requirements of the dry buffalo herd (Bertoni et al., 1994).

Dry matter (kg)	10-12
NE_L (Milk FU/kg DM)	0.63-0.65
CP (% DM)	10-11
NDF (% DM)	52-58
Starch + Sugars (% DM)	8-10

Table 3. Nutrient requirements during gestation of the dry buffalo herd (multiparous: 600 kg; primiparous: 500 kg) in relation to the gestation months (Technical-scientific Committee, 2002)

Months of pregnancy		**Milk FU/d**	**CP (g/d)**	**Ca (g/d)**	**P (g/d)**
8	multiparous	5-7	700	40	35
	primiparous	6-7	830	40	35
9	multiparous	6-7	700	40	35
	primiparous	6-7.5	830	40	45
10	multiparous	6-7	800	40	45
	primiparous	7-8	900	40	45

Table 4. Indicative characteristics of requirements of the dry buffalo herd (Bartocci et al., 2002).

Dry matter (kg)	10.61
NE_L (Milk FU/kg DM)	0.63
CP (% DM)	7.90
NDF (% DM)	49.10
NSC (% DM)	33.10

Table 5. Energy and protein requirements for the production of 1 kg of buffalo milk relative to the fat and protein content (Proto, 1993)

Energy equirements (Milk FU/kg of milk)													
Milk fat	6.5	7.0	7.5	8.0	8.3	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0
NE_L	0.61	0.64	0.67	0.70	0.72	0.73	0.76	0.79	0.82	0.85	0.87	0.90	0.93
Protein requirements (g/kg of milk)													
Milk protein (%)			3.5	3.7	3.9	4.1	4.3	4.5	4.7	4.9	5.1	5.3	5.5
CP			99	105	111	116	122	128	134	139	145	151	157

Table 6. Indicative characteristics of rations for the lactating buffalo herd (Bertoni et al., 1994).

Milk yield	**DM (kg)**	**NE_L (Milk FU /kg DM)**	**CP (% DM)**	**NDF (% DM)**	**Starch + Sugars (% DM)**
>8-9 kg/d	15.5-16.5	0.80-0.85	13.5-14.5	42.0-46.0	14.0-16.0
<8-9 kg/d	14.5-15.5	0.76-0.80	12.5-13.5	46.0-50.0	12.0-14.0

Table 7. Energy and quality of cattle and buffalo milk and indicative requirements (Zicarelli, 1999).

	Cattle milk 4% (FCM)	**Buffalo milk**	**Buffalo milk (same energy as cattle milk 4% FCM)**
Energy and quality			
Energy (kcal/kg)	740	1258	740
Milk protein (g/kg)	31	45	26.47
Milk fat (g/kg)	40	87	51.18
Ca (g/kg)	1.2	2.0	1.18
P (g/kg)	0.9	1.2	0.71
kcal/g protein	23.9	28	28
Requirements/kg of milk			
Crude protein (g)	85	123	73
NE_L (Milk FU)	0.44	0.74	0.44
Ca (g)	3.5	5.80	3.43
P (g)	1.7	2.3	1.33

Table 8. Conversion factors to calculate the milk yield normalized 8.30 percent fat and 4.73 percent protein (Technical-scientific Committee, 2002).

	Fat (%)											
	6.0	**6.5**	**7.0**	**7.5**	**8.0**	**8.3**	**8.5**	**9.0**	**9.5**	**10.0**	**10.5**	**11.0**
Protein (%)												
3.8	0.779	0.813	0.847	0.881	0.915		0.943	0.984	1.019	1.053	1.087	1.121
4.0	0.792	0.827	0.860	0.845	0.929		0.964	0.998	1.032	1.066	1.101	1.135
4.2	0.806	0.840	0.874	0.909	0.943		0.977	1.012	1.045	1.080	1.114	1.149
4.4	0.820	0.853	0.888	0.923	0.957		0.991	1.025	1.060	1.094	1.128	1.162
4.6	0.833	0.868	0.902	0.936	0.971		1.005	1.039	1.073	1.108	1.142	1.176
4.73												
4.8	0.847	0.881	0.916	0.950	0.984		1.019	1.052	1.087	1.121	1.156	1.190
5.0	0.861	0.895	0.929	0.964	1.000		1.032	1.066	1.101	1.135	1.170	1.204
5.2	0.875	0.909	0.943	0.977	1.012		1.046	1.080	1.114	1.149	1.184	1.217
5.4	0.888	0.923	0.957	0.991	1.025		1.060	1.094	1.128	1.163	1.197	1.231
5.6	0.902	0.936	0.971	1.005	1.039		1.070	1.180	1.142	1.177	1.210	1.245

Table 9. Indicative characteristics of rations for the lactating buffalo herd (average live weight= 650 kg; normalized milk: fat=8.30 percent and protein=4.73 percent) (Technical-scientific Committee, 2002).

	Production of normalized buffalo milk (kg/d)							
	<6	6	7	8	9	10	11	12
Advised intake (kg DM/d)	13.3	14.2	14.7	15.1	15.6	16.1	16.5	17.0
NE_L (Milk FU/d)	0.75	0.79	0.82	0.84	0.86	0.88	0.90	0.92
CP (%DM)	13.0	13.9	14.3	14.6	15.0	15.3	15.6	15.9
NDF (%DM)	52.0	47.0	46.0	44.0	43.0	42.0	40.0	39.0
NSC (%DM)	25.0	27.0	28.0	29.0	30.0	30.0	31.0	32.0

Table 10. Indicative characteristics of rations for the lactating buffalo herd (average live weight= 650 kg; normalized milk: fat=8.30 percent and protein=4.73 percent) (Bartocci et al., 2002).

	Production of normalized buffalo milk (kg/d)					
	7	8	9	10	11	12
Advised intake (kg DM/d)	16.00	16.25	16.50	16.75	17.00	17.00
NE_L (Milk FU/d)	0.74	0.76	0.79	0.82	0.85	0.89
CP (%DM)	10.16	11.16	12.13	13.06	13.97	15.08
NDF (%DM)	46.70	44.76	42.87	41.05	39.27	38.10
NSC (%DM)	36.35	36.71	37.07	37.41	37.75	38.63

Table 11. Performances of buffalo heifers during different trials until puberty (Borghese et al., 1996).

Exper.	Groups	N	Initial age (days)	Initial weight (Kg)	gain/d before trial (g)	Puberty age (days	N	Puberty weight (days)	gain/d (g)	DM/d (Kg)	Milk FU/d	Milk FU/d Kg DM	Milk FU/Kg gain
1	TM	30	371	274^{a}	631^{A}	598	30	421^{a}	679^{a}	6.5	5.00	0.76	7.36
	D	30	383	267^{a}	593^{A}	612	28	392^{ab}	525^{b}	601	4.65	0.76	8.86
	J	29	385	235^{b}	506^{B}	624	7	385^{ab}	472^{b}	5.5	4.09	0.75	8.66
	S	30	372	204^{c}	441^{C}	658	30	358^{b}	538^{b}	6.6	4.61	0.70	8.57
2	Low level	12	237	143	435	767	12	388	465^{b}	6.4	4.42	0.69	9.50
	High level	12	241	141	419	736	12	410	562^{a}	7.3	5.56	0.76	9.89
3	Maize silage 1	6	319	280	752	490	6	402	693^{a}	5.1	4.34	0.85	6.09
	Pasture 1	4	333	285	736	513	4	380	488^{b}	5.8	3.92	0.67	7.41
	Maize silage 2	9	132	107	508	602	9	426	679	5.8	4.93	0.85	7.26
	pasture 2	8	138	109	523	569	8	387	637	6.5	4.65	0.70	7.30

Different letters in the same column of the same trial mean significant differences between groups (A, B, C: <0.01; a, b, c: P<0.05)

Table 12. Weight and age at puberty, feeding efficiency in buffalo heifers (Borghese et al., 1997).

	Maize silage	Pasture	Unifeed	RMSE
Initial age (d)	267	260	258	28.236
Initial weight (kg)	195.8	164.7	197.2	37.919
Weight at puberty (kg)	425.7	386.6	423.1	38.130
Age at puberty (d)	603^{a}	569^{ab}	532^{b}	39.361
Daily gain (g)	684	718	824	0.088
DM (kg/d)	5.8	6.5	5.7	
Milk FU/d	4.93	4.65	4.80	
Feed efficiency Milk FU/kg	7.21	6.48	5.83	

Different letters mean significant differences for $P<0.05$

Table 13. Average requirements in Italian heifers

	WEIGHT (kg)			
	100-200	200-300	300-400	400-500
Dry Matter kg	3.5-4.5	4.5-7	7.9-9	9.5-11
Crude protein % DM	15-16	15-16	15-16	13
Neutral Det. Fibre % DM	35	35	38	40
Mcal/kg DM	1,4	1,4	1,4	1,3
Calcium % DM	0,6	0,6	0,48	0,45
Phosphorus % DM	0,4	0,4	0,32	0,3
vit A UI/kg DM	300	300	3 400	3 200
vit D UI/kg DM	1 100	1 100	1 300	1 200
vit E UI/kg DM	31	31	34	32

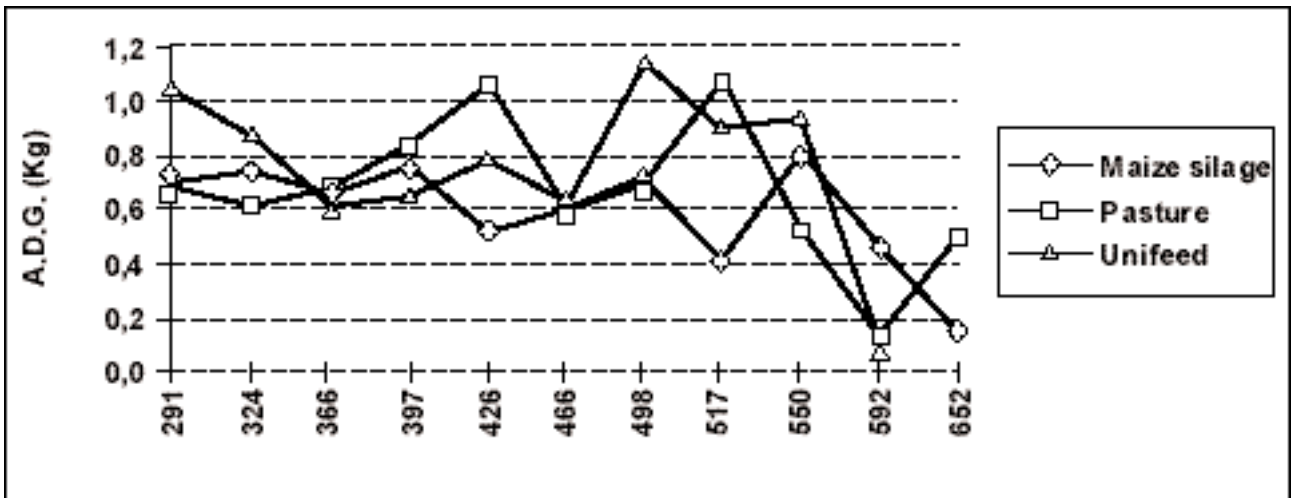


Figure 1. Average daily gain trend in buffalo heifers (Borghese et al., 1997)

References:

Abdullah, N., Ho, Y.W., Mahyuddin, M. and Jaladuin, S. 1990. Comparative studies of fibre digestion between cattle and buffaloes. Domestic buffalo production in Asia: 75-87 International Atomic Energy Agency, Vienna.

Asgha, A.A., Saghar, M.S. and Rehman, K.U. 1983. Effect of intensive feeding of buffalo on age at maturity, conception rate and age of first calving. Fourth Ann.Rep. Direct. LPRI, Bahadarnagar, Okara: 91-93.

Bartocci, S., Amici, A., Verna, M., Terramoccia, S. and Martillotti, F. 1997. Solid and fluid passage rate in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. Livestock Production Science, 52: 201-208.

Bartocci, S., Tripaldi, C. and Terramoccia, S. 2002. Characteristics of foodstuffs and diets, and the quanti-qualitative milk parameters of Mediterranean buffaloes bred in Italy using the intensive system. An estimate of the nutritional requirements of buffalo herds lactating or dry. Livestock Production Science, 77: 45-58.

Bertoni, G., Di Lella, T. e Bartocci, S. 1994. Nuove acquisizioni nel campo dell'alimentazione dei bufali. Agricoltura Ricerca, 153: 159-172.

Borghese, A., Terzano, G.M., Barile, V.L., Annichiarico, G. and Parmeggiani, A. 1993. Onset of puberty in Italian buffalo heifers. Note II: Influence of bull exposure on age at puberty. Intern. Symp. On Prospect of Buffalo Prod. In the Mediterranean, Middle East, Cairo, EAAP Publ. N. 62 Pudoc Sci. Ed.: 370-373.

Borghese, A., Terzano, G.M., Barile, V.L. and Parmeggiani, A. 1994. Season and feeding level effects on the onset of puberty in buffalo heifers. Fourth World Buffalo Congress, Sao Paolo, Brazil: 525-528.

Borghese, A., Barile, V.L., Terzano, G.M., Pilla, A.M. and Parmeggiani, A. 1995. Melatonin trend during seasons in heifers and buffalo cows. Bubalus bubalis, I: 61-65.

Borghese, A., Terzano, G.M., Barile, V.L., Catalano, A. and Malfatti, A. 1996. Onset of puberty in buffalo heifers in different feeding and management systems. Intern. Symp. Buff. Resources and Prod. Systems, Cairo, 14-17 October: 41-46.

Borghese, A., Barile, V.L., Galasso, A., Marchiori, E. and Terzano, G.M. 1997. Feeding system effect on reproduction performances in buffalo heifers. Fifth World Buffalo Congress, Caserta, Italy, 13 16 October: 697-701.

Campanile, G, Di Palo, R., Di Meo, C. e Boni, R. 1989. Effetti dell'integrazione di P durante l'asciutta sui livelli ematici di Ca, P e Mg nella bufala. Atti SISVet, XLIII:261-265.

Campanile, G., Di Palo, R., Esposito, L., Boni, R. e Di Meo, C. 1995. Variazioni di alcuni costanti ematiche in bufale in lattazione. Atti XI Cong. ASPA, Grado: 77-78.

Chaudhary, M.A., Haider, S.I., Ahmed, H.I. and Rasool, G. 1983. Effect of concentrate feeding on growth rate, age and weight at puberty, first calving and milk production in Sahiwal, Friesian x Sahiwal crossbreed and Nili-Ravi buffalo heifers. Fourth Ann . Rep: Direct. LPRI, Bahadarnagar, Oakara: 88-90.

Di Lella, T. 2000. Alimentazione della bufala in asciutta. Bubalus bubalis II: 32 36.

Di Palo, R. 1992. Produzione lattea nella bufala con diete tradizionali e con l'impiego di acidi grassi. Research Thesis, Fac. Med. Vet. Napoli, Italy.

Dollah, M.A., Ramakrishan, N., Nordin, Y. and Adullah Seni, R. 1989. Reproductive responses to climatic heat induced by management systems in Swamp buffaloes. IDEA RC 325 3/13: 155-166.

Esposito, L., Di Palo, R., Campanile, G., Boni, R. and Montemurro, N. 1993. Onset of ovarian activity in Italian buffalo heifers. Note III. Prospect of Buffalo Prod. In the Mediterranean Middle East, Cairo, EAAP Publ. (62), Pudoc Sci. Ed.: 374-377.

INRA. 1988. Alimentation des Bovins, Ovins et Caprins. INRA Publications. Parigi.

Kaur, H. and Arora, S.P. 1989. Growth and puberty as influenced by the plan of nutrition in Murrah buffaloes. Buffalo J., 1: 57-64.

Kennedy, P.M. 1990. Digestion and passage of tropical forages in swamp buffalo and cattle. Proceedings of "Domestic buffalo production in Asia", Rockhampton, Australia, 20-24 February 1989: 21-40.

Proto, V. 1993. L'alimentazione della bufala. Giornata di studio "Alimentazione zootecnica e qualità del latte bovino e bufalino". 29 Ottobre 1993, Eboli (SA): 1-42.

Puppo, S., Bartocci, S., Terramoccia, S., Grandoni, F. and Amici, A. 2002. Rumen microbial counts, in vivo digestibility in buffaloes and cattle given different diets. Animal Science, 72: 323-329.

Technical-scientific Committee of the consortium for the protection of Campania buffalo mozzarella cheese, 2002. "Regolamento per la gestione igienica ed alimentare dell'allevamento bufalino in relazione alla produzione della Mozzarella di bufala campana DOP" Ed.: Consorzio Tutela Mozzarella di Bufala Campana, S. Nicola la strada (CE), Italy

Terramoccia, S., Bartocci, S., Amici, A. and Martillotti, F. 2000. Protein and protein-free dry matter rumen degradability in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. Livestock Production Science, 65: 185-195.

Terzano, G.M., Barile, V.L., Mongiorgi, S. e Borghese, A. 1993. Effeto di diversi livelli alimentari sulla pubertà in bufale di razza Mediterranea. Atti 47o Conv. S.I.S. Vet., Riccione: 1803-1807.

Terzano, G.M., Barile, V.L., Francia, U., Malfatti, A., Todini, L. and Borghese, A. 1996. Onset of puberty in buffalo heifers bred on pasture or in intensive feeding systems. Bulgarian J. Agric.Sci., 2(1): 89-92.

Zicarelli, L., Intrieri, F., De Franciscis, G. e Squillacciotti, A. 1982. Il profilo metabolico nella bufala gravida in relazione al regime alimentare adottato: indagine in allevamenti con diversa incidenza di prolasso vaginale. Atti del II Conv. Int. sull'allevamento bufalino nel mondo, Caserta, 29 sett./2 ott.: 262-288.

Zicarelli, L. 1999. Nutrition in dairy buffaloes. In: Tionhati H., Barnabe V.H., Baruselli P.S. (Eds.), Perspectives of buffalo husbandry in Brazil and Latin America. Funep, Jabutical: 157-178.

Zicarelli, L. 2000. Considerazioni sulla profilassi del prolasso vaginale e uterino nella bufala mediterranea italiana. Bubalus bubalis, III: 71-90.

Chapter VIII

NEW ACQUISITIONS ON THE DIGESTIVE PHYSIOLOGY OF THE MEDITERRANEAN BUFFALO

Settimio Bartocci, Stefano Terramoccia and Simonetta Puppo

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

The high demand for buffalo mozzarella cheese and the problem of milk quotas in cattle breeding has led to an increased diffusion of the buffalo species, with about 230 000 head bred in Italy in 2001, according to recent estimates reported by the National Association of Buffalo Breeders. This present situation is a radical transformation of the original circumstances dating back to 1950, when the buffalo population reached its minimum historical number of 5 000 head (Lucifero, 1998). At that time the nutritional requirements of dry and lactating buffaloes were almost completely unknown. In recent years studies have been published by researchers from various institutions (Proto, 1993; Bertoni et al., 1994; Zicarelli, 1999; Bartocci et al., 2002) and by the Scientific Committee of the Consortium for the Protection of Campania buffalo mozzarella cheese (2002) which give estimates of the nutritive requirements and suggested suitable diets for buffaloes in their different physiological conditions.

In order to ascertain an exact definition of such requirements it is also necessary to know the digestive physiology of the species and different research centres have carried out studies in this field (Masoero et al., 1994; Infascelli et al., 1995; Di Francia et al., 2000). In addition the Istituto Sperimentale per la Zootecnia has made its own contribution with a research activity covering the following aspects: the passage rate of fluids and solids in the gastro-intestinal tract, the rumen degradability of feeds and the rumen microflora content in relation to its in vivo digestibility. As a result of this research activity four studies have been published in such scientific reviews as Animal Science (Amici et al., 1997; Puppo et al., 2002) and Livestock Production Science (Bartocci et al., 1997; Terramoccia et al., 2000), in order to contribute to the knowledge regarding the digestive physiology in buffaloes.

The research activity was carried out using four Mediterranean buffaloes (*Bubalus bubalis* L.) bulls and four Holstein Friesian bulls, two year of age; the average live weight was 417.1 and 509.2 kg respectively. Eight weeks prior to the research all the animals were fitted with a rumen silicon cannula. The animals involved in this trial were supervised in compliance with the Italian laws and regulations regarding experimental animals. Adequate procedures to minimize pain and discomfort were adopted during the operative and post operative periods. Four isoproteic diets (about 14 percent of crude proteins, CP) were administered differing in the following way with regard to forage:concentrate ratio: Diet 1= 87.5:12.5; Diet 2= 75:25; Diet 3= 62.5:37.5; Diet 4= 50:50. The forage of the diets were alfalfa hay and maize silage mixed in a ratio of 65 to 35 on a dry matter (DM) basis. The concentrate contained in a decreasing amount: barley, maize, extracted soya bean meal and a vitamin-mineral supplement. The components of the diets were accurately mixed together. Each animal was fed each of the four diets, over four consecutive periods, according to a Latin square design.

The administration of the feed was at maintenance level twice daily, at 08:00 and 16:00 hours, with an amount equal to 50 g of dry matter/kg of metabolic weight. Each period, lasting 21 days, was divided into a first stage of feeding adaptation of 14 days and a second one of seven days, which was used for the whole collection of faeces for the determination of in vivo digestibility (ASPA, 1982). Samples of feeds and faeces were analysed for: DM, CP, crude fibre (CF), ether extract (EE), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL). The analyses were carried out according to AOAC methods (1984) and

according to Goering and Van Soest (1970). The chemical composition of feeds and diets used during the research is reported in Table 1.

Buffaloes and cattle, after the period of diet adaptation, received directly in the rumen, through the rumen cannula, 300 g of hay mordanted with $Na_2Cr_2O_7$ (Udén et al., 1980) and 50 g of Co-EDTA diluted in 300 ml of distilled water, in order to determine the passage rate of the solids and fluids; the markers were administered the morning before the first meal. 28 samples of faeces were taken from each animal, directly from the rectum, from 0 to 152 hours after the administration of markers; in order to determine the amount of chrome and cobalt. The samples were analysed by atomic absorption spectroscopy (Williams et al. 1962). In order to describe the digesta movements in the gastrointestinal tract the individual faecal concentration curves were evaluated with three gamma (2, 3, 4) age-dependent one-compartment models (Pond et al. 1988), one gamma age dependent/age-independent two-compartment model (Matis, 1972; Pond et al. 1988) and one multicompartment model (Dhanoa et al. 1985). The coefficients in the various models have the following meaning: k_1 and k_2 represent the constant outflow rate per hour; $1/k_1$ and $1/k_2$ are constants which are associated with the passage rate in the reticulo-rumen and in the caecum-proximal colon; (passage time) is the time delay in hours from the administration of the marker to its first appearance in the faeces. The total retention time (TTR) in the whole gastro-intestinal tract is calculated as follows: $TTR = 1/k_1 + 1/k_2 + \tau$ in the multicompartment model (age independent); $TTR=n/l + \tau$ in one compartment models (age dependent) where n corresponds to the distribution gamma order and l to the passage rate per hour ($k_1=l*0.59535$ for Gamma2, $k_1=l*0.47454$ for Gamma3, $k_1=1*0.40857$ for Gamma 4); $TTR = n/l + 1/k_2 + \tau$ in the two mixed compartment age dependent and independent models.

After the period of diet adaptation, ground maize silage, concentrate and hay, were separately introduced into the rumen by nylon bags (3 g of DM for each sample). The bags were removed from the rumen after 2, 4, 8, 24, 48 and 72 hours (and 120 hours only for hay); the value of degradability at 0 time was obtained by immersing the bags in the rumen fluid for three minutes. In order to determine the feed degradability at different times of incubation, the content of CP and of protein-free dry matter (PFDM) of each bag was calculated. The CP rumen degradability was obtained by $((CP_1-CP_2)/CP_1)*100$, while the PFDM degradability (degradability of structural and non structural carbohydrates) was obtained, at the different times, by $(((DM_1-CP_1)-(DM_2-CP_2))/(DM_1-CP_1))*100$, where DM_1 and CP_1 are the amounts of dry matter and crude protein of the feed before the incubation, while DM_2 and CP_2 after incubation. The data of CP and PFDM rumen degradability at different times of incubation were processed by the procedure SAS/NLIN (SAS, 1993), with the exponential model of Ørskov and McDonald (1979): $dg(t)=a + b * (1 - exp(-c*t))$, where dg(t) is the rumen degradability at time t, a is the rapidly soluble fraction at zero time, b the potentially degradable fraction, c the degradation rate constant of fraction b and t the incubation time. By using the three previously calculated parameters (a, b, c), and the reticulo-rumen passage rate constant of solids (k_1) we obtained the effective rumen degradability of CP and PFDM by the following equation: $a + (b * c/ (c + k_1))$; the single values of k_1 for species and for diets, used in the above equation, were experimentally calculated and are reported in Table 3.

In order to determine the total viable counts of rumen bacteria, samples of the whole rumen content were withdrawn, after the period of diet adaptation, at 08:00 hours before the morning meal, for three consecutive days. They were immediately gassed with CO_2 and treated with a blender-homogenizer to detach bacterial cells from the feed particles. The anaerobic technique used was that of Hungate (1950) modified by Bryant (1972), which provides an anaerobic glove-box (atmosphere: 95 percent CO_2 and 5 percent H_2). The incubation lasted five days at 39°C and the total viable counts were determined according to Harrigan and McCance's procedure (1976).

During the research the weight of the buffaloes did not undergo relevant changes, thus

demonstrating that the amount of daily dry matter administered (50 g/kg of metabolic weight) is a suitable amount for the maintenance level in buffaloes as already fixed for cattle.
From the two by two comparison of the residual deviations of the five models taken into consideration, the multi compartment model proved to be the best for the study of faeces elimination kinetics of the marker (Cr) of solids both in cattle and the buffaloes, while for the study of the faeces elimination kinetics of the marker (Co-EDTA) of fluids the best model proved to be, in both species, the Gamma4.

The reticulo-rumen passage rate in the first compartment (k_1) of the marker of the solid particles (Table 2), evaluated by the multicompartment model, is significantly higher in cattle compared to buffalo (2.99 *vs* 2.46 percent h_{-1}, $P<0.05$), so the slower passage rate of the marker of solid particles causes a longer average retention time ($1/k_1$) in the buffalo rumen than that of cattle (40.65 *vs* 33.44 h, $P<0.05$). Ponappa et al. (1971) confirmed a longer residence time of feeds in the buffalo rumen; Colucci et al. (1990) found an average rumen retention time in cattle equal to 32.25 h, similar to ours. The difference in the average rumen retention time of the marker of solid particles in the two species shows a different rumen digestion of feeds. The passage rate (k_2) of the marker of the solid particles in the caecum-proximal colon (the second compartment), which can be considered the digestive tract following the abomasum, in which the blend of digested feed takes place, shows values significantly higher in buffaloes than in cattle (11.37 *vs* 10.02 percent h_{-1}, $P<0.05$). The mean retention time ($1/k_2$) of this tract is equal to 8.79 *vs* 9.98 h, with a difference of only 1.2 h and even if it is significant, this datum marginally influences the difference between the two species with regard to the total retention time in the whole gastro-intestinal tract. The time between the administration of the marker of solids and its first appearance in the faeces (passage time, τ) resulted to be significantly lower in buffalo than in cattle (6.98 *vs* 19.06 h, $P<0.05$); Colucci et al. (1990) found similar results in cattle. The total retention time in the whole gastrointestinal tract ($1/k_1+1/k_2+\tau$) is significantly longer in cattle than in buffalo (64.55 *vs* 57.73 h, $P<0.05$) and such difference is specifically caused by the passage time (τ). Buffalo have a longer average residence time of the marker of solid particles in the rumen compared to cattle and a shorter residence time in the post-rumen tract, consequently it can be deduced that there is a different residence time of feeds and so a different behaviour of the two species with regard to their digestive physiology.

The reticulo-rumen passage rate (k_1) of fluids marker, evaluated with the Gamma4 model (Table 2) does not show any difference between the two species: 6.98 percent h_{-1} in the buffalo and 6.57 percent h_{-1} in the cattle; with a retention time ($1/k_1$) of 14.33 h for buffalo and of 15.22 h for cattle. As regards this coefficient, Hume and Sakaguchi (1991) did not find any difference between the two species, while Kennedy (1990) found that the fluids passage rate in buffalo is higher than in cattle; in our opinion, this difference could come from the different models used for evaluating this coefficient. There is no significant difference between the species, also with regard to the time between the administration of the fluids marker and its first appearance in the faeces (τ), which is equal to 4.76 h in buffalo and 6.19 h in cattle; however the total retention time ($n/l + \tau$) is significantly different favouring the cattle (31.59 *vs* 28.93 h, $P<0.05$). Another important data is represented by the rumen volume which was calculated by the determination of the marker concentration (Co-EDTA) in samples of rumen fluid withdrawn at different times, using an exponential model to describe the disappearance of the marker. The volume of the buffalo rumen resulted significantly greater than that of cattle (65.80 *vs* 59.10 l, $P<0.05$); the outflow rate of rumen fluid also resulted to be significantly higher in buffalo (4.34 *vs* 3.77 l/h, $P<0.05$).

The values of reticulo-rumen passage rate (k_1) of the solid particles used for the calculation of the effective CP and PFDM degradability are reported in Table 3. The effective CP rumen degradability of the feeds used, is reported in Table 4. Significant differences in favour of buffalo were observed in the degradability of the concentrate (64.8 *vs* 58.8 percent, $P<0.05$); in cattle, a value close to ours was obtained by Murphy and Kennelly (1987). When changing the

diet, we noticed a higher degree of variability in the CP degradability of concentrate in cattle (standard deviation:±6.2 for cattle and ±1.1 for buffalo), showing a higher sensitiveness to the increase of concentrate. The effective CP degradability of hay is always higher in buffalo (62.7 *vs* 57.0 percent, $P<0.01$); values similar to ours were obtained for cattle by Erdman et al. (1987). If we consider the diets, with regard to the CP degradability of hay, buffalo showed a lower variability (standard deviation: ±2.5 for cattle and ±0.8 for buffalo). The CP rumen degradability of maize silage is significantly higher in buffalo than in cattle (68.6 *vs* 58.7 percent, $P<0.01$); with regard to the latter species, values comparable with ours were obtained by Miller (1981) and Susmel et al. (1990). Infascelli et al. (1995) obtained a higher CP rumen degradability in buffalo but in a comparison study with sheep. Table 5 reports the effective PFDM rumen degradability of the three feeds used by the two species. As regards concentrate and maize silage, we notice significant differences ($P<0.01$) in favour of buffalo (70.0 *vs* 64.1 percent and 64.8 *vs* 56.0 percent, respectively); in the case of hay no significant difference can be noted: 49.2 percent for buffalo and 48.2 percent for cattle, however also in this case the highest value is that of the buffalo.

If we globally consider the degradability response, we can deduce a different ability of the two species of degrading both CP and PFDM of feeds; therefore the percentage of carbohydrates and amino acids, available in the small intestine, is higher in cattle, while in buffalo the level of ammonia and energy available for rumen micro-organisms is higher.

The results regarding the total viable bacteria in buffalo and cattle are reported in Table 6; the significances are referred to the transformed data ($\log_{10}$), since they do not have a normal distribution. Buffaloes have a higher and significant number of rumen bacteria (11.88×10^{10} *vs* 1.61×10^{10} cells/g of dry rumen content, $P<0.01$). As the concentrate in the diet increases, we notice an increase in bacterial number in buffalo, with a significant difference between diet 1 and the other three diets (2.03×10^{10} *vs* 8.75×10^{10}, 11.66×10^{10} and 25.10×10^{10} cells/g of dry rumen content, $P<0.01$). These values are similar in buffalo and cattle only for diet 1; as regards the latter species, as the concentrate increases, we do not notice any significant difference in the total number of rumen bacteria. By comparing the four diets between species, the total viable bacteria of buffalo shows higher values compared to cattle; such difference is significant for diet 2 ($P<0.05$) and for diets 3 and 4 ($P<0.01$). The higher microbial synthesis in buffalo comes from a higher ammonia rumen level; in this study, even if the ammonia level was not measured, a higher CP rumen degradability by buffalo was observed: therefore we can affirm that the rumen ammonia level is different in the two species, and it is higher in buffalo, as it was also reported by Bittante et al. (1994), Sangwan et al. (1990) and Kennedy et al. (1992a). Bertoni et al. (1993), in a research in which cattle and buffalo were fed isoproteic diets with different energetic levels, found that the level of urea in blood is quite constant in buffalo; by contrast in cattle this value significantly decreased when the dietary energy increased. This trend is attributable to the decline of ammonia in the cattle rumen when the concentrate increases, presumably resulting from a limited ability to recycle blood urea to the rumen. Also the level of available energy in the rumen is higher in buffalo because of the higher degree of degradability of the protein-free dry matter.

Considering the apparent digestibility of the most significant parameters (Table 7), the digestibility of the organic matter results to be higher in cattle (69.6 *vs* 67.6 percent, $P<0.05$). Di Francia et al. (2000) found an average digestibility in buffalo, with regard to this parameter, equal to 68.5 percent, similar to the data reported by us. The better digestibility of the organic matter by cattle is caused by the longer residence time of ingesta in the post-ruminal tract. The average time of rumen retention of feeds in buffalo is significantly longer compared to cattle, while the total retention time in the whole gastro-intestinal tract is significantly longer in bovine (Table 2). By comparing the four diets between the two species the digestibility values of the organic matter are always higher for cattle, but significantly ($P<0.01$) only for diet 4, while within the species, there is a significant difference ($P<0.05$) between diet 1 and diet 4 either for buffalo (66.1 *vs* 68.8 percent) or for cattle (67.6 *vs* 71.4 percent). Pannu and Kaushal (1985), found that the Hariana cattle digest the organic matter better than the buffalo Murrah

with a diet 50:50 of forage: concentrate ratio. Settineri and Puppo (1998), by using eight different feeds, found that the in vitro digestibility values of the organic matter were significantly higher favouring cattle, for six of the feeds.

The crude protein digestibility data show that the Mediterranean buffalo and the Holstein Friesian cattle have the same utilization of proteins (67.1 and 66.7 percent respectively). By comparing the diets within each species, cattle show a significant difference between diet 1 and the other three (63.2 *vs* 66.0, 68.7, 68.8 percent, $P<0.05$). On the contrary, the CP digestibility in buffalo is almost constant for all diets. The CP digestibility values of diet 1 are significantly higher in buffaloes than in cattle (66.2 *vs* 63.2 percent $P<0.05$) and diet 2 also shows a CP digestibility, which even if not significant, higher in the buffalo species: therefore we can affirm that the best utilization of proteins can be found in buffaloes with diets with a high content of structural carbohydrates. This result is confirmed by Sangwan et al. (1987), who, with a forage:concentrate ratio equal to 77:23, found higher CP digestibility in buffalo (76.5 *vs* 70.3 percent, $P<0.05$). Furthermore, Moran et al. (1979) feeding Swamp buffaloes with sorghum hay ad libitum, found that they had a better CP digestibility compared to Shorthorn cattle.

Cattle show a better aptitude to the NDF digestive utilization (54.8 *vs* 51.1 percent, $P<0.05$), because of the better digestibility of cellulose (62.1 *vs* 50.9 percent, $P<0.01$), while no differences were found with regard to the digestibility of hemicelluloses. Contradictory results can be found in literature: Kennedy et al. (1992b) found that NDF digestibility is higher in cattle, on the contrary Hussain and Cheeke (1996) found a better NDF digestibility in buffalo. By comparing the species for each diet, the NDF digestibility is significantly ($P<0.05$) higher for cattle only for diet 1, while the cellulose digestibility is significantly higher ($P<0.05$) with regard to diets; no differences can be noted with regard to the digestibility of hemicelluloses.

Fig.1 shows the digestibility trend of cellulose and hemicelluloses in the two species in relation to the increase of forage in the diet. The regression equations regarding cellulose and hemicelluloses are significant ($P<0.05$) for both species. The straight lines of cellulose tend to be parallel when the forage percentage is higher than 62.5 percent, so cattle digest cellulose in higher percentage and quite constantly as the forage increases. The values of hemicelluloses digestibility are similar and tend to be convergent when the contribution of forage is over 75 percent.

In buffaloes, the increase in rumen micro-organisms and the decrease in the structural carbohydrate digestibility show that the number of micro-organisms does not reflect the digestibility of NDF and cellulose. In a comparison test between buffalo and cattle, regarding the rumen degradability of the NDF, with a diet consisting of 75 percent of hay and 25 percent of concentrate, Settineri et al. (1994) found that the rumen degradability of only hay is similar in the two species, while that of hay + concentrate is higher in buffalo (69.1 *vs* 66.0 percent, $P<0.05$). Buffaloes, therefore, are more capable of degrading the most digestible structural carbohydrates in the rumen, consequently the higher degree of digestibility of NDF and cellulose in cattle, as already found with regard to the digestibility of the organic matter, is due to a longer residence time of the structural carbohydrates in the post-rumen tract. In spite of the higher ruminal microbial numbers in the buffalo for all diets, the cattle have higher, even if not always significant, digestibility coefficients for most nutrients except crude protein (diets 1 and 2) and hemicelluloses.

In conclusion, for the two animal species considered in this research, the best fit for the marker associated with solid particles was obtained with the multicompartment model while the Gamma4 model was more appropriate for the fluid marker. Buffaloes retain particles in the rumen longer than cattle, although the retention time in the whole digestive tract was shorter because of their lower residence time in the gut. The mean retention time of fluids was shorter in buffalo than for cattle. The buffalo degrades a greater amount of both crude protein and protein-free dry matter than cattle. The total number of rumen bacteria is higher in buffalo and does not reflect the trend in organic matter digestibility which is always higher in cattle as it

is also for NDF and cellulose. The crude protein digestibility is similar in both species, but it is higher in buffalo fed diets with a higher content of structural carbohydrates.

Table 1. Dry matter (percent as fed) and chemical composition (percent DM) of feeds and of four experimental diets.

	DM	CP	CF	EE	NSC	Ash	NDF	ADF	ADL
Feeds									
Concentrate	90.20	14.42	8.02	2.40	49.15	8.70	25.33	10.87	3.14
Alfalfa hay	87.34	16.25	34.41	1.20	19.55	8.98	54.02	40.92	9.94
Maize silage	33.67	9.03	22.45	2.71	32.43	5.13	50.70	28.52	4.15
Diets (Forage:Conc.)									
1] (87.5 : 12.5)	71.00	13.81	27.38	1.81	26.88	7.77	49.73	33.37	7.32
2] (75.0 : 25.0)	73.94	13.89	24.67	1.89	30.42	7.90	45.90	30.15	6.71
3] (62.5 : 37.5)	76.20	13.98	21.89	1.96	33.57	7.98	42.51	26.87	6.11
4] (50.0 : 50.0)	79.37	14.07	18.92	2.06	36.70	8.17	39.00	23.73	5.53

DM=dry matter; CP = crude protein; CF = crude fibre; EE = ether extract; NSC = non-structural carbohydrates; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin.

Table 2. Effect of animal species on excretion patterns of solid marker (Cr) and fluid marker (Co-EDTA) in faeces.

		Passage rate of solid particles (multicompartment model)		Passage rate of fluids (Gamma4 model)	
		Buffalo	**Cattle**	**Buffalo**	**Cattle**
1° compartment (reticulo-rumen)					
Passage rate	$k1$ (%h^{-1})	2.46^{b}	2.99^{a}	6.98	6.57
Constant outflow rate	$1/k_1$ (h)	40.65^{a}	33.44^{b}	14.33	15.22
2° compartment (caecum-proximal colon)					
Passage rate	(%h^{-1})	11.37^{a}	10.02^{b}	-	-
Constant outflow rate	$1/k_2$ (h)	8.79^{b}	9.98^{a}	-	-
Time delay between marker administration and its first appearance in the faeces	τ(h)	6.98^{b}	19.06^{a}	4.76	6.19
Total time of retention in the gastro-intestinal tract	TTR (h)	57.73^{b}	64.55^{a}	28.93^{b}	31.59^{a}

a, b: $P<0.05$

Table 3. Solid marker (Cr) and fluid marker (Co-EDTA) passage rate constant k1 (% h^{-1}) of the four diets in buffalo and cattle reticulo-rumen.

Diets	Buffalo	Cattle
	Cr marker	
1]	2.80	3.57
2]	2.42	2.82
3]	2.39	2.86
4]	2.24	2.67
Mean	2.46^{b}	2.99^{a}
	Co-EDTA marker	
1]	7.95	7.52
2]	7.44	6.37
3]	6.43	6.40
4]	6.12	6.01
Media	**6.98**	**6.58**

a , b : $P<0.05$

Table 4. Effective crude protein rumen degradability of the three feeds.

	Concentrate			Alfalfa hay			Maize silage	
Diets	**Buffalo**	**Cattle**		**Buffalo**	**Cattle**		**Buffalo**	**Cattle**
1]	63.3	51.8		61.5	54.5		67.8	56.3
2]	64.8	56.0		63.0	55.4		68.4	57.1
3]	64.9	61.7		63.1	58.3		68.5	59.7
4]	66.0	65.8		63.1	59.9		69.8	61.9
Mean	**64.8^{a}**	**58.8^{b}**		**62.7^{A}**	**57.0^{B}**		**68.6^{A}**	**58.7^{B}**

A, B: $P<0.01$ a, b: $P<0.05$

Table 5. Effective protein-free dry matter rumen degradability of the three feeds.

	Concentrate			Alfalfa hay			Maize silage	
Diets	**Buffalo**	**Cattle**		**Buffalo**	**Cattle**		**Buffalo**	**Cattle**
1]	69.1	61.5		48.0	47.1		67.8	53.2
2]	70.1	62.2		48.7	48.4		64.4	56.8
3]	70.3	66.0		49.0	48.6		65.3	57.0
4]	70.4	66.6		51.2	48.8		67.1	57.0
Mean	**70.0^{A}**	**64.1^{B}**		**49.2**	**48.2**		**64.8^{A}**	**56.0^{B}**

A, B: $P<0.01$ a, b: $P<0.05$

Table 6. Total viable bacteria (n x 1010 cells per g dry rumen content) in buffalo and cattle given four diets.

	Buffalo	**Cattle**
Mean	$11.88^{\#}$	$1.61^{\#}$
1]	2.03^{B}	1.72
2]	$^{a}8.75^{A}$	$^{b}2.52$
3]	$^{A}11.66^{A}$	$^{B}1.36$
4]	$^{A}25.10^{A}$	$^{B}0.82$

Means in the same column followed by different superscripts are significantly different (a,b,c: P<0.05; within each species).
Means in the same column preceded by different superscripts are significantly different (a,b: P<0.05; A,B: P<0.01; between species, for all diets and for each diet).

Table 7. Apparent digestibility coefficients (percentage) of organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), cellulose and hemicelluloses in buffalo and cattle given four diets

	OM	CP	NDF	Cellulose	Hemicelluloses
Buffalo	$^{b}67.6$	67.1	$^{b}51.1$	$^{B}50.9$	66.6
Cattle	$^{a}69.6$	66.7	$^{a}54.8$	$^{A}62.1$	65.3
Buffalo					
1]	66.1^{b}	$^{a}66.2$	$^{b}53.1^{a}$	$^{B}54.6^{a}$	68.4
2]	67.0^{ab}	67.0	52.2^{ab}	$^{B}52.5^{a}$	66.5
3]	68.5^{ab}	67.8	50.7^{bc}	$_{B}50.5^{ab}$	65.9
4]	$^{B}68.8^{a}$	67.4	48.3^{c}	$^{B}46.0^{b}$	65.4
Cattle					
1]	67.6^{b}	$^{b}63.2^{b}$	$^{a}57.8^{a}$	$^{A}66.0^{a}$	67.6^{a}
2]	69.1^{ab}	66.0^{a}	55.5^{ab}	$^{A}63.0^{ab}$	66.6^{ab}
3]	70.2^{ab}	68.7^{a}	53.6^{b}	$^{A}60.5^{b}$	63.7^{ab}
4]	$^{A}71.4^{a}$	68.8^{a}	52.4^{b}	$^{A}58.8^{b}$	63.3^{b}

Means in the same row with same superscripts differ significantly (P<0.01)
Means in the same column followed by different superscripts are significantly different (A,B: P < 0.01; within each species).
Means in the same row preceded by different superscripts are significantly different (a, b: P < 0.05; A, B: P < 0.01; between species, for each diet).

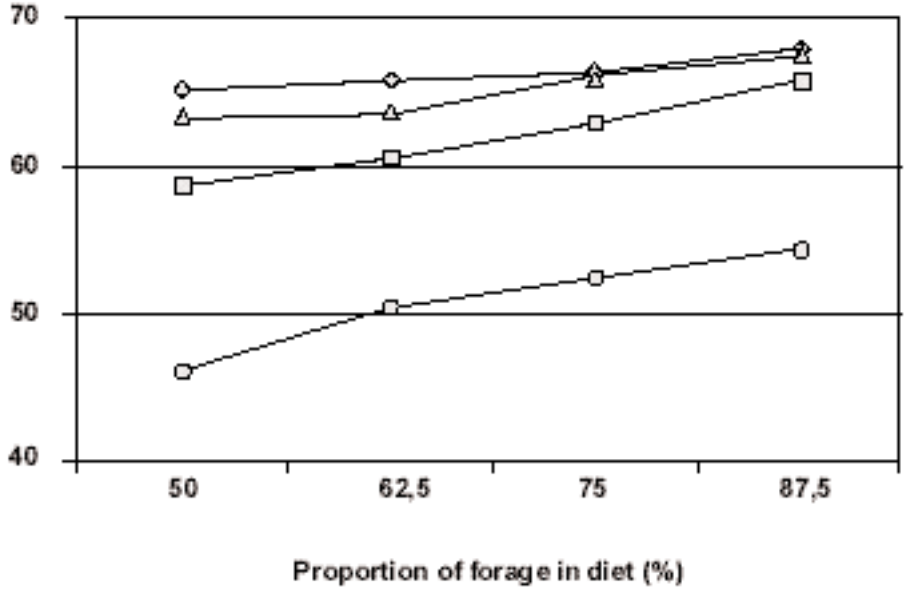


Figure 1. Cellulose (buffalo=□; cattle=□) and hemicellulose (buffalo◆; cattle=△) digestibility in the two species in relation to the increase of forage in the diets.

References

Amici, A., Bartocci, S., Terramoccia, S. and Martillotti, F. 1997. Passage rate of solids and fluids in the digestive tract of buffaloes, cattle and sheep: selection of non-linear model. Animal Science, 64: 63-69.

AOAC. 1984. Official Methods of Analysis, 14th Edn. Association of Official Analytical Chemists, Washington, DC.

ASPA. Commissione Valutazione degli alimenti, 1982. Valutazione degli alimenti di interesse zootecnico. 2) Aspetti metodologici della digeribilità in vivo. Zootecnica Nutrizione Animale, 8: 387-394.

Bartocci, S., Amici, A., Verna, M., Terramoccia, S. and Martillotti, F. 1997. So1id and fluid passage rate in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. Livestock Production Science, 52: 201-208.

Bartocci, S., Tripaldi, C. and Terramoccia, S. 2002. The characteristics of foodstuffs and diets, the quanti-qualitative milk parameters of Mediterranean buffaloes bred in Italy using the intensive system. An estimate of the nutritional requirements of buffalo herd lactating or dry. Livestock Production Science, 77: 45-58.

Bertoni, G., Amici, A., Lombardelli, R. and Bartocci, S. 1993. Variations of metabolic profile and hormones in blood of buffalo, cattle and sheep males fed the same diets. In Pudoc Scientific Publisher, Proceedings of the International Symposium "Prospects of buffalo production in the Mediterranean and Middle East", Wageningen, Netherlands. EAAP Publication 62: 345-348.

Bertoni, G., Di Lella, T. e Bartocci, S. 1994. Nuove acquisizioni nel campo dell'alimentazione dei bufali. Agricoltura Ricerca, 153: 159-172.

Bittante, G., Ramanzin, M., Bailoni, L., Simonetto, A. e Bartocci, S. 1994. Confronto fra alcuni parametri ruminali di bufali, bovini e ovini alimentati con diete diverse. Agricoltura Ricerca, 153: 135-142.

Bryant, M.P. 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Animal Journal Clinical Nutrition, 25: 1324-1328.

Colucci, P.E., McLeod, G.K., Grovum, W.L., McMillan, I. and Barney, D.J. 1990. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at low and high intake. Journal Dairy Science, 73: 2143-2156.

Dhanoa, M.S., Siddons, R.C., France, J. and Gale, D.L, 1985. A multicompartmental model to describe marker excretion patterns in ruminant faeces. British Journal of Nutrition, 53: 663-671.

Di Francia, A., Masucci, F., Infascelli, F., Gioffrè, F. and Proto, V. 2000. Digeribilità in vivo e valore energetico in bufali ed ovini. 6. Diete a base di paglia di frumento trattata o no con ammoniaca. Zootecnica Nutrizione Animale, 26: 211-217.

Erdman, R.A., Vandersall, J.H., Russek-Cohen, E. and Switalski, G. 1987. Simultaneous measures of rates of ruminal digestion and passage of feeds for prediction of ruminal nitrogen and dry matter digestion in lactating dairy cows. Journal Animal Science, 64: 565-577.

Goering, H.K. and Van Soest, P.J. 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). Agriculture handbook n° 379, ARS USDA, Washington, DC.

Harrigan, W.F. and McCance, M.E. 1976. Laboratory methods in food and dairy microbiology. Harrigan, W.F. (Ed.), Academic Press, London: 383-389.

Hume, I.D. and Sakaguchi, E. 1991. Patterns of digesta flow and digestion in foregut and hindgut fermenters. In: ed. Tsuda T., Sasaki Y., Kawaschima R. "Physiological aspects of digestion and metabolism in ruminants", Academic Press, San Diego: 427-451.

Hungate, R.E. 1950. The anaerobic, mesophilic, cellulolytic bacteria. Bacterial Review, 14: 1-49.

Hussain, I. and Cheeke, P.R. 1996. Evaluation of animal ryegrass straw: corn juice silage with cattle and water buffalo: digestibility on cattle v. buffalo and growth performance and subsequent lactational performance of Holstein heifers. Animal Feed Science and Technology, 57: 195-202.

Infascelli, F., Di Lella, T. and Piccolo, V. 1995. Dry matter, organic matter and crude protein degradability of high protein feeds in buffaloes and sheep. Zootecnica Nutrizione Animale, 21 (Suppl.): 89-94.

Kennedy, P.M. 1990. Digestion and passage of tropical forages in swamp buffalo and cattle. Proceedings of "Domestic buffalo production in Asia", Rockhampton, Australia, 20-24 February 1989: 21-40.

Kennedy, P.M., McSweeney, C.S., Ffoulkes, D., John, A., Schlink, A.C., LeFeuvre, R.P. and Kerr, J.D. 1992a. Intake and digestion in swamp buffaloes and cattle. 1. The digestion of rice straw (Oryza sativa). Journal of Agricultural Science, 119: 227-242.

Kennedy, P.M., Boniface, A.N., Liang, Z.J., Muller, D. and Murray, R.M. 1992b. Intake and digestion in swamp buffaloes and cattle. 2. The comparative response to urea supplements in animals fed tropical grasses. Journal of Agricultural Science, 119: 243-254.

Lucifero, M. 1998. L'allevamento bufalino in Italia: evoluzione storica. Convegno "L'allevamento bufalino in Italia. Ieri, oggi e domani", Accademia dei Georgofili, 30/05/98, Firenze: 7-36.

Masoero, F., Cabiddu, A., Fiorentini, L., Moschini, M. e Piva, G. 1994. Effetto di diete a diverso contenuto energetico sulla digeribilità intestinale di proteine alimentari nel bufalo. Agricoltura Ricerca, 153: 107-122.

Matis, J.H. 1972. Gamma time dependency in Blaxter's compartment model. Biometrics, 28: 597-602.

Miller, E.L. 1981. Methods of assessing protein for ruminants including laboratory methods. In: Miller E.R., Pike I.H., Van Es A.J.K. (Eds) "Protein contribution of feedstuffs for ruminants", Butterworths, London.

Moran, J.B., Norton, B.W. and Nolan, J.V. 1979. The intake, digestibility and utilization of a low-quantity roughage by Brahman cross, buffalo Banteng and Shorthorn steers. Australian Journal of Agricultural Research, 30: 333-340.

Murphy, J.J. and Kennelly, J.J. 1987. Effect of protein concentration and protein source on the degradability of dry matter and protein in situ. Journal Dairy Science, 70: 1841-1849.

Ørskov, E.R. and McDonald, L. 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. Journal Agricultural Science, 92: 499-503.

Pannu, M.S. and Kaushal, J.R. 1985. Effect of roughage:concentrate ratio on the rumen volume,

rumen degradation and the digestibility of proximate principles in cattle and buffalo. Indian Journal of Animal Nutrition, 2: 61-64.

Pond, K.R., Ellis, W.C., Matis, J.H., Ferreiro, H.M. and Sutton, J.D. 1988. Compartment models for estimating attributes of digesta flow in cattle. British Journal of Nutrition, 60: 571-595.

Ponnappa, C.G., Uddin, M.N. and Raghavan, G. 1971. Rate of passage of food and its relation to digestibility of nutrients in Murrah buffaloes and Hariana cattle. Indian Journal Animal Science, 41: 1026-1031.

Proto, V. 1993. L'alimentazione della bufala. Giornata di studio "Alimentazione zootecnica e qualità del latte bovino e bufalino". 29 Ottobre 1993, Eboli (SA): 1-42.

Puppo, S., Bartocci, S., Terramoccia, S., Grandoni, F. and Amici, A. 2002. Rumen microbial counts, in vivo digestibility in buffaloes and cattle given different diets. Animal Science, 72: 323-329.

Sangwan, D.C., Pradhan, K. and Sagar, V. 1987. Effect of dietary fibre and protein sources on rumen metabolites and nutrient digestibility in cattle and buffalo. Indian Journal of Animal Sciences 57: 562-569.

Sangwan, D.C., Pradhan, K., Bhatia, S.K., Sagar, V. and Sadhana, S. 1990. Associative effect of wheat straw or oat hay with protein supplements on rumen metabolites and nutrient digestibility, in cattle and buffalo. Indian Journal of Animal Sciences 60: 472-479.

SAS Statistical Analysis System Institute, 1993. SAS User's Guide, Statistics, SAS Institute, Cary (NC), USA.

Scientific Committee of the Consortium for the Protection of Campania buffalo mozzarella cheese, 2002. Pattern of regulation for sanitary and feeding management of buffalo herds for the production of buffalo mozzarella cheese. Ed.: Consorzio Tutela Mozzarella di Bufala Campana, S. Nicola la Strada (CE), Italy

Settineri, D., Pace, V. e Marzoli, C. 1994. Degradazione della fibra e attività cellulosolitica nel rumine di bufali e bovini. Agricoltura Ricerca, 156: 99-106.

Settineri, D. and Puppo, S. 1998. In vitro comparative digestibility by cow, buffalo and sheep rumen fluids. Buffalo Journal 14: 21-29.

Susmel, P., Stefanon, B., Wills, C.R. e Piasentier, E. 1990. Impiego di modelli matematici diversi ed effetto della macinazione e della setacciatura sulla degradabilità in situ della sostanza secca e dell'azoto. Zootecnica Nutrizione Animale, 16: 157-166.

Terramoccia, S., Bartocci, S., Amici, A. and Martillotti, F. 2000. Protein and protein-free dry matter rumen degradability in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. Livestock Production Science, 65: 185-195.

Udén, P., Colucci, P.E. and Van Soest, P.J. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. Journal Science Food Agriculture, 31: 625-632.

Williams, C.H., David, D.J. and Ismaa, O. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. Journal of Agricultural Science, 59: 381-385.

Zicarelli, L. 1999. Nutrition in dairy buffaloes. In: Tionhati H., Barnabe V.H., Baruselli P.S. (Eds.), Perspectives of buffalo husbandry in Brazil and Latin America. Funep, Jabutical: 157-178.

Chapter IX

BUFFALO MILK QUALITY

Carmela Tripaldi

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

1. Introduction

In recent years, the buffalo population in Italy has increased from 200 000 head reared in 2001 (Zicarelli, 2001a) to the 265 000 head of today (ANASB, 2003). The principal motive for this trend, which began in the 1950s/1960s, was the potentiality for converting the buffalo farming system from extensive to intensive utilizing the structures and management systems in place for the dairy cow. Another reason was the high market demand for mozzarella cheese, a typical Italian cheese made from fresh buffalo milk, recognized as DOP and called "Mozzarella di Bufala Campana", when produced in traditional buffalo-rearing areas. Table 1 indicates the positive trend of milk yield and quality over the last years. The improvement in buffalo milk production is principally due to new feeding criteria, changed diets and rearing conditions and selective breeding (Di Palo, 2002).

Almost all buffalo milk is assigned to cheese making, mainly to mozzarella cheese, therefore it is important to produce milk which in turn will yield a good quality cheese in high quantities. A characteristic of buffalo milk is the very high fat content and the fat to protein ratio is about 2 : 1. Another characteristic is the high casein to protein ratio (81-84 percent) (Tripaldi et al., 1997; Tripaldi et al., 2003) compared to bovine milk (78 percent). Moreover the high calcium content of casein micelles results in a faster rennet coagulation, increased curd tension and a faster syneresis (Addeo et al., 1996) and its rennet ability is considered to be very good. Mozzarella from buffalo milk is richer in fat and presents sensorial characteristics very different from the more common bovine Mozzarella (Addeo et al., 1993). The present paper analyses the main factors influencing buffalo milk quality.

2. Feeding and milk quality

Buffalo feeding has been the object of numerous studies which have often been aimed at defining the appropriate requirements of this species. A lack of such defined requirements makes it more difficult to explain the results of research.

2.1. Energy content of the diet.

The results of two investigations on some commercial herds are shown in Table 2 including data relating to the whole lactation where diets with different energetic levels were administered, respectively, 0.78-0.82 vs 0.70-0.72 Milk FU/kg DM, (Bertoni et al., 1991) and 0.82 vs 0.77 and 0.73 Milk FU/kg DM (Bartocci et al., 2002). The dietary energy positively and significantly affected milk yield when 0.82, 0.77 and 0.73 Milk FU/kg DM diets were compared (Bartocci et al., 2002). Milk protein and fat content in both studies (Bertoni et al., 1991; Bartocci et al., 2002) were significantly higher consistent with the higher energetic levels of diets. Milk from buffaloes fed with rations with a higher energetic content also had better renneting ability and a higher estimated yield of Mozzarella cheese ($P < 0.001$) (Bartocci et al., 2002).

The results of the experimental trials carried out on multiparous buffalo cows at early-mid lactation (Verna et al., 1994; Tripaldi et al., 1997) were as follows: no significant differences in milk yield but a positive effect of high energy content (0.78 vs 0.68 and 0.83 vs 0.77 Milk FU/kg DM) on milk protein and fat. However the differences between high and low energy diets were

not significant.

In the same experimental trials when energy was increased there was a corresponding increase in casein content, even if not significant, and an improvement in the milk coagulation parameters, in particular clotting and curd firming time decreased while curd firmness increased; these are the characteristics of milk necessary for the production of good quality cheese (Tripaldi et al., 1997). The estimated yield of "Mozzarella cheese" was also higher for higher energy diets.

It has been largely demonstrated that the protein and casein content of cow's milk is positively affected by the energy level of diets (Remond, 1985). While, other authors when referring to bovine milk, indicate that total N, protein and casein content increased with energy supplies equal to or lower than INRA recommendations (Vertes et al., 1989; Macheboeuf et al., 1993). Whereas if the animals are fed energy supplies equal to or higher than INRA recommendations, the previous nitrogen fractions do not differ (Laurent et al., 1992).

In dairy cows it has been shown that fat content decreases when the energy level in the diet is higher (Journet and Chilliard, 1985). The higher fat content of buffalo milk when the diet has a higher energy level can be explained by a greater mobilization of lipidic reserves (Bertoni et al., 1991; Bertoni and Piccioli Cappelli, 1994). Masoero et al. (1994) found that, when the dietary energy was higher, rumen fermentations were oriented more towards butyric acid content and this acid favours mammary synthesis of fat. Zicarelli (2001b) asserted that if the dietary energy was in excess of the requirements, milk quality would be modified. Based on existing knowledge, the increase of fat in buffalo milk, due to an increase of energy level in the diet, is difficult to interpret. It is evident that the subject requires further investigation.

According to Tripaldi et al. (1997), the energy level of diets affected the composition of milk fat, as observed in the milk of dairy cows (Grummer, 1991). The short chain fatty acids increased when energy availability was higher, otherwise the long chain fatty acids prevailed when food energy was lower. The latter condition favoured fat mobilization and a higher long chain fatty acids content in the blood, than in the milk.

Numerous trials concerning the addition of fat to buffalo diets have been carried out and their effects on milk quality have been analysed. In Table 3 it can be noted that if the fat, calcium salts or crio-crystallized fatty acids, were added to diets in the first two months of lactation, milk yield and ECM milk increased, fat percentage was not significantly different and protein percentage was significantly lower only in one trial. When fat was administered after the first two months of lactation, the results of the two reported trials were contradictory. According to Di Palo et al. (1997) and Zicarelli (2001b) the increase of the dietary energy after the peak lactation phase had a positive effect only on milk fat percentage and not on milk yield. The possible explanation of these authors is the change from a phase characterized by energy deficit, where fat addition increased milk yield, to a phase where dry matter intake is regularized and that an increase in dietary energy only produces an increase of milk fat content. Polidori et al. (1997) observed a higher milk yield and a decrease in protein content in mid-late lactation, while milk fat content was not modified. According to these authors the lack of increase in body condition score and in milk fat content indicates that the administered fat was probably utilized to support the increased oxidation processes related to higher milk production.

The beneficial impact on milk yield of adding fat in the first two months of lactation is evident, the effect in the second part of lactation needs further investigation.

There was a significantly higher content of short and medium chain fatty acids in the milk fat of buffaloes fed on calcium soaps in the first two months of lactation (Cheli et al., 1991), but it has not yet been established what would be the effects on the sensory and texture characteristics of the cheese.

The feeding also affected milk acidity: the higher dietary energy caused an increase in milk protein content and a decrease of pH values (Bertoni and Piccioli Cappelli, 1994; Tripaldi et al., 1997). According to Zicarelli (2001b) in two farms where the dietary energy, but mainly protein content, was increased, milk titratable acidity increased respectively from 6,1 and 6,6 to 8,8, and 8,4 °SH percent, these latter values being normal in buffalo milk.

2.2. Protein content of the diet.

Table 4 records the results of an experimental trial where levels of 12 and 14 percent of dietary protein were compared in buffaloes at the beginning of lactation, yielding an average of 10 kg/day of milk. The only effect was an increase in NPN content corresponding to the higher protein content of the diets (Tripaldi et al., 1997).

Dietary protein of 12 percent compared with 9 percent in buffaloes 132 days in milk and with 10 kg/day of milk yield, increased milk yield, protein content and protein quantity (Campanile et al., 1998). In another trial using the same levels of dietary protein on buffaloes 164 days in milk, yielding 7 kg/day of milk, no effects on milk yield and quality were observed (Campanile et al., 1998).

According to Bertoni et al. (1993), buffaloes seem to be more adaptable than dairy cows to lower dietary protein. However, very low dietary protein, in theory not meeting buffalo requirements, can affect milk yield and quality. In fact in both the above-mentioned trials (Campanile et al., 1998) the higher level of dietary protein caused an increase in blood urea content and the stabilization of the milk freezing point (-0.54 vs -0.52°C), which with lower dietary protein proved to be above the contractual level.

If a dietary protein level of 17 and 19 percent was used on multiparous buffalo cows between 45 and 165 days in milk and yielding an average of 14 kg/day of milk, there were no differences in milk yield and characteristics (Sarubbi et al., 2000). Milk urea content was very high (47.7 and 51.8 mg/100 ml, respectively) when compared with the milk urea content in the above trials using 12 percent dietary protein (35.0 mg/100 ml) (Campanile et al., 1998).

In a survey lasting fifteen months covering nine buffalo herds fed 13.4 percent protein and yielding an average of 8.5 kg/day of milk, the average milk urea content observed was 40.8 mg/100 ml (Di Francia et al. 2003). These results confirm that buffalo milk urea content is higher than that of the dairy cow as already indicated (ASPA, 1999) and can be justified by higher amino acid catabolism and/or more efficient renal urea reabsorption.

3. Somatic cell count and milk quality

Somatic cell count is usually utilized as a sanitary control of milk and specifically as an indicator in the presence of sub-clinical mastitis. Inflammation of mammary epithelium, in addition to reducing milk yield, modifies milk composition, and this in turn affects cheesemaking properties, cheese yield and composition. Some studies have been carried out on cow milk (Politis and Ng-Kwai-Hang, 1988a; 1988b; 1988c; Auldist et al., 1996), but little is known about buffalo milk either with regard to the effects of somatic cell count on milk quality or on the physiological threshold of the somatic cell count (Esposito et al., 1997; Tripaldi et al., 2003).

Italian regulations regarding the hygienic and sanitary characteristics of buffalo raw milk only established a limit for the total bacterial count while no limit was set for the somatic cell count. European standards have established a limit of 400 000 somatic cells/ml for buffalo milk assigned to raw milk products, as is the case for cow's milk. The European directives have established the same limit both for cow and buffalo milk, and therefore it is likely that Italy will soon set a threshold also for buffalo milk.

According to Galiero et al. (1996), who studied 28 buffalo farms, 79 percent of herds produced

milk having less than 400 000 somatic cells/ml. The average value of somatic cell count observed in 37 farms of Italian buffalo from 1997 to 2000 was 191 808/ml (APA Latina, 2000). During a one-year survey on 20 farms, the average value of somatic cell count was 221 280/ml (Tripaldi et al., 2003). The somatic cell number observed in Surti, Murrah and Sri Lankan buffaloes varied from 50 000 to 375 000/ml with an average of 140 000/ml (Silva and Silva, 1994). The average value of somatic cell count revealed in 2 693 Murrah buffaloes' milk samples, obtained monthly from 1997 to 2000, was 63 610/ml. (Cerón Muñoz et al., 2002).

In Table 5 the average values of milk yield, pH, protein and casein content, casein to protein ratio and coagulating properties according to different somatic cell classes are reported. Milk yield decreased when the somatic cell number increased, contrarily, the milk pH increased. The protein and casein content and casein to protein ratio decreased when the somatic cell count increased. The coagulating properties deteriorated when the somatic cell count increased. According to Pasquini et al. (2003) the casein content of buffalo milk increases by about 10 percent when the somatic cell count decreases from 1 500 000 to 13 000/ml.

In another trial (Di Bernardini, 2004) the somatic cell classes were <200 000, 200 000-1 000 000 and >1 000 000. Milk yield started to decrease significantly when the somatic cell count was higher than 200 000/ml (4.51 vs 3.42 kg in morning milking); if the somatic cell count was higher than 1 000 000/ml pH value increased significantly (6.76 vs 6.88), otherwise, lactose content decreased significantly (4.66 vs 4.10). Fat, protein and casein content were affected mainly by the lactation phase. Rennet clotting time and curd firming time were significantly different when the somatic cell count passed from <200 000 to more than 1 000 000, curd firmness appeared not to be affected by udder health.

In Table 6 it can be noted that the milk samples with poor and very poor rennet ability were characterized by a higher somatic cell count with respect to milk samples with good and fairly good rennet ability (respectively 314 330 and 385 850 vs 203 260 and 231 330/ml).

It appears that the higher milk yield and the better chemical and technological characteristics were obtained when the somatic cell count was somewhere between 100 000 and 200 000/ml.

4. Bacterial count and milk quality

With regard to the bacterial count, European limits are the same as for Italy, i.e. 500 000/ml for products from raw milk. It has been reported that often raw milk delivered to cheese farms contains a high number of total germs (Galiero et al., 1996; Amante et al., 2001).
In Italy the price of buffalo milk is not dependent upon its fat and protein content and somatic cell and bacterial count, as is generally applied to cow's milk, but a poor hygienic quality of the milk could be one of the many factors affecting the shelf-life of Mozzarella, which is a fresh cheese. During the preparation of Mozzarella, a thermic treatment of curd provokes a decrease in the bacterial count, but it is not known if the shelf-life of the finished product varies according to the number and the type of germs present in the raw milk.

5. Conclusions

In buffaloes a high energy diet increases fat content as well as protein content. Fat added to diets in the first phase of lactation increases the milk yield. A very low level of dietary protein can cause a decrease in milk yield, protein content, and protein quantity and a destabilization of the milk freezing point. When the protein level of the diet is higher, the only effect on milk quality is an increase in the NPN content of the milk. The average somatic cell count of buffalo milk is not very high; a higher milk yield and better chemical and technological characteristics are obtained when the somatic cell count is approximately 200 000/ml. The high bacterial count is a critical issue with regard to buffalo milk and requires greater vigilance on the farm.

Table 1. Milk average yield(1), fat and protein content of buffaloes controlled in Italy (AIA).

Years	Head controlled	Yield (kg)	Fat (%)	Protein (%)
1977 - 1981	2 220	1 669	7.09	-
1982 - 1986	6 673	1 658	7.88	-
1987 - 1991	9 852	1 818	8.13	4.4
1992 - 1996	13 994	1 935	8.23	4.56
1997 - 2001	20 786	2 096	8.31	4.72

(1) Average yield of lactation length higher than 150 days

Table 2. Effect of different energy content of the diet on the milk yield and quality of the Italian buffalo.

	Monitoring		Monitoring			1st Experimental trial		2nd Experimental trial	
Period of treatment	**Whole lactation**		**Whole lactation**			**2nd to 5th month of lactation**		**2nd to 5th month of lactation**	
Milk FU/kg DM	0.78 0.82	0.70 0.72	0.82	0.77	0.73	0.78	0.68	0.83	0.77
Milk (kg/d)	9.77	9.39	10.46A	8.21B	7.27C	10.97	11.59	10.59	10.58
Fat (%)	8.79A	7.97B	8.83A	8.47B	8.83A	8.60	8.13	9.04	8.97
Protein (%)	4.53A	4.22B	4.77A	4.80A	4.70B	4.41	4.28	5.02	4.85
References	Bertoni et al., 1991		Bartocci et al., 2002			Verna et al., 1994; Tripaldi et al., 1997		Verna et al., 1994; Tripaldi et al., 1997	

A,B = P<0,001; a,b = P<0,01

Table 3. Effect of administration of fat to diet on milk yield and quality of the Italian buffalo (Zicarelli, 2001b, modified).

Type of fatty acids	**Calcium salts of long chain fatty acids**		**Crio-crystallized fatty acids**							
Period of treatment	**First two months of lactation**		**Mid-late lactation**		**First two months lact.(fixed diet)**		**First two months lact. (cross group)**		**50-110 days in lactation**	
References	Di Palo, 1992		Polidori et al., 1997		Di Palo et al., 1997		Di Palo et al., 1997		Di Palo et al., 1997	
Milk FU/kg DM	0.905	0.866	0.935	0.906	0.923	0.851	0.923 0.851		0.944	0.875
Milk (kg/d)	14.02	12.63	7.70A 6.31B		9.83a	7.60b	10.31A	8.39B	8.71	8.50
ECM (kg/d) (1)	23.35a	20.39b	13.40A(2)	10.96B	16.09a	12.07b	15.85a 14.33b		14.14	12.5
Fat (%)	8.14	7.80	9.12	9.04	8.21	7.71	7.76	8.65	8.04a	7.49b
Fat (g/d)	1141a	985b			811a	588b	781	734	704a	621b
Protein (%)	4.72	4.62	4.69b	4.79a	4.41	4.48	4.18A	4.48B	4.36	4.42
Protein (g/d)	662	584			429a	339b	430	376	380	372
Estimated Mozzarella (kg/head/d)	3.60	3.14			2.42	1.85	2.40	2.13	2.11	2.02

A,B = P < 0,001; a,b = P < 0,01

(1) ECM = equivalent correct milk; (2) 4% FCM (kg/d) = fat corrected milk.

Table 4. Effect of different protein content of diet on milk yield and quality of the Italian buffalo (experimental trials).

Days in milk	Milk average yield (kg/d)	Protein content of diet (%)	Effect on milk yield and quality	References
30-150	10	12		(Verna et al., 1994; Tripaldi et al., 1997)
		14	NPN content	
132-214	10	9		(Campanile et al., 1998)
		12	→ milk yield → milk protein Increase of blood urea content and stabilization of milk freezing point	
164-246	7	9		
		12	Increase of blood urea content and stabilization of milk freezing point	
45-165	14	17		(Sarubbi et al., 2000)
		19	No significant differences in milk yield and quality	

Table 5. Daily average production, physical-chemical and technological characteristics of the Italian buffalo milk according to the somatic cell number (Tripaldi et al., 2003).

Somatic cells (n*103/ml)	<50	50÷99	100÷199	200÷299	300÷399	400÷499	500÷999	≥1000	RMSE
Milk yield (kg/d)	9.32ab	9.93a	9.55ab	8.96abc	8.62bc	8.61bc	7.89c	7.99c	3.70
pH	6.73dc	6.71dc	6.70d	6.72dc	6.74bc	6.77ab	6.80a	6.79a	0.11
Protein (%)(1)	4.84a	4.72ab	4.77ab	4.30abc	4.15bc	3.66c	3.64c	3.62c	0.45
Casein (%)(1)	3.90a	3.81ab	3.90a	3.26bc	3.07c	2.74c	2.71c	2.69c	0.35
Casein/ Protein (%) (1)	80.79a	80.31a	82.12a	79.36ab	77.18bc	74.86c	74.75c	74.31c	1.98
Rennet clotting time, r (min)	14.64b	14.27b	14.53b	14.73b	16.68a	16.93a	17.42a	17.03a	4.63
Curd firming time, K_{20} (min)	3.57bcd	3.45cde	3.05e	3.12de	3.48cde	3.95ab	3.66bc	4.20a	1.31
Curd firmness, A_{30} (mm)	42.64dc	45.77bc	50.28a	48.37ab	45.17bc	44.25dc	43.60dc	40.58d	12.12

a, b, c. d, e : $P<0.05$ (1)Data from 5 percent of samples

Table 6. Daily average production, physical-chemical and health characteristics and coagulating properties of the Italian buffalo milk samples regrouped according to their renneting ability (Tripaldi et al., 2003).

	Group 1 Good rennet ability	**Group 2 Fairly good rennet abil.**	**Group 3 Poor rennet ability**	**Group 4 Very bad rennet ability**	**RMSE**
% Samples	71.92	18.64	3.76	5.68	
Daily average yield (kg/d)	9.51A	9.09A	7.86B	6.72C	3.55
pH	6.70C	6.78B	6.86A	6.87A	0.09
Somatic cells ($n*10^3$/ml)	203.26B	231.33B	314.33A	385.85A	296.86
Clotting time (min)	13.61C	18.71B	25.11A		3.59
Curd firming time (min)	2.81C	4.46B	5.92A		0.87
Curd firmness (mm)	52.24A	36.27B	17.44C		8.35

A, B, C: $P<0.01$

References

Addeo, F., Chianese, L. and Masi, P. 1993. The influence of processing conditions on the quality of water buffalo mozzarella cheese. Prospects of buffalo production in the Mediterranean and the Middle East, EAAP Publication, 62: 214-222.

Addeo, F., Emaldi, G.C. and Masi, P. 1996. Tradition and innovation in the "mozzarella di bufala campana cheese" production. International symposium on buffalo products, EAAP Publication, 82: 23-39.

Amante, L., De Rosa, C., Fasano, L., Banchelli, L., Midea, D. e Di Palo, R. 2001. Valutazione dei punti critici della mungitura in aziende di bufale di pianura e di collina del basso Lazio. Atti I° Congresso Nazionale sull'Allevamento del Bufalo, Eboli (SA), 3-5 Ottobre: 251-255.

ANASB 2003. Statistical data.

APA Latina 2000. Statistical data.

ASPA 1999. Guida all'interpretazione dei profile metabolici. Univ. Perugia.

Auldist, M.J., Coats, S., Sutherland, B.J., Mayes, J.J., Mc Dowele, G.H. and Roogers, G.L. 1996. Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of Cheddar cheese. J. Dairy Res., 63: 269-280.

Bartocci, S., Tripaldi, C. and Terramoccia, S. 2002. Characteristics of foodstuff and diets, and the quanti-qualitative milk parameters of Mediterranean buffaloes bred in Italy using the intensive system. An estimate of the nutritional requirements of buffalo herds lactating or dry. Livest. Prod. Sci. 77: 45-58.

Bertoni, G., Piccioli Cappelli, F., Bernabucci, U. and Di Stefano, E. 1991. Some effects of feeding management on milk production and metabolism of dairy buffaloes. Proceedings of Third World Buffalo Congress, Varna, Bulgaria, 13 18 May: 861-868.

Bertoni G., Amici, A., Lombardelli, R. and Bartocci, S. 1993. Variations of metabolic profile and hormones in the blood of buffaloes, cattle and sheep males fed the same diets. Prospects of buffalo production in the Mediterranean and the Middle East, EAAP Publication, 62: 214-222.

Bertoni, G. and Piccioli Cappelli, F. 1994. Influenza dell'alimentazione sulle condizioni metaboliche e produttive delle bufale. Inf. Agr.: 18, 29-33.

Campanile, G., De Filippo, C., Di Palo, R., Taccone, W. and Zicarelli, L. 1998. Influence of dietary protein on urea levels in the blood and milk of buffalo cows. Livest. Prod. Sci., 55: 135-143.

Cerón-Muñoz, M., Tonhati, H., Duarte, J., Oliveira, J., Muñoz-Berrocal, M., and Jurado-Gàmez, H. 2002. Factors affecting somatic cell counts and their relations with milk and milk constituent yield in buffaloes. J. Dairy Sci., 85: 2885-2889.

Cheli, F., Boni, R., Pastorino, P. e Dell'Orto, V. 1991. Impiego dei saponi di calcio nell'alimentazione della bufala nella prima fase di lattazione. Nota II: Effetti sulla composizione acidica del grasso del latte. Atti SISVet, 45: 1791-1795.

Council Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk based products.

Di Bernardini, R. 2004. Effetto del numero di cellule somatiche sulla produzione quali-

quantitativa del latte di bufala. Tesi di laurea.

Di Francia, A., Masucci, F., Maresca di Serracapriola, M.T., Gioffré, F. and Proto, V. 2003. Nutritional factors influencing milk urea in buffaloes. J. Anim. Sci., vol 2 (suppl. 1): 225-227.

Di Palo, R. 1992. Produzione lattea nella bufala con diete tradizionali e con l'impiego di acidi grassi. Ph.D Thesis, University of Naples, Italy.

Di Palo, R., De Filippo, C., Principalli, D., Campanile, G. and Zicarelli, L. 1997. Effect of dietary supplementation with cryo-cristallized fatty acids on milk production and metabolic profile in buffalo cow. Proc. of the Fifth World Buffalo Congress, Caserta, Italy, October: 372-377.

Di Palo, R. 2002. Modern technologies applied to buffalo farming for milk production. 53rd Annual Meeting of the European Association for Animal Production, 1-4 September, Cairo, Egypt.

D.P.R. N. 54 14/1/1997 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products.

Esposito, L., Di Palo, R., De Barros Pinto, H.M., Ricci, G. and Zicarelli, L. 1997. Variations in lactodynamometric characteristics of Mediterranean buffalo milk from individual animals. Proceedings of the Fifth World Buffalo Congress, Caserta, Italy, Oct. 13-16: 225-230.

Galiero, G., Lai, O., Fenizia, D., Palladino, M. e Cuoco, E. 1996. Stato sanitario delle aziende bufaline nella provincia di Salerno: indagini chimiche e batteriologiche sul latte destinato alla trasformazione. Veterinaria Italiana, 20: 29 34.

Grummer, R.P. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci., 74: 3244-3257.

Journet, M. and Chilliard, Y. 1985. Influence de l'alimentation sur la composition du lait. 1. Taux butireux: facteurs généraux. Bull. tech. CRZV, Theix, INRA, 60: 13-23.

Laurent, F., Coomans, D., Gardeur, J.N. et Viognon, B. 1992. Composition azoté et characteristiques technologiques du lait de vache en relation avec le nature et le niveau d'apport de l'aliment concentré. Le Lait, 72 175-183.

Macheboeuf, D., Coulon, J.B. and D'hour, P. 1993. Effect of breed, protein genetic variants and feeding on cows' milk coagulation properties. J. Dairy Res., 60: 43 54.

Masoero, F., Cabibbu, A., Fiorentini, L., Meschini, M. e Piva, G. 1994. Effetto di diete a diverso contenuto energetico sulla digeribilità intestinale di proteine alimentari nel bufalo. Agricoltura ricerca, 153: 107-122.

Pasquini, M., Tommei, B. and Mattii, S. 2003. Buffalo milk: proteins electrophoretic profile and somatic cell count. Ital. J. Anim. Sci., vol 2 (suppl. 1): 299-301.

Polidori, F., Sgoifo Rossi, C.A., Senatore, E.M., Savoini, G. and Dell'Orto, V. 1997. Effect of recombinant bovine somatropin and calcium salts of long-chain fatty acids on milk from Italian buffaloes. J. Dairy Sci., 80: 2137-2142.

Politis, I. and Ng-Kwai-Hang, K.F. 1988a. Effects of somatic cell count and milk composition on cheese composition and cheese making efficiency. J. Dairy Sci., 71: 1711-1719.

Politis, I. and Ng-Kwai-Hang, K.F. 1988b. Association between somatic cell count of milk and cheese yielding capacity. J. Dairy Sci., 71: 1720-1727.

Politis, I. and Ng-Kwai-Hang, K.F. 1988c. Effects of somatic cell counts and milk composition on the coagulating properties of milk. J. Dairy Sci., 71: 1740-1746.

Remond, B. 1985. Influence de l'alimentation sur la composition du lait de vache. 2. Taux protéique: facteurs généraux. Bull. Tech. CRVZ, Theix INRA, 62: 53-67.

Sarubbi, F., Di Lella, T., Galiero, G., Infascelli, F., Bovera, F. and Durante, G. 2000. Feeding diets varying in crude protein concentration and ruminal degradability to lactating Mediterranean buffalo cows. Zoot. Nutr. Anim., 26: 181-188.

Silva, I.D. and Silva, K.F.S.T. 1994. Total and differential cell counts in buffalo (Bubalus bubalis) milk. Buffalo J., 2: 133-137.

Tripaldi, C., Catillo, G., Martillotti, F. and Angelucci, M. 1997. Influence of some characteristics of diet on the milk quality of water buffalo. Buffalo J., 1: 1-13.

Tripaldi, C., Terramoccia, S., Bartocci, S., Angelucci, M. and Danese, V. 2003. Effect of the somatic cell count on yield, composition and coagulating properties of Mediterranean buffalo milk. Asian-Australasian J. Animal Sci., 16: 738-742.

Verna, M., Bartocci, S., Amici, A. e Agostini, M. 1994. Effetto di diete diverse sulle prestazioni produttive di bufale in lattazione. Agricoltura ricerca, 153: 73-78.

Vertes, C., Hoden, A. et Gallard, Y. 1989. Effet du niveau d'alimentation sur la composition chimique et la qualité fromagère du lait de vaches Holstein et Normandes. Prod. Anim., 2 (2) : 89-69.

Zicarelli, L. 2001a. La bufala Mediterranea Italiana: esempio di una razza autoctona in espansione. Sci.Tecn. Latt.-Cas., 52: 279-284.

Zicarelli, L. 2001b. Alimentazione della bufala da latte. Facoltà di Medicina Veterinaria, Dipartimento di Scienze Zootecniche e Ispezione degli Alimenti. Università degli Studi di Napoli Federico II.

Chapter X

BUFFALO CHEESE AND MILK INDUSTRY

Antonio Borghese

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

The buffalo products market is increasing in the same countries where buffalo numbers are increasing since both of these factors are linked to consumer demand. In Italy, in particular, the price of buffalo milk is much higher (€1.20/kg) than that for bovine milk (€0.30). Moreover mozzarella cheese consumption is increasing in Italy and in the world: 14 percent of the Italian production is exported to Germany, France, UK, Switzerland, USA and Japan.

This increase in demand is due to several factors: the D.O.P. (Denomination of Protected Origin) "Mozzarella di Bufala Campana" registered in the E.U., the high quality of mozzarella (Fig.1), very soft and tasty, rich in milk and flavours, and the spread of Italian cooking style using mozzarella in pizza, caprese and other dishes.

Figure 1. Mozzarella di Bufala Campana cheese
(Bubalus bubalis photo, 1999)

The market limits are linked with the organization of the cheese industry and with distribution, therefore many farmers or cooperatives manage and process their production in order to achieve additional and alternative profits, to be sure to sell the milk and also for producing different cheeses, not only mozzarella, which is limited by a very short life span, but other products, such as treccia, ricotta, crescenza, robiola, caciocavallo, butter and yoghurt.

One of the main marketing problems is due to the diversity between typical mozzarella, which is produced by small-scale industries, with natural yeasts and microbes and with a shelf-life of only three to five days, not preserved in the refrigerator, and large-scale industry and distribution that must produce long shelf-life mozzarella (30 days) for supermarkets and export, preserved in the refrigerator, but without live yeasts and microbes, and less soft and

juicy. Both these products have the same denomination and D.O.P. but are very different; both are useful for the market but can produce confusion for the consumer.

In the other European and Near East countries, no typical cheese exists which demands a good price on the global market: this is a limiting factor for the economic expansion of the animal network for the diffusion of quality products and for the technical development of buffalo livestock. In Bulgaria, Romania and Albania buffalo milk is almost completely processed into yoghurt which is the most requested product on local markets. Other countries produce butter and creams from the fat and following processing they also drink the skimmed milk: in Egypt a cream, called Queshta Mosakhana is the floating cream removed after boiling milk; Gaymar in Iraq is obtained both from spontaneous floating and from spinning; Quishada in Syria is obtained from raw or boiled milk and is sometimes heated to make it more concentrated. Ghee is obtained by boiling butter and is much appreciated in Egypt and Azerbaijian.

Many farms undertake their own processing of cheese and cream and sell the products directly.

Classifying the types of cheese according to water content (Borghese and Moioli, 2002), the following are typical products:

1. Soft cheese (water content > 45 percent): Karish, Mish and Domiati in Egypt; Madhfor in Iraq; Mozzarella in Italy; Alghab in Syria; Vladeasa in Romania.
2. Semi-hard cheese (water content 40-45 percent): Beyaz peyneri in Turkey.
3. Hard cheese (water content < 40 percent): Braila in Romania; Rahss in Egypt; White brine in Bulgaria; Akkawi in Syria.

Table 1 (Borghese et al., 2000) summarizes the cheeses of the Mediterranean area by dividing them according to their origin and the different stages of their technology and types of classification.

The most common classification of cheese is made according to the type of coagulation: either enzymatic coagulation (due to the rennet), or acid coagulation (after natural acidification or due to lactic bacteria). Many cheeses undergo a mixed coagulation (both acid and enzymatic) though in some of them the acid coagulation prevails, while in others the enzymatic prevails. Most of the cheeses produced in the Mediterranean area belong to the acid-enzymatic category, meaning that the acid coagulation prevails, which can be envisaged since the milk is left to acidify before adding rennet and because the coagulation times are long. Looking at Table 1, it is evident that the acid coagulation prevails in all cheeses. A general consideration should now be mentioned. Since technologies are the result of many factors and must fit with the overall conditions of each environment. Each technology is affected by climate, by the availability of animal or vegetable rennet and by the characteristics of the raw milk, which is also affected by climate and the sanitary conditions of the herds. The Italian buffalo cheese called Mozzarella originated from southern Italy, where temperatures are high and buffalo rearing systems were mainly on pastures; therefore the technology related to this cheese had to be adapted to a kind of milk that, when reaching the dairy plant, was already acidified. The same has probably occurred for most Mediterranean cheeses.

In some cheeses, milk undergoes only a spontaneous acidification (Domiati, Karish, Mish, Madhfor, Alghab). In other cheeses, acidification is encouraged by adding starters, i.e. lactic bacteria cultures (Vladeasa, Beyaz peyneri) or natural whey cultures (Mozzarella). Starters are also used in cheeses where enzymatic coagulation prevails, in order to favour rennet activity and following the processing stages (White brine cheese, Fresh cheese of Iraq, Braila).

Table 1 also includes other important aspects regarding the treatment of milk before processing and characteristics of the technology.

Pasteurization, i.e. heating treatment of milk with the purpose of killing pathogenic germs and reducing microflora, that can damage and result in losses in the final product, is only

performed in a few instances (White brine cheese, Domiati, Braila, Vladeasa, Beyaz peyneri).

Some cheeses are consumed fresh, i.e. only a few days after processing (Karish, Fresh cheese of Iraq, Mozzarella, Ricotta, Alghab), others are ripened and consumed even after several months. In this case, Mediterranean countries adopted the very wise practice of preserving cheese in brine, in order to guarantee excellent conservation without expensive investments, such as refrigerators. In fact, the ripening and preserving of cheese without refrigerators in hot climates, could not only damage cheese, but also be risky for the health of the consumer and might cause considerable losses of products. In one cheese (Mish) it was observed that acid buttermilk, skimmed milk and whey are added to the brine.

In Table 1 a few technology peculiarities are made evident. In Domiati and Akkawi cheese, salt is added to the milk before processing. This practice is very common in Egypt and Syria, deriving from the need to add bacteriostatics to milk, in order to limit spontaneous microflora during processing. In two cheeses (Madhfor, in Iraq, and Mozzarella, in Italy) after the curd has been cut into pieces, it is left to acidify. Acidification is strictly dependent on the temperature, because it is caused by lactic bacteria which grow best at high temperatures. After acidification, in the case of Mozzarella, the curd which has reached a pH of 4.8 4.9, is stretched and then it is moulded. The stretching phase is typical of Mozzarella: no other buffalo cheese is produced in this way. The stretching phase includes several actions: hot water is poured on the curd while the curd is kneaded and stretched. Then whey is removed and further hot water is added several times, while the kneading and stretching continue. This technology phase is the crucial step in the making of Mozzarella and produces the typical stretched texture of the cheese. Stretching can be done either manually (Fig. 2) or mechanically; however, even in the bigger dairy plants, manual stretching is preferred because it improves both the quality and yield of the product. The stretching phase is also important because the hot water together with the curd acidity help to improve the sanitary conditions of the product. The subsequent phase, the moulding in pieces of various weight (from 15 g to 500 g) and shapes (egg-shaped or braided) can also be performed either manually or mechanically (Fig. 3). During the stretching phase, either salt can be added or the moulded pieces can be left in brine at 10-18 percent NaCl for a short time (from a few minutes to a few hours). Mozzarella is then preserved for a few days in acidified brine (2-3 percent NaCl).

Figure 2. Mozzarella manual stretching (Borghese photo, 2003)

Figure 3. Mozzarella mechanical moulding (Borghese photo, 2003)

Interesting by-products from dairy plants are Ricotta (produced only in Italy, where the production regulation was approved in order to obtain the DOP, Fig. 4) and similar products in Syria and Egypt (Alkarish). They are made from the whey after the processing of cheese. It is surprising that these by-products, which exploit the proteins lost in the whey (very rich in sulphurate amino-acids), are not produced in other countries. It is possible to speculate that either the residual whey is too acid to let whey proteins precipitate or that, being too fresh and with low acidity, it is subject to alterations.

In Turkey a drink with water and yoghurt (AYRAN) is widely consumed. Creams also show a variability in production; in Egypt, Queshta Mosakhana is the floating cream removed after boiling milk. Gaymar in Iraq is obtained both from spontaneous floating or from spinning, in this case it is then pasteurized.
In Italy, cream from buffalo milk is obtained after spinning; then it is pasteurized. After thermo-acid coagulation (citric acid) of cream, another dairy product is obtained which is called Mascarpone.
Quishada (a product of Syria) is obtained from raw or boiled milk; sometimes, this cream is heated to make it more concentrated.

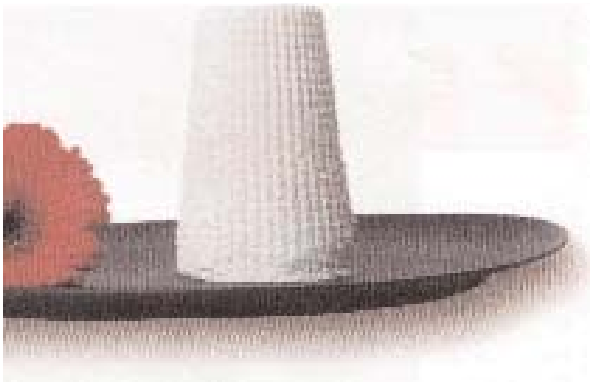


Figure 4. Italian ricotta (Bubalus bubalis, 2004)

Figure 5. Azerbaijan ghee and soft cheese (Borghese photo, 2003)

Industrial butter is produced by churning cream, often after pasteurization. The home-made product is obtained simply by churning acidified milk. The peculiarity of buffalo butter is its colour, which is much whiter than cow butter, due to the lack of carotenoids.
Ghee is obtained by boiling butter. It is very popular in Egypt and valued by the baking industry. In Azerbaijan, Ghee is the only product obtained from buffalo milk fat. Some very simple soft cheeses are also produced in Azerbaijan (Fig.5).

It is evident that the dairy products of the Mediterranean area need to be studied more closely: the variability of their technologies is in fact an important part of global biodiversity.

Among all Mediterranean cheeses, the Italian Mozzarella is the cheese which registers the highest quantity of production and forecasts predict that production is going to increase. At present the actual production is about 38 million kg/year. The success of this cheese is correlated on the one hand to the consumer appreciation of this kind of cheese which includes three types: the whole buffalo milk Mozzarella, the mixed (buffalo-cow), and the whole cow milk Mozzarella and, on the other hand, to the evolution of buffalo farming. In fact, the increasing demand for buffalo milk together with the excessive production of cow milk all over Europe, have encouraged buffalo farmers to increase buffalo milk production through the improvement of management and feeding conditions, as well as through milk recording and the selection of best breeding animals. In fact, in Italy, 27.8 percent of buffaloes (ANASB, 2003) are submitted to official milk recording and genealogy registration. The average milk production of milk recorded buffaloes during standard lactation is over 2 100 kg milk and a good number exceed 5 000 kg. It is only recently that the buffalo sector has started to develop, and relations between buffalo farming, milk processing and scientific expertise are very intense.

Over the past few years, the consumption and market demand for fresh cheeses such as Mozzarella has increased considerably. In order to distinguish and protect the Mozzarella made from buffalo milk, buffalo milk producers have created an association that has drafted official regulations for the production of Buffalo Mozzarella cheese, and has succeeded in obtaining the formal approval of the Italian Government with two laws in 1979 and 1993. Regulations now impose the following requirement in order to utilize the name "Mozzarella di Bufala Campana"

D.O.P. (Denomination of Protected Origin): "Only raw, whole buffalo milk must be used, the origin of the milk must be from areas where buffaloes have been raised for centuries, and the particular processing that has been performed for centuries in these areas must be followed". This regulation was approved in Brussels for all countries in the European Union. Therefore cheeses made from bovine milk or mixed milk cannot be called Mozzarella D.O.P. The yield of buffalo mozzarella is 24 percent in comparison to the 13 percent of the bovine variety and the buffalo mozzarella is richer in fat and proteins.

Buffalo Mozzarella is different from other types of Mozzarella because of its typical texture and juicy consistency, apart from its special taste. Outside the original area of production (Naples and nearby provinces) Buffalo Mozzarella is considered a top quality product, to be consumed on special occasions. Thanks to the official stamp (green and red), printed on the wrapping paper, the buyer can immediately differentiate Buffalo Mozzarella from other similar cheeses in the shop. Furthermore, the existence of approved regulations in addition to the special stamp allows the detection and repression of fraud.

The efforts of buffalo milk processing plants have led to the expansion of demand for this cheese on international markets. Mozzarella is a component of a typical Italian food, the Pizza, which is known and appreciated all over the world and the success of Mozzarella was boosted by the increase of Pizza consumption everywhere.

The development of the buffalo sector in Italy has contributed to the creation of new job opportunities both at the farm and at the dairy processing level. The development of buffalo in an area of Italy, where unemployment was high due to it being less industrialized, has favoured, in particular, the employment of young people who in Italy were not consistently present in agriculture (Borghese, 2003). Research in dairy technology has demonstrated that buffalo milk is suitable for processing into various dairy products which could be exported all over the world. In Latin America over the last few years the number of farms that supply milk to produce "Caso Blanco", mature cheese and Mozzarella, besides producing buffaloes for slaughter, has steadily increased, even if the magnitude of increase is hard to estimate: Amazonia, where the species is continuously reproductive, is the area most suited to Mozzarella production (Zicarelli, 2001). In Asia, where 96 percent of total world buffalo milk is produced, the cheese industry is also developing to satisfy food demands particularly those for human protein requirements. Dairy technology is expanding substantially in India, where, following the "White revolution" milk production is the highest in the world (about 134 million tons), mostly due to buffalo farming: 55 percent of total milk produced in the country and 65 percent of global buffalo milk. The chemical superiority of buffalo milk over that of other species makes it preferable for processing as fluid milk and for use in the manufacture of several Indian and western dairy products. Generally speaking, buffalo milk is more suitable for the manufacture of the following dairy products (Patil and Nayak, 2003).

Concentrated milk

Buffalo milk is as stable to heat as cow milk in its concentrated form; kheer is an indigenous cereal-based concentrated milk product mainly prepared from buffalo milk for immediate consumption.

Fat-rich milk products

Buffalo cream churns much faster at higher fat levels and gives higher overrun than cow cream. Due to the bigger size of globules and higher proportion of solid fat in buffalo milk, the separation of the cream and the churning of the cream is easier and the loss of fat in skimmed milk and buttermilk is far less. Buffalo milk produces butter with a significantly higher yield due to its higher fat content compared to cow milk. Further, in keeping quality tests, butter from buffalo cream displayed more stability than that from cow cream, due to the more solid fat and slower rate of fat hydrolysis in the former cream. This might explain why during storage, cow milk fat is more vulnerable to hydrolytic rancidity. The texture of buffalo ghee is better than cow ghee due to its bigger grain size, which, in turn, may be due to a higher proportion

(9-12 percent) of high melting triglycerides compared to only about 5 percent in cow milk fat (Patil and Nayak, 2003).

Heat-desiccated milk products

Buffalo milk is preferred in India for the manufacture of heat-concentrated milk products like khoa, rabri, kheer and basundi. Evidence has revealed that buffalo milk always results in high yields and a superior quality of condensed milk products compared to cow milk. Khoa (a heat-desiccated indigenous milk product), a product of great commercial importance due to its use as a base for the preparation of a variety of indigenous milk sweets such as burfi, peda, milk cake, gulabjamun, etc. throughout the country. Since buffalo milk gives greater yield and has a more desirable softer body and smooth texture because of the presence of a proportionately higher fat content, the quality of khoa made from buffalo milk is superior to that made from cow milk as the product has a moist surface and a sticky and sandy texture (Reddy, 1985). Ramamurthy (1976) claimed that the higher emulsifying capacity of buffalo milk fat is due to the presence of higher proportions of butyric acid (50 percent) containing triglycerides compared to only 37 percent in cow milk fat, a factor responsible for the smooth and mellow texture of buffalo milk khoa. In addition, the standards for khoa prescribed under the Prevention of Food Adulteration (PFA) rules in India, is heavily slanted towards the use of buffalo milk. Moulick et al. (1996) reported that, in terms of chemical, microbiological and sensory attributes, the overall quality of kalakand was superior from buffalo milk to that from cow milk.

Heat-acid coagulated milk products

The quality of buffalo milk paneer (an acid coagulated milk product) is superior to that of cow milk paneer. The cow milk paneer is too soft, weak and fragile and after cooking its pieces loose their identity (Sachdeva et al., 1985). The low proportion of solid fat, the smaller size of casein micelles and fat globules, and the lower colloidal calcium could be the reason for the inferior quality of paneer from cow milk. Indian paneer is produced through acidifying milk by adding acidified curd or citric acid or lemon juices; following this it is boiled for a few minutes and coagulation is obtained; the curd is filtered and pressed without salt. Paneer (Fig. 6) must be consumed within three days, preferably mixed with spicy sauces made from various vegetables and spices: pepper, chilly, ginger, cumin, garlic, tamarind, Greek hay etc. Indian people normally do not use cutlery to consume paneer and eat it with a special flattened, round and low leavened bread, called "ciabatti". An addition of 0.3 percent sodium citrate to buffalo milk was found to be effective in producing chhana similar to chhana from cow milk in terms of springiness and quality of Rasogolla prepared from the same (Rao, 1986).

Fermented milk products

The superior body and texture of buffalo milk dahi could be attributed to the higher total solids content, especially fat and protein, the casein micelles and the large fat globules and higher calcium content in the colloidal state (Sindhu and Singhal, 1988). Ghosh (1986) reported that misti dahi made from buffalo milk is popular in the Eastern belt of India. Buffalo milk is also appropriate for making yoghurt with improved body and texture, because of its higher total solids content (13-17 percent) as compared to cow milk. In addition, when buffalo milk is used it requires no prior concentration or addition of milk powder to obtain optimum body. It is reported that the growth of yoghurt starter culture is faster in buffalo milk and produces more acetaldehyde, a key flavour component, than in cow milk and thus has a high organoleptic quality in the final product (Singh and Kaul, 1982). Chakka, a base material of Shrikhand, is preferentially prepared from buffalo milk since the curd obtained from cow milk is soft, weak and of low curd tension but the curd from buffalo milk is hard, smooth and mellow. The yield of Shrikhand from buffalo milk is about 15-20 percent higher than that from cow milk. Shrikhand and chakka made from buffalo milk are extremely nutritious and are popular among the Indian population (Patil and Nayak, 2003).

Frozen milk products

In comparison to cow milk, ingredients from buffalo milk viz. skim milk powder and whey solids, produce better body and texture in ice cream (Patel and Mathur, 1982). The higher

protein content in buffalo milk may help to make ice cream more compact and smooth and has a tendency to prevent a weak body and coarse texture. Hence, use of buffalo milk solids in ice cream may improve sensory appeal especially in vanilla ice cream where no colouring is added.

Dehydrated milk products

Buffalo milk and cream are intrinsically whiter and more viscous. Hence, buffalo milk is more appropriate for the production of tea and coffee whitener powders. The whey proteins of buffalo milk are more resistant to heat denaturation compared to the whey proteins of cow milk and thus dried buffalo milk may be preferred to dried cow milk for those technological applications where higher levels of undenatured whey proteins are more desirable.

Figure 6. Indian Paneer with spices (Borghese photo, 2003)

Figure 7. Indian soft cheese (Borghese photo, 2003)

Figure 8. Pastillas de leche and other Philippine milk products (Barile photo, 2004)

Figure 9. Italian mozzarella and ricotta (Borghese photo, 2003)

Cheeses

In India there are many simple buffalo soft cheeses (Fig. 7) obtained from direct acid coagulation, without adding salt, that must be consumed fresh within three days of their preparation. No old tradition of cheese making exists in India, either with regard to technologies or to ripening techniques and hence cheese consumption is also a relatively new habit.

In the Philippines the Nueva Ecija Federation of Dairy Carabao Cooperatives (NEFDCCO) has been established, this is a federation which collects milk, and processes and sells the various products: raw milk, pasteurized fresh milk, aromatized milk, ice creams, pastillas de leche (milk pastilles), kesong puti and paneer cheeses (Fig.8).

Table 1. Dairy products in European and Near East Countries (Borghese et al., 2000)

Type of cheese	Country of origin	Employed milk	Type of production	Pasteurization	Acidification of milk	Type of coagulation	Pressing	Technology peculiarity	Ripening	Water content
White brine cheese	Bulgaria	Buffalo	Industrial	yes	Through starters	Enzymatic-acid	3-4 hours		In brine at 22-23% NaCl, for 35-40 days	Hard cheese
Domiati	Egypt	Buffalo or cow+buffalo	Industrial	yes	Light acidification before coagulation	Acid-enzymatic	yes	Salt is added to milk (6-14%)	In brine for 9 months	Soft cheese
Karish	Egypt	Buffalo + skimmed cow milk or buttermilk after acidification of cream			Natural acidification for 1-3 days	Acid				Soft cheese
Mish cheese	Egypt		Home-made		Natural acidification for 1-3 days	Acid		Preserved in acid brine either with buttermilk, acid skimmed milk, or whey	In acid brine	Soft cheese
Rahss	Egypt	Buffalo or cow+buffalo	Industrial			Acid-enzymatic	yes		2-3 months at 12-18 °C	Hard cheese
Fresh cheese	Iraq	Skimmed buffalo milk			Through starters	Enzymatic	yes			Soft cheese
Madhfoor or Dhafayer	Iraq				Light acidification before coagulation	Acid-enzymatic	yes	Acidification of curd till pH 5.2	In brine at 10% NaCl, for 2 months	Soft cheese

Type of cheese	Country of origin	Employed milk	Type of production	Pasteurization	Acidification of milk	Type of coagulation	Pressing	Technology peculiarity	Ripening	Water content
Mozzarella	Italy	Buffalo	Home-made and industrial		Through addition of whey from the processing of the previous day. Whey undergoes a natural acidification at room temperature.	Acid-enzymatic		Acidification of curd till pH 4.8-4.9 and stretching in hot water	Preserved only a few days in its whey + 2-3% NaCl, lightly acidified	Soft cheese
Ricotta	Italy	Whey from mozzarella processing	Home-made and industrial			Thermo-acid		Protein precipitation of whey at 85-90 °C	4-6 °C for a few days	Over 60% water content
Braila cheese	Romania	Buffalo		yes	Through starters	Enzymatic	yes		In brine at 10-12% NaCl, for 1 month	Hard cheese
Vladeasa, Bucedis, Home made cheese	Romania	Buffalo or cow+buffalo		yes	Through starters	Acid-enzymatic		There are two types: high and low fat content	At 5-10 °C for 3 weeks	Soft cheese

Type of cheese	Country of origin	Employed milk	Type of production	Pasteurization	Acidification of milk	Type of coagulation	Pressing	Technology peculiarity	Ripening	Water content
Alghab/Hama cheese	Syria	Buffalo or cow+buffalo			Natural acidification for 3-4 hours	Acid-enzymatic	yes	Coagulation for 3-4 hours		Soft cheese
Akkawi	Syria	Buffalo or cow+buffalo						Salt is added to milk (10-12%)	In brine	Hard cheese
Al Karish	Syria - Egypt	From whey after Alghab processing				Thermo-acid		Protein precipitation of whey through boiling		By product with higher water content
Beyaz peyneri	Turkey	Buffalo or buffalo+ sheep/goat		yes	Through starters	Acid-enzymatic		Coagulation for 1.5-2.5 hours	In brine at 12-14% NaCl, for 4-6 months	Semi-hard cheese

References:

ANASB. 2003. Statistical data.

Borghese, A., Moioli, B. and Tripaldi, C. 2000. Buffalo milk: processing and product development in mediterranean countries. Proc. Third Asian Buffalo Congress, Kandy, Sri Lanka, March 27-31: 37-46.

Borghese, A. and Moioli, B. 2002. Buffalo husbandry/Mediterranean Region. In Encyclopaedia of Dairy Science. Elsevier Science Ltd.: 193-197.

Borghese, A. 2003. Buffalo production systems in Europe and the Near East. Proc. Fourth Asian Buffalo Congress, New Delhi, India, Feb. 25-28: 13-21.

Ghosh, J. 1986. Production, packaging and preservation of misti dahi. M. Sc. Dissertation, Kurukshetra University, Kurukshetra.

Moulick, S., Ghatak, P.K. and Bandyopadhyay, A.K. 1996. A comparative study on the quality of market and laboratory-made kalakand. lndian J. Dairy Sci., 49: 406.

Patel, J.N. and Mathur, B.N. 1982. Production of hydrolysed lactose whey for utilization in ice cream manufacture. Indian J. Dairy Sci., 35: 228.

Patil, G.R. and Nayak, S.K. 2003. Competitive advantages of using buffalo product manufacture. Proc. Fourth Asian Buffalo Congress, New Delhi, India, Feb. 25-28: 183-190.

Ramamurthy, M.K. 1976. Technological problems encountered with buffalo milk fat in the manufacture of milk products. Indian Dairyman, 27: 415.

Rao, M.S. 1986. Studies on the preparation of chhana from treated buffalo milk and its suitability for rasogolla-making. M. Sc. Dissertation, Andhra Pradesh Agricultural University, India.

Reddy, C.R. 1985. Process modifications for production of khoa-based sweets. Ph. D. Thesis, Kurukshetra University, Kurukshetra.

Sachdeva, S., Singh, S. and Kanawjia, S.K. 1985. Recent developments in paneer technology. Indian Dairyman, 37: 501.

Sindhu, J.S. and Singhal, O.P. 1988. Qualitative aspects of buffalo milk constituents for product technology. Buffalo production and Health: A compendium of latest research information based on Indian studies. Proc. Fourth Asian Buffalo Congress, New Delhi, India, Feb. 25-28: 263.

Singh, J. and Kaul, Y. 1982. Activity of yoghurt starter in different types of milk. Milchwissen., 37: 731.

Zicarelli, L. 2001. Buffalo milk production worldwide. Proc. Sixth World Buffalo Congress, Maracaibo, Zulia, Venezuela, May 20-23: 202-230.

Chapter XI

BUFFALO MEAT AND MEAT INDUSTRY

Antonio Borghese

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

In the 1990s the average consumption of meat was 12 kg/ head per year for sub-Saharan Africa, 18 kg/head per year for Asia and 45 kg/head per year for Latin America (FAO, 1998) compared to an average of 76 kg/head per year in developed countries. Although a number of factors affect the long-term estimates for per capita demand for livestock products, the scenario predicted for changes in consumption patterns based on economic development has been considered (Bouwman, 1997) and the per capita demand (kg/year) for all the developing countries will increase from 17 kg in 1989/91 to 25 kg in 2010 and to 30 kg in 2025. It is considered that buffalo meat has a strong potential for meeting this requirement for increased per capita consumption. (Kondaiah and Anjaneyulu, 2003).

The production of buffalo meat has high growth possibilities and poses a minimal level of risk from pesticides and veterinary drugs when compared to beef production in developed countries. Buffalo meat is produced primarily in Asia. The contribution of buffalo meat to world total meat production is only 1.3 percent. India produces 1.43 million tonnes of buffalo meat annually and accounts for 36 percent of total meat production contributing significantly to human nutrition. As the meat produced is mainly from spent animals, it is coarse and fibrous. The demand for buffalo meat is high as it is relatively lean with a fat content below 2 percent and it is free from Mad Cow Disease as the animals are only fed grass and farm by-products. The functional proprieties of buffalo meat for product processing could be improved by increasing its popularity on the Indian market. For these reasons the future potential for buffalo meat and meat products is promising for India both on the domestic and international markets (Murty and Prince Devadason, 2003).

The quality and quantity of buffalo meat depend on many factors, the most important of which are the water buffalo type and breed, age, feeding intensity, management system and environmental conditions.

Generally, cattle are superior to buffaloes in their growth rate and there are also differences between the two water buffalo subspecies: River and Swamp.

The possibility of producing buffalo meat in Italy was initially studied by Bartolo Maymone (Maymone, 1945), the first Director of the Istituto Sperimentale per la Zootecnia (Animal Production Research Institute), and later by Beniamino Ferrara at the Veterinary University in Naples (Ferrara et al., 1964).

Studies relating to early weaning and growth rate in water buffalo calves were reported (De Franciscis and Zicarelli, 1974) during the First International Congress on Buffalo Livestock held in Caserta in 1974. The authors found a 154-163 kg live weight at 180 days of age with daily gains between 639 and 707 g without significant differences at different feed levels, with about 2.5-3.0 FU/kg live weight as conversion ratio. However, many animals presented myodistrophic disease and died or did not show acceptable performances. The first problem which was tackled in Italy was the question of early weaning in order to save buffalo milk for the market (the actual price is €1.20/litre) and spend less on milk replacers.

Many studies were carried out on calf early weaning and it was found that calf adaptation to

artificial feeding was easier if the calf never suckled the mother's milk. It was also observed that feeding and health control in individual boxes during the first month of life can reduce calf mortality.

But the main problem affecting the success of early weaning was the chemical composition of milk powders produced at that time for the calve bovine market, which, when reconstituted, had a chemical composition similar to cattle milk: crude protein 19 percent and fat 14 percent on dry matter. So Romita and Dias da Silva (1975) developed a milk powder with 21 percent crude protein and 25 percent fat on DM and obtained a daily weight gain of 919 g in males and 933 g in females up to 220 kg live weight and 59.72 percent and 58.60 percent as dressing percentage respectively. Many studies were carried out in order to ascertain the effects of different milk powders, different concentrations, varying ages at weaning and integrations with pre-starter concentrates or lactobacillus in comparison to bovine or buffalo milk (Palladino et al.,1993, Di Lella et al., 1998, Tripaldi et al., 2001, Roncoroni et al., 2001). Presently, acidified milk, with 25 percent crude protein and 23 percent fat on D.M., with probiotics and without copper because of its toxicity in buffaloes (Zicarelli et al.,1981) are used at normal climatic conditions, without causing problems of preparation (16-18 percent concentration) or mortality in calves that can be weaned within three months of age, with 700-800 g daily gains and with about 100 kg live weight.

Giovanni De Franciscis organized the Second International Congress on Buffalo Livestock at the Royal Palace in Caserta in 1982, where Ferrara (Ferrara et al., 1982) reported on the first review of the results of the different trials to produce buffalo calves and young bulls for meat production in Egypt, Romania, Yugoslavia, India, Indonesia and Italy. These results revealed a high daily gain (904 g) for a low slaughter weight (320 kg), and a low daily gain (200 g) for a high slaughter weight (683 kg); and the dressing percentage was increased with increasing live weight changing from 52.7 to 60 percent (Di Lella et al., 1975). These authors suggested that it was not economical to produce animals heavier than 450 kg because of the reduction in growth and efficiency in both males and castrated animals, and particularly because of the organoleptic characteristics of the meat, which smelt badly of musk. In Italy many trials were carried out by the Istituto Sperimentale per la Zootecnia on water buffaloes and Friesian bovine males, under the same feeding and environmental conditions, and at different slaughter ages in order to compare the meat performance and these were also referred to at the Second International Congress on Buffalo Livestock. Six trials were carried out on 116 males: 58 Mediterranean Italian buffaloes and 58 Friesian bovines which were fed milk substitutes and concentrates and slaughtered at 20, 28 and 36 weeks, or fed milk substitutes, hay and concentrates and slaughtered at 36, 52 and 64 weeks of age. The Friesians showed better performances than the buffaloes, the latter realized the following daily gains at 20, 28, 36(1), 36(2), 52, and 64 weeks of age: 795, 807, 746, 963, 930, 949 g/d (Romita et al., 1982; Table 1), and the following respective net dressing (the net dressing is about 4.5 percent more than the normal dressing because it is the ratio carcass/net live weight, without stomach and intestine contents) percentages: 59.6, 58.2, 57.6, 55.3, 57.0, 56.6 percent (Romita et al., 1982, Table 2). These trials proved that buffaloes could be bred until 15 months of age following an early weaning with good performances and a similar body growth trend in comparison to Friesians. The head and skin of the buffaloes were always heavier than that of the bovines, which showed higher intestine length and circumference; the carcasses were heavier in the bovines at all ages, but buffaloes showed a significantly higher percentage of meat on the carcasses at 20, 28, and 36(1) weeks of age. However at later ages these differences disappeared with the fattening increasing in buffaloes. In particular, buffaloes showed significantly more subcutaneous fat and less intermuscular and intramuscular fat at 36(2), 52, and 64 weeks of age, so the meat percentage on the carcass transpired as similar (62-64 percent) in both species (Gigli et al.,1982, Table 3).

As in the previous trials the buffaloes were fed hay and concentrates. In the 1990s the buffalo fattening was effected by using maize silage because of the lower cost/UFC (Meat Feed Unit). Gigli et al., (1994) fed 24 male Mediterranean Italian buffaloes with maize silage ad libitum,

0.9 kg/d soya bean meal 50 percent CP, 0.1 kg/d vitamin mineral nucleus (group A) in comparison to 24 animals fed the same diet (group B) with 50 percent maize silage of the quantity fed to group A. Eight animals from each group were slaughtered at 10, 14 and 18 months respectively: the buffaloes fed ad libitum realized a similar growth (Table 4) to the previous trials with about 970 g/d gain up until 14 months, reduced to 798 g/d at 18 months; the lower feeding level strongly affected the animal growth rate; the conversion ratio (feed intake/gain) increased with increasing age as did the net dressing percentage with values of more than 60 percent. Conformation and fatness scores also increased with the age of the animal as did the subcutaneous and intermuscular fat percentage on the carcass (Table 5), and consequently the meat percentage on the carcass decreased. The carcasses of the animals of group B registered lower fat and more meat than the group A buffaloes. Di Lella et al., (1998) using three different isoenergetic-isoproteic diets, obtained significantly higher daily gains (977 g) with 50 percent maize silage, 15 percent alfalfa hay and 35 percent concentrates on DM, 0.84 UFC/kg DM, 14.2 percent CP, between 6 and 12 months, in comparison with a diet with 25 percent corn silage, 50 percent concentrates and 25 percent NH3 treated straw. In the period between 360 and 480 days with 0.80 UFC/kg DM, 12 percent CP again the best daily gain (887 g) was obtained in the first group in comparison with the second one (818 g).

Silva et al., (1997), in a study undertaken at the Instituto Agronomico do Paraná (IAPAR) (Pinhais/PR), involving 18 Murrah animals with isoproteic and isocaloric feeds, in three proportions of volume: concentrate (75:25, 65:35 or 55:45) achieved DWG (Daily Weight Gain) of 1.23, 1.23 and 1.21 kg/day, respectively. Restle et al., (1990) established DWG of 1.032 and 1.345 kg/day in buffaloes and cattle, respectively. In a study with calves of different genetic groups, Jorge et al., (1997a) observed similar results, with buffaloes, the DWG was smaller than two cattle breeds and greater than another one. However, these results differed from those of Moletta and Restle (1992), who evaluated the performance of calves of different genetic groups, finding an average DWG for buffaloes of 1.032 kg/day and for cattle of 1.029 kg/day, with all the animals being subjected to similar treatments under confinement. Villares et al., (1979a) observed an average daily weight gain of 0.991 kg, with 18 month old animals from three different buffalo breeds (Jafarabadi, Mediterranean and Murrah), when all of them were subjected to the same nutritional management. In weight gain tests of young Mediterranean buffaloes, Nascimento and Veiga (1973) demonstrated the great meat potential of this breed, which demonstrated an average daily gain of 0.857 kg. Velloso et al., (1994), comparing the DWG of buffaloes and zebu cattle, obtained values of 1.027 and 0.808 kg/day, respectively, which represents a difference of 20 percent. In a test involving buffaloes fed with different diets from 26 months (330 kg) and slaughtered at about 30 months of age (408 kg), Tonhati et al., (2001a), working with Murrah buffaloes, fed with sugarcane as volume and three protein sources (amiferm, poultry litter and wastes, and soya bean bran), observed a DWG of 0.39, 0.88 and 0.78 kg, respectively, with an overall average of 0.68 kg.

Johnson and Charles (1975), in a comparative study involving confined (132 days) buffaloes and Holstein, Angus and Hereford cattle, matured to ages between 20 and 30 months with a diet rich in concentrate, concluded that the buffaloes had a lower yield (53.3 percent) than the cattle (58.4 percent; 63.3 percent and 62.1 percent in the three genetic groups). Felicio et al., (1979) evaluated the carcass composition and meat quality of eight whole male buffaloes, of Jafarabadi breed, matured to approximately 24 months, with a slaughter weight of 400.6 kg and an empty weight of 349.5 kg. The results indicated a carcass yield of 48.7 percent in relation to slaughter weight and 55.8 percent in relation to the empty weight. Villares et al., (1979b), in Botucatu (SP), analysed the meat production of 15 Mediterranean buffaloes, 10 males and 5 females, matured to about 24 months, in a free stall regimen and with an average weight before slaughter of 364.07±46.10 kg. The weight of the warm carcass was 183.36±13.65 kg, representing a 50.36 percent yield in relation to the live weight before slaughter. Cockrill (1974) reported a yield of 49 percent for Italian female buffaloes at eighteen months of age, and also reported that in the former USSR, female buffaloes had greater yields than the males, 48.70 percent and 47.80 percent, respectively. Mattos et al., (1998) obtained an average warm carcass yield (WCY) of 52.20 percent, 52.05 percent and 53.10 percent, for females, non

castrated and castrated males, respectively. The lower carcass yield of buffaloes in relation to cattle was observed by several authors and can be attributed to the fact that these animals have a thicker skin and a larger percentage of head, horns, hoofs and guts. Jorge et al., (1997b) observed an average carcass yield of 49.44 percent in buffaloes slaughtered at different stages of maturity, fed with a diet containing 2.4 Mcal metabolizable energy/kg DM. Oliveira et al., (1991) observed an average carcass yield of 49.30 percent in confined buffaloes , in addition to differences in body composition between buffaloes and zebus. Afif et al., (1974) observed an average carcass yield between 50 and 55 percent for male buffaloes at different ages and weights at slaughter. Pillai et al., (1988) studied the carcass yield of 15 male whole buffaloes and 30 females, of different breeds, originating from two different regions of India. In one of the regions, the male and female buffaloes produced, respectively, 410.57±104.92 kg and 470.18±72.74 kg of slaughter weight; 41.58±1.83 percent and 43.06±1.01 percent of carcass yield in relation to the slaughter weight. In similar studies, Lorenzoni et al., (1986) found a carcass yield of 53.2 percent for buffaloes and 58.7 percent for Nelore. Mattos et al., (1997) evaluated carcass characteristics for eight Mediterranean buffaloes and six Nelore cattle, confined for 120 days, obtaining a smaller carcass yield for buffaloes (52.09 percent) and greater for Nelore (56.28 percent). In a study undertaken by Franzolin et al., (1998), 15 male Mediterranean buffaloes, fed with three different energy levels, produced an average carcass yield varying from 49.66 percent to 50.76 percent, with the first average as the least energetic and the last one as the most energetic. Lourenço Jùnior et al., (1987), comparing carcass yield in relation to empty body weight (EBW), found Mediterranean, Carabao and Jafarabadi buffaloes, respectively, 54.08, 53.76 and 53.32 percent, which showed little difference between them. Gazzeta et al., (1995), evaluating 12 Jafarabadi buffaloes, 12 Mediterranean and 6 Nelore cattle, obtained carcass yields of 51.45 percent, 51.44 percent and 57.18 percent, respectively. Tonhati et al., (2001b) showed an average carcass yield of 48.65 percent for Murrah buffaloes slaughtered at 30 months. Preston and Willis (1974) demonstrated that several factors can affect carcass yield values, such as the assessment basis (in relation to live weight or in relation to empty body weight). When live weight is used, the yield is affected by the diet type and fasting period that the animals have been subjected to prior to slaughter. Jorge (1993), considered that carcass yield, often does not provide a good estimation of quality meat yield, especially when considering animals that have been excessively finished, due to the dilution effect of the fat tissue on other carcass components: i.e. muscles and bones (Jorge and Fontes, 1997). In a study covering Mediterranean animals, Jorge et al., (1997) showed values of 55.86, 27.64 and 16.51 percent for muscles, fat and bones, respectively. In the same presentation order, Tonhati et al., (2001c) found averages of 52.01, 30.50 and 17.24 percent for Murrah 30 months old. In Egypt, Abdallah et al., (1981) investigated the comparative performance of buffaloes and cattle weighing 167 kg of warm carcass, and found values of 65.6 percent and 60.0 percent for muscles, 10.1 percent and 8.1 percent for fat, and 17.1 percent and 15.9 percent for bones, respectively (Tonhati and Ferreira Lima, 2003).

In South American and Asian countries buffalo meat production is generally undertaken utilizing extensive systems, using pasture or poor crops, with no incentive to use high energy diets, realizing low daily gains (500 g) and producing bulls weighing 400 kg at about two years. In Italy the aim is to obtain a minimum daily gain of 800 g and up to 900 g, and to produce young bulls weighing 400 kg within 15 months. Therefore, specific diets are used, as shown in Tables 6, 7 and 8 with an energetic concentration varying from 0.86 to 0.80 UFC (Meat Feed Unit)/kg DM (Dry Matter) and with about 10 percent of DCP (digestive crude protein). 40 kg prior to slaughter a fattening period is effected adding 1 kg cereal meals/pro die/pro capite.

The buffalo performances for meat production i.e. growth, feed efficiency, conversion ratio, dressing percentage, carcass evaluation and composition and meat quality cuts, are very important in economic terms but the priority focus for expanding the buffalo meat market is meat quality, which means chemical, physical, organoleptical and hygienic characteristics and a good presentation to the consumer.

Many years ago a study was undertaken on meat quality in buffalo males slaughtered at different ages. This trial covered 30 Friesian male calves and 30 Mediterranean Italian buffaloes reared under identical feeding and environmental conditions and slaughtered at 20, 28 and 36 weeks of age (Borghese et al.,1978b). The water buffalo meat, upon the visual inspection of the judges, was lighter than the bovine meat and a colorimeter confirmed this fact; it became darker with the increasing age of the animals. Cooking losses also decreased with the age of the animal. The meat tenderness using the Warner Bratzler Shear machine and according to a panel taste decreased as the age increased, as did flavour scores, while juiciness scored better after 36 weeks of age.

Many studies have been undertaken in this field comparing buffaloes to Friesian bovines up to 52 and 64 weeks of age, including analysis of the fatty acid composition of subcutaneous, intermuscular, intramuscular, perivisceral and perinephric lipids at different ages (Borghese et al., 1978a) but only a few of these results are reported here covering the meat quality of Italian buffaloes fed with hay and concentrates and slaughtered at 52 weeks of age compared with Friesian bovines reared under the same conditions (Borghese et al.,1996).

Muscle pH was approximately 6.2 at slaughter, 5.7 after two hours, and 5.5 after six hours. Only the *longissimus dorsi* in bovines showed a significantly ($P<0.05$) higher value (6.2 after two hours and 5.8 after six hours) than other muscles (*semimembranosus* and *semitendinosus*) and than in buffalo *longissimus dorsi*. After 24 hours, the pH was about the same (5.5-5.6) for all the muscles in both species, with a slight increase (5.7) from the sixth day on. Therefore, after ageing, pH characteristics are practically the same in both species.

The trial demonstrated that normally there were no significant differences between species in the nine studied muscles for all the physical parameters, using the Instron machine (Table 9). The hardness of the raw meat tested by the Warner Bratzler Shear machine was significantly ($P<0.05$) higher in buffalo only in the *iliopsoas* muscle, while in bovine bulls it was higher than in buffaloes only in the *semitendinosus* (Fig. 1), while significant differences were found in the force used only in the *caput longum tricipitis brachii* (Fig. 2). With the compression test only gumminess in the *supraspinatus* was significantly higher in the bovine (Table 9).

The chewiness, that is the synthesis of physical parameters, shows a tendency to be higher in bovine bulls. This could explain why generally people say that buffalo meat is more tender.

The results of the Warner Bratzler Shear tests on cooked meat are reported in Table 10: *longissimus dorsi* (Fig. 3) was more tender than in the raw meat, particularly if baked; after baking, both species showed the same values, while buffalo meat appeared more tender after being cooked in boiling oil ($P<0.09$).

Figure 1. *Semitendinosus* muscle in buffalo young bull
(Borghese photo, 2004)

Figure 2. *Caput longum tricipitis brachii* in buffalo young bull
(Borghese photo 2004)

Figure 3. *Longissimus dorsi*
(Borghese photo, 2004)

Figure 4. *Gluteobiceps*
(Borghese photo 2004)

The cooking losses, when the meat temperature reached 70°C, were about 21 percent after boiling in oil, and about 12 percent after baking, with no differences between species (Table 10).

Further quantity was lost, ten minutes after cooking with liquids: seven percent after cooking in boiling oil, and approximately six percent after baking, with the same trend for both bovine and buffalo meat.

The percentage of judges that identified the species was 22.9 percent for meat slices cooked in the open pan and only 7.5 percent for meat cooked by pressure cooking, less than casual probability. The percentage was significantly ($P<0.01$) higher for slices cooked in the open pan, where the meat was less cooked and quite natural. A large number of judges declared it impossible to identify the meat (52.0 to 74.5 percent for the open pan and pressure cooker respectively), while 25.1 and 18.0 percent respectively mistook the identification. No judge identified the species in all the tests. No difference was found with regard to the evaluation of tenderness, flavour and juiciness (Table 11).

Only in two taste tests was buffalo meat significantly overscored by one judge on the panel. The judges always gave better scores ($P<0.05$) to the meat cooked in the pressure cooker than that cooked in the open pan (Borghese et al., 1996).

Regarding the tests on tenderness values undertaken with the Instron machine on several muscles of Italian buffaloes slaughtered at 190 days, Failla et al. (2001) found values in raw meat varying from 2.98 to 4.87 kg/cm, while Tonhati et al. (2001d) found a mean of 4.52 kg/cm in Murrah 30 months old.

With respect to the nutritional characteristics of buffalo meat new data (Infascelli et al., 2003) confirmed the very low lipid content (1.36 ± 0.1 percent), correlated to the low energy value of the diet (0.84 UFC/kg DM) fed to the animals. The cholesterol content (48.8 ± 2.9 mg/100 g) was lower than that reported for Italian bovine genotypes with an aptitude for meat production. The content of myristic, palmitic and stearic acids, the first two with both atherogenic and thrombogenic activity, was also very low. Thus, despite the low values of oleic acid and polyunsaturated acid of the ω-6 and ω-3 series, both the atherogenic and thrombogenic indexes were very low (0.53 and 1.48, respectively). Based on these results, the nutritional quality of buffalo meat can be considered of great interest. Many researches are being carried out in Italy regarding the physical, chemical and organoleptic characteristics of industrial products derived from buffalo meat, due to the marketing and managerial interest in this field. Buffalo Beef is a company in Capua (Campania region) that produces a lot of typically Italian buffalo meat products: bresaola (salted rump lean muscle, particularly *Semitendinosus*, Fig. 1), salami, sausages, cacciatorini (very little salami), buffalo cheese rolls containing salami, ham or dry meat etc. (Fig. 5).

Figure 5. Bresaola and typical salami produced by Buffalo Beef

In Milan also, Paleari et al. (2000) studied characteristics of buffalo bresaola as compared with the typical IGP (Indication of Protected Geographic Origin) bovine bresaola of Valtellina (Prealpine Lombard region), which is just entering several international markets. There were no significant differences in the percentage weight loss (32.3 percent) and pH after thawing and curing (5.67) for the buffalo and beef samples. Significant differences were found for all the chemical composition parameters (moisture % 62.94 *vs* 60.95, protein % 29.79 *vs* 31.96, collagen % 0.9 *vs* 0.78 in buffalo and in bovine respectively) except the fat (1.75 percent) and ash (5.43 percent). In terms of brightness and hue there were no significant differences, either inside or outside; however, the saturation value revealed the buffalo bresaola to be darker. Furthermore, the buffalo bresaola was less tender than the beef one, tenderness being expressed as shear force (0.189 *vs* 0.157 kg/mm^{-2}). The investigation demonstrated the possibility of transforming cuts of buffalo rump into a product similar to that of beef. Thus buffalo beef can be transformed into various cured products, especially that of the male animal, which would be of increased value, with clear advantages to the breeder. In this way a range of typical products with their own niche in the market place could be created. This could also satisfy consumer demand for lean products with a low energy content which could be well integrated into the modern diet.

At present Italian breeders (particularly three cooperatives are of particular interest: Consorzio Alba, Consorzio La Baronia and Buffalo Beef) are trying to produce high quality meat for the luxury market (restaurants and gourmet food) adopting a production protocol in accordance with the IGP symbol (Indication of Protected Geographic Origin) "Carne di bufalo mediterraneo" (Mediterranean buffalo meat). The geographic territory where the buffaloes are reared is the same as the D.O.P. "Mozzarella di bufala Campana": Campania and the south Lazio regions and some parts of the Puglia and Molise regions.

The calves can be weaned with milk substitutes or by nursing bovine cows. Following this they can be fed fresh or conserved (silages or hay) forages and meals or concentrates until they reach no more than 450 kg live weight in order to avoid carcasses which are too fat or bad smelling. The daily gain must be between 800 and 950 g for young bulls in order to avoid sick animals or hormone treatments. Four months prior to slaughter the use of maize silage and of particular feeding stuffs is prohibited in order to avoid bad flavour in the meat and the animals must be reared on slattered floors or on floors where the straw is changed each week to avoid the smell

of urine and faeces. Stress before and during slaughter must be avoided for the quality of meat. The carcasses must be included in the medium and abundant classes for fatness and in the good and optimum classes for conformation. Ageing must be effected for nine days at least and the quality characteristics of the *longissimus dorsi* muscle must be: PH 5.5 5.9, intramuscular fat <3 percent, protein >20 percent, cholesterol <50 mg/100 g, iron > 1.5 mg/100 g, and total mesophil bacteria< 10^5 Units/cm^2. These regulatory measures guarantee the high quality of the meat and of the products which are produced in the different industries such as: bresaola, salami, cacciatorini, and buffalo cheese rolls with salami or ham.

Therefore, the aim of the Italian market is to develop products of good nutritional and organoleptic quality. In South America also, particularly in Brazil where most of the South American buffalo population can be found (3 000 000 head), buffalo meat is recognized as a differentiated product as compared to bovine meat. Buffalo meat is also leaner and presents 40 percent less cholesterol and 55 percent less calories, 11 percent more protein and 10 percent more minerals (Table 12). Buffalo meat, which is to be placed on the market, should derive from young animals, preferably from 18 to 24 months of age, since older animals have fibrous meat and meat with lower organoleptic qualities. Therefore, due to these advantages, there is an open and guaranteed market for buffalo meat, not only in Brazil, but all over the world. In Brazil buffalo meat is already sold in special kits, with cuts ready to be used. At present, the demand for this product comes mainly from specialized restaurants (Rocha Loures, 2001).

Clearly the priority in Asia is for food production to satisfy human needs, particularly for animal proteins, and the possibility to produce buffalo meat could strongly assist in finding a solution to this planetary problem. India is the highest producer of buffalo meat in Asia.

Meat production in India is estimated at 4.7 million tons, taking eighth place in the ranking of the world's meat production. Buffalo in India contribute about 30 percent of the total Indian meat production with 1.43 million tons annually. The contribution from cattle, sheep, goats, pigs and poultry is 31 percent, 5 percent, 10 percent, 10 percent and 13 percent, respectively (FAO, 2003). The trends in livestock population, slaughter rate (number slaughtered as percentage of population), carcass weight and meat production in India in 2003 are shown in Table 13. The major export of meat is from buffaloes, as is shown in Table 14 (Ranjhan, 2004).

It can be seen that exports of buffalo meat have increased significantly over the last three years. The export of buffalo meat in 1997-98 was only 176 328 MT, and this increased to 243 355 MT in 2001-2002 accounting for an increase of 43 percent. In 2002-2003, exports were almost 300 000 MT valued at 13,054 million Indian rupees equivalent to about US$300 million. In addition, the export of meat from small ruminants (sheep and goats) has increased over the last three years. India also has a large number of dogs and cats, which are kept as pets but there is no scientific production of pet food. The international market is vast and the demand for pet food runs into billions of dollars. Slaughterhouses produce large quantities of raw material for pet food and the technology for its commercial utilization is evolving in India. In the next five years this sector is poised for a quantum leap with many world leaders in this field working towards joint ventures with Indian companies (Ranjhan, 1996, 2004).

In India the intensive feeding of male buffalo calves for meat production was never visualized and was considered a taboo. However, intensive feeding has been implemented over the last five years (1999-2004) for the first time in a commercial feedlot for the production of quality meat. In a village demonstration farm with HAIL, a commercial feedlot housing 5 000 male calves has been established. The facilities include environmentally controlled animal houses with slatted floors where urine and dung are collected in the Keller and are regularly pumped out for spraying on the forage field. The male calves are purchased from the farmers at the age of eight to ten months and are then quarantined for 15 days during which time vaccinations and de-worming are provided. Thereafter, they are brought to the main farm and are fed on a high protein/high-energy diet in order to gain an additional weight of 120 kg in four months and produce quality meat. The composition of the feed is as follows:

Composition of the feed (pelleted)

Straw	50%
Rape Seed	30%
Bran	5%
Urea	2%
Molasses	10%
Mineral Mix	1%
Salt	2%

The above mixture is pelleted and contains above 18 percent crude protein equivalent and 60 percent TDN. Each animal is fed 2.5 - 3.0 kg of pellets; 3 kg of spent brewer's grains and 2 kg whole sugar cane/sorghum per day. This above ration provides 20 percent crude protein and 65 percent TDN with a dry matter intake of 4.0 to 5.0 kg per animal per day. The above composition of the feed can be changed according to the local availability of feed resources (Ranjhan, 2004).

The Murrah calves grow at the rate of 900 to 1 000 g per head per day with a feed conversion ratio of 5.0:1. The cost of 1 kg live weight gain comes to around Rs.25.00 (US$0.5) per kg live weight gain (feed cost Rs.20.00+overhead cost Rs.5.00 per day). The dressing percentage of such animals is around 65 percent. A 250 kg live animal produces a carcass of about 150 kg fetching a price of Rs.65.00 to 90.00 (US$1.5 to 2.0) per kg on the international market. The quality of the buffalo meat is excellent, since it is lean, tender and juicy. It is no exaggeration that buffalo is black gold. The other positive aspects are that there is identification and certification of origin and traceability which are essential tools for accomplishing control of diseases, for improving production standards and providing the consumer with a more transparent and reliable market. These are the hallmarks of the Codex Alimentarius. Buffalo are also never fed on antibiotics, hormones and growth promoters. It should not be forgotten that with the globalization of the meat trade, the expansion of animal production will lead to global warming and an indiscriminate use of medication, increasing the risks for human beings. These factors could also disrupt the existing eco balance.

In India about 40 million people are engaged in the meat sector in the trade of live animals, hides, bones, horns, hooves etc. (Ranjhan, 2004).

Table 1. Weight, gain and feed intake at different ages (Romita et al., 1982)

Age (weeks)	20		28		36 (1)		36 (2)		52		64	
	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes
Starting weight (kg)	48.4	46.1	39.6	45.6	48.5	48.1	48.1	50.7	48.6	50.8	49.1	50.0
Final weight (kg)	161.5*	151.8	229.3***	203.7	291.0***	231.1	285.7	273.7	402.8	385.0	481.1	466.4
Total gain (kg)	113.1*	105.7	189.7***	158.1	242.5***	183.0	237.6	224.7	354.2	334.2	432.0	416.4
Daily gain (kg)	0.850*	0.795	0.968***	0.807	0.988***	0.746	1.007	0.963	0.980	0.930	0.987	0.949
Milk (kg)	199.6	199.6	292.3	292.3	456.2	456.2	39.4	41.8	43.7	43.7	43.7	43.7
Concentrate (UF)			170.8***	142.3	274.3***	188.7	772.0	733.5	1448.6	1381.3	1970.7	1885.0
Hay (UF)							260.0	243.0	431.7	395.1	653.1**	552.1

(* p≤5%, ** p≤1%, *** p≤0.1% between species, within ages)

Table 2. Fast live weight, net live weight, carcass and dressing at different ages (Romita et al., 1982)

Age (weeks)	20		28		36 (1)		36 (2)		52		64	
	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes
Fast live weight (kg)	161.5*	151.8	228.3***	203.4	291.4***	230.2	285.7	273.7	402.2	384.5	479.2	465.7
Net live weight (kg)	145.8	141.0	201.7**	185.3	263.0***	210.1	257.5	251.1	360.6	353.9	433.2	428.4
Carcass weight (kg)	92.9**	84.0	125.5***	108.0	159.2***	121.0	152.6**	138.9	225.0**	201.8	262.3*	242.7
Dressing (%)	57.52	55.33	54.97	53.09	54.63	52.56	53.41	50.74	55.94	52.48	54.73	52.11
Net dressing (%)	63.70**	59.60	62.40***	58.25	61.24***	57.65	59.28***	55.33	62.31***	57.03	60.56***	56.64

(* p≤5%, ** p≤1%, *** p≤0.1% between species, within ages)

Table 3. Composition of the carcass (Gigli et al., 1982)

Age (weeks)	**20**		**28**		**36 (1)**		**36 (2)**		**52**		**64**	
	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes	Bovines	Buffaloes
Half carcass weight (kg)	43.7**	39.0	59.2***	50.7	75.6***	57.3	71.7*	65.3	108.5**	97.5	125.0	117.3
Meat (%)	65.0**	67.6A	64.16**	66.36AB	63.26*	65.40B	64.35	63.80C	62.34	61.41D	61.67	62.00D
Bone (%)	26.4**	24.9A	23.16	22.09B	22.65	21.96B	21.38*	19.5C	19.48***	17.57D	20.20***	16.87D
Total Fat (%)	8.6	7.51D	12.68	11.55C	14.04	12.64C	14.37*	16.70B	18.19*	21.02A	18.12**	21.13A
Other tissues (%)							0.91	0.99	0.86	0.92	1.17	1.22
Subcutaneous fat (%)							4.56***	7.28	5.20***	8.86	5.25***	9.41
Intermuscular fat (%)							6.48	6.78	9.52	9.13	8.57	8.26
Perineal fat (%)	1.9**	0.7	2.23***	0.87	2.30***	0.97	2.42*	1.65	2.61	2.11	3.13**	2.23

(* p≤5%, ** p≤1%, *** p≤0.1% between species, within ages)
Different letters mean significant differences ($p<0.05$) between ages

Table 4. Performances in vivo and at slaughtering (Gigli et al., 1994)

	n	Daily gain (kg)	ME/d (MJ)	Conversion ratio (MJ/kg)	PDI (g/d)	n	Net live weight (kg)	Net dressing (%)
6 months						10	128.7F	56.23C
10 months								
A	24	0.962A	53.34C	55.79C	577.54C	8	212.8D	59.63B
B	24	0.594CD	31.66E	54.07C	439.96E	8	178.0E	59.48B
14 months								
A	16	0.979A	64.46B	66.36B	649.86B	8	326.3B	60.58AB
B	16	0.647C	35.27DC	54.88C	463.87C	8	251.5C	59.73B
18 months								
A	8	0.798B	72.06A	87.42A	696.45A	8	409.1A	61.68A
B	8	0.550D	37.21D	65.35B	473.01D	8	316.6B	60.22AB
Mean	96	0.772	46.98	60.40	537.45	58	255.9	59.53
Error variance		0.009	34.763	55.380	1645.676		77537	2.194

Different letters mean significant differences ($p<0.05$)

Table 5. Composition of the carcass (%*) (Gigli et al., 1994)

	Half carcass (kg)	Total meat	Subcutaneous fat	Inter-muscular fat	Total fat	Bone
6 months	33.9E	62.06AB	3.98E	5.91D	9.89D	24.93A
10 months						
A	60.2C	63.79A	5.37D	8.01BC	13.38BC	20.20C
B	50.2D	63.86A	4.74DC	6.40CD	11.15ED	22.14B
14 months						
A	93.2B	60.37C	9.00B	11.02A	20.02A	17.47D
B	70.5C	62.76A	6.48C	7.68BC	14.16BC	20.33C
18 months						
A	118.4A	59.16C	10.69A	10.93A	21.62A	17.17D
B	91.1B	62.37AB	6.93C	8.31B	15.25B	19.98C
Mean	72.5	62.04	6.65	8.24	14.89	20.48
Error variance	76.81	4.826	1.208	2.636	5.674	1.436

* Without other tissues
Different letters mean significant differences ($p<0.05$) between ages

Table 6. Diet for weaned calves from 150 kg

	kg t.q.	kg DM	UFC	g DCP	g CF	Cost
Maize silage	2	0,62	0,56	29	100	€ 0,10
Italian Ryegrass hay	2	1,76	1,1	146	644	€ 0,28
Barley grain	1	0,9	1	63	51	€ 0,20
Leguminous seed	1	0,9	0,94	220	78	€ 0,20
Total	**6**	**4,18**	**3,6**	**458**	**873**	**€ 0,78**

Table 7. Diet for weaned calves from 200 kg

	kg t.q.	kg DM	UFC	g DCP	g CF	Cost
Maize silage	3	0,93	0,84	43	150	€ 0,15
Italian Ryegrass hay	3	2,64	1,65	219	966	€ 0,42
Barley grain	1	0,09	1	63	51	€ 0,20
Leguminous seed	1	0,9	0,94	220	78	€ 0,20
Total	**8**	**5,37**	**4,43**	**545**	**1245**	**€ 0,97**

Table 8. Diet for weaned calves from 300 kg

	kg t.q.	kg DM	UFC	g DCP	g CF	Cost
Maize silage	4	1,24	1,12	58	200	€ 0,20
Italian Ryegrass hay	4	3,52	2,20	292	1288	€ 0,56
Barley grain	1	0,9	1	63	51	€ 0,20
Leguminous seed	1	0,9	0,94	220	78	€ 0,20
Total	**10**	**6,56**	**5,26**	**633**	**1617**	**€ 1,16**

Table 9. Myorheological parameters determined with the Instron instrument on raw meat (Borghese et al., 1996)

Muscle	Species	n	Warner Bratzler Shear		Compression test				
			Hardness (kg)	Work (kgm)	Hardness (kg)	Cohesiveness (max=1)	Springiness (cm)	Gumminess (Hard.XCohe.)	Chewiness (Gum.XSpring.)
C.l.tricipitis brachii	Bovine	9	11.53 c	0.33 Bbcde	31.62 ab	0.35 b	0.19 ab	11.47 a	2.27 a
	Buffalo	9	13.36 ab	0.42 Aab	26.85 ab	0.35 a	0.22 ab	8.80 c	2.00 bc
Supraspinatus	Bovine	9	16.12 b	0.45 bc	24.05 b	0.46 a	0.19 ab	11.07 Aa	2.21 a
	Buffalo	9	14.15 ab	0.40 ab	18.72 c	0.39 a	0.22 ab	7.45 Bc	1.73 bc
Rectus femoris	Bovine	9	10.66 c	0.32 bcde	26.87 b	0.39 ab	0.19 ab	10.93 a	2.17 a
	Buffalo	9	10.20 b	0.30b	24.70 bc	0.35 a	0.20 b	8.86 c	1.83 bc
Guteus medius	Bovine	9	10.55 c	0.33 bc	29.37 ab	0.40 ab	0.20 ab	11.68a	2.47 a
	Buffalo	9	10.27 b	0.30 b	22.26 c	0.38 a	0.21 ab	8.35 c	1.79 bc
Gluteobiceps	Bovine	9	16.15 b	0.42 bcd	39.90 a	0.35 b	0.23 a	13.88 a	3.04 a
	Buffalo	9	13.11 ab	0.36 ab	35.43 ab	0.37 a	0.22 ab	12.86 b	2.88 b
Semitendinosus	Bovine	9	21.34 Aa	0.66 a	35.36 ab	0.42 ab	0.23 a	14.47 a	3.33 a
	Buffalo	9	16.36 Ba	0.54 a	37.55 a	0.43 a	0.26 a	16.97 a	4.31 a
Semimembranosus	Bovine	9	13.56 bc	0.45 bcd	32.39 ab	0.34 b	0.22 ab	10.46 a	2.33 a
	Buffalo	9	13.07 ab	0.46 ab	28.51 ab	0.34 a	0.20 b	9.42 c	1.99 bc
Longissimus dorsi	Bovine	9	9.74 c	0.29 dce	15.31 c	0.33 b	0.18 b	5.14 b	0.94 b
	Buffalo	9	10.69 b	0.32 bc	18.30 c	0.37 a	0.20 b	6.81 c	1.44 c
Iliopsoas	Bovine	9	7.26 Bc	0.24 e	9.24 c	0.26 c	0.22 ab	2.48 b	0.55 b
	Buffalo	9	9.59 Ac	0.28 c	7.83 c	0.23 b	0.20 b	1.75 d	0.36 d

Different letters mean significant differences ($p<0.05$) between muscles if small; between species if capital

Table 10. Cooking losses and hardness (Warner Bratzler) of cooked meat. (*longissimus dorsi* muscle) (Borghese et al., 1996)

	N	BOILING OIL			BAKING		
		at 70°C%	after 10'%	Hardness kg	at 70°C%	after 10'%	Hardness kg
Bovine	10	19.2	26.5	7.0	11.1	17.3	5.9
Buffalo	10	22.2	29.7	6.1	12.6	18.5	5.7
ST. E.		2.41	1.97	0.37	0.91	0.72	0.37

Table 11. Taste panel mean scores (Borghese et al., 1996)

		Open pan				Pressure cooker			
	n	Bovine		Buffalo		Bovine		Buffalo	
		x	cv%	x	cv%	x	cv%	x	cv%
Tenderness	882	6.00b	22.0	6.14b	21.7	7.29a	15.2	7.40a	13.6
Flavour	878	6.28b	17.6	6.18b	18.5	7.25a	11.8	7.35a	11.2
Juiciness	880	4.88b	29.3	4.85b	28.6	5.55a	27.1	5.75a	26.4

Different letters mean significant differences for $P<0.05$.

Table 12. Comparison of some characteristics of bovine and buffalo meat (Rocha Loures, 2001)

Characteristics	Buffalo	Bovine
- Calories (Kcal)	131.00	289.00
- Protein (N x 6.25)	26.83	24.07
- Total fat (g)	1.80	20.69
- Fatty acid:		
- Saturated (g)	0.60	8.13
- Monosaturated (g)	0.53	9.06
- Polysaturated (g)	0.36	0.77
- Cholesterol (mg)	61.00	90.00
- Minerals		
Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium, Zinc, Copper and Manganese (total mg)	641.80	583.70
- Vitamins		
Ascorbic acid, Thiamine, Riboflavin, Niacin, Pantotenic acid, Vit.B6, Folacin and Vit. B12 (total mg)	20.95	18.52

Table 13. Trends in Livestock Production and Meat Production in India (FAO, 2003)

Livestock Species	Population in Millions	Animals slaughtered (millions)	Percent slaughtered (%)	Carcass weight (kg)	Meat production (million tons)	Share in total meat production (%)
Cattle	180.1	14.2	7.9	103	1.46	31.1
Buffalo	103.0	10.3	10.0	138	1.43	30.5
Sheep	40.1	19.2	47.9	12	0.23	4.9
Goats	124.0	47.0	37.9	10	0.47	10.0
Pigs	18.0	16.0	88.9	31	0.47	10.0
Poultry	820.0	604.0	73.6	0.8	0.63	13.4
Total					**4.69**	**100**

Table 14. Export of Buffalo Meat (MT) from India to some major countries (Ranjhan, 2004)

Countries	2000-2001	2001-2002	2002-2003
Malaysia	77 135	67 251	79 421
Philippines	47 447	50 356	46 971
UAE	41 516	19 988	27 635
Yemen	3 738	3 938	5 604
Qatar	617	8 520	1 334
Mauritius	3 192	3 004	3 382
Oman	7 631	8 101	10 106
Lebanon	4 130	2 980	4 518
Jordan	12 442	15 327	16 212
Iran	12 576	10 741	7 617
Egypt	48 716	17 808	19 524
Total	**268 027**	**243 355**	**297 897**

References

Abdallah, O.Y. et al., 1981. J. Agric. Sci., London, vol. 97: 205-212.

Afif, Y.A. et al., 1974. Agric. Res. Ver. Vol. 52: 1-20.

Borghese, A., Gigli, S., Romita, A., Di Giacomo, A. and Mormile, M. 1978a. Fatty acid composition of fat in water buffalo calves and bovine calves slaughtered at 20-28 and 36 weeks of age. Seminar EEC, Patterns Growth Development in Cattle, Ghent, Belgium, Martinus Nijhoff Ed.: 267-276.

Borghese, A., Romita, A., Gigli, S. and Di Giacomo, A. 1978b. Eating quality of buffalo and bovine calves slaughtered at 20-28 and 36 weeks of age. Seminar EEC, Patterns Growth Development in Cattle, Ghent, Belgium, Martinus Nijhoff Ed.: 603-611.

Borghese, A., Di Giacomo, A. and Mormile, M. 1996. Meat eating quality in bovine and buffalo young bulls of one year of age. International Symposium on buffalo products, Paestum (Salerno), Italy, Dec 01-04/1994, EAAP Publ. 82: 247-254.

Bouwman, A.F. 1997. Long-term scenarios of livestock crop-land use interactions in developing countries. FAO Land and Water Bulletin, 6, FAO, Rome.

Cockrill, W.R. 1974. The husbandry and health of the domestic buffalo. Editor FAO, Rome.

De Franciscis, G. e Zicarelli, L. 1974. Prove di svezzamento precoce in vitelli bufalini. Atti II Convegno Internazionale sull'allevamento bufalino nel mondo, Caserta, Italia, Nov. 7-9: 175-186.

Di Lella, T., de Franciscis, G. e Zicarelli, L. 1975. Acta Medica Vet. XXI: 3-4.

Di Lella, T., Cutrignelli, M.I., Calabrò, S. and Infascelli, F. 1998. Influence of the feeding programme on growth dynamics of buffalo young bulls until 16 months of age. Bubalus Bubalis, IV/Jun: 81-90.

Failla, S., Settineri, D., Bisegna, V. e Mormile, M. 2001. Produzione del vitello a carne bianca alimentato con due diversi tipi di latte. Nota II: Qualità della carne. Primo Congresso Nazionale sull'allevamento del bufalo, Eboli (SA), Italia, Oct. 3-5: 281-284.

FAO Stat. 1998. Food and Agriculture Organization of the United Nations. FAOSTAT database, http://faostat.faoorg.

FAO. 2003. Production Year Book.

Felìcio, P.E. et al. 1979. ITAL, Campinas, SP. Boletim Técnico, 3: 33-66.

Ferrara, B., Minieri, L., de Franciscis, G. e Intrieri, F. 1964. Acta Medica Vet. X, 5.

Ferrara, B., Minieri L., de Franciscis G. e Intrieri F. 1982. La produzione della carne nella specie bufalina. Atti II Convegno Internazionale sull'allevamento bufalino nel mondo, Caserta, Italia, Sett. 29-Ott. 2: 620-634.

Franzolin, R. et al. 1998. Reunião Anual da Soc. Bras Zootec., 35, Botucatu, SP. Anais: 395-397.

Gazzeta, M.C.R.R. et al. 1995. Boll. Ind. Anim., 52 (1): 76-77.

Gigli, S., Romita, A., Borghese, A. and Mormile, M. 1982. Water buffaloes and Friesian bovine

males performances at different ages. Fifth quarter and carcass characteristics. Atti II Convegno Internazionale sull'allevamento bufalino nel mondo, Caserta, Italia, Sett. 29-Ott. 2: 593-609.

Gigli, S., Failla, S., Carretta, A., Iacurto, M., di Giacomo, A. e Mormile, M. 1994. Fabbisogni energetici e performances in vita, alla macellazione ed alla dissezione in maschi bufalini in accrescimento. Agricoltura e Ricerca, 153, Gen.-Mar.: 63-72.

Infascelli, F., Cutrignelli, M.I., Bovera, F., Piccolo, G., Tudisco, R., Calabrò, S., Zicarelli, F. and Piccolo, V. 2003. Nutritional characteristics of buffalo meat: Cholesterol content and fatty acid composition. Bubalus bubalis, 4 : 51-57.

Johnson, E.R. and Charles, D.D. 1975. Aust. J. Agric. Res., vol. 26 : 415-422.

Jorge, A.M. 1993. Dissertaçao de Mestrado em Zootecnia, UFV, MG, 97.

Jorge, A.M. and Fontes, C.A.A. 1997. Feedlot performance of buffalo and cattle bulls, slaughtered at two stages of maturity. Proc. Fifth World Buffalo Congress. Caserta, Italy, Oct. 13-16: 428-432.

Jorge, A.M. et al. 1997a. Rev. Soc. Bras. Zootec., vol. 26, 4: 806-812.

Jorge, A.M. et al. 1997b. Reunião Anual da Soc. Bras Zootec., 34, Juiz de Fora. Anais: 323-325.

Kondaiah, N. and Anjaneyulu, A.S.R. 2003. Potential of buffalo meat to processing different products. Proc. of Fourth Asian Buffalo Congress, New Delhi, India, Feb. 25-28: 200-204.

Lorenzoni, W.R. et al. 1986. Rev. Soc. Bras. Zootec. vol. 15, 6 : 486-497.

Lourenço Jùnior, J.B. et al. 1987. Boletim de Pesquisa, 81 EMBRAPA-CPATU, Belém, PR.

Mattos, J.C. et al. 1997. Reunião Anual da Soc. Bras Zootec., 34, Juiz de Fora. Anais : 346-348.

Mattos, J.C. et al. 1998. Reunião Anual da Soc. Bras Zootec., 35, Bocatau. Anais : 88-90.

Maymone, B. 1945. I bufali allevati in Italia. Annali dell'Istituto Sperimentale Zootecnico di Roma, vol. III: 5-65.

Moletta, J.L. and Restle, J. 1992. Ciencia Rural, vol. 22-2: 227-233.

Murthy, T.R.K. and Prince Devadason, I. 2003. Buffalo meat and meat products - An overview. Proc. of Fourth Asian Buffalo Congress, New Delhi, India Feb 25 - 28: 194-199.

Nascimento, C.N.B. and Veiga, J.B. 1973. Boletim de Pasquisa, 56. EMBRAPA-CPATU, Belém, IPEAN.

Oliveira de A.L., Veloso, L. and Schalch, E. 1991. Carcass characteristics and yield of zebu steers compared with buffalo. Proc. Third World Congress, Varna, Bulgaria, May 13-18: 1019-1026.

Paleari, M.A., Beretta, G., Colombo, F., Foschini, S., Bertolo, G. and Camisasca, S. 2000. Buffalo meat as a salted and cured product. Meat Science, 54: 365-367.

Palladino, M., di Meo, C., Esposito, L. e Zicarelli, L. 1993. Prove di svezzamento di vitelle bufaline con l'impiego di un latte ricostituito acidificato. Atti X Congr. Naz. ASPA, Bologna, Mag. 31-Giu. 3: 261-266.

Pillai, S.R. et al. 1988. Indian J. of Anim. Sci., New Delhi, vol. 58, 4: 516-521.

Preston, T.R. and Willis, M.B. 1974. 2nd Ed. Oxford, Pergamon Press.

Ranjhan, S.K. 1996. Livestock Industry Prospects in India, Issues and Policies Proceedings on Agricultural Development Prospectives for the Ninth Five Year Plan, Indian Institute of Management, Ahmadabad, 13-15 June.

Ranjhan, S.K. 2004. Commercial production of buffalo meat with social agenda. Proc. of Seventh World Buffalo Congress, Manila, Philippines, Oct. 20-23: 1-7.

Restle, J. et al. 1990. In: Congresso Mundial de Buiatria, 16, Salvador: 939-944.

Rocha Loures, R. 2001. Buffalo production systems in the Americas. Proc. of the Sixth World Buffalo Congress, Maracaibo, Venezuela, May 20-23, vol. I: 74-86.

Romita, A. e Da Silva Dias, A.V. 1975. Accrescimenti e indici di conversione di bufali allevati fino a sei mesi con tre regimi alimentari diversi. Annali dell'Istituto Sperimentale per la Zootecnia, vol. VIII: 79-87.

Romita, A., Gigli, S., Borghese, A. and di Giacomo, A. 1982. Water buffaloes and Friesian bovine males performances at different ages. I°- In vivo and at slaughtering characteristics. Atti II° Convegno Internazionale sull'Allevamento Bufalino nel Mondo, Caserta, Italia, Sett. 29-Ott. 2: 573-592.

Roncoroni, C., Failla, S., Bisegna, V., Tripaldi, C. e Pasqui, E. 2001. Effetto della somministrazione di fermenti lattici durante l'allattamento dei vitelli bufalini. Nota I: Accrescimenti e stato sanitario. Atti I Congr. Naz. sull'allevamento del Bufalo, Eboli (Salerno), Italia, Ott. 3-5: 404-408.

Silva, M.E.T. et al. 1997. In: Renuião Anual da Soc. Bras. Zootec., 34, Juiz de Fora Anais: 314-316.

Tonhati, H., Spironelli, A.L., Ferriani, L., Tseimazides, S.P., De Oliveira, J.A. and Berchielli, T.T. 2001a. Weight gain, feed conversion ratio and intake of dry matter of buffaloes finished in feedlot. Atti Primo Congr. Naz. sull'allevamento del Bufalo, Eboli (Salerno), Italia, Ott. 3-5: 273-276.

Tonhati, H. et al. 2001b. Proc. Sixth World Buffalo Congress, Maracaibo, Venezuela, May 20-23, vol. 2: 90-95.

Tonhati, H., Spironelli, A.L., Ferriani, L., Tseimazides, S.P., Dutra de Resende, F., De Oliveira, J.A. and De Oliveira, H.N. 2001c. Body composition in carcass of buffalo. Atti I Congr. Naz. sull'Allevamento del Bufalo, Eboli (Salerno), Italia, Ott. 3-5: 265-268.

Tonhati, H., Resende, F., Spironelli, A.L., Ferriani, L., Tseimazides, S.P., De Oliveira, J.A. and Arrigone, M. 2001d. Correlation between softness and some physical and chemical characteristics of carcasses of buffaloes. Atti I Congr. Naz. sull'allevamento del Bufalo, Eboli (Salerno), Italia, Oct. 3-5: 269-272.

Tonhati, H. and Ferriera Lima, A.L. 2003. Buffalo meat: production and quality. Atti Secondo Congr. Naz. sull'allevamento del Bufalo, Monterotondo (Roma), Italia, Aug. 28-30: 67-79.

Tripaldi, C., Failla, S., Verna, M. and Roncoroni, C. 2001. Allattamento dei vitelli bufalini: composizione e concentrazione del latte ricostituito. Atti I Congr. Naz. sull'allevamento del Bufalo, Eboli (Salerno), Italia, Ott. 3-5: 399-403.

Velloso, L., Scalch, E., Zanetti, M.A., Felicio, P.E. and Higaschi, H. 1994. Comparative performances on buffalo, zebu (Nelore) and Holstein steers, fed crude soya bean meal, dry cassava meal and ground sugar cane in a feedlot trial. Proc. Fourth World Buffalo Congress, Sao Paulo, June 27-30: 266-268.

Villares, J. B. et al. 1979a. In: Fundaçao Cargill. Campinas, SP: 235-252.

Villares, J. B. et al. 1979b. In: Fundaçao Cargill. Campinas, SP: 133-146.

Zicarelli, L., Macrì, A., Vittoria, A., Padula, P., Costantini, S., Rania, V. and Giordano, R. 1981. Riv. Zoot. Vet., 9: 246-251.

Chapter XII

METABOLIC AND HORMONAL PARAMETERS IN BUFFALOES

Giuseppina Maria Terzano [1], Simona Allegrini [1], Antonio Borghese [1], Cristina Roncoroni [2] and Lavinia Alfieri [2]

[1] Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

[2] Istituto Zooprofilattico Sperimentale Lazio e Toscana
(Animal Prophylaxis Research Institute for Lazio and Toscana regions)
Via Appia Nuova 1411, 00178 Capannelle (Rome), Italy

The biochemical characteristics in livestock were initially investigated in order to establish their physiological ranges. In 1970 Payne et al., suggested the possibility of ascertaining nutritional disorders in livestock through blood analysis. Since then many studies have been undertaken in order to set reference values for haematochemical parameters of animals and to discover relationships between blood parameters and the pathological phenomena related to nutrition.

Many studies have also been undertaken in buffalo by several authors, demonstrating the differences with other species (Table 1) and contributing to the advancement of knowledge regarding:

1) the pattern of changes in different physiological states (i.e. growth, dry milk period, pregnancy, lactation);
2) the pattern of changes before and after meals;
3) the pattern of changes due to diets which have a different energy and protein content;
4) the pattern of changes due to transport, handling and the stress connected to slaughter;
5) the pattern of changes due to different environmental factors (housing systems, season, management).

The comparison between species (Table 1) shows that buffaloes are different from cattle and sheep, particularly with regard to:

a. Hematocrit, glucose, creatinine, calcium, phosphorus, magnesium, AST and bilirubin. These are all higher in buffalo than in cattle and sheep.
b. Cholesterol, zinc, total protein and albumen, which are lower in buffalo than in cattle but higher in buffalo than in sheep;
c. Urea, GGT and ceruloplasmin which are higher in sheep, while in buffalo the values are between cattle and sheep;
d. T3 values are similar in the three species, while insulin and cortisol are higher in buf falo and sheep than in cattle.

With regard to reproductive hormonal parameters, it is generally considered that the changes in the ovarian steroids and gonadotrophins blood concentrations during the cyclic ovarian activity in buffalo cows are comparable to those of bovine cows (Jainudeen and Hafez, 2000). Also Seren et al., (1994) when monitoring dynamic ovarian changes by transrectal palpation and ultrasound, found that the observed perioestrus endocrine changes did not indicate clear differences throughout the year and were essentially similar to those recorded in the cow. On the other hand, in the past decade, lower levels of several sexual hormones and some differences in the oestrus behaviour and in other reproductive aspects in buffalo cows and bulls in comparison to that of bovines, have been reported.

Table 1. Mean values of some endocrine-metabolic parameters in buffaloes *vs* cattle and sheep (Bertoni et al.,1994b, modified)

	Variations in		
Parameters	**Buffaloes**	**Cattle**	**Sheep**
Hematocrit (l/l)	0.41	⇓	↓
Glucose (mmol/l)	4.43	⇓	⇓
Cholesterol (mmol/l)	2.43	⇑	⇓
Urea (mmol/l)	5.52	⇓	⇑
Calcium (mmol/l)	2.69	↓	↓
Phosphorus (mmol/l)	2.42	↓	⇓⇓
Magnesium (mmol/l)	1.16	⇓	↓
Total protein (g/l)	76.3	↑	↑
Globulin (g/l)	42.1	↑	⇑
Albumin (g/l)	34.2	⇑	⇑
AST (U/l)	101.2	⇓	⇓
GGT (U/l)	21.2	↑	⇑
Bilirubin (mcmol/l)	5.35	↓	⇓
Zinc (mcmol/l)	10.9	⇑	↓
Ceruloplasmin (mcmol/l)	3.74	↓	↑
Insulin (mcU/l)	11.94	↓	⇑
T3 (ng/ml)	1.33	⇓	↓
Cortisol (ng/ml)	10.47	⇓	⇑

If ↑↓ the variations are small
If ⇑⇓ the variations are great

Parameters of energetic metabolism

Glucose - In all species glucose is used by various tissues and organs for free energy (i.e. ATP) production. In addition, glucose may be converted either into glycogen or triacylglycerols which are subsequently stored within tissues (liver, adipose tissues, muscles) or into lactose which is subsequently incorporated into milk in the case of lactating females.

The destination of glucose is regulated by various hormones such as insulin, cortisol, glucagone, somatotropin and adrenalin, and consequently blood glucose levels depend on the nutritive values of the diets, on social or environmental stress conditions as well as on physiological phases.

In previous studies Campanile et al., (1991) and Borghese (1994) established glucose reference values for productive buffaloes (4.00-4.16 mmol/l) and buffalo heifers (4.20 mmol/l). A number of authors have studied the effect of calving distance on metabolic profile (Campanile et al., 1997) and, according to Bertoni et al., (1994a), the buffalo metabolic response to lactation shows a different pattern compared to other ruminants, as also demonstrated by the low incidence of metabolic disorders. Several researches on buffaloes during the dry milk period and lactation indicated that, among energetic metabolism indicators, serum glucose levels were very constant (Bertoni et al.,1994a; Satriani et al., 2001). Several authors (Setia et al., 1992; Campanile et al., 1997; Montemurro et al., 1997), subsequently, reported increased evidence that nutritional status plays a major role in determining variations of the circulating glucose concentration levels. Low serum glucose levels have been found in buffaloes intaking less than

1 020 Kcals/l of milk of NE/l (net energy/lactation), inversely correlated with the quantity of milk produced, but positively correlated with the distance from calving (Zicarelli et al., 1982; Elthohamy et al., 1994). Other studies have shown an increase of glucose following a rise of T3 and T4 values in cold climates (Campanile et al., 1994). It is well known that early lactation is characterized by a negative energetic balance, less intense in buffalo than in bovines: plasma glucose concentrations are lower during the catabolic phase of lactation, and are higher during the anabolic phase of lactation when energy intake is equal or superior to the energy release (De Rosa et al., 2001). Terzano et al. (1997), studying the effect of feeding systems and puberty onset on blood metabolites in buffalo heifers, found that blood glucose levels remained within physiological ranges with a mean value (4.08 mmol/l) similar to that reported by Campanile et al. (1991) and Bertoni et al. (1994a) in lactating buffalo cows or by Borghese (1994) in buffalo heifers, while Montemurro et al. (1995a) reported lower glucose values in heifers bred in two different farms (2.80-3.44 mmol/l). In the same study the feeding system significantly affected the glucose level as found by other authors (Montemurro et al.,1995a; Zicarelli et al., 1982), according to the energy level of diet: heifers bred in a feedlot and fed maize silage ad libitum (DM 33 percent, crude protein 8 percent, crude fibre 21 percent, 0.85 MFU/kg DM) plus hay (about 20 percent on fed maize silage) and protein-mineral-vitamin supplement, showed higher daily gains and higher glucose concentration than heifers fed natural pasture (50 percent graminaceae, 40 percent leguminosae and 10 percent other species; DM 20-70 percent, crude protein 10-21 percent, crude fibre 18-35 percent, 0.50-0.85 MFU/kg DM). The same authors reported that glucose levels were significantly affected by the onset of puberty since this probably involves a more intensive energy metabolism. Zia-Ur-Rahman et al. (1997), studying the changes in hormones and haematochemical parameters in buffaloes undergoing transport (150-320 km) handling and slaughter stress, found a strong positive correlation between glucose concentration and struggling time and, specifically, glucose levels rose from a basal value of 3.20 mmol/l to 3.80 mmol/l (after transport), to 3.40 mmol/l (after handling) and to 3.80 mmol/l (after slaughter), due to an increase in cortisol levels.

Triglycerides - The values of serum triglycerides are usually considered as indicators of good nutrition, they increase with high-fat diets (Bertoni, 1989), in the presence of an altered regulatory mechanism of the lipid metabolism or due to degenerated hepatic function. Buffalo cows show higher triglycerides values during pregnancy (0.275 mmol/l) (Zicarelli et al., 1986) and in the dry period (0.29 mmol/l) (Bertoni et al.,1994b) than during lactation (0.17 mmol/l); Piccioli Cappelli et al., (2001) reported plasma triglycerides values of 0.19-0.25 mmol/l during the dry period and 0.10 - 0.15 mmol/l during lactation. Serum triglycerides concentrations increase during lactation and show a positive correlation with milk fat levels (Zicarelli, 1988). One study carried out on 306 buffaloes with different days in milk and fed 17 different diets evidenced that triglycerides represent a good index of meeting energy requirements in the early stage of lactation (1-31 days) while total cholesterol and HDL in the intermediate phase (31-110 days) and glycaemia after 110 days of lactation, are also positively correlated with the energy supplied by the diet (Di Palo et al., 1990). It is well known, in fact, that the NEFA which are released at early lactation following intense fat mobilization, are used by the liver for the synthesis of the triglycerides only if the balance between energy absorbed in the diet and that emitted due to production is not especially deficit. Bertoni et al. (1997), clarified the pattern of changes of blood metabolites and hormones, in relation to the energy content and protein degradability of diets (0.17-0.20 mmol/l) for lactating dairy buffaloes showing that plasma triglycerides levels were dependent on the energy level (ranging between 0.10 mmol/l and 0.12 mmol/l) and varied before and after meals (ranging between 0.07 and 0.17 mmol/l). However, serum triglycerides increase in buffaloes under different environmental conditions (low temperatures and season) (Satriani et al., 2001).

Not esterified fatty acids (NEFA) - The concentration of NEFA in blood reflects the degree of adipose tissue mobilization, therefore, the greater the extent of negative energy balance, the more NEFA are released from body fat and the higher the concentration of NEFA in the blood. Animals adapt to negative energy balance by mobilizing energy from adipose tissue in the form of NEFA: metabolic and endocrine factors regulate the rate of NEFA release but with a low

degree of sensitivity. The energy balance is the main determinant of plasma NEFA concentration but other factors have an important influence on plasma NEFA concentration (impending parturition, stress, previous nutritional history, etc.). Buffalo cows in early lactation have higher energy requirements that cannot be supported by dietary intake. As a result, the cow must utilize body fat as a source of energy. Buffalo fat mobilization begins towards the end of gestation (Campanile et al., 1997; Grasso et al., 2004) and in early lactation plasma concentrations of NEFA are high, but never reach the levels found in the cow. In postpartum buffalo cows the plasma NEFA concentration was reported to be highest at d 20, (0.48 mmol/l) then decreasing and returning at about d 110 to the plasmatic levels of the dry milk period (0.17 mmol/l). Bertoni et al. (1997), clarified the pattern of changes of blood metabolites and hormones, in relation with the energy content and protein degradability of diets (0.17-0.20 mmol/l) for lactating dairy buffaloes showing that plasma NEFA levels were dependent on the energy level and varied before and after meals (ranging between 0.07 and 0.17 mmol/l).

Cholesterol - The values of serum cholesterol are usually considered as an indicator of good hepatic lipoproteins production used as carriers of triglycerides, synthesized from NEFA (NEFA-AcilCoA-glicerphosphate---trygliceride s). Total cholesterol and HDL-fractions indirectly reflect the degree of exogenous energy availability and the hepatic functionality: its levels rise owing to a moderate negative energetic balance: lactation, low temperatures and high thermal ranges (Campanile et al., 1994). In a recent study Grasso et al. (2004), observed a marked effect of calving distance on this parameter (ranging between 2.05 and 3.01 mmol/l); in the same study the authors showed that housing systems (intensive vs traditional system) did not markedly affect the plasma cortisol level of buffalo cows. Terzano et al. (1997), studying the effect of feeding systems and puberty onset on blood metabolites in buffalo heifers, reported that serum cholesterol level was not affected by the feeding system (1.93 mmol/l) but it was significantly affected by puberty, showing an increasing trend with a significant difference after puberty (1.83 vs 2.03 mmol/l). The higher concentration of cholesterol with the advancement of age is probably a physiological adjustment to meet growth requirements.

Beta-hydroxybutyrate - Acetoacetate, beta-hydroxybutyrate (BOHB), and acetone are collectively called ketone bodies. Acetone is formed by spontaneous decarboxylation of acetoacetate while the first two are synthesized from acetyl CoA, in the mitochondria of liver cells. Acetyl-CoA can be oxidized to carbon dioxide in the citric acid cycle for the production of energy but during periods of increased fat metabolism and decreased carbohydrate metabolism (fasting, early lactation, diabetics), oxaloacetate may not be available for use in the Tricarboxylic acid cycle (TCA) as it is consumed by gluconeogenesis. The liver mitochondria can convert acetyl-CoA to ketone bodies that, unlike fatty acids and triglycerides, are water-soluble and after having been exported from the liver, are taken up by other tissues, notably the brain, skeletal and cardiac muscles; they are the preferred energy source for the heart muscle and the kidney cortex. There, they are broken down to acetyl-CoA which is oxidized via the TCA cycle to yield energy. Under normal physiological conditions, the production of ketone bodies occurs at a relatively low rate. If the production of ketone bodies exceeds the ability of peripheral tissues to oxidize them, the result is a lowering of the pH of the blood, and a high anion gap metabolic acidosis due to an excessive blood concentration of keto-anions. Blood acidification is dangerous, chiefly as it impairs the ability of hemoglobin to bind oxygen. Beta-hydroxybutyrate is stable, and it is usually present in higher concentrations than the other ketones, it is a more sensitive test to use when monitoring for ketoacidosis and it is stable in whole blood for up to 48 hours at room temperature.

Lean et al. (1992) found that in multiparous cows, milk yield was positively associated with BOHB while dry matter intake was negatively correlated with it. Free fatty acids (FFA) are positively associated with BOHB production, glucose concentrations are negatively cross-correlated with BOHB concentrations and the estimated net energy balance is negatively cross-correlated with beta hydroxybutyrate. In buffaloes, Campanile et al. (1997) found an increase in BOHB blood levels when a greater quantity of protein was fed in the diet, because

of a "relative" energy deficiency. BOHB blood levels found in buffaloes during early lactation period have been 2.69 mg/dl in summer and 4.40 mg/dl in winter (Fagiolo et al., 2004). In buffalo calves it is lower until weaning (1.3-2.4 mg/dl) (Cavallina et al., 2003).

Free radicals and antioxidants

Free radicals - Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Free radicals are very unstable and react quickly with other compounds to capture the electron needed for stability. Generally, free radicals attack the nearest stable molecule making it lose its electron and become a free radical itself. A chain reaction begins. Once the process is started, as a cascade, it can finally result in the disruption of a living cell. Their chief danger comes from the possibility of reaction with important cellular components such as DNA or cell membranes. Cells may function poorly or die if this occurs.

Free radicals arise normally during metabolism and sometimes are purposely created by the body's immune system's cells to neutralize viruses, bacteria and parasites. This has been detected in the buffalo species too: after activation with opsonized zymozan or lipopolysaccharide, buffalo polymorphonuclear cells increase H_2O_2/O_2-production (Singh et al., 1997). Some free radical blood levels found in buffaloes are 94.62 U car during the dry milk period and 80-69 U car in lactation during the catabolic and anabolic phase (Fagiolo et al., 2004; unpublished data).

However, environmental factors such as pollution, radiation and herbicides can also spawn free radicals. To prevent free radical damage the body has a defence system of antioxidants.

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.
Antioxidative status consists of two mechanisms: nonenzymatic and enzymatic mechanisms. Nonenzymatic mechanisms are composed of antioxidants, scavengers of free radicals, transition metal ions, sequester transition metal ions, albumins, ceruloplasmin, and metallothioneins. The principal micronutrient antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, these mechanisms depend on the nutritional status of antioxidant minerals, especially copper, zinc, iron, selenium, silicon, and manganese. The nutritional status of cattle in different regions of the world is often characterized by a lack of these minerals; therefore, there is a great potential for changes in the activity of defence mechanisms against free radicals (Kleczkowski et al., 2003). These micronutrients must be supplied in the diet as in buffaloes, vitamin A, beta-carotene and selenium proved to enhance in vitro phagocytic and kill activities of polymorphonuclear leukocytes isolated around parturition (Ramadan et al., 2001). Moreover immunopotentiation in late gestation with vitamin E and Selenium reduced the calving to first oestrus interval and the length of the postpartum service period, shortening also the uterine involution period (Qureshi et al., 1997). Buffaloes show a deficiency in β–carotene, probably due to rapid transformation into vitamin A. Average values of vitamin A and E are 597 g/l and 175 g/l, respectively. They present the same trend with cholesterol, increasing with the distance from calving and reducing after 120 days of lactation (Campanile et al., 1997).

The most important enzymatic mechanisms which protect an organism against oxidative stress are superoxide dismutase (SOD), peroxidase (Px), e.g. glutathione peroxidase (GSH-Px) and ascorbate peroxidase, catalase and glutathione reductase. Their activity depends on many trace elements. Normally, those mechanisms allow the body to handle free radicals, but if antioxidants are unavailable, or if the free-radical production becomes excessive, damage can occur. New and re-occurring metabolic and infectious diseases of cattle emerge when there is a disproportion in the balance between reactive oxygen species and the antioxidative enzymatic barrier (Kleczkowski et al., 2004). Buffalo polymorphonuclear cells (PMN) present oxygen-dependent and oxygen independent antimicrobial systems (Singh et al., 1997).

Reddy et al. (1988) examined Leucocyte-superoxide dismutase (SOD), GSH peroxidase (GSH-Px), GSH-reductase (GR), GSH-S-transferase (GSH-S-t) and arginase in samples from buffaloes infected with Anaplasma marginale: SH S t, GSH- and glutathione-reductase (GR) levels in leucocytes decreased in infected animals suggesting a decline in the efficiency of the GSH-oxidant defence system. SOD levels increased but there was no change in leucocyte-arginase activity due to infection. Infection caused no significant changes in red cell SOD, GSH-Px, GR and GSH. However, GSH-S-t significantly decreased ($P < 0.05$).
Buffaloes suffering from post-parturient haemoglobinuria showed a drastic reduction in the red cells glutathione content compared to healthy control buffaloes. They also exhibited severe hypophosphataemia (Chugh et al., 1998).
Retinol and alpha-tocopherol levels in milk are correlated to the plasma level of triiodothyronine that enhances the transport of both antioxidants through the blood-mammary barrier (Spagnuolo et al., 2003).

Proteic metabolism

Total protein, albumin, globulin - Unlike lipid metabolism, protein metabolism is not markedly influenced by the energy-protein content in diets or by different environmental conditions. When the protein level of the diets is high, animals enhance gluconeogenesis by amino acids from protein degradation; on the contrary, when the protein level of the diets is low, animals reduce production (meat and milk) and afterwards enhance hepatic protein synthesis and the production of microbial protein which may represent a significant part of total amino acid entering the small intestine of host animals. Thus, microbial protein contributes to satisfying the protein requirement of the animal for tissue maintenance and growth and for milk and wool production.
In a previous study Bertoni et al. (1994b), studying the serum emoprotein levels of lactating buffaloes reported no significant variations during lactation. Campanile et al. (1991), setting the metabolic conditions of healthy and affected buffaloes in farms with a high incidence of endometritis, found that the endometritis affected animals tended to have a significant increase of γ-globulins; the latter, therefore, can represent a useful indicator of this pathological phenomena. Montemurro et al. (1997) reported low serum total protein and globulin levels in buffaloes before calving and after 45 days of lactation. The same authors reported high serum albumin levels at the end of pregnancy, while the serum total protein levels have been found to show very little variation during lactation, as reported by Campanile et al. (1997). Montemurro et al. (1995a) showed that, in heifers, different seasons and feeding did not markedly affect the serum total protein, albumin and globulin levels. On the contrary, Setia et al. (1992) and Campanile et al. (1994) found a significant effect of feeding systems on serum total protein levels. In the study of Terzano et al. (1997), feeding systems affected serum total protein levels as heifers fed natural pasture benefited from more protein than the heifers bred in the feedlot and fed maize silage ad libitum (69.4 g/l vs 73.2 g/l, respectively). The same authors reported serum total protein levels to be significantly affected by puberty, showing an increasing trend with significant differences after puberty (69.0 g/l vs 73.6 g/l). Zia-Ur-Rahman et al. (1997), in a study on hormonal and haematological profiles in buffaloes after transport, handling and slaughter stress, found higher serum total protein and albumin levels after transport, higher serum total protein after handling, higher serum albumin levels and lower serum globulin levels after slaughter.

Urea - The concentration of urea in the blood reflects the degree of protein catabolism and is synthesized in the liver from CO2 and NH3. Serum blood urea levels are influenced not only by renal function but also by external factors. In fact, when the protein level of diets is excessive or when the energy/protein ratio is very low an insufficient ruminal protein synthesis follows; a high quantity of ammonia is formed and absorbed through the ruminal wall into the portal blood and is converted to urea in the liver. This, in the long run, may cause hepatic failure and degeneration causing infertility, mastitis, puerperal collapse, lameness and steatosis. When the scarcity of protein is heavy and lengthened, the protein synthesis is reduced and changes in haematochemical parameters and in productive and/or reproductive functions follow. The

values of serum urea are also considered as indicators of stress conditions as they rise after a too high protein degradation under the stimulus of adrenalin and cortisol hormones (Maianti et al., 1990). In buffaloes serum urea levels rise during lactation, whether by high protein intake or by tissue protein mobilization. In fact, Montemurro et al. (1997) found that serum urea levels in buffaloes rose from a basal value of 5.48 mmol/l (before calving) and of 5.15 mmol/l (at 45 days of lactation) to a peak of 10.29 mmol/l (at 160 days of lactation). Bertoni et al. (1994b) reported urea values of 5.48 mmol/l during the dry milk period and 6.14 mmol/l during lactation. In buffalo cows, as well as in dairy cows, dietary protein characteristics and protein/energy (P/E) ratio influence urea levels in blood serum and milk (Campanile et al., 1996). As the buffalo cow adapts itself better to a lack of protein than the dairy cow, Bertoni et al., 1993 and Campanile et al. (1996), reported low values of serum and milk urea levels, compared to the cow, when the protein content in the diet decreased from 12 percent to 9 percent. The same authors found that longterm diets with protein concentrations of less than 9 percent did not determine a further lowering of serum urea levels, but rather caused an increase in the milk freezing point, especially if the diet showed a high amount of fermentable energy. It would seem relevant, therefore, to evaluate the protein/energy ratio in order to obtain a correct diet. In buffalo such a ratio can be increased: diets with a high protein concentration, in fact, determine less harmful effects in buffalo compared to that in bovine milk cows. Buffalo cows make better use of ingested nitrogen than bovine cows, even if there is a carbohydrate deficit, since buffalo ruminal milieu is more favourable to the growth of microrganisms, using non proteic nitrogen (Langer et al., 1969). Serum and milk urea levels are also affected by the crude protein/non structural carbohydrate (CP/NSC) ratio in buffalo cows. Specifically, Smith (1969) reported that nitrogen deficiency, in ruminants living in tropical areas, reduces renal clearance of urea increasing its return to the rumen and decreasing its haematic levels. This, in turn, would promote a better urea utilization in the digestive tract and a better protein synthesis by ruminal bacteria (Houpt, 1970).

Serum blood urea is influenced by the days in milk (Campanile et al., 1997; Grasso et al., 2004), by the diet (Campanile et al., 1997) and by the season (Satriani et al., 2001). The values of serum blood urea are considered to be an indicator of total protein intake and its determination with creatinine is important in order to exclude renal damage, which is fairly frequent in this species.

In the study of Terzano et al. (1997), the feeding system affected the serum blood urea levels as heifers fed natural pasture benefited by more protein than heifers bred in the feedlot and fed maize silage ad libitum (9.82 mmol/l vs 3.60 mmol/l). The same authors reported that serum blood urea levels were not significantly affected by puberty (mean value: 6.71 mmol/l).

Blood serum enzymes activity

The high variability in the blood activity of serum enzymes suggests considering age, physiological conditions and lactating period in establishing reference values (Pizzuti and Salvatori, 1993).

Asparagine aminotransferase (AST or GOT) - This enzyme transfers the amminic group from aspartate to -ketoglutaric acid, forming glutamate and oxaloacetate. In ruminants, AST is present in greater concentrations in the muscles and heart as opposed to the liver, and increases in serious cases of prolonged fasting and infectious, infestious and nutritional liver disease. In fact it is localized at the mitochondrial level, so that it increases only in the case of extensive hepatic necrosis. It can be referred to muscular cells damage and tissue changes related to the neonatal phase in buffalo calves (Campanile et al., 1997). In buffalo heifers near to puberty, values of 216 U/l have been found (Borghese, 1994). Finally in adult buffaloes AST varied from 120 to 145 U/l in the dry milk and lactating period, respectively; higher levels were measured at 30 and 190 days of lactation (Bertoni et al., 1994a). In the early months of lactating, De Rosa et al. (2001) detected values ranging from 143 to 160 U/l. Decreasing metabolism is linked to lower AST hematic concentrations, while thyroid hormones,

particularly T4, induce a greater emission of the enzyme increasing mitochondria membrane permeability (Campanile et al., 1997).

Alanine aminotransferase (ALT or GPT) - This enzyme transfers the aminic group from alanine acid to ketoglutaric acid, forming glutamate and piruvate. In ruminants, ALT is present in small quantities in the liver and in various tissues, particularly in the muscles. It is in fact referred to, together with AST, as an index of muscular integrity. In buffalo heifers Borghese (1994) found values of 60 U/l, in adult buffaloes at different pre- post- partum time intervals, Pizzuti and Salvatori (1993) found ALT values ranging from 176 to 219 U/l. In the early months of lactation, De Rosa et al. (2001) detected values ranging from 83 to 116 U/l. ALT and AST, together with hemoglobin, increased in autumn, in buffaloes fed at pasture until early autumn. Decreasing metabolism is linked to lower ALT hematic concentrations, while thyroid hormones, particularly T4, induce a greater emission of the enzyme, increasing mitochondria membrane permeability (Campanile et al., 1997).

g-glutamyltransferase (GGT) - GGT is a membrane linked enzyme that transports amino acids inside the cells. It is present in various tissues in the kidney, pancreas, mammary gland, liver and so on. In water buffalo mature females, the enzyme GGT was significantly higher compared to that in immature females (Canfield et al., 1984). This has been verified by Borghese (1994) who found values of 24.7 U/l in buffalo heifers, and by Bertoni et al. (1994a), who recorded, in adult buffaloes, a GGT variation from 29 to 35 and 41 U/l in the dry milk period and 30 and 230 days of lactation, respectively. In the early months of lactation, De Rosa et al. (2001) detected values ranging from 37 to 52 U/l. It is an important index in liver diseases as it is the first serum enzyme that increases even in mild liver disease. GGT decrease during the growth period has been recorded from 35 U/l (at 30 days) to 18 U/l (at 50 days of age). This progressive lowering in GGT and g-globulines values, after the increase due to absorption during the colostral phase, is an index of good liver and kidney function (Campanile et al., 1997).

LDH - This is a blood test that measures the amount of lactate dehydrogenase (LDH). LDH is a cytoplasmic enzyme that converts piruvic acid into lactic acid. The enzyme comes from the myocardium, skeletal muscles, liver, kidneys, pancreas, red blood cells and the lungs. In adult healthy buffaloes, LDH values (U/l) ranging from 1272 to 1741 and from 713 to 1047, have been found in different housing and seasonal conditions (Terzano et al., 2000; Fagiolo et al., 2004). Lower values have been found for calves: 1325 UI/l (Cavallina et al., 2003, unpublished data). Higher-than-normal levels may indicate: intestinal ischemia (blood deficiency) and infarction (tissue death); liver disease (for example, hepatitis); muscle injury; muscular dystrophy; neoplastic (new abnormal tissue formation) states; pancreatitis; pulmonary infarction (tissue death); heart attack; hemolytic anaemia; hypertension. In the neonatal phase, the growth and changes that involve a turbulent synthesis activity, are responsible for the progressive increase of the LDH serum levels in buffalo calves. In adult buffaloes, higher LDH values are a proof of hepatic sufferance induced for example by acidosis, that causes a greater effort to be made by this organ in transforming lactic acid into propionic acid (Campanile et al., 1997).

Alkaline phosphatase - ALP is an enzyme made in the liver, bone, and the placenta and is normally present in high concentrations in growing bone and in bile. The enzyme is termed alkaline phosphatase because it works under alkaline (non-acidic) conditions, as opposed to acid phosphatase. Alkaline phosphatase is released into the blood during injury and during such normal activities as bone growth and pregnancy. Abnormally high blood levels of ALP may indicate disease in the bone or liver, bile duct obstruction, or certain malignancies. However, higher values are found in buffaloes during the first 40 days of life due to the more intense bone remodelling. An increase of ALP during early lactation in buffaloes is proof of speedy parathyroid activation (Campanile et al., 1997). Pizzuti and Salvatori (1993) found differences in the ALP mean values of buffaloes at various distances from partum, increasing in the advanced phases of pregnancy and decreasing before parturition; they ranged from 159 to 228 U/l. Terzano et al. (2000) reported ALP values ranging from 200 to 650 U/l, in adult

buffaloes under different housing conditions.

Micro - and macroelements

The body uses over 80 minerals for its maximum function. Every living cell depends on minerals for its proper structure and function. Nutritionally, minerals are grouped into two categories: bulk or essential minerals, also called macrominerals, and trace minerals or microminerals. Macrominerals such as calcium and magnesium are needed by the body in larger amounts. Although only minute quantities of trace minerals are needed, they are nevertheless important for good health. Microminerals include boron, chromium, iron, zinc, and many others. Essential trace elements range from metals to non-metals. During the buffalo dry period, even minimum but long-term deficiencies can cause damage that will influence the health state of the following lactation (Campanile et al., 1997).

Ca - Most calcium in the body, about 90 percent, is in the bones, where it can be reabsorbed by blood and tissue, but about one percent is used for nerve impulses and muscle contractions (including the heart, kidney, and other organs). Calcium participates in the protein structuring of RNA and DNA, and also contributes to the formation of intracellular cement and cell membranes. It helps in normalizing blood clotting action, to metabolize the body's iron and it is more effective when combined with: vitamins A, C and D. Buffalo calcium blood levels show limited variability during lactation and dry milk period (Bertoni et al., 1994a); higher levels have been found in the last month of pregnancy and lower ones at the end of the lactation period (Montemurro et al., 1997). A seasonal variation has been evidenced with higher values during the winter. Ca/P ratio is maintained at 1.4-1.6 (Montemurro et al., 1995a). Campanile et al. (1997) found constant values of about 10 mg/dl, confirmed by Terzano et al. (2000) who found 8.87-10.63 mg/dl of Ca blood levels in adult buffaloes. In buffalo species calcium excesses could alter the Ca/P ratio during the dry milk period, inducing parathyroid hypoactivity which would cause magnesium to increase and calcium to decrease at the beginning of the lactation due to a non immediate calcium mobilization by the bones. The altered Ca/Mg ratio favours utero-vaginal muscular release, responsible for uterus atony and eventually uterine prolapse (Campanile et al., 1997).

Phosphorus - Phosphorus is the second most plentiful "essential mineral" in the body and is a key component of DNA, RNA, bones, teeth, and many other compounds required for life. It plays an important role in the energy metabolism of cells, affecting carbohydrates, lipids and proteins. Phosphorus also stimulates muscle contraction and contributes to tissue growth and repair, nerve-impulse transmission, central nervous system health, and proper heart and kidney function. Phosphorus deficiency during buffaloes' dry milk period is responsible for the most frequent causes of vaginal and/or uterine prolapse in this species. If the diet is deficient in P before calving, calcium levels decrease upon calving while phosphatemia is normal (Campanile et al., 1997). On the other hand, diets rich in silage and/or concentrates (>59 percent), have been associated with high P and Cu hematic values and subclinical metabolic acidosis, that is frequently connected with uterine prolapses and endometritis (Campanile et al., 1997). Phosphorus levels in buffaloes have been found to be quite stable at six mg/dl (Campanile et al., 1997). An increasing trend has been evidenced starting from the pre-partum period (6.3 mg/dl) to 160 days of lactation (7.9 mg/dl) (Montemurro et al., 1997).
In water buffalo mature females, inorganic phosphate was significantly higher compared to that of immature females (Canfield et al., 1984). Buffalo heifers showed higher P blood levels in winter (Montemurro et al., 1995a). Buffaloes suffering from post-parturient haemoglobinuria showing a decrease in the reduced glutathione content in the red cells, also exhibited severe hypophosphataemia (Chugh et al., 1998).

Potassium - Potassium is the third most abundant mineral in the body, after calcium and phosphorus. Potassium works closely with sodium and chloride to maintain fluid distribution and pH balance and to augment nerve-impulse transmission, muscle contraction, and regulation of heartbeat and blood pressure. Potassium is also required for protein synthesis,

carbohydrate metabolism, and insulin secretion by the pancreas. It works with sodium to regulate the body's water balance. Deficiencies are rare in ruminants while excesses in the diet can reduce Mg, Ca and P digestion. Low blood levels have been observed in cattle fed with high concentrate levels and have also been associated with stress conditions. In buffaloes physiologic values range from 4 to 5 mmol/l (Bertoni et al., 1999).

Sodium - Sodium is one of the three main electrolytes in the body. All body fluids - including blood, tears, and perspiration - contain sodium. Together with potassium and chloride, sodium maintains fluid distribution and pH balance; together with potassium, sodium also helps in the control of muscle contraction and nerve function. Blood levels are strictly controlled as a result of the surrenalic hormone aldosterone, so alterations are usually only possible in cases of homeostasys problems. Buffaloes' hematic values are 144-146 mmol/l during the dry milk period and 143-147 mmol/l in early lactation (Bertoni et al., 1999).

Magnesium - Magnesium is involved in more than 300 enzymatic reactions. Magnesium is essential for the conversion of vitamin D to its biologically active form which helps the body absorb and use calcium. The highest magnesium concentration is found in the tissues that are most metabolically active including the brain, heart, liver and kidney. Magnesium is a key substance in the proper functioning of nerves and muscles. It is also required for the healthy maintenance of bones. Mg deficiency is well known as grass tetany, while the excess during post partum puerpueral collapse seems to be caused by renal failure. Physiologic values in buffaloes are 1.1-1.3 mmol/l in the dry milk period and 1.2-1.4 mmol/l during lactation (Bertoni et al., 1999).

Iron - Iron mainly works in the red blood cells hemoglobin, which transport oxygen from the lungs to the body's tissues, including the muscles and the brain. Iron is also a component of myoglobin, a similar protein in the muscle, that stores and provides oxygen during muscle exertion and is found in the part of the cell involved in energy production and as a co-factor for several enzymes. The diet of ruminants usually provides enough iron and it is well utilized in digestion (Bertoni et al., 1999). Iron deficiency generally occurs during the growth period or when intakes fail to replace the iron loss associated with blood losses. Excessive amounts of phosphates, calcium, zinc and manganese can also inhibit iron absorption. When iron stores are depleted and there is an inadequate production of heme (the portion of hemoglobin associated with iron), the red blood cells become small (microcytic) and have a decreased capacity to carry oxygen. There is also a drop in iron-containing enzymes that are important in cellular metabolism. This results in decreased work capacity, fatigue, paleness, dizziness, sensitivity to cold, irritability, heart palpitations and altered behaviour.
Because iron strengthens the immune function, its deficiency may also increase susceptibility to infection. Iron is also an important nutrient for bacteria, that is why one of the body's natural defence mechanisms during infections is to reduce plasma iron, in order to inhibit bacterial growth. Cavallina et al. (2003) found mean iron blood levels in buffalo calves at one and three months of age of 96 and 143 mmol/l, respectively (unpublished data). In lactating buffaloes Fagiolo et al., (2004) found seasonal differences in iron blood levels: higher in winter than in summer (150.07-61.81 mmol/l) though not significantly. Iron blood level analysis can be altered by different factors, it is sometimes preferable to evaluate it indirectly by means of hemoglobin and packed cell volume.

Selenium - Selenium is an essential non-metallic element. Selenium is important for the function of several proteins. One of these is glutathione peroxidase, an enzyme that prevents cellular oxidative damage from a variety of peroxides. It is said to stimulate the metabolism. Together with vitamin E, it is extremely important in preventing free radical damage to cell membranes.
Selenium also supports the immune function and neutralizes certain poisonous substances such as cadmium, mercury, and arsenic. Selenium proved to enhance phagocytic activity in buffalo polymorphonuclear leukocytes starting from parturition up until three weeks post-partum (Ramadan et al., 2001).

Animals grown for the purposes of meat production, in areas with soil deficient in selenium, develop "white muscle disease." Selenium was not detectable in many pregnant buffalo cows suggesting that the prevention of myodistrophy in buffalo calves must be effected in the prenatal period (Pizzuti and Salvatori, 1993).
Symptoms of selenium deficiency include muscle weakness and pain, inflammation of the muscles, fragile red blood cells and degeneration of the pancreas.
Animals grazing on plants that have accumulated selenium show acute or chronic selenium poisoning. Chronic selenium toxicity (alkaline disease) is characterized by muscle degeneration, rough coat, laboured breathing and cardiovascular failure. Selenosis induced in male buffalo calves was associated with an increased activity of the erythrocyte glutathione peroxidase (GSH-Px) and signs of selenium toxicity at Se levels of about 2.0 µg/ml (Deore et al., 2002).

Zinc - Zinc is a part of every cell in the body and forms part of over 200 enzymes that have functions ranging from proper action of body hormones to cell growth.
Zinc is an extremely important mineral for many body functions, down to the very core structure of cells. Zinc is integral to the synthesis of RNA and DNA, the genetic material that controls cell growth, division and function. In various proteins, enzymes, hormones, and hormone-like substances called prostaglandins, zinc contributes to many body processes such as: bone development and growth, development of the testicles, skin integrity, appetite, aiding enzymes in digestion and energy metabolism, cell respiration; wound healing; the liver's ability to remove toxic substances from the body; immune function and the regulation of heart rate and blood pressure. Zinc is a co-factor for many enzymes, which means that it is necessary for the proper functioning of these enzymes. ALP is rapidly inhibited by Zn deficiencies.
Zinc is a critical nutrient of immunity, being involved in so many immune mechanisms including cell-mediated and antibody-mediated immunity, thymus gland function and thymus hormone action. When zinc levels are low, the number of T cells is reduced and many white blood functions critical to the immune response are severely lacking. Like vitamin C, zinc also possesses direct antiviral activity, including activity against several viruses. It is also present in members of a class of proteins called the metallothioneins that are believed to provide antioxidant protection by scavenging free radicals. In buffaloes it normally ranges from 10 to 12 µmol/l in the dry period and from 9 to 12 µmol/l during lactation (Bertoni et al., 1999).
Zinc deficiency may be associated with long-term hypo-nutrition, higher requirements during pregnancy or diseases of the intestine such as paratuberculosis. Excessive zinc interferes with the function of copper and iron.

Hemogram (CBC)

This test evaluates the number and status of red blood cells, white blood cells, and platelets. It screens for anaemia, leukemia, polycythemia, and other disorders that affect blood cells. Buffalo haematological values are comparable with those found in adult cattle (Ciaramella et al., 2005).

Hematocrit (HCT) - This test, also called packed cell volume (PCV), measures the amount of space (volume) red blood cells occupy in the blood. The value is given as a percentage (%, vol/vol) of red blood cells in a volume of blood. For example, a hematocrit of 38 means that 38 percent of the blood's volume is composed of red cells. It is the quickest and most accurate measure of the red cell component of blood. PCV is higher in heifers than in adult buffaloes (Ciaramella et al., 2005). It has shown seasonal differences in lactating buffaloes, proving to be higher in the summer (40.75 percent) than in the winter (32.63 percent) (Fagiolo et al., 2004).

Hemoglobin - Hemoglobin is the major substance in red blood cells. It carries oxygen and gives the red colour to blood cells. The hemoglobin test measures the amount of hemoglobin in blood and is a good indication of the blood's ability to carry oxygen throughout the body. Hemoglobin concentration (Hb) is reported as grams of hemoglobin per decilitre of blood (g/dl). In lactating buffaloes, Hb was found to be higher in the summer (13.62 g/dl) than in the winter (11.37 g/dl)

(Fagiolo et al., 2004).

Mean Corpuscular Volume (MCV) - MCV measures the average size of red blood cells. The mean cell volume indicates the volume of the "average" red cell in a sample. It is expressed in femtolitres (fl; 10-15 litres). In lactating buffaloes mean values in different seasons were about 53 and 56 fl, without significant differences (Fagiolo et al., 2004).
Red cell populations with an MCV above reference range are termed macrocytic; if the volume is normal: normocytic. Common causes of macrocytosis are reticulocytosis and myelodysplastic syndrome (MDS). Common causes of microcytosis are iron deficiency anaemia and chronic liver disease.
A regenerative response can be seen as a population of macrocytic hypochromic cells extending off the normal red cell population. Agglutination can be seen as a macrocytic normochromic cluster of cells that is discrete from the normal red cell population.

Mean Corpuscular Hemoglobin (MCH) - The MCH value is the amount of hemoglobin in an average red blood cell. MCH is the mean cell hemoglobin. This represents the absolute amount of hemoglobin in the average red cell in a sample. Its units are picograms (pg; 10-12 liters) per cell. In lactating buffaloes mean values in different seasons were about 17.8 and 19.5 pg, without significant differences (Fagiolo et al., 2004). It is normally lower in heifers (Ciaramella et al., 2005); in adults a low MCH could be due to smaller than normal cells with normal Hb concentration or normal sized cells with lower than normal Hb concentration. It is preferable to have exact information regarding cell volume and Hb concentration directly.

Mean Corpuscular Hemoglobin Concentration (MCHC) - The MCHC measures the concentration of hemoglobin in an average red blood cell. MCHC is the mean cell hemoglobin concentration, expressed in g/dl. In lactating buffaloes mean values in different seasons were about 33.5 and 34.8 g/dl, without significant differences (Fagiolo et al., 2004). It is lower in heifer buffaloes (Ciaramella et al., 2005). Red cell populations with values below the reference range can be termed "hypochromic". This can occur in strongly regenerative anaemia, where an increased population of reticulocytes with low Hb content "pull" the average value down. Low MCHC can also occur in iron deficiency anaemia, where microcytic, hypochromic red cells are produced as a result of the lack of iron to support hemoglobin synthesis. Values for MCHC significantly above the reference range are not physiologically possible due to limitations on the solubility of Hb. Lipemia or other causes of turbidity in the lysate can cause falsely high values, which raises the apparent MCHC. Furthermore, hemolysis (in vitro or in vivo) will cause a lowering of the HCT and increase the MCHC.

Red Cell Distribution Width (RDW) - The RDW is an index of the variation in cell volume within the red cell population. The RDW indicates whether all the red cells are about the same width, size, and shape. This helps further classify the types of anaemia.
Red cell populations with higher than normal RDW are termed heterogenous; those with normal RDW are homogeneous. For example, increased numbers of reticulocytes will cause an increased RDW. In some instances, the RDW is the first test result to increase with changes in red cell population sizes. For example, in early iron deficiency, there are only low numbers of microcytic red blood cells. This will increase the standard deviation and the RDW, but the mean cell volume is unchanged because there are insufficient numbers of microcytic cells to change the mean volume. In lactating buffaloes mean values in different seasons were about 17.5 and 16.2 percent, without significant differences (Fagiolo et al., 2004).

Red Blood Count - The red blood cell count on the routine CBC is the concentration of red cells, expressed in millions/µL of whole blood (106/µl). In lactating buffaloes this parameter showed seasonal variations from $7.7\text{-}10^6$/µl in summer to $5.8\text{-}10^6$/µl in winter (Fagiolo et al., 2004).

White Blood Count - White blood cells protect the body against infection and are bigger than red blood cells and normally fewer in number. In case of bacterial infection, the number of

white cells can increase dramatically, so the number of white blood cells is sometimes used to identify an infection White cell count (WBC), the total number of leukocytes in a volume of blood, is expressed as thousands/µl (106/µl). In buffaloes above eight years of age it is lower (Ciaramella et al., 2005).

Neutrophils - Cells of the neutrophil line are classified by the shape of their nuclei. Cells with nuclei whose sides are parallel, or nearly so, or that have smooth nuclear outlines are classified as band neutrophils. Band neutrophils and metamyelocytes are blood neutrophils less mature than segmented neutrophils. Segmented neutrophils have nuclei with focal areas that are distinctly narrower than the width of the widest points and usually have irregular nuclear outlines. Fagiolo et al. (2004) found seasonal changes in early lactating buffaloes neutrophil percentage: from 64 percent in summer to 7 percent in winter.

Lymphocytes - Lymphocytes are the most numerous cell type in the buffalo species. Characteristic features include a dense, round nucleus and a scant rim of pale blue cytoplasm. Healthy ruminants have a wide range of lymphocytes in peripheral blood; many are quite large. In addition, in all species, there are low numbers of lymphocytes with small red cytoplasmic granules, so-called granular lymphocytes. These are either natural killer cells or cytotoxic T-lymphocytes and are involved in cell-mediated immunity. Lymphocytes, unlike the other leukocytes, are produced in lymphoid tissue and in bone marrow. Most of the lymphocytes in blood are long-lived cells that recirculate between blood and tissue. Changes in blood lymphocyte number usually reflects changes in distribution rather than changes in production or loss. Early lactating buffaloes showed a decrease in lymphocytes during the summer (41 percent) with respect to winter values (77 percent) (Fagiolo et al., 2004). In buffaloes above eight years of age there is a significant reduction in the absolute values of lymphocytes (Ciaramella et al., 2005).

Monocytes - Monocytes share a common committed stem cell with neutrophils. They are produced in the marrow, circulate briefly in the blood, and migrate into the tissues where they differentiate further to become macrophages.
There is no storage pool of monocytes in the marrow; their numbers in the marrow at a given time are very small. Monocytes in blood are distributed between a marginated and circulating pool. Cytokines (e.g. M-CSF, GM-CSF) produced at sites of inflammation can increase monocyte production. In buffaloes mean values are usually near to 0 percent (Fagiolo et al., 2004).

Eosinophils - Eosinophils are produced in the marrow, circulate in the blood for a few hours, and migrate into the tissues where they survive for several days. Increased production of eosinophils is mediated by interleukin-5 and interleukin-3, which are produced by several cell types, but especially T lymphocytes and mast cells. Corticosteroids decrease the blood eosinophil number but increase the marrow pool of eosinophils.
Increased numbers of circulating eosinophils may be seen in hypersensitivity reactions, as with certain forms of parasitism and allergic conditions. The presence of mast cell tumours in an animal may also be associated with eosinophilia. Increased numbers of basophils (basophilia) sometimes occurs concurrent with eosinophilia. Fagiolo et al. (2004) found the following mean percentage values for early lactating buffaloes in different seasons: 1.64 - 0.16 percent. In water buffalo mature females, eosinophils were significantly higher compared to those in immature females (Canfield et al., 1984) and buffaloes over ten years of age show higher absolute values of eosinophil levels (Ciaramella et al., 2005).

Basophils - Basophils, in general, contain dark purple granules in the cytoplasm. Basophils are produced in the marrow. The number in the blood is very small in all species as in the buffalo species (Fagiolo et al., 2004).

Platelet Count - Platelets (thrombocytes) are the smallest type of blood cell. They play a major role in blood clotting. When bleeding occurs, the platelets swell, clump together, and form a sticky plug that helps stop the bleeding. If there are too few platelets, uncontrolled bleeding

may be a problem. If there are too many platelets, there is a risk of a blood clot forming in a blood vessel. Platelet counts can be performed manually or using automated cell counters. Platelet clumping lowers the platelet count when determined by any method. Platelet clumping is usually due to a sample collection problem and can be minimized by collecting blood from a large peripheral vein (jugular). The blood should be mixed with an anticoagulant as soon as possible after collection, by gentle rotation or inversion. Platelet clumping increases with time, so platelet counts should be done as soon as possible after collection to maintain accuracy. In lactating buffaloes mean values in different seasons were about 251.8 and 201 10^3/µl, without significant differences (Fagiolo et al., 2004).

Mean Platelet Volume (MPV) - Individual platelets can vary markedly in size within a given sample. Little is documented in literature regarding the clinical interpretation of this parameter. In very general terms, increased MPV might be expected in "regenerative" thrombocytopenia, i.e., that caused by increased peripheral loss, destruction, or utilization of platelets and accompanied by increased production of platelets by marrow (megakaryocytic hyperplasia). Accelerated thrombopoiesis tends to result in the release of larger platelets (which also have enhanced functional capabilities). Fagiolo et al. (2004) found the following mean values in early lactating buffaloes in different seasons: 8.8 and 9.7 fl.

The immune system

The immune system is an incredibly intricate mechanism that prevents infections and diseases by moderating malignant and foreign cells within the body. To evaluate the immune responses of water buffalo to infectious agents and potential vaccines, it is necessary to characterize the immune system and elucidate the changes in the immune response that account for the development of protective immunity (Davis et al., 2001).
The immune system shows two types of response: non-specific (innate) and specific (adaptive/acquired).
The first one mainly depends upon monocytes, neutrophils (granulocytes, eosinophils, basophil), complement, interferon and lysozyme activity whereas the second one relies on T-cells and B-cells production. The immune system is composed of several organs and systems, as well as various types of immune cells. Organs that constitute the immune system - known as lymphoid organs -include the spleen and the thymus. Additional components of the immune system are lymph nodes and bone marrow. Within the bone marrow, lymphocytes (white blood cells) are created which function as immune cells. Neutrophils are the main circulating white blood cells, which seek out invaders when they are summoned into action by other immune cells. They secrete toxins that kill antigens (invading proteins) and devour them. The two major classes of lymphocytes are B-cells, which mature in the bone marrow and reside in the lymph system, and T-cells, which mature in the thymus and circulate throughout the body. B-cells are responsible for producing antibodies (immunoglobulins), which are proteins designed to recognize and mark a specific antigen, while T-cells are charged with destroying antigens that are tagged with an antibody.
There are three types of T-cells: cytotoxic T-cells, helper T-cells and suppressor T-cells. Cytotoxic T-cells attach themselves to malignant or infected cells. They secrete interferons, which stop viruses from reproducing. Helper T-cells including TH1 and TH2 assist cytotoxic T-cells by recognizing an attack on the body, at which point cytotoxic T-cells are sent to fight the infection. Helper T-cells also help the body's B-cells produce antibodies. Suppressor T-cells are responsible for regulating the body's immune response to invasions; they stop cytotoxic T-cells from releasing cytokines (immuno-regulatory substances) and prevent the production of immunoglobulins. Five types of immunoglobulins are normally distinguished but in water buffalo and in the cow four classes exist.

IgA, IgG1 and IgG2, IgM and IgE - IgA is responsible for holding off invaders or pushing them out of the body, which is why it exists in tears, milk, sweat and saliva. IgG is specifically designed to kill certain bacteria and viruses, and activates enzymes that digest invaders. Tripaldi et al., (2003) reported an IgG titre of 0.07 ± 0.03 OD_{450} in adult buffalo cows.

IgM circulates in the blood stream to kill bacteria. IgE plays a remarkable role in ruminant colostrum, since this class is transmitted to newborn calves during the first days of lactation providing a passive immunity against several parasite species.
Additional immune cells are scavenger cells called phagocytes which include granulocytes and macrophages. These cells seek out and devour invading cells. Macrophages are usually stationary and protect a specific area, although they are also known to travel to a point of infection to assist in warding off an antigen. They release pyrogen, a substance that alerts the body to increase temperature to induce fever, which is often useful for killing pathogens. Another scavenger, the natural killer (NK) cell, destroys invaders without seeking out B-cells' tags. NK cells search for foreign cells and kill them by releasing toxic enzymes and interferons. Just as NK cells secrete interferons, other immune cells secrete interleukins, another type of cytokine, or immuno-regulatory substance.

These substances, which include monokines (secreted by monocytes) and lymphokines (secreted by lymphocytes), are responsible for regulating the body's immune response, such as the magnitude of an inflammation. Interleukins, which are secreted by macrophages, monocytes and some T-cells, include more than 30 types. Interleukin-1 is produced by macrophages and is involved in inducing fever, which can kill or slow down a virus or bacterium. Interleukin-2 assists helper T-cells in encouraging cytotoxic T-cells to kill invaders. Interleukin-4 enhances the B-cells' ability to produce antibodies (IgG and IgE in particular), and it stimulates helper T-cells and cytotoxic T-cells. Interleukin-6 is released by macrophages, monocytes and some T-cells, and induces B-cells to produce antibodies. Tumour necrosis factor (TNF) is released by macrophages to induce fever. It can kill cancer cells and promotes the production of lymphokines. With all of these cells and processes for fending off invading microbes, fungi, bacteria and viruses, the immune system has its work cut out for it, and there are numerous external factors that can disrupt the body's process for fighting off infection such as anxiety and depression, as well as malnutrition. Since malnutrition adversely affects immune function, ensuring proper nutrition through the diet is essential for protecting and maintaining the immune system. In addition to nutrient intake, several botanical and nutritional substances work to enhance the various activities that make up immune function (Abbas-Lichtman, 2000). Analysis of leukocytes in peripheral blood of young and adult buffaloes has revealed that the composition of leukocyte populations in water buffalo is similar to that in cattle. One of the unique features to emerge from the study of the immune system of cattle is the presence of two complex sub populations of gdT cells, one sub population that is similar to gdT cells as observed in humans and other species and a second sub population that has only been identified in sub orders of Artiodactyla, Ruminantia, Suiformes, and Tylopoda. As in cattle, the WC1+ population of gdT cells in buffaloes was comprised of subsets that express the WC1-N3 and WC1-N4 isoforms. The frequency of WC1+ gdT cells was high in young animals and low in adults. There were corresponding differences in the frequency of CD2+ abT cells in young and adult animals. There was no apparent correlation in the frequency of B cells with the frequency of WC1+ gdT cells in young and adult animals (Davis et al., 2001). Recently, Tripaldi et al., (2003), studying the effect of two different housing systems on a range of behavioural and physiological variables, found no difference in the immune response following a percutaneous injection of phytohaemagglutinin (7.73 ± 0.4 mm - 6.32 ± 0.4 mm); 40 days after injection, the IgG titre increased from 0.07 ± 0.03 OD_{450} to 1.33 ± 0.03 OD_{450}.

Lysozyme - Lysozyme is a protein present in many tissues and secretions which was able to interfere with the growth of some specific bacterial colonies. This lysing element was called "lysozyme" by Fleming himself who went on to study its different characteristics and in 1922 isolated the enzyme from hen egg white, other tissues and biological secretions of living organisms. Some years later the bactericidal activity of lysozyme was widely substantiated and after 1930 many studies revealed how in nature every living organism, both in the animal and the plant kingdoms, produces lysozyme. The term "lysozyme" (or rather lysozymes considering their ubiquity and their various structural differences) refers to an enzyme with well-defined hydrolasic activity. In nature different types of lysozyme exist with different characteristics according to their origin. It is difficult to distinguish between human lysozyme, which is

contained in various secretions such as tears and saliva, and the lysozyme present in products belonging to the animal and vegetable kingdom. In animal serum lysozyme destroys bacteria, mostly Gram positive. In fact, this enzyme preferably hydrolyzes the b1,4 glucosidic linkages between N-acetylmuramic acid and N acetylglucosamine which occur in the mucopeptide cell wall structure of Gram positive microorganisms. An elevated level of serum lysozyme signifies a good non specific immune response. Cavallina et al. (2003) found a decreasing trend in buffalo calves' lysozyme from the first to the third month of age and following weaning, with mean values ranging from 3.8 to 0.33 µg/ml. In water buffalo during lactation, Fagiolo et al. (2004) detected seasonal differences with higher lysozyme levels in summer (2.7 µg/ml) than in winter (0.84 µg/ml).

Bactericide activity - Among aspecific immune responses there is bactericide activity. This particular serum activity permits the elimination of some antigens, mostly Gram negative microrganisms.
Furthermore, these antibodies may be prevalently produced from birth as a response to stimulations, both from intestinal flora and heterogenic antigens of non-bacterial origin.
Naturally the defensive power and therefore the natural resistance of an organism during first infection, depend upon the casual affinity that the pathogenic strain shows towards this antibody. In any event these antibodies show their best disposition against Gram negative bacteria.
The bactericide test is based on the principle that exposing numerous Gram negative strains to human and animal serum cause the lysis of a percentage of germs. In this procedure, serum samples are tested with E. coli culture, in S phase and incubated at 37°C for four hours.
Serum bactericide activity is determined by measuring the percentage of eliminated microrganisms and comparing these results with the starting culture percentage (Poli, 1996). In buffalo calves bactericide activity significantly increases from 41 percent in the first month of age to 64 to 68 percent over three months (Cavallina et al., 2003). In lactating buffaloes Fagiolo et al. (2004) did not find any seasonal variation with mean values ranging from 52 to 56 percent in winter and summer, respectively.

Electrophoresis

Electrophoresis is the migration of charged molecules like proteins in an electrical field. The separation of proteins in an electrical field is based on the size, shape, and charge (Figure 2). The charges are provided by the side chains of the amino acids of which the proteins are composed. The charge of the protein depends on the pH of the protein and the pH of the surrounding buffer. Most electrophoretic methods use a supporting media, such as starch, paper, polycrylamide, or Agarose. It should be remembered that the actual environment through which the proteins migrate is composed of a 50 percent buffer solution. The term zone electrophoresis refers to electrophoresis which is carried out in a supporting medium, whereas moving-boundary electrophoresis is carried out entirely in a liquid phase. When proteins are visualized on gels and the migration distances are compared to standards the isoelectric pH (isoelectric focusing) and molecular weights (SDS electrophoresis) of various proteins can be measured. The isoelectric pH and molecular weights are useful in identifying and purifying proteins.
Serum proteins are separated into albumin and globulins. In other words, total protein (albumin + globulin). Albumin is the protein with the highest concentration in the serum. It carries many small molecules, but it is also of prime importance in maintaining the oncotic pressure of the blood.
Globulins are roughly divided into alpha-1, alpha-2, beta, and gamma globulins. These can be separated and quantified in the laboratory by electrophoresis and densitometry.
Usually, alpha-1 and alpha-2 protein levels increase in the presence of inflammation. The beta fraction includes transferrin, plasminogen, and beta lipoproteins. The gamma fraction includes the various types of antibodies (immunoglobulins M, G and A). Using this method we can detect any change in the protein fractions concentration due to several health alterations.
Buffalo calves (aged four to six months) serum protein electrophoresis evidenced the following

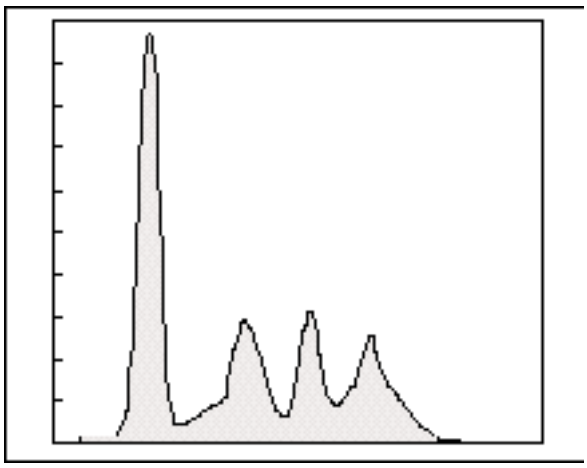


Figure 2. A Buffalo serum protein electrophoresis as a pattern in polyacrilamide gel (IZS Latium and Tuscany).

percentage results in females: albumin 45; α-globulin 16; β-globulin 17; γ-globulin 22, and in males: albumin 44.8; α-globulin 15.5; β-globulin 14.9; γ-globulin 24.9. In adult buffaloes the following percentage results were found: albumin 35.5; α-globulin 25.5; β-globulin 11; γ-globulin 28 (females), and albumin 37.7; α-globulin 27.4; β-globulin 8.5; γ-globulin 26.4 (males) (Satija et al., 1979).

Flow cytometry

Flow cytometry is a method to measure certain physical and chemical characteristics of cells or particles as they travel in suspension (blood, bone marrow, body fluids or tissue cell suspensions) one by one past a sensing point.
Flow cytometry employs instrumentation that scans single cells flowing past excitation sources in a liquid medium. The technology can provide rapid, quantitative, multiparameter analyses on single living (or dead) cells based on the measurement of visible and fluorescent light emission. Flow cytometry is a widely used method for characterizing and separating individual cells. Physical characteristics such as cell size, shape and internal complexity can be measured and, of course, any cell component or function that can be detected by a fluorescent compound can be examined.
A number of different antibody panels are used, depending on the clinical question to be resolved.
The relative fluorescence intensity of the positive cells indicates the amount of antibody bound to specific binding sites on the cell.
The specific panel of antibodies used is selected by the laboratory based on the patient's clinical history, referring physician information, and the morphologic appearance of the cells present in the specimen. Antibodies of particular interest may be requested by the referring physician. In addition, printouts of flow histograms will be provided upon request.
So the applications of flow cytometry are numerous, and this has led to the widespread use of these instruments in biological and medical veterinary fields.
The following analyses can be executed using flow cytometry:

- Immunophenotyping
- Tumour Antigen Markers
- Apoptosis
- Enzyme activity
- DNA/RNA content and Cell Cycle Analysis
- Cytokine receptors and their synthesis
- Phagocytosis
- Identification of WBC in milk

It has been used for an initial screening in buffaloes on leukocyte differentiation molecules that are differentially expressed on one or more lineages of leukocytes: 200 monoclonal antibodies (mAbs) yielded 138 with patterns of reactivity similar or identical to those in cattle (Davis et al., 2001).

Clinical applications

Immunophenotyping has proved to be an important tool in the diagnosis and classification of leukemias and lymphomas. Morphology and immunophenothyping generally provide sufficient information for making a reliable diagnosis in patients with a clinically suspect lymphoproliferative disease.
With the advent of monoclonal antibody technology and availability of fluorochromes, immunophenotyping of leukocyte subsets in animals has become more routine. Flow cytometry is also used to analyse mastitic milk and to define a leukemic population.
All these applications can be employed in the examination of buffalo peripheral blood and milk.

Hormonal parameters

Cortisol - Cortisol is a corticosteroid hormone synthesized in the zona fasciculata of the cortex of the adrenal glands. It is synthesized from cholesterol and its production is stimulated by the pituitary adrenocorticotropic hormone (ACTH) which is regulated by the corticotropin releasing factor (CRF). ACTH and CRF secretions are inhibited by high cortisol levels in a negative feedback loop. In plasma a preponderance of cortisol is connected with a high affinity to corticosteroid binding globulin (CBG or transcotin). Cortisol acts through specific intracellular receptors and affects numerous physiologic systems including immune function, glucose counter regulation, vascular tone and bone metabolism.
Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir at night. The factor controlling this rhythm has not been completely clarified and can be disrupted by a number of physical and psychological conditions. ACTH and cortisol are secreted independently from the circadian rhythm in response to physical and psychological stress. For this reason it has attracted widespread attention as the so-called "stress hormone." Serum cortisol levels fluctuate in response to a number of different variables, apart from ACTH levels, including psychological stress and such physiological strains as hypoglycemia, illness, fever, trauma, pain, fear, physical exertion or extremes of temperature. Cortisol is usually released in response to long-term stress. In a previous study, Borghese et al., (1991) found cortisol levels in buffaloes significantly correlated with estradiol-17b (r=0.45) and with the quantity of milk produced (r=0.38), with values of 13-14 ng/ml in the early stage of lactation and 4-6 ng/ml in the subsequent stages. Bertoni et al., (1994b) clarified the pattern of changes of cortisol level showing that it varied before (10.4 ng/ml) and after meal (9.3 ng/ml). Later, Bertoni et al., (1997) and Terzano et al., (unpublished data) confirmed the significant cortisol level reduction following a meal (4.7 ng/ml vs 3.3 ng/ml; 3.67 ng/ml vs 2.97 ng/ml, respectively). Zia-Ur-Rahman et al. (1997), in a study on hormonal and haematological profiles in buffaloes subjected to different stress conditions, found higher serum cortisol levels before and after transport (23.1 *vs* 80.6 mmol/l, respectively), before and after handling (23.1 *vs* 86.0 mmol/l, respectively) and before and after slaughter (23.1 *vs* 92.0 mmol/l, respectively). The findings were indicative of different hormonal changes during different types of stress in buffaloes.

Triiodothyronine (T3) and Thyroxine (T4) - The thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are tyrosine-based hormones produced by the thyroid gland. They act on the body to increase the basal metabolic rate, heat production and the O2 consumption; moreover they affect carbohydrate absorption, glycogenolysis, gluconeogenesis, lipid metabolism, protein synthesis and increase the body's sensitivity to catecholamines (such as adrenaline). The major type of thyroid hormone in the blood is T4. This is converted within cells to the active T3 by deiodinases. The thyroid is directly regulated by the anterior pituitary and indirectly regulated by the hypothalamus. The hypothalamus secretes TRH, which stimulates

the anterior pituitary to synthesize and secrete TSH. TSH enters the blood stream and eventually stimulates the thyroid gland to undergo hyperplasia, increase iodine uptake, and synthesize and secrete thyroid hormones (T3 and T4) into the blood stream where they act on tissues. The plasma thyroid hormone levels are highly affected by various factors: species, breed, sex and individual characteristic (Borghese et al.,1991). The authors reported significantly higher plasma T3 values in buffalo cows less than five years old (1.39 ng/ml) than the oldest (1.08 ng/ml). Moreover the same authors found that the calving period had a significant effect on T4 values, these latter values also increased during lactation and resulted inversely correlated with milk production (r=-0.30). Moreover their levels depend on physiological phases (Campanile et al.,1997), the nutritive value of diets (Bertoni et al., 1997), housing systems and environmental conditions. Montemurro et al. (1995b) reported the following T3 plasma values in heifers bred in two different farms (0.91-1.88 ng/ml), the same values which were found by other authors (Ghaly, 1987; Dixit et al., 1984; Sharawi et al., 1987). The same authors also reported the mean value of T4 plasma levels which range between 3.35-5.27 g/dl. Terzano et al., (unpublished data) found different plasma T3 levels between, before and after meals (1.70 ng/ml *vs* 1.0 ng/ml). The circadian variation of serum T3 and T4 concentrations was investigated by Vulcano et al., (1997) in pregnant and non pregnant Murrah buffalo cows; they found no differences in serum T3 and T4 concentrations during the cycle in any group. The serum T3 concentration was higher at 2 a.m. (1.88 ng/ml) in non-pregnant cows and serum T4 concentration was higher at 2 p.m. (5.68 g/dl) and 6 p.m. (6.0 g/dl) in pregnant cows, confirming the effect of pregnancy and circadian rhythms on thyroid activity. Zia-Ur-Rahman et al. (1997), in their study found higher serum T3 levels after transport (3.5 pmol/l,), handling (3.8 pmol/l,) and slaughter (3.8 pmol/l,), as compared to before transport (2.2 pmol/l).

Reproductive hormones

Gonadal steroid hormones - Steroid hormones are secreted by the ovary, testes, placenta and adrenal cortex. The two classes of hormones produced by the ovaries are *progestins and estrogens* and have cholesterol as a common precursor.

- *Progestins* are a group of hormones with similar physiological activity, the most important being progesterone (P4). This latter has a dominant role in regulating the oestrus cycle. In buffaloes, during the normal cycles, plasma P4 levels drop sharply two or three days before the luteinizing hormone (LH) peak, start to rise two to four days after the LH surge reaching the highest characteristic mid-luteal phase (dioestrus) levels (ranging between 5 and 12 ng/ml in Mediterranean buffalo cows or between 4-6 ng/ml in Murrah buffalo cows). P4 blood concentration can change during the seasons: Srivastava et al. (1999) during the fresh season reported the highest progesterone levels of the luteal phase in comparison to that recorded during the hot dry or hot wet seasons. Hattab et al. (2000) have shown that the P4 metabolites levels measured in faeces are a good medium for the monitoring of the corpus luteum function, reflecting the blood P4 levels with a high significant correlation coefficient (r=0.77). Therefore providing a simple tool for determining luteal status in buffalo. Moreover P4 have been reported to hasten the transport of oocytes in the oviduct, to have a dominant role in the maintenance of pregnancy, particularly during the early stage, and to be able to synergize with estrogens in stimulating the udder alveolar development and growth. P4 blood level is considered a good indicator of the onset of puberty and heifers are usually considered to have achieved puberty when plasma P4 levels exceed 1.5 ng/ml. A number of environmental factors have a pronounced effect on the age at puberty. In general, any factor which slows growth rate, thus preventing expression of full genetic potential, will delay puberty. A buffalo heifer on a good level of nutrition will reach puberty at about 19 months (Terzano et al., 1993, 1997; Borghese et al., 1994).

- *Estrogens* are hormones produced by the ovary and are transported in the body by binding proteins. Estrogens act on the Central Nervous System in order to induce behavioural oestrus in females and the most important of these hormones is estradiol. In buffalo the general pattern

of secretion of the estradiol-17ß indicates a surge which takes place on the day preceding the LH peak (Singh et al., 2001; Malfatti, 2003) or frequently very close to the LH peak with blood levels ranging between 9 and 13 pg/ml (Seren et al., 1994) respectively in Murrah and Mediterranean buffaloes. Following the LH surge, there is a rapid decline in estradiol secretion and this results in the cessation of oestrus after a relatively constant time interval (about 12 hours). The basal estradiol levels during the luteal phase of the cycle range between three and eight pg/ml, but some minor elevations can be observed in the early luteal phase, up to 1.3 pg/ml (Singh et al., 2001; Malfatti, 2003). Oestrus symptoms are much less obvious than in cattle; it is significant to note that in Italian studies silent oestrus was recorded in more than two thirds of the animals, all presenting endocrine changes comparable to that of the cattle showing overt oestruses.

Pituitary gonadotrophins - The *luteinizing hormone* (LH) is important in studies of ovarian activity since its preovulatory surge is responsible for the rupture of the follicle wall and ovulation. The peak values are always well defined in respect to the basal blood levels; the duration of LH surge was estimated by different authors and was calculated at 6 to 12 hours (Seren et al., 1994) or 6 to 9 hours (Maurel et al., 1995) or 8 to 12 hours (Barile et al., 1998) in Mediterranean buffalo cows. Buffalo cows are characterized by the occurrence of two peaks of the hormone during the oestrus time; this phenomenon was recorded by Seren et al. (1994) in 25 percent of the 24 observed oestruses. The double LH peak was frequently followed by double ovulation that also occurred after a single peak in an additional 8 percent of the animals. LH peak time (in relation to oestrus symptoms and to ovulation time) is more important than the LH peak. Seren et al., (1994), monitoring ovulation time by ultrasound, found that the mean time of LH peak-ovulation was 35.5 hours (in buffalo cows with single ovulation) and 60 hours (in buffalo cows with double ovulation). Moioli et al. (1998), in spontaneous oestruses of buffalo cows, found a mean interval LH peak-ovulation (this latter detected by rectal palpation considering follicle changes from turgid to flaccid) of 25.2 ± 13.1h and of 46.1 ± 18.8h respectively in oestruses followed by pregnancy and in those not followed by pregnancy. The same authors found that the mean time of LH peak end of the continuous courtship was 2.4 ± 10.4h and 14.7 ± 15.2h respectively in the two groups. Several authors (Barile et al., 1998, Malfatti et al., 2003) studying buffalo cows after PRID + PMSG treatment, found that the mean time from PRID removal to LH peak ranged from 54.7 ± 12.3h to 61.0 ± 12.05h and that it was nearly 85 hours from PRID removal to ovulation time. Malfatti et al. (2003), evaluating the LH peak in buffalo cows synchronized with two different hormonal protocols for fixed time artificial insemination, found no difference between the PRID + PMSG group and PRID + GnRH group, in the interval from PRID removal to LH peak (57.3 ± 13.4h and 54.4 ± 9.6h respectively) nor in the interval from PRID removal to ovulation time (86.4 ± 13.1h and 90.0 ± 11.1h respectively). Following this previous trial, Malfatti et al. (2004), evaluated LH peak and ovulation time in buffalo cows submitted to two different hormonal protocols (PRID and Ovsynch) for fixed time artificial insemination, during the lower breeding season; no differences were found between treatments. Particularly average LH peak values occurred at 51.30 ± 13.94h and ovulation at 85.14 ± 13.63h and the mean time interval detected between LH peak and ovulation was 33.71 ± 4.30h. Moreover in the PRID group the times of LH peaks and ovulations were notably more scattered and highly significant than in the Ovsynch group (CV%= 37.50 vs 3.55 and 21.41 vs 6.73). The authors concluded that both treatments were able to synchronize oestrus and ovulation in buffaloes and that the Ovsynch protocol appears more suitable for use in fixed time artificial insemination programmes due to a better LH peak and ovulation synchronization. The basal level of LH did not register differences during pregnancy. The *follicle stimulating hormone* (FSH) promotes follicle growth and estrogen production through granulosa cells in the ovarian follicles. This hormone has been studied by several authors in buffalo cows over the past few years. Seren et al. (1994) found the FSH surge (1.6-6.0 ng/ml) to be coincident with the LH peak and to have a duration time of six to nine hours, with a lack of evident additive peaks during the luteal phase of the oestrus cycles, when the hormone levels ranged between 0.2-1.5 ng/ml. Singh et al. (2001) also found the FSH surge to be coincident with the LH peak, averaging near 25 ng/ml, but reported the major surge of this hormone on the tenth day after oestrus and a minor increase on days 4 and 15 after

oestrus. Palta and Madan (1995, 1997a) recorded FSH peak means of 70-80 ng/ml and a duration time of 5.8 ± 0.07h (during pregnancy) and 5.5 ± 0.02h (during postpartum) after GnRH stimulation. It has now been established also in buffalo that a transient rise in serum concentration of FSH begins each follicular wave (Baruselli et al. 1997; Singh et al. 2001; Presicce et al. 2003) and a decreased episodic secretion of LH is associated with loss of dominance and with the end of a nonovulatory follicular wave. Palta and Madan (1995, 1997a) found alterations of the gonadotrophins release by hypophysis in pregnant and postpartum buffalo cows. The basal level of FSH presented significant differences during pregnancy; in fact it was higher at day 60 than at day 240 of pregnancy. During postpartum the basal levels of LH and FSH increased significantly from day 2 to day 20, but only the LH levels were higher at day 35 in comparison to day 20. The authors pointed to a progressive reduction of the hypophysial responsiveness to the GnRH during pregnancy and this could be due to the chronic negative feedback exerted by gonadal steroids, together with a probable reduction of GnRH receptors in the pituitary, both verified in other ruminants (Nett, 1987; Schoenemann et al., 1985). After delivery a progressive reestablishment of the positive feedback by estrogens was observed and a rise of the basal gonadotrophins levels was recorded.
Prolactin synergizes with LH by increasing LH receptor sites in the corpus luteum. It also has a stimulating effect on the development of the mammary gland and the synthesis of milk. In buffalo cows prolactin blood concentrations during the oestrus cycle have not been extensively studied. Seren et al. (1994) reported a pulsatile secretion starting before the luteolysis and lasting during the oestrus, ending near the ovulation time. The plasma levels were very variable, ranging from 10-20 to 150-200 ng/ml.

Inhibin - Inhibins are heterodimeric protein hormones secreted by the granulosa cells of the ovary in the female and Sertoli cells of the testis in the male. It is recognized that inhibin plays an important role in regulating FSH secretion (Baird et al., 1993; Singh et al., 2001) and also has local paracrine actions in the gonads (Burger H.G., 1992). Two forms of biologically active inhibin (inhibin A and B) have been identified and most data regarding changes in circulating inhibin concentrations come from human studies (Fried et al., 2003; Eldar-Geva, 2000). These hormones have been studied in buffalo cows by only a few authors over the past few years. Palta et al. (1996) validated a specific RIA method to assay immuno reactive inhibin (ir-inhibin) in the plasma of buffalo cows measuring the hormone blood levels during the oestrus cycle. They observed an increase of inhibin concentrations during the follicular phase with a peak occurring on day 0 (oestrus day defined by the lowest P4 value); the differences occurring between the concentrations recorded during the cycle were not significant, ranging from 0.40 ± 0.07 to 0.67 ± 0.13 ng/ml. Based on their tissue localization (Muttukrishna et al., 1994), it is believed that inhibin A is a product of large follicles, whereas inhibin B is produced by a cohort of antral recruited follicles. These data suggested that inhibin A may provide an indication of follicular development (Lockwood et al., 1996), whereas inhibin B may be a suitable marker for ovarian follicle reserve (Seifer et al., 1997). The inhibin concentrations increase notably when superovulatory treatments are performed, due to the greater number of large follicles growing. Palta et al. (1997b) recorded concentrations up to 1.01 ± 0.31 ng/ml (nearly double the physiological values at oestrus) when PMSG superovulatory treatment was performed. Terzano et al. (2004a) evaluated the relationship of plasma inhibin A (analyzed in duplicates by a human sandwich type immunoassay) to ovarian follicular development in prepuberal Mediterranean Italian buffaloes subjected to two different ovarian stimulation protocols. The data suggested that the medium/large follicles are the most important source of hormone production and that serum inhibin A determined during FSH treatment may provide a useful marker in the control of ovarian hyperstimulation.

Pregnancy-associated glycoproteins (PAGs) - The pregnancy-associated glycoproteins constitute a large family of glycoproteins synthesized by trophoblast binucleate cells and released in the maternal blood circulation after implantation until birth. During the last decade they have been isolated from the placenta of various ruminant species (Terzano et al., 2004b). In recent years, it has become evident that there may be more than 100 PAG genes and that many of them are expressed (Gonzalez et al., 2000). Among these the best known is the

Pregnancy Specific Protein B (PSPB), first detected in bovine placenta (Butler et al., 1982), and now widely used for pregnancy diagnosis in most animals. It represents, as do other PAGs, a reliable "foetal-placental welfare marker" and could be a very useful technique for early detection of animals with a high risk of pregnancy failure (Willard et al., 1995; Zoli et al., 1995; Garbayo et al., 1998). The first study of the presence of PAGs in pregnant buffalo cows was by Debenedetti et al. (1997), and this investigation showed that its blood concentration is clearly pregnancy-linked. Malfatti et al. (2001), through a double antibody RIA (Humblot et al., 1998) utilizing bovine PSPB antibody and standards, found that in this species the hormone became detectable in 33 percent of animals between the 20th and 25th day after fertilization. On the 30th day it resulted measurable in all animals (1.6 ± 1.1 ng/ml) and on the 35th day, 91 percent of the animals had PAGs blood level > 1.0 ng/ml. The hormone concentration reached values of 6.6 ± 3.2 ng/ml on the 50th day and at the end of pregnancy the values were similar (6.28 ± 1.87). The PAGs decreased markedly after birth: five days after, their levels were 45 percent less. The successive decrease was much slower: the mean calculated halving time is nearly 10 days; 50 days after birth the PAGs plasma concentrations were not detectable (< 0.3 ng/ml). Barbato et al. (2003) have described the first isolation and the partial characterization of PAGs from the buffalo placenta which enabled a study of the PAGs blood concentration and its trend during pregnancy and led to a reliable pregnancy test for the buffalo species.

Melatonin - Melatonin is a brain hormone, produced and stored in the pineal gland during daylight and then secreted during darkness, beginning a short time after sunset and ending at sunrise. Its secretion constitutes the endocrine signal of the light-dark rhythm in the environment. The best known role of melatonin is the regulation of the circadian as well as of the annual rhythms in many species, from the more primitive species to man. In the endocrinology of ruminants its role has been extensively explored with regard to the induction of seasonal ovarian cyclicity in ewes and goats, especially at high latitudes. Buffalo is considered a seasonal species and this seasonality does not seem to depend on the diet, food availability or metabolic status, while climate and photoperiod appear to play a pivotal role. The strong influence of photoperiod seems to be further corroborated by the finding that the period of higher reproductive efficiency is reversed in the two opposite hemispheres (Borghese et al., 1994; Zicarelli, 1997; Baruselli et al., 2001). Parmeggiani et al. (1993, 1994), assayed melatonin blood levels in buffalo cows reared on Italian farms where the reproductive activity was characterized by strong seasonality and on farms where parturition frequency tended to be more uniformly distributed throughout the year. The season-linked buffaloes evidenced a melatonin profile reflecting the photoperiodic changes, with hormone concentrations below 20 pg/ml during the light time and systematic rises after sunset (averages of near 60 pg/ml). Conversely the less season-linked buffaloes presented melatonin concentrations frequently high during the light time (30 40 pg/ml), with a lack of evident melatonin increases during the night. The author's view, is that the different melatonin trends are the result of extensive selection carried out in the latter farms aimed at eliminating the seasonal breeder cows (Seren et al., 1995). Borghese et al. (1995) assayed melatonin blood levels, every two hours for 24 hours at equinoxes and at solstices, in 16 buffalo cows and in 16 buffalo heifers in natural daylight conditions in order to better understand melatonin's role in hypothalamus-pituitary-ovarian axis processing. Blood melatonin level was less than 10 pg/ml during the day, while it was 30-100 pg/ml during the night, showing considerable differences between seasons and between cows and heifers. In March, in particular, the buffalo cows showed much higher melatonin values at night than heifers.

References

Abbas, A., Lichtman, A.H. and Pober, J.S. 2000. Cellular and Molecular Immunology, 4th edition.

Baird, D.T. and Smith, K.B. 1993. Inhibin and related peptides in the regulation of production. Oxf. Rev. Reprod. Biol., 15: 191-232.

Barbato, O., Sousa, N.M., Malfatti, A., Beckers, J.F., De Santis, G. and Debenedetti, A. 2003. Purification of pregnancy-associated glycoproteins (Pags) in buffalo (Bubalus bubalis): preliminary results. Atti Secondo Congresso Nazionale sull'Allevamento del Bufalo, Monterotondo, Roma: 279-283.

Barile, V.L., Galasso, A., Terzano, G.M., Malfatti, A. e Barbato, O. 1998. Valutazione del picco di LH in bufale sottoposte a sincronizzazione dell'estro ai fini della inseminazione artificiale. Atti S.I.S.Vet., 52: 85-86.

Baruselli, P. S., Mucciolo, R.G., Visintin, J.A., Viana, W.G., Arruda, R.P., Madureira, E.H., Oliveira, C.A. and Molero-Filho, J.R. 1997. Ovarian follicular dynamics during the oestrus cycle in buffalo (Bubalus bubalis). Theriogenology, 47: 1531 1547.

Baruselli, P.S., Bernardes, O., Braga, D.P.A.F., Araujo, D.C. and Tonhati, H. 2001. Calving distribution throughout the year in buffalo raised all over Brazil. Proc. Fourth World Buffalo Congress, Maracaibo, Venezuela, 2: 234-240.

Bertoni, G. 1989. Effetti e livello ematico e produttivo della somministrazione di grasso animale a bovine da latte ante e post partum. Zoot. Nutr. Anim., 15 (4): 341-354.

Bertoni, G., Amici, A., Lombardelli, R. and Bartocci, S. 1993. Variations of metabolic profile and hormones in blood of buffaloes, cattle and sheep males fed the same diets. Proc. of the Intern. Symp Prospect of Buffalo Production in the Med/Middle East, Cairo, Egypt. EAAP Pubbl. n.62, Pudoc. Sci. Ed: 345-348.

Bertoni, G. e Piccioli Cappelli, F. 1994a. Indagine sull'allevamento delle bufale in provincia di Latina: influenza dell'alimentazione sulle condizioni metaboliche e produttive. Inf. Agrario, 18: 19-33.

Bertoni, G., Lombardeli, R., Piccioli Cappelli, F., Bartocci, S. e Amici, A. 1994b. Alcuni fattori che influenzano le condizioni endocrino-metaboliche della specie bufalina. Agr. Ricerca, 16: 87-98.

Bertoni, G., Bartocci, S., Piccioli Cappelli, F. e Amici, A. 1997. Blood metabolites and hormone changes in lactating buffaloes fed diets different for energy content and protein degradability. Proc. Fifth World Buffalo Congress. Caserta, Italia: 961-965.

Bertoni, G. et al. 1999. Guida all'interpretazione dei profili metabolici. ASPA.

Beutler, B. 2004. Innate Immunity Molecular Immunology, 40 (22): 845-859.

Borghese, A., Terzano, G.M., Debenedetti, A., Lucaroni, A. e Malfatti, A. 1991. Indagine sull'attività endocrina delle bufale in lattazione. Atti IX Congresso Nazionale ASPA. Tavola Rotonda: 1235-1250.

Borghese, A. 1994. Studio dei profili ematici come indicatori dello stato fisiopatologico degli animali in produzione zootecnica. Biologia Oggi VIII (1 2): 91-112.

Borghese, A., Barile, V.L., Terzano, G.M., Pilla, A.M. and Parmeggiani, A. 1995. Melatonin trend during season in heifers and buffalo cows. Bubalus bubalis, vol. 1: 61-65.

Burger, H.G. 1992. Inhibin. Reprod. Med. Rev., 1: 1-20.

Butler, J.E., Hamilton, W.C., Sasser, R.G., Ruder, C.A., Hass, G.M. and Williams, R.J. 1982. Detection and partial characterization of two bovine pregnancy-specific proteins. Biol. Riprod., 26: 925-933.

Campanile, G., Bonavoglia, C., Perrucci, G.F. e Di Palo, R. 1991. Caratteristiche ematochimiche in bufale appartenenti ad aziende con differente fertilità. Atti Fe. Me. S. P. Rum., 1: 93-98.

Campanile, G., Di Palo, R., Di Meo, C., Staiano, M. and Zicarelli, L. 1994. Metabolic profile in ltalian Mediterranean Buffaloes. Proc. Fourth World Buffalo Congress, Sao Paolo, Brazil: 257-259.

Campanile, G., Di Palo, R., De Filippo, C., Taccone, W. and Zicarelli, L. 1996. Influence of dietary protein on urea levels in blood and milk of buffalo cows. Eighth Intern. Symp. of Vet. Lab. Diagn., 48 (Abstract).

Campanile, G., Di Palo, R. e D'Angelo, A. 1997. Profilo metabolico nel bufalo. Suppl. n. 4 Bubalus Bubalis: 236-249.

Canfield, P.J., Best, F.G., Fairburn, A.J., Purdie, J. and Gilham, M. 1984. Normal haematological and biochemical values for the swamp buffalo (Bubalus bubalis). Aust. Vet. J Mar, 61(3): 89-93.

Cavallina, R., Alfieri, L. and Lai, O. 2003. Metabolic, endocrine and immune profile in buffalo calves from birth to weaning. Atti Secondo Congresso Nazionale sull'Allevamento del Bufalo. Monterotondo, Roma: 279-283.

Chugh, S.K, Bhardwaj, R.M and Mata, M.M. 1998. Lowered antioxidant status of red blood cells in post-parturient haemoglobinuria of buffaloes. Vet. Res. Commun., Sep, 22 (6): 383-388.

Ciaramella, P., Corona, M., Ambrosio, R., Consalvo, F. and Persechino, A. 2005. Haematological profile on non-lactating mediterranean buffaloes (Bubalus bubalis) ranging in age from 24 months to 14 years. Research in Veterinary Science, Article in Press.

Davis, W.C., Khalid, A.M., Hamilton, M.J., Ahn, J.S., Park, Y.H. and Cantor, G.H. 2001. The use of crossreactive monoclonal antibodies to characterize the immune system of the water buffalo (Bubalus bubalis). J. of Vet. Sci., 2 (2): 103-109.

Debenedetti, A., Malfatti, A., Borghese, A., Barile, V.L. and Humblot, P. 1997. Pregnancy specific protein B (PSPB) detection by RIA in buffalo cows. Proc. Fifth World Buffalo Congress. Caserta, Italy: 771-775.

Deore, M.D., Srivastava, K.A. and Sharma, S.K. 2002. Blood selenium levels during different stages of selenosis in buffaloes and its evaluation as a diagnostic tool. Vet. Hum. Toxicol., 44 (5): 260-263.

De Rosa, C., Campanile, G., Amante, L., Banchelli, L., Midea, D. e Zicarelli, L. 2001. Modificazioni endocrino-metaboliche nella fase di transizione e nei primi mesi di lattazione. Atti Primo Congresso Naz. Allevam. Bufalo. Salerno, Italia: 293-297.

Di Palo, R., Campanile, G., Boni, R., Di Meo, C. e Zicarelli, L. 1990. Interrelazione tra caratteristiche della sostanza secca della dieta, produzione lattea ed alcune costanti

ematochimiche nella bufala. Atti XLLIV Conv. Naz. S.I.S.Vet.: 1501 1505.

Dixit, N.K., Agarwal, S.P., Agarwal, W.K. and Dwaraknat, P.K. 1984. Seasonal variation in serum levels of thyroid hormones and their relation with semen quality and libido in buffalo bulls. Theriogenology, 22 (5): 497-507.

Eldar-geva, T., Robertson, D.M., Cahir, N., Groome, N., Gabbe, M.P., Maclachlan, V. and Healy, D.L. 2000. Relationshìp between serum inhibin A and B and ovarian follicle development after a daily fixed dose administration of recombinant follicle stimulating hormone. Clinic Endoc. and Metab., 85 (2): 607 613.

Eltohamy, M.M., Ahmed, W.M. and Abdon, A.S.S. 1994. Ovarian activity in relation to some blood composition. Proc. Fourth World Buffalo Congress. Sao Paolo, Brazil, 3: 612-614.

Fagiolo, A., Lai, O., Alfieri, L., Nardon, A. and Cavallina, R. 2004. Environmental factors and different managements that influence metabolic, endocrine and immuno responses in water buffalo during lactation. Proc. Seventh World Buffalo Congress, Manila, Philippines: 24-26.

Fried, G. et al. 2003. lnhibin H predicts oocyte number and the ratio IGF I/IGFBP-I may indicate oocyte quality during ovarian hyperstimulation for in vitro fertilization. J. of Ass. Reprod. and Gen., 20 (5): 167-176.

Garbayo, J.M., Remy, B., Alabart, J.L., Folch, J., Wattiez, R., Falmagne, P. and Beckers, J.F. 1998. Isolation and partial characterization of a pregnancy associated glycoprotein (PAG) family from the goat placenta. Biol. Reprod., 58 (1): 109-115.

Ghaly, H.A.M. 1987. Egypt. J. An. Prod., 25 (1): 83-92.

Gonzalez, F., Calero, P., Quesada, E., Garbayo, J.M. and Beckers, J.F. 2000. The Pregnancy Associated Glycoproteins (PAGs) in the caprine species: fundamental and clinical approach. International Conference on Goats. Tome 1: 413-415.

Grasso, F., Terzano, G.M., De Rosa, G., Tripaldi, C. and Napolilan, F. 2004. Influence of housing conditions and calving distance on blood metabolites in water buffalo cows. Italian J. Anim. Sci., vol.3: 273-287.

Hattab, S.A., Kadoom, A.K., Palmo, R. and Bamberg, E. 2000. Effects of Crestar and the relationship between faecal and plasma concentrations of progestagens in buffalo cows. Theriogenology, 54: 1007-1017.

Houpt, R. 1970. Transfer of urea and ammonia to the rumen. Physiology of Digestion and Metabolism in the Ruminant, Oriel Press Limited, New Castle Upon Tyne, Royaume-Uni: 119-131.

Humblot, P., Camous, S., Martal, J., Charlery, J., Jeanguyot, N., Thibier, M. and Sasser, R.G. 1988. Pregnancy-specific protein B, progesterone concentrations and embryonic mortality during early pregnancy in dairy cows. J. Reprod. Fert., 83: 215-223.

Jainudeen, M.R. and Hafez, E.S.E. 2000. Cattle and buffalo in reproduction in farm animals. Ed. Hafez E.S.E. and Hafez B., 7 ed., Lippincot Williams & Wilkins, Baltimore USA.

Kleczkowski, M., Klucinski, W., Sikora, J., Zdanowicz, M. and Dziekan, P. 2003. Role of antioxidants in protection against oxidative stress in cattle non-enzymatic mechanisms (Part 2). Pol. J. Vet. Sci., 6 (4): 301-308.

Kleczkowski, M., Klucinski, W., Sikora, J. and Zdanowicz, M. 2004. Role of antioxidants in

protection against oxidative stress in cattle-trace elements and enzymatic mechanisms (Part 3). Pol. J. Vet. Sci., 7 (3): 233-240.

Langer, P.N., Sidhu, G.S. and Bhatia, J.S. 1969. A study of the microbial population in the rumen of buffalo (Bos bubalus) and zebu (Bos indicus) on a feeding regimen deficient in carbohydrates. lndian J. Vet. Sci., 38: 333-336.

Lean, I.J., Farver, T.B., Troutt, H.F., Bruss, M.L., Galland, J.C., Baldwin, R.L., Holmberg, C.A. and Weaver, L.D. 1992. Time series cross-correlation analysis of postparturient relationships among serum metabolites and yield variables in Holstein cows. J. of Dairy Sci., 75: 1891-1900.

Lockwood, et al. 1996. Circulating inhibins and activin A during GnRH-analogue down-regulation and ovarian hyperstimulation with recombinant FSH for in vitro fertilization-embryo transfer. Clin. Endocrin. (Oxf.) 45: 741-748.

Maianti, M.G., Bertoni, G., Lombardelli, R. e Cappa, V. 1990, Variazioni ematiche in bovine sottoposte a fattori diversi di stress. Zoot. Nutr. Anim., 16: 19-27.

Malfatti, A., Debenedetti, A., Barbato, O., Mancini, L., Borghese, A. e Terzano, G.M. 2001. II dosaggio delle glicoprotoine associato alla gravidanza (PAG, PSPB) nella bufala. Atti Primo Congr. Naz. sull'Allevamento del Bufalo, Eboli, ltaly, 3-5 Ott.: 332-336.

Malfatti, A. 2003. Recent advances in buffalo endocrinology. Atti Secondo Congresso Nazionale sull'Allevamento del Bufalo. Monterotondo, Roma, 28 - 30 Ag.: 161 176.

Malfatti, A., Todini, L., Barbato, O., Barile, V.L., Pacelli, C., Terzano, G.M., Allegrini, S., Mazzi, M. and Borghese, A. 2004. LH peak and ovulation in buffalo cows treated for oestrus synchronization using two different hormonal schedules. Proc. (abstracts) Seventh World Buffalo Congress, Manila, Philippines, 20 - 23 Oct.: 70.

Maurel, M.C., Malfatti, A., Debenedetti, A., Catalano, A. and Barile, V.L. 1995, Detection of the preovulatory LH surge in the buffalo by an enzyme-immunologic assay. Reproduction and Animal Breeding advances and strategy, Ed. Enne G., Greppi G.F., Lauria A. Elsevier Amsterdam 405-406.

Moioli, B.M., Napolitano, F., Puppo, S., Barile, V.L., Terzano, G.M., Borghese, A., Malfatti, A., Catalano, A. and Pilla, A.M. 1998. Patterns of oestrus, time of LH release and ovulation and effects of time of artificial insemination in Mediterranean buffalo cows. Animal Science, 66: 87-91.

Montemurro, N., Pacelli, C. and Borghese, A. 1995a. Metabolic profiles in buffalo heifers bred in two farms with different feeding and climatic conditions. Egyptian J. Anim. Prod., vol. 32 (1): 1-12.

Montemurro, N., Pacelli, C., Todini L., Malfatti, A. e Borghese, A. 1995b. Studi preliminari dell'attività tiroidea in manze bufaline. Atti Soc. Ital. Sci. Vet., vol. XLIX: 345-346.

Montemurro, N., Pacelli, C. and Borghese, A. 1997. Blood metabolites change in milking buffalo cows. Bubalus Bubalis, vol. III: 69-78.

Muttukrishna, et al. 1994. Serum concentration of dimeric inhibin during the spontaneous human menstrual cycle and after treatment with exogenous gonadotropin. Hum. Reprod., 9: 1634-1642.

Nett, T.M. 1987. Function of the hypothalamic hyphophysial axis during the postpartum period in ewes and cows. J. Reprod. Fertil., 34 (suppl.): 201-213.

Palta, P. and Madan, M.L. 1995. Alterations in hypophysial responsiveness to synthetic GnRH at different postpartum intervals in Murrah buffalo (Bubalus bubalis). Theriogenology, 44: 403-411.

Palta, P., Prakash, B.S. and Madan, M.L. 1996. Peripheral inhibin levels during the oestrus cycle in buffalo (Bubalus bubalis). Theriogenology, 45: 655-664.

Palta, P. and Madan, M.L. 1997a. Effects of gestation on GnRH-induced LH and FSH release in buffalo (Bubalus bubalis). Theriogenology, 46: 993-998.

Palta, P., Kumar, M., Jailkani, S., Manik, R.S. and Madan, M.L. 1997b. Changes in peripheral inhibin levels and follicular development following treatment of buffalo with PMSG and Neutra-PMSG for superovulation. Theriogenology, 48: 233-240.

Parmeggiani, A., Seren, E., Esposito, L., Borghese, A., Di Palo, R. and Terzano, G.M. 1993. Plasma levels of melatonin in buffalo cows. Proc. Intern. Symp Prospect of Buffalo Production in the Med/Middle East, Cairo, Egypt. EAAP Pubbl. n.62. Pudoc. Sci. Ed: 401-403.

Parmeggiani, A., Di Palo, R., Zicarelli, L., Campanile, G., Esposito, L., Seren, E., Accorsi, P.A. e Soflai, S.M. 1994. Melatonina e stagionalità riproduttiva della bufala. Agric. Ricerca n.16: 41-48.

Payne, J.M., Dew Sally, M., Manston, R. and Faulks, M. 1970. The use of a metabolic profile test in dairy herds. Vet. Rec., 8: 150-158.

Piccioli-Cappelli, F., Satriani, A., Polimeno, F., Trevisi, E., Ferrara, L. e Bertoni, G. 2001. Fattori fisiologici e manageriali causa di variazioni endocrino metaboliche nella bufala da latte. Atti 1° Congresso Nazionale sull'allevamento del bufalo. Eboli (SA): 289-292.

Pizzuti, G.P. and Salvatori, G.C. 1993. Some blood parameters of water buffalo in different physiological conditions. Boll. Soc. Italian. Sper., 69 (10): 649-654.

Poli, G. e Rocchi, M. 1998. Microbiologia e Immunologia Veterinaria ed. UTET.

Presicce, G.A., Parmeggiani, A.M., Senatore, E.M., Stecco, R., Barile, V.L., De Mauro, G.J., De Santis, G. and Terzano, G.M. 2003. Hormonal dynamics and follicular turnover in prepuberal Mediterranean Italian buffaloes. Theriogenology: 1-9.

Qureshi, Z.I., Lodhi, L.A. and Sattar, A. 1997. An apparent effect of immunopotentiation during late gestation on the postpartum reproductive performance of Nili-Ravi buffaloes (Bubalus bubalis). Vet. Res. Commun., 21 (5): 375-380.

Ramadan, A.A., Ghoniem, A,A., Hassan, H.M. and Youssef, A.E. 2001. Effects of beta-carotene, selenium and vitamin A on in vitro polymorphonuclear leukocytic activity in peripartal buffalo (Bubalus bubalis). Theriogenology, 55 (3): 693-704.

Satija, K.G., Rajpal, S. and Pandey, R. 1979. Electrophoresis of buffalo (Bos bubalis) serum proteins including immunoglobulins. Infection and Immunity: 567-570.

Satriani, A., Piccioli-Cappelli, F., Palimeno, F., Lambardelli, R., Ferrara, L. e Bertoni, G. 2001. Fattori ambientali causa di variazioni endocrino-metaboliche nella bufala da latte. Atti I Congresso Naz. Allevam. Bufalo. Salerno, Italia, 3 5 Ott.: 285 288.

Schoenemann, H.M., Brown, J.L. and Reeves, J.J. 1985. LHRH receptors, LH and FSH concentrations in anterior pituitary of cycling, non-cycling and early pregnant heifers. J. Anim. Sci., 60: 532-536.

Seifer, D.B., Lambert-Messerlian, G., Gerdiner, A.C., Blazer, A.S. and Berk, C.A. 1997. Day three serum inhibin B is predictive of assisted reproductive technologies outcome. Fertil. Steril, 67: 11-15.

Seren, E., Parmeggiani, A., Mongiorgi, S., Zicarelli, L., Montemurro, N., Pacelli, C., Campanile, G., Esposito, L., Di Palo, R., Borghese, A., Barile, V., Terzano, G.M., Annicchiarico, G. e Allegrini, S. 1994. Modificazioni endocrine durante il ciclo estrale nella bufala. Agric. Ricerca, 16: 17-24.

Seren, E., Parmeggiani, A. and Campanile, G. 1995. The control of ovulation in Italian buffalo. Reproduction and Animal Breeding advances and strategy, ed. Enne G., Greppi G. F. and Lauria A., Elsevier Amsterdam: 265-275.

Setia, M.S., Duggel, R.S. and Singh, R. 1992. Biochemical constituents of blood in buffaloes and cows during late pregnancy and different feeding and climatic conditions. Egypt. J. Anim. Prod., 25 (1): 1-12.

Sharawi, S.M., El-Azab, M.A., Labib, F.M. and Heshmat, H.A. 1987. Serum levels of thyroxine (T4) and triodothyronine (T3) in the normal cycling buffaloes with inactive ovaries before and after PMSG treatment. J. Egypt. Veter. Med. Ass., 477: 299-306.

Singh, V.K., More, T. and Singh, S. 1997. The effect of activation of granulocytes on enzyme release and hydrogen peroxide and superoxide production in buffaloes. Vet. Res. Commun., 21 (4): 241-247.

Singh, B., Dixit, V.D., Singh, P., Georgie, G.C. and Dixit, V.P. 2001. Plasma inhibin levels in relation to steroids and gonadotrophins during the oestrus cycle in buffalo. Reprod. Dom. Anim., 36: 163-167.

Smith, R.H. 1969. Reviews of the progress of dairy science. J. Dairy Res., 36: 313-331.

Spagnuolo, M.S., Cigliano, L., Sarubbi, F., Polimeno, F., Ferrara, L., Bertoni, G. and Abrescia, P. 2003. The accumulation of alpha-Tocopherol and Retinol in the milk of water buffalo is correlated with the plasma levels of triiodothyronine. Biofactors, 19 (3-4): 197-209.

Srivastava, S.K., Sahni, K.L., Uma Shaker, Sanwal, P.C. and Varshney, V.P. 1999. Seasonal variation in progesterone concentration during the oestrus cycle in Murrah buffaloes. lndian J. Anim. Sci., 69 (9): 700-701.

Terzano, G.M., Barile, V.L., Mongiorgi, S. e Borghese, A. 1993. Effetto di differenti livelli alimentari sulla pubertà in bufale di razza mediterranea. Atti S.I.Vet. 47, Riccione: 1803-1807.

Terzano, G.M., Galasso, A., Barile, V.L., Pacelli, C., Montemurro, N. and Borghese, A. 1997. Effect of feeding system and puberty on blood metabolites trend in buffalo heifers. Proc. Fifth World Buffalo Congress, Caserta, Italia, 13-16 Oct.: 951-956.

Terzano, G.M., Tripaldi, C., Allegrini, S., Pasqui, E., Roncoroni, C., Satà, M.C. e Terramoccia, S. 2000. Benessere e riproduzione: approccio sperimentale sulla specie bufalina. Proc. Workshop Stato di benessere ed efficienza riproduttiva negli animali di interesse zootecnico. Viterbo, Italia: 147-153.

Terzano, G.M., Catone, G., Todini, L., Malfatti, A., Pacelli, C., D'Alessandro, A. and Borghese, A. 2004a. Plasma inhibin A level and follicular development in prepuberal buffalo heifers superovulated with FSH or FSH/eCG: preliminary results. Proc. Seventh World Buffalo Congress, Manila, Philippines: 644-647.

Terzano, G.M., Taibi, L., Terramoccia, S., Annicchiarico, G., Tripaldi, C. and Borghese, A. 2004b. Effect of mating season and shade/no shade on pregnancy specific protein B (PSPB) levels in sheep. I.C.A.R., Porto Seguro, Brasil: 75.

Tripaldi, C., Napolitano, F., Scatà, M.C., Grasso, F., De Rosa, G:, Pasqui, E., Roncoroni, C. and Bordi, A. 2003. Housing system and welfare of buffalo cows. Book of Abstracts 54th Annual Meeting of the European Association for Animal Production, Rome, Italy: 184.

Vulcano, L.C., Mamppim, M.J., Oba, E. and Munziz, L.M.R. 1997. Circadian variation of serum triodothyronine (T3) and thyroxine (T4) concentrations in non pregnant Murrah buffalo cows. Proc. Fifth World Buffalo Congress. Caserta, Italia: 767-770.

Willard, S.T., White, D.R., Wesson, C.A.R., Stellflug, J. and Sasser, R.G. 1995. Detection of fetal twins in sheep using a radioimmunoassay for pregnancy specific proteins. J. Anim. Sci., 73: 960-966.

Zia-Ur-Rahman, I.U., Haq, I., Javed, Mushtaq-Ul-Hassan, Z.H., Naqvi, M.M. and Asif M. Jaivi. 1997. Hormonal and haematological profiles in buffalo after transport, handling and slaughter stress. Proc. Fifth World Buffalo Congress. Caserta. Italia: 961-965.

Zicarelli, L., Intrieri, F. e De Franciscis, G. 1982. Variazioni stagionali ed aziendali del profilo metabolico nei bufali. Zoot. Nutr. Anim., 8: 321-356.

Zicarelli, L., Avallone, L., Salvatore, M. e Pizzuti, G.P. 1986. Comportamento di alcune costanti ematiche nelle bufale in gravidanza e lattazione. Atti S.I.S.Vet., 40: 569-572.

Zicarelli, L. 1997. Reproductive seasonality in buffalo. Proc. of the Third Course on Biotechnology of Reproduction in Buffaloes. Issue II. Caserta, Italy: 29-52.

Zoli, A.P., Beckers, J.F., et Ectors, F. 1995. Isolement et caractérization partielle d'une glycoproteine associée à la gestation chez la brebis. Ann. Medicine Veterinaire, 139: 177-184.

Chapter XIII

BUFFALO PATHOLOGIES

Antonio Fagiolo [1], Cristina Roncoroni [1], Olga Lai [1] and Antonio Borghese [2]

[1].Istituto Zooprofilattico Sperimentale Lazio e Toscana
(Animal Prophylaxis Research Institute for Lazio and Toscana regions)
Via Appia Nuova 1411, 00178 Capannelle (Rome), Italy

[2].Istituto Sperimentale per la Zootecnia (Animal Production Research Institute)
Via Salaria 31, 00016 Monterotondo (Rome), Italy

The water buffalo is susceptible to most diseases and parasites that afflict cattle, although the effects of disease on the buffalo and its productivity are sometimes less evident. Generally *Bubalus bubalis* is a healthy animal, in spite of a natural habitat consisting of hot and humid regions that are very favourable to micro organism and parasite proliferation.
The diseases affecting buffaloes have been subdivided as follows:

Viral diseases

- Foot-and-mouth disease
- Rinderpest
- Malignant catarrhal fever
- Infectious bovine rhinotracheitis/ infectious pustular vulvovaginitis
- Blue tongue
- Bovine viral diarrhoea
- Rabies
- Ephemeral Fever
- Buffalo pox

Neonatal diarrhoeal diseases

- Rotavirus
- Salmonellosis
- Colibacillosis
- Cryptosporidiosis

Bacterial diseases

- Bovine brucellosis
- Tuberculosis
- Paratuberculosis
- Haemorrhagic septicaemia
- Chlamydiosis
- Leptospirosis
- Contagious bovine pleuropneumonia
- Anthrax

Parasitic diseases

- Trypanosomiasis

- Ascaridiosis
- Fasciolosis
- Babesiosis
- Theileriosis
- Strongilosis
- Coccidiosis
- Echinococcosis/hydatidosis
- Mange

Fungal diseases

- Deg Nala disease

VIRAL DISEASES

Foot-and-mouth disease

Etiology- Foot-and-Mouth Disease (FMD) is an infection caused by the Aphtovirus, an RNA virus pertaining to the Picornaviridae family that affects cloven hoofed animals. The virus occurs in seven main serotypes: O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1. Every serotype comprises various immunological subtypes of different virulence. Hajela and Sharma (1978) reported that Asia 1 is more severe in buffaloes than in cattle.

Epidemiology- The disease is present worldwide except in North and Central America (north of Panama), Australia, New Zealand, Japan, Great Britain and Scandinavia. The European Union (EU) countries are generally free from FMD. Severe forms of the disease have been reported in the indigenous Swamp buffaloes of India, Egypt and Romania. The disease is acute and highly contagious and may spread over huge areas due to movement of infected or contaminated animals, products, objects, and people.

The susceptibility of buffaloes to FMD has shown to vary according to the country and the various strains of virus. Cattle and buffaloes are mainly infected by inhalation due to aerosol transmission, often from pigs, which excrete large amounts of virus by respiratory aerosols and are considered highly significant in the diffusion of the disease. It also spreads through ingestion or direct contact. Large amounts of virus are excreted by infected buffaloes and cattle before clinical signs are evident, and winds may spread the virus over long distances. During the acute stage of infection, the disease has been transmitted from cattle to buffalo and vice versa (Gomes et al., 1997); occasionally buffaloes remain unaffected although in direct contact with cattle. In Nepal the clinical disease was comparatively mild and mainly affected buffaloes as a severe decrease in milk production, even for those in close contact with cattle, that exhibited the classical lesions.

People can be infected through skin wounds or the oral mucosa by handling diseased stock, the virus in laboratories, or by drinking infected milk, but not by eating meat from infected animals. The human infection is temporary and mild, so FMD is not considered a public health problem, but, due to the range of species affected, the high rate of infectivity, and the fact that the virus is shed before clinical signs occur, FMD is one of the most feared reportable disease. An outbreak of FMD involves very high costs due to lost production, which includes milk and draught power, loss of export markets, and loss of animals during the eradication of the disease. The significance of many other reportable diseases is due to their similarity to FMD and the importance of differentiating between them at the first indication of an unusual disease outbreak.

The incubation period is 2 to 21 days (average three to eight). The rate of infection (morbidity) can reach 100 percent, however mortality can range from 5 percent (adults) to 75 percent (suckling pigs and sheep). It may rise up to 20 to 25 percent in buffaloes. Persistent infection

in buffaloes during the first 35 days following infection is similar to that in cattle (Gomes et al., 1997). Recovered cattle and buffaloes may be carriers for 18 to 24 months, sheep for one to two months, while pigs are not carriers. Vaccinated buffaloes may also be carriers whenever exposed to infection.

Clinical findings- Clinical signs in cattle are salivation, depression, anorexia and lameness caused by the presence of painful vesicles in the skin of the lips, tongue, gums, nostrils, coronary bands, interdigital spaces and teats. Fever and decreased milk production usually precede the appearance of vesicles. Vesicle rupture leaves large denuded areas which are then liable to infection. Foot-and-mouth disease has been described in Indian buffalo (*Bubalus bubalis*) with the same features as that in cattle in terms of temperature, viraemia, virus replication in pharyngeal area, excretion, antibodies cynetics and titres, and persistent infection; differences were the presence of minor tongue lesions and initial scaly foot lesions eventually becoming vescicular in buffalo (Gomes et al., 1997). Milk production can be affected by up to 30 percent in buffaloes. All age groups are equally affected but in suckling buffalo calves the disease may result as more severe and a high mortality rate may sometimes ensue (Sharma and Kumar, 2003). Post mortem lesions are characterized by vesicular eruptions and erosions. In calves there can be hyaline degeneration of the myocardium (tiger heart).

Diagnosis- The suspect deriving from clinical findings can be confirmed by foot-and-mouth virus isolation in cell culture and laboratory animals. Up to five days are necessary before a negative tissue culture result can be given. Serological methods consist of complement fixation (CFT), plaque reduction assay, virus neutralization, radial immunodiffusion, virus infection associated antigen test and ELISA. With the latter, results may be available within four hours, differentiating FMD from the Swine vesicular disease (SVD) antigen and, if positive for FMD, it will indicate the serotype of the FMD antigen involved. ELISA has been found to be more sensitive than the microserum neutralization test.

Negative results may be due to poor samples rather than to freedom from infection, so the quality of the submitted material is extremely important. The FMD viral genome can be detected through the PCR technique. Polymerase chain reaction: This technique relies on the amplification of a selected region of the FMD viral genome. A pair of nucleotide primers is selected with sequences related to the nucleotide sequence at the start and end of the selected region. Following reverse transcription of the viral RNA into DNA, the selected region between the pair of primers is amplified by repeated cycles of heating and cooling in the presence of a heat-stable DNA polymerase. The resulting mixture is run on an agarose gel and a positive result is recorded if a band of the appropriate size is observed. Confirmation of the result can be obtained by nucleotide sequencing of the proposed FMD-specific band. The advantage of such a technique is that no live virus presence is required. FMD viral RNA can therefore be identified in material which has been inactivated or poorly preserved. The above technique is extremely sensitive and allows the FMD viral genome to be identified when insufficient virus is present to initiate infection in tissue culture. However, using current techniques, the presence of inhibitory substances in some samples means that not all samples that contain virus or viral genome will give a positive result by PCR.

Therapy- FMD lesions should be disinfected and treated with emollients; the administration of antibiotics could prevent secondary bacterial infections. Sometimes non-specific immunomodulators proved useful (Sharma and Kumar, 2003).

Prophylaxis- Strategies for the control of foot-and-mouth disease can be based on different measures depending on the objectives and on the existing sanitary situation. Eradication implies a policy in which the presence or possible incursion of the virus is not tolerated, while control implies that the presence of the virus might be tolerated but the effects of the disease are minimized by vaccination and other zoosanitary measures. Control by 'Stamping Out' is applied when outbreaks occur in countries which are otherwise free from FMD. It is also applied as the final stage in an eradication campaign to eliminate the virus once the disease

has been controlled. Control of the movement of animals, import controls, removal of the source of infection (slaughter of all infected and in-contact stock), and epidemiological investigations are essential elements in the eradication of FMD.

Methods of immunization have been studied taking into account the antigenic diversity among serotypes. The control of the disease should be effected through inoculation, twice a year, with a cocktail vaccine containing the specific locally circulating serotypes. Molecular studies pointed towards VP1 capsid protein as the major immunogenic site, and it has been tested for vaccines with encouraging results (Sharma et al., 2001).

Rinderpest

Etiology- The causative agent is an RNA virus, belonging to the Paramyxoviridae family, Morbillivirus genus. The disease is characterized by a high morbidity rate. The mortality rate is also high with virulent strains but variable with mild strains, that have almost identical immunological properties.

Epidemiology- Hosts are represented by cattle, zebus, water buffaloes, sheep and goats and many species of wild animals e.g. African buffaloes, eland, kudu, wildebeest, various antelopes, bushpigs, warthog, giraffes, etc. Buffalo susceptibility is variable: Egyptian and Turkish buffaloes seem to be reasonably resistant, while the Far East species appears to be highly susceptible. In India buffaloes are three times more susceptible than cattle; this is perhaps due to host specificity of strains of the virus (Sharma and Kumar, 2003). The disease has been eradicated in most parts of the world. However, among countries involved in buffalo breeding, exceptions are represented by India, Pakistan, the Philippines and Turkey. The virus has never been encountered either in the Americas nor in Australia or New Zealand.

Transmission is due to direct or close indirect contacts through tears, nasal secretions, saliva, urine and faeces, vaginal exudes and milk. Blood and all tissues are infectious before the appearance of clinical signs and excretion is usually limited to two to three weeks after infection. The primary site of invasion is the epithelium of the upper or lower respiratory tract. The incubation period is 3 to 15 days, in buffaloes three to seven days have been reported, but this may vary due to the differences in innate resistance (Adlakha S.C. and Sharma S.N. 1992).

Clinical findings- A febrile period (40-42°C) with depression, anorexia, reduction of rumination, rough hair coat, and an increase in the respiratory and cardiac rate. After two to three days a mucous membrane congestion (oral, nasal, ocular and genital tract mucosae), intense mucopurulent lacrimation and abundant salivation, anorexia, necrosis and erosion of the oral mucosae can be witnessed. Following this gastrointestinal signs appear as the fever drops, with profuse haemorrhagic diarrhoea containing mucus and necrotic debris. Severe tenesmus, dehydration, abdominal pain, abdominal respiration, weakness, recumbency and subnormal temperature occur a few hours prior to death, which transpires within seven to twelve days. The mortality rate is high. In India it is approximately 77.5 percent (Sharma and Kumar, 2003). In rare cases, clinical signs regress by day ten and recovery occurs by day 20 to 25. In the peracute form no prodromal signs or high fever (>40-42°C) are observed; but sometimes the mucous membranes are congested, and death ensues. This form occurs in highly susceptible young and newborn animals. The subacute form is characterized by only one or more of the classic signs and has a low mortality rate. Finally the atypical form is characterized by irregular pyrexia, and mild or absence of diarrhoea, cutaneous eruptions on the perineum, around the udder, scrotum and between legs, abortions and neurologic signs. The lymphotropic nature of the rinderpest virus favours recrudescence of latent infections and/or increased susceptibility to other infectious agents. Lesions are represented by either areas of necrosis and erosions, or congestion and haemorrhage in the mouth, intestines and upper respiratory tracts; enlarged and oedematous lymph nodes; white necrotic foci in Peyer's patches; 'zebra striping' in the large intestine; carcass emaciation and dehydration.

Diagnosis- Laboratory diagnosis involves identification of the agent by antigen detection (Agar gel immunodiffusion test; direct and indirect immunoperoxidase tests; counter immunoelectrophoresis; immunohistopathology); virus isolation and identification in VERO or bovine kidney cell cultures; virus RNA detection (cDNA probes; PCR); serological tests (enzyme-linked immunosorbent assay (ELISA), virus neutralization, agar gel precipitation test, neutralization of inhibition of haemoagglutination, counterimmunoelectrophoresis (CIEP), complement fixation test (CFT), fluorescent antibody test (FAT)). Using the agarose immunodiffusion test (AGID), serum should be taken three to five days following the onset of fever (Sharma and Kumar, 2003). Differential diagnosis is necessary for the mucosal virus disease complex that resembles rinderpest but that has no antigenic relationship to it.

Therapy- No specific treatment. When diarrhoea is present, lost fluids should be replaced by saline or lactated Ringer's solution.

Prophylaxis- Prevention and control is possible thanks to sanitary prophylaxis, isolation or slaughtering of sick and in-contact animals, destruction of cadavers, disinfection, protection of free zones, medical prophylaxis. The virus is in fact very fragile and sensitive to common disinfectants, heat treatment and drying. Cell-culture attenuated virus vaccines are highly effective.

The commonly used vaccine is an attenuated strain of rinderpest virus. In some countries a mixed rinderpest/contagious bovine pleuropneumonia vaccine is used. Immunity lasts at least five years and is probably life-long. Annual revaccination is recommended in order to obtain a high percentage of immunized animals in an area.

Genetically engineered thermostable recombinant vaccines have passed all the safety and efficacy trials recommended by the OIE and are currently undergoing limited field trials. Animals immunized with recombinant vaccinia virus developed immunity and were resistant to infection with virulent RP virus. The immunity lasts for at least one year following one single vaccination (Sharma et al., 2001).

Malignant catarrhal fever

Etiology- Also known as malignant head catarrh, malignant catarrhal fever (MCF) is a generalized viral disease caused by a highly cell-associated lymphotropic herpes virus of the subfamily Gamma herpesvirinae. Two viral strains have recently been designated: alcelaphine herpes virus-1 (AHV-1) and alcelaphine herpes virus-2 (AHV-2), although some continue to designate this agent as bovid herpes virus-3. In addition, a sheep associated form (SA-MCF) identified as ovine herpes virus-2 (OHV-2), represents a worldwide problem in cattle and buffaloes. Seroepidemiological studies implicate goats as possible carriers of SA-MCF (Sunil-Chandra, 2000).

Epidemiology- The virus can be carried as a latent infection by African antelope of the family Bovidae, subfamily Alcelaphinae which includes wildebeest (Connochaetes sp.), hartebeest (Alcelaphus sp.), and topi (Damaliscus sp.), that are considered carriers of the alcelaphine MCF virus. There is serologic evidence that several other African wild ruminants, such as various species of oryx and addax, may also be reservoir hosts, although the MCF virus has not been isolated from these species. Domestic and wild sheep and goats are also considered reservoir hosts for the MCF virus. Sheep-associated MCF occurs worldwide. In cattle the alcelaphine antelope-associated form chiefly occurs in Africa, in the natural habitat of wildebeest, hartebeest, and topi. This form of MCF has, however, been observed in zoos and wild animal parks that keep wildebeest. There is increasing serologic evidence that cattle may develop low levels of neutralizing antibodies following exposure to MCF, especially of sheep or goat origin, without manifesting the clinical disease. There is evidence that stress or some other immunosuppressive factors may be necessary as a precursor of clinical sheep-associated MCF. The MCF virus in wildebeest, hartebeest, and topi is largely cell-associated in adult animals

and hence rarely transmissible. However, neonatal wildebeests have been found to shed cell-free MCF virus through nasal and ocular secretions and faeces. Cell-free MCFV has also been evidenced in nasal secretions of captive adult wildebeests after stress or the administration of corticosteroids. Transmission to cattle or other susceptible species may occur by inhalation of cell-free virus in infectious aerosol droplets, ingestion of food or water contaminated with infectious secretions or faeces, or possibly mechanically by arthropods. The mode of transmission of sheep-associated MCF remains unknown, although relatively close contact between cattle and sheep, especially lambing ewes, is believed necessary. Sheep associated MCF is of major economic concern in Indonesia where susceptible animals, Balinese cattle and water buffalo, are commonly housed together with sheep and goats (Sunil-Chandra, 2000). MCF-affected cattle appear to shed only cell-associated virus, and thus cattle to cattle transmission is thought to be rare or nonexistent, although there are documented instances where this has occurred. There is no evidence that MCF is infectious for humans. Many exotic ruminant species in zoos have been reported affected with MCF, including several wild bovines such as bison, water buffalo, gaur and banteng, and several deer (including white-tailed deer) and antelope species. Morbidity in nonalcelaphine MCF outbreaks in Malaysia ranged from 28 percent to 45 percent. MCF affects all ages, breeds, and sexes; buffaloes are more susceptible than cattle with a morbidity ranging from 20 to 50 percent; the disease is particularly common in the late winter/spring months (Sharma and Kumar, 2003). The prognosis in MCF is poor. Once clinical signs are observed, mortality is usually greater than 95 percent (90-100 percent). The incubation period in natural cases is not known, but epidemiologic evidence indicates it may be as long as 200 days. Experimentally, the incubation period varied from 9 to 77 days. The disease has been reported from most countries breeding buffaloes.

Clinical findings- Clinical signs in domestic cattle and buffaloes and in many species of wild ruminants are characterized by high fever, profuse nasal discharge, corneal opacity, ophthalmia, generalized lymphadenopathy, leukopenia, and severe inflammation of the conjunctival, oral, and nasal mucosas with necrosis in the oral and nasal cavities sometimes extending into the esophagus and trachea. Occasionally central nervous system (CNS) signs, diarrhoea, skin lesions, and non-suppurative arthritis are observed.

Clinical MCF in cattle can be divided into four types: 1. Peracute: Fever, severe inflammation of the oral and nasal mucosas and haemorrhagic gastroenteritis with a course of one to three days. 2. Intestinal: Fever, diarrhoea, hyperemia of oral and nasal mucosas with accompanying discharges, and lymphadenopathy with a course of four to nine days. 3. Head and eye: This is the typical syndrome of MCF with fever, nasal, and ocular discharges progressing from serous to mucopurulent and purulent. Encrustation of the muzzle and nares occurs in later stages, causing obstruction to the nostrils, dyspnea, open-mouthed breathing, and drooling. There is intense hyperemia and multifocal or diffuse necrosis of the oral mucosa (usually on the lips, gums, and hard and soft palate) and buccal mucosa. Erosion of the tips of buccal papillae, leaving them reddened and blunted, is often encountered. Ocular signs referable to ophthalmia include lacrimation progressing to purulent exudation, photophobia, hyperemia, and edema of the palpebral conjunctiva and injection of scleral vessels. Corneal opacity, starting peripherally and progressing centripetally, results in partial to complete blindness. Hypopyon may also be seen. Corneal opacity is usually bilateral but can occasionally be unilateral. Fever is common and usually high (104-107°F [40-41.6°C]) until the animal turns moribund, at which time it shifts to hypothermy. Clinical features at early onset of the disease have included reddening of the udder skin, the coronary bands and interdigital spaces, and marked hyperemia of the oral cavity. Increased thirst accompanies fever, and anorexia is seen in late stages. Constipation is common in this form of MCF, but terminal diarrhoea is sometimes observed. Nervous signs are not frequently seen but may be manifested by trembling or shivering, uncoordinated gait, and terminal nystagmus. Necrotic skin lesions are occasionally seen, and horn and hoof coverings may be loosened or sloughed in some cases. The course of the "head and eye" form, which is invariably fatal, is usually 7 to 18 days. 4. Mild: These are syndromes caused by the experimental infection of cattle with attenuated viruses and are usually nonfatal.

The manifestations of the "head and eye" form of MCF are considered the typical syndrome in cattle, but clinical signs in exotic ruminants are often less dramatic and not usually diagnostic. In buffaloes the disease is seen in head and eye or intestinal forms (Sharma and Kumar, 2003). In Indonesian swamp buffaloes, hyperaemia of the skin, enlargement of the lymph nodes and depression were described (Hoffmann et al.,1984; Adlakha and Sharma, 1992). Gross lesions vary considerably, depending on the form or severity and course of the disease. Animals that die with peracute disease may have few lesions other than a haemorrhagic enterocolitis.

In the more protracted acute to subacute disease (intestinal and head and eye forms), the carcass may be normal, dehydrated, or emaciated. The muzzle is often encrusted and raw. Cutaneous lesions sometimes occur as a generalized exanthema with exudation of lymph causing crusting and matting of the hair. Where skin is unpigmented, hyperemia is apparent. These lesions are frequently seen in the ventral thorax and abdomen, inguinal region, perineum and loins, and sometimes on the head. Enlarged lymph nodes are characteristic findings in MCF. All nodes may be involved, but those in the head and neck and periphery are the most consistently prominent. Affected nodes are grossly enlarged and edematous and sometimes have patchy reddened or beige-brown areas on cut surfaces. Hemolymph nodes are also enlarged and prominent. The spleen is slightly enlarged, and Malpighian corpuscles are prominent. Pale areas may be seen in the heart muscle; serofibrinous epicarditis and myocarditis were found in Indonesian swamp buffalo (Hoffmann et al., 1984). Lesions in the respiratory system range from mild to severe. When the clinical course is short, there is slight serous nasal discharge and hyperemia of the nasal mucosa. Later the discharge becomes more copious and mucopurulent to purulent and is accompanied by intense nasal mucosal hyperemia, edema, and small focal erosions. Occasionally a croupous pseudomembrane formation is seen. Lesions in the nasal passages and turbinates may extend to the frontal sinuses. The pharyngeal and laryngeal mucosas are hyperemic and edematous and later develop multiple erosions, often covered with grey-yellow pseudomembranes. Inflammation and sometimes petechiation and ulceration are seen in the tracheobronchial mucosa. The lungs are often edematous and sometimes emphysematous but in some cases may appear normal. A bronchopneumonia may complicate chronic cases. The alimentary tract mucosa may have no gross lesion in peracute cases. When the course of the disease is protracted, the alimentary lesions are commensurately more severe and include mild to severe mucosal inflammation (hyperemia and edema), erosions, and ulcerations - especially on the dental pad and gingival surfaces, the palate, tongue, and buccal papillae. Mucosal inflammation, haemorrhage, and erosions may also be found in the rest of the digestive tract including the esophagus, rumen, omasum, abomasum, small intestines, colon, and rectum. Petechiation may be seen. Faeces are usually scant, dry, pasty, or blood stained. Urinary tract lesions include hyperemia and sometimes marked distention and prominence of bladder mucosal vessels and mucosal edema, perhaps with petechial to severe hemorrhage and occasionally epithelial erosion and ulceration. Kidneys may appear normal or mottled with patches of beige, discoloured raised areas. Petechiae or ecchymoses may occur in the renal pelvis and ureters. The liver is usually slightly enlarged, and, upon close examination, has a prominent reticular pattern. There may be hemorrhages and erosions in the gall bladder mucosa. In most cases, small arterioles are very prominent and tortuous and have thickened walls. This is usually seen in subcutaneous vessels and those in the thorax, abdomen, and CNS. Fibrinous polyarthritis is seen in many cases of MCF.

Diagnosis- Presumptive diagnosis of MCF can be based on clinical history and gross necropsy lesions.

A history indicating contact with sheep, goats, or alcelaphine antelope, especially around the period of parturition, associated with typical clinical features of MCF, provides grounds for a tentative diagnosis of MCF. The inability to differentiate the alcelaphine clearly from the sheep-associated MCF by clinical observations, lesions, or laboratory means presents an enigma in evaluating the possibility of a foreign animal disease. Based on existing knowledge of the disease, history of association with sheep, goats, or with alcelaphine antelope remains the only practical means of differentiating one form from another. Laboratory diagnosis can

support the field diagnosis if there are microscopic lesions of an extensive fibrinoid necrotizing vasculitis, perivasculitis, and lymphoreticular proliferation in lymphoid organs with mononuclear infiltrations in the kidney, liver, adrenals, CNS, etc., that are pathognomonic for MCF and are a sound, practical basis for a confirmed diagnosis. The disease should be distinguished from BVD mucosal disease, bluetongue, rinderpest, vesicular diseases (FMD, vesicular stomatitis (VS)), infectious bovine rhinotracheitis, haemorrhagic septicemia, ingested caustics and some poisonous plants and mycotoxins.

Virologic and serologic examinations provide additional information that may also ultimately lead to a better understanding of the epizootiology and differences between viral strains and the clinical manifestations. Methods used consist in virus isolation in calf thyroid tissue culture, identification of viral isolates, demonstration of the appearance or rising titers of MCF antibodies and molecular techniques using viral DNA probes, or target DNA amplifying methods such as the polymerase chain reaction (PCR). A single antibody positive serologic sample is of limited value in establishing an etiologic diagnosis. The PCR method for demonstrating MCF DNA segments is proving to be useful for identifying MCF carriers as well as diagnosing overtly diseased animals from formalin-fixed and paraffin-embedded tissue blocks.

Therapy- No specific treatment; supportive treatment can be effected with the use of broad-spectrum antibiotics, fluid therapy and non steroid anti-inflammatory drugs to relieve discomfort.

Prophylaxis- The control of the disease is possible by separating cattle and buffaloes from potential reservoir hosts such as sheep, goats, and wildebeest - especially during lambing, kidding, or calving seasons, respectively. The stocking of cattle ranches with alcelaphine antelope, wild sheep, or goats should be discouraged; in any event a negative MCF serologic test should be required and this preferably by the serum-virus neutralization method. A negative PCR test for any wild ruminant assigned to such a facility should be exacted. Similar testing of such wild ruminants before being placed in, or transferred between, zoos is also recommended as a means to prevent the introduction of potential carriers of the MCF virus.

Containment of an outbreak usually means the immediate separation of cattle or susceptible host from sheep and goats in the case of domestic disease and the susceptible host from alcelaphine or wild ruminants in the case of alcelaphine MCF.

No effective vaccine is available for MCF. Some viral strains have undergone limited attenuation after serial passage in cell cultures and represent a prospect for a future modified live virus vaccine. Experimental killed virus vaccines have been inconsistent in inducing protection against this virulent virus challenge, although some have induced significant titers of serum virus neutralizing antibodies.

Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis

Etiology- The Infectious Bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis (IBR/IPV), sometimes called Red Nose, is an infectious disease of cattle due to Bovine Herpes virus 1, belonging to the Herpesviridae family, sub family Alphaherpesvirinae. The virus can infect the upper respiratory tract or the reproductive tract. Mortality is low but the economic loss can be considerable. In Australia buffaloes' alphaherpesvirus has been differentiated from BHV-1 on a viral DNA restriction profile, indicating that it could be a distinct species (Sunil Chandra N.P., 2000). Like the homologous human herpes viruses, BHV-1 spreads through monocytes and other white blood cells and through peripheral nerves producing a latent infection in neuronal cells of the trigeminal and sacral ganglia. The latent infection allows the virus to persist in the infected hosts for indefinite periods. Reactivation may occur either spontaneously or induced by natural or artificial immunosuppressive stimuli (parturition, transport, dexamethasone). It leads to virus replication and re-excretion with its spread in the environment.

Epidemiology- Susceptible species are domestic and wild bovines; under experimental conditions goats, sheep and pigs have also demonstrated infection.

Transmission occurs through aerosols, direct and indirect (over short periods of time) contact and venereal viae, also artificial insemination, since large quantities of virus are shed in respiratory, ocular and reproductive secretions (amniotic liquid, placenta, foetus and semen) of the infected animals, for ten to fourteen days after infection, even if asymptomatic. During latent infections shedding is not so abundant and long lasting as during the acute phases. BHV 1 transmission is also possible due to milking machine cups (Sunil Chandra, 2000). The incubation period ranges from two to twenty days. It is a worldwide disease. The virus was first isolated in Australia (1972), then in Malaysia, India and Egypt. In Brazil, in the central Amazon region, antibodies against IBR/IPV were detectable in 59 percent of serum samples using the ELISA test (Munchow and Pisarz, 1994), in India 21.1 percent of buffaloes were positives (Costa, 2002). Serological investigations in Northern and South Central Italy evidenced seroprevalence rates of 78.4 percent and 51.1 percent for herds and animals, with a more active viral circulation where buffaloes and cows were reared together (Cavirani et al. 1997). In Southern Italy the prevalence was 82.9 percent among farms and 66.1 percent for the animals (Galiero, 1998). Finally, in Central Italy, the prevalence was 59 percent and about 30 to 80 percent (Fagiolo et al., 2001; Fagiolo and Roncoroni, 2003). A diffuse circulation of the virus is observed in the buffalo species.

Clinical findings- The severity of symptoms very much depends on the strain of the virus and the susceptibility of the cattle. In the respiratory form the symptoms include: fever (up to 42°C), general depression, drop in milk production, anorexia and emaciation, severe hyperaemia of the nasal mucosa (Red nose) with numerous clusters of greyish foci of necrosis on the mucous membranes, abundant serous discharge from nose and eyes, conjunctivitis, hyper salivation, tachypnea and tachycardia, sometimes mastitis, short explosive cough and, rarely, death due to obstructive bronchiolitis or bronchopneumonia from secondary bacterial infection. An abortion form can complicate the respiratory form with late abortion (between the fifth and eighth month of pregnancy) and placenta retention. It can be the only manifestation.

In the genital form of the disease, it lasts for two to three weeks and symptoms include: moderate fever, hyperaemia of genital mucosa with vesicles of one to two mm., white discharge of the vulva, pollakiuria, and reduction in milk yield. Males exhibit a balanoposthitis.

In the young calf, less than six months old, the disease is more severe: meningoencephalitis (lack of coordination, hyperexitation and depression), salivation, blindness, and a high mortality rate.

In newborn calves the disease causes: fever and lack of appetite, salivation, inflammation of the nasal mucosa, conjunctivitis, erosions of the mouth mucous membranes covered with mucopurulent exudate, and respiratory distress is common due to swelling of the larynx and pneumonia. Some calves may develop diarrhoea. Pathological manifestations have been observed mainly in bovines, while the pathogen role in buffaloes is less clear.

Lesions are usually restricted to the upper respiratory tract and include: swelling and congestion of mucosa, sometimes with necrotic foci, petechiae, profuse and fibrinopurulent exudate in severe cases. Differential diagnosis for the respiratory form: enzootic bronchopneumonia, Bovine Virus Diarrhoea/Mucosal Disease, gangrenous coryza, rinderpest, theileriosis; for the abortion form: Bovine Virus Diarrhoea/Mucosal Disease, brucellosis, listeriosis, leptospirosis, coxiellosis.

Diagnosis- The virus can be isolated from blood on EDTA, nasal, pharyngeal, conjunctival swabs, aborted foetus, placenta, vaginal swab, prepuce washing fluid and semen. Techniques include neutralization or antigen detection methods using monospecific antisera or monoclonal antibodies; PCR is also used for detection on semen. Samples should be stored in a transport

medium (cell culture medium containing antibiotics and two to ten percent foetal bovine serum to protect the virus from inactivation), cooled at 4°C, and rapidly submitted to the laboratory. Serum can be submitted for virus neutralization test and ELISA. The ELISA test allows the detection of antibodies in milk. A delayed cutaneous hypersensitivity test has also been proposed for IBR/IPV diagnosis.

Therapy- No specific treatment. Broad spectrum antibiotics can prevent bronchopneumonia from secondary bacterial infection.

Prophylaxis- IBR/IPV is most likely to be introduced by importation of infected animals and semen. Once introduced IBR/IPV is difficult and expensive to eradicate especially due to the fact that when the disease is established, animals tend to become unapparent carriers. Systematic testing and elimination of positives has been successful in some countries such as Denmark and Switzerland (Ackermann et al. 1990).

Different types of inactivated vaccines are available. Officially free countries restrict the use of them.

IBR is present worldwide, very few countries have eradicated it (Austria, Denmark, Finland, Sweden, Switzerland). Many Pacific countries or territories have reported serological evidence without clinical cases (Fiji, Guam, Niue, Samoa, the Solomon Islands, Tonga, and Vanuatu), New Caledonia has identified clinical cases.

Blue tongue

Etiology- Blue tongue is a vector-borne disease of ruminants caused by a virus of the Reoviridae family (24 serotypes have been identified), genus Orbivirus and biologically transmitted by five species of Culicoides mosquitos, small biting midges. It is not transmitted by direct or indirect contact between animals in the absence of these insects. The vector competence of all the different species of Culicoides has not yet been totally explored however new species are regularly found to be potential vectors.

Epidemiology- The virus may rarely be excreted in the semen when males are viraemic. Contaminated semen may infect recipient cows but would be unlikely to settle in an area unless abundant vectors were present. Blood is also an infective material. Hosts are represented by all domestic and wild ruminants: sheep, goats, cattle, buffaloes, dromedaries and wild ruminants. The incubation period ranges from five to twenty days. The mortality rate is normally low in sheep but can be up to 10 to 30 percent in some epizooties. The distribution of Blue tongue disease is substantially related to the distribution of the Culicoides vector. It can be introduced to new regions by the importation of infected animals, but it will not survive unless competent vectors are present and sufficient susceptible hosts are available.

Blue tongue occurs as a clinical disease in Africa, the Middle East, the Indian subcontinent, China, USA, Mexico and southern Europe. Serological evidence has also been found in South East Asia, northern South America, northern Australia, the Solomon Islands and Papua New Guinea and, on buffaloes, in Egypt, India, Botswana and Papua New Guinea, while all buffalo serum samples tested resulted negative in Iraq and India during the outbreaks of catarrhal fever of the ovine (Adlakha and Sharma, 1992; Capezzuto and Galiero, 2001).

Clinical findings- The disease is characterized by the inflammation of mucous membranes, congestion, swelling and haemorrhages. Sheep are generally the worst affected. The disease can be quite variable showing commonly: fever (42 °C), loss of condition and emaciation, inflammation, ulcers and necrosis in and around the mouth (gums, cheeks and tongue) and, in a small percentage of cases, cyanotic tongue that appears purplish-blue, reddening and haemorrhages of the coronary band (above the hoof) causing lameness. Abortions and congenital malformations can also occur and sometimes pneumonia.

Infection is generally sub-clinical in cattle and buffaloes. Cattle can remain a source of infection for sheep for some time. In about 5 percent of cases, fever, salivation, congestion and swelling and ulcers inside the mouth may occur. The varying reactions of buffaloes to the infection could depend on the serotype, on the infectant dose and on the involved species infecting the buffalo, whether it is only a carrier-amplifier of the virus or a serological responder without viraemia (Capezzuto and Galiero, 2001).

In sheep, most deaths occur as the result of secondary pneumonia. Hence severe, bilateral pneumonia is a common finding. Other findings may include: haemorrhages in the heart, swelling and necrosis of the muscles, enlarged lymph nodes and swelling and congestion of the spleen and liver.

Diagnosis- Serum can be used for the competitive ELISA, a serological test which is the OIE reference test, or Agar Gel Immunodiffusion. Virus neutralization and Complement Fixation can also be performed. Serum samples should be paired to demonstrate a rising antibody titre. Isolation of the agent is possible from organs and whole blood, by inoculation of sheep, intravascular inoculation in ten to twelve day old embryonated chicken eggs and in tissue cultures. The same matrices can be used for identification by PCR. For identification plaque reduction serum neutralization can be used but for serotyping there are too many cross-reactions.

Therapy- No efficient treatment is available.

Prophylaxis- In disease-free areas prevention consists in quarantine and serological survey and vector control. In infected areas sanitary prophylaxis can only be represented by vector control, for example putting sensitive animals during the night into closed rooms protected by mosquito nets. A medical prophylaxis is possible by vaccination with modified live virus vaccine or deadened polyvalent vaccines. Serotypes incorporated into the vaccine must be the same as those causing infection in the field. Attenuated vaccines are widely and effectively used in southern Africa, Europe and the USA, but present a number of disadvantages. Vaccination of pregnant ewes should be avoided because of the risk of foetal abnormalities and abortions. Inactivated vaccines are not used in endemically infected countries, since effective ones are yet to be developed. New immunizing drugs, based on the use of proteic viral fractions will probably prove to be a valid alternative to the traditional vaccine (Capezzuto and Galiero, 2001).

Bovine viral diarrhoea

Etiology- The BVD virus belongs to the Pestivirus genus (Flaviviridae family) and is closely related to the classical swine fever virus, the border disease of sheep and hepatitis C. These viruses are related antigenically as well as genetically. It is a group of small enveloped viruses with a single stranded, positive sense RNA genome. They all have a similar genomic structure and protein composition, the virus particle comprising a single capsid protein surrounded by an envelope containing two or three glycoproteins. The bovine viral diarrhoea virus has the ability to develop many different variants. If the virus finds itself in another situation, another virus type may take over the population with shifts in its pathogenic or antigenic properties.
BVDV also presents different biotypes: one producing cytopathologic effects (CPE) in cell culture, versus another one that does not (non-CPE). This is important as persistent infections are always due to non-CPE viruses that also cause foetal malformations, while CPE viruses are responsible for mucosal disease (MD) (Farina and Scatozza, 1998). Type I and Type II BVDV are related, but distinctly separate. Some Type II isolates do not cause severe clinical disease, and some Type I isolates indeed do, and both Type I and Type II BVD viruses present cytopathic and non-cytopathic forms. In both cases, the non-cytopathic form is the natural, more common state in cattle.

Epidemiology- The bovine viral diarrhoea (BVDV) infection is a major worldwide problem affecting different species of ruminants (Pringle 1999). Two biotypes of the virus, cytopathic

and non-cytopathic, are identifiable based on their lythic activity in vitro cultures (Meyers and Thiel, 1996). The disease is associated both with acute and persistent infections and, depending on epidemiological circumstances, may manifest as outbreaks affecting large numbers of animals or a continual low incidence of cases within endemically infected herds. The significant economic impact inflicted is due to productive and reproductive losses: by a reduced milk yield, reduced conception rate, abortion, foetus mummification, congenital malformations, weak calves and increased animal mortality.

Apart from cattle, other species that have been infected with BVDV include sheep, goats, lamas, pigs, giraffe, captive and free-ranging deer, antelope, elk, buffalo, water buffalo, reindeer and wildebeest. Persistently infected calves derive by in utero exposure at a certain period of gestation. These calves are persistently infected as they become immunotolerant, so they recognize the virus as "self" and never clear the viral infection. For that reason, they shed considerable quantities of virus, however this may not be done continuously. BVDV can be transmitted in many ways among the cattle population, such as through foetal infection and the shedding of virus in secretions. BVDV is primarily maintained in the population by persistently infected animals. Persistent infection (PI) is probably the primary mode of transmission among herds, but acute infections can also be involved in the transmission. The high prevalence of cattle herds infected with BVDV in many countries throughout the world is believed to be a consequence of the ability of non-cytopathic BVDV (ncpBVDV) to establish lifelong infections following in utero infection in early pregnancy, thus generating a reservoir of persistently infected animals (Brownlie et al. 1989).

This biotype, which is most frequently isolated during investigations is characterized by a vertical virus transmission and is responsible for a permanent BVDV circulation in the cattle population (Brownlie, 1991 and Booth et al. 1995). The investigations on the spread of BVDV, its manifestation and distribution depend not only on the properties of the agent, ways of transmission or immune status of the animal, but can also be associated with demographic risk factors.

Clinical findings- BVDV infection can be described as an acute, chronic, persistent, mucosal disease and haemorrhagic syndrome. It is now possible to find a much broader spectrum of disease than before. The clinical aspects of the infection are influenced by host factors such as immune status to BVDV, that is immunocompetence or immunotolerance of the animal, the gestation period, and by the BVDV-genotype involved. Estimates of economic losses due to BVDV infection vary, depending on the immune status of the cattle population and the pathogenicity of the virus strains (Houe 1999). In buffaloes, clinical signs are mild compared to those in cattle, but they are similar in chronology. In a study on buffalo calves experimentally infected with BVDV, an initial diphasic rise in temperature of about 40°C was evidenced as well as nasal and lacrimal discharge; only some of them had transient diarrhoea during and after the febrile phase; erosions and/or ulcers were seen in the surface of the lips and dental pad associated with congestion of the nasal and gum mucosa. In the first 11 days of infection a mild leukopenia (neutropoenia and lymphopoenia) was evidenced later turning to leukocytosis (neutrophilia) from day 15 to 32. The phagocytic activity decreased starting from the first day post infection and the T-lymphocyte population was severely affected (Hegazy et al. 1991).

Diagnosis- The primary dilemma for veterinary clinicians and diagnosticians is the detection of the virus in samples obtained from clinically ill, as well as subclinically infected cattle (Bolin, 1995) and the control of the viral disease, especially in reproductive-age cattle. Although acute infections with non-CPE BVDV are often asymptomatic or produce only mild clinical symptoms, there is evidence that they result in immunosuppression. The problems associated with the BVDV infections during pregnancy are complex. Pregnant, immunologically naive cattle are at risk of acquiring BVDV infections early (<100 days gestation), resulting in abortion; or mid-gestation (100-125 days), resulting in calves that are congenitally infected and are born persistently infected (PI); or later in gestation (>125 days), resulting in weak calves. There are at least two factors associated with the virus and two associated with the pregnant

animal that contribute to the vulnerability of the host to infection and disease. The two viral factors are immunosuppression and strain variation while the two host factors are the immune status of the heifer entering pregnancy and the physiologic immunosuppression which occurs during pregnancy. Laboratory tests can confirm clinical diagnosis and detect persistent infections. The samples are represented by oral swabs, faecal samples and blood with EDTA; spleen, brain, lung and kidney from foetuses and spleen, lymph nodes, gut and abomasum from adult animals. The samples are submitted to cell cultures with labelled antibodies. Sereological diagnosis can be performed with serum neutralization and ELISA.

Therapy- The primary treatment for the BVD virus arises from prevention since no good therapy exists.

Prophylaxis- Careful culling with proper diagnosis is therefore most important. Prevention focuses on good management and a vaccination programme. Management involves minimizing exposure to the virus from potential sources:

1. All ruminants; large or small (sheep and goats), wild (deer) or domestic movement of animals into a closed herd.
2. Modified live vaccines have been sources in the past.
3. Careful embryo transplant technique.

Additionally, good management must minimize stress in order to enable an optimum immune response to vaccines. Identifying persistent carriers in the herd is a good procedure but may not be practical for most herds. Whenever a case of BVD is confirmed or suspected, especially in a pregnant animal, identification of suspect carriers and culling is necessary.

A perfect vaccination programme has not been effected to date. However, careful administration of quality vaccine products with precise handling will provide a good deal of protection. Traditionally modified live vaccines have proved best in stimulating antibody response. The protection provided by the vaccines is a function of the strains in the product as well as the strains encountered in the field. Although only partial protection is accomplished with vaccines, they will in any case reduce the incidence. A good vaccine schedule should start at four to six months of age after colostral protection is ending and this should be repeated prior to breeding. A killed product should be used in pregnant animals. A killed product can be used if the facility permits easy handling of livestock and allows vaccination to be repeated since the duration of immunity is shorter than a modified live (ML) product. Since no pathognomonic clinical signs of infection with BVDV are to be evidenced, diagnostic investigations rely on either laboratory-based detection of the virus, or virus-induced antigens or even antibodies in submitted samples. In unvaccinated dairy herds, serological testing of bulk milk is a convenient method for BVDV prevalence screening. Alternatively, serological testing of young stock may indicate if BVDV is present in a herd. In BVDV positive herds, animals persistently infected (PI) with BVDV can be identified by the combined use of serological and virological tests for examination of blood samples. ELISA has been used for rapid detection of both BVDV antibodies and antigens in blood, but should preferably be supported by other methods such as virus neutralization, virus isolation in cell cultures or amplification of viral nucleic acid.

Rabies

Etiology- The Rhabdovirus (genus Lyssavirus) responsible for the disease is a truly neurotropic virus that causes lesions only in nervous tissue. It may be eliminated by the use of standard disinfectants and heat treatment.

Epidemiology- The source of infection is always represented by infected animals and it spreads mostly by saliva through contamination of wounds or bites or ingestion. The excretion in milk is low and does not cause the disease. Reports of this disease in buffaloes are not very numerous since they defend themselves well from rabid animals and no cases of rabies have

been reported due to transmission by bats as is the case in cattle. However mortality in buffaloes is 100 percent. Rabies occurs in most countries of the world except on islands where rigid quarantine measures are guaranteed. The incubation period is almost three weeks.

Clinical findings- The symptoms resemble those in cattle. The disease may present a paralytic (drooling of saliva, eructation, grinding of teeth, tail movement, anorexia, stiffness of hind limbs, paralysis and recumbency, death in two to three days) or furious form (alert state, hypersensitivity, sexual excitement, inability to swallow, ramming of head on fixed objects, loud bellowing, collapse and death) (Adlakha and Sharma, 1992).

Diagnosis- It can be made based on clinical symptoms. For confirmation: negri bodies evidenced by brain microscopic examination, impression smears from brain tested by FAT, CFT and ELISA, direct immunofluorescence test and PCR. The biologic test on mice is also important.

Therapy- Wounds should be irrigated with a soap solution and water. Post exposure vaccination can be performed. Suspected animals should be kept under close observation avoiding euthanasia.

Prophylaxis- Destruction of wild fauna around animal holdings and vaccination of cats and dogs are important. Vaccines from chick embryo origin and tissue culture origin can be used (Sharma and Kumar, 2003).

Ephemeral Fever

Etiology- Bovine Ephemeral Fever (BEF) is a viral disease of cattle and buffalo, caused by an arthropod-borne rhabdovirus that features four serovars (Sharma and Kumar, 2003). It is also referred to as an arbovirus in so much as biting insects spread it.

Epidemiology- The most likely insects to transmit the disease are blood sucking flies or mosquitoes, such as Culex annulirostris. Biting midges may also play a role in disease spread, and it is possible that some vectors have still not been identified. Adult buffaloes are those primarily affected while those below six months of age are not.

The distribution of the above mentioned insects varies according to climatic conditions; this, in turn, will influence the pattern of disease spread and time of occurrence. In fact most cases occur under hot and humid conditions. Mortality is low, although the morbidity rate is considerable, which entails enormous economic losses in terms of a significant reduction in production, disruption of national and international trade resulting in a variety of complications and such inconveniences have drawn appreciable attention to this disease. Subtropical and temperate regions of Africa, Asia and Australia have experienced the main epidemics of bovine ephemeral fever (Nandi and Negi, 1999).

Clinical findings- A sudden onset of fever (41°C) can be observed. The first sign in milking cows is a sudden and severe drop in milk production. Buffaloes in advanced stages of pregnancy may sometimes abort, this is probably due to the fever, rather than to a specific effect of the virus. Animals stop eating and drinking and become depressed, start drooling saliva, and develop a stringy nasal discharge. Lameness may not appear prior to the second day of illness but may cause the typical posture of laminitis. Muscular areas over the shoulder, back and neck regions show swelling. Shivering, stiffness and clonic muscular movements are also manifest (Sharma and Kumar, 2003). By day three the affected animal is usually standing again and will begin to eat. However, lameness and weakness may last for another two or three days. The disease can vary in severity. Some animals may show only slight symptoms for about 24 hours, while a small number may be affected for many weeks. The disease is usually milder in calves below 12 months of age. Milk production should return nearly to normal after about three weeks, but cows which are affected late in lactation, often become dry. Mastitis sometimes

develops, with a marked rise in the somatic cell count.

Diagnosis- When an outbreak occurs in unvaccinated cattle not previously exposed to the virus, a diagnosis of BEF can often be accomplished based on clinical signs and the brevity of the illness. Blood analysis evidences leukocytosis, neutrophilia, lymphopenia and increased fibrinogen.

Laboratory confirmation is possible by agar gel precipitation, complement fixation, ELISA and fluorescent antibody tests (Sharma and Kumar, 2003). This is usually undertaken by taking two blood samples - one during the very early stages of the illness, and another one three weeks later. If BEF is responsible, BEF antibody levels will be much higher in the second test than in the first one.

Therapy- Medical treatment is often unnecessary for non-lactating stock. However, bulls and high-producing cows, in early to peak production, should have supportive treatment. It consists in relieving temperature and muscular stiffness with paracetamol and phenylbutazone. Broad spectrum antibiotics (streptopenicillin, tetracyclines) prevent secondary bacterial complications.

Affected animals should not be drenched or force fed as BEF can impair the swallowing reflex, so this may result in the inhalation of food or water and pneumonia.

Prophylaxis- Both live and inactivated vaccines against BEF are available: In cattle the live vaccine gives at least 12 months' protection following two doses, the killed one only gives about six months' protection. Cattle can be vaccinated beginning from six months of age and should then be revaccinated each year to ensure continued protection.

Buffalo pox

Etiology- Orthopox virus, family Pox Viridae. It is resistant to inactivation by ether but sensitive to a change of pH, chloroform and bile salts. It only affects buffaloes. Rabbits and infant mice can be experimentally infected. The infection has been reported in man, among persons handling affected animals.

Epidemiology- The disease is usually present in an endemic form. Morbidity can be as high as 70 percent but the mortality rate is known to be low. It has been reported in India, Indonesia, Italy, Pakistan, Russia and Egypt (Sharma and Kumar, 2003).

Clinical findings- It can be observed in both a localized and generalized form. The pox skin lesions are mainly on the teats, udder and thighs and manifest the typical stages and heal in three to four weeks. About 50 percent of affected animals show mild to severe mastitis. Signs of the disease are represented by fever, anorexia, dullness, depression and congestion of conjunctivae (Adlakha and Sharma, 1992).

Diagnosis- The presence of lesions on the udder and teat could lead to suspicion. Isolation can be performed on chick embryo or cell culture. Confirmation is performed by precipitation, complement fixation and neutralization tests. ELISA has been found to be more sensitive.

Therapy- No specific treatment is applicable. Antibiotics can control secondary bacterial contamination

Prophylaxis- The control of the disease is based on strict hygienic measures since no suitable vaccine is available (Sharma and Kumar, 2003).

NEONATAL DIARRHOEAL DISEASES

Etiology- The greatest buffalo losses are often among calves. Farm records show that losses in buffalo calves can reach 22.2 percent in the first month of life or in the fourth to sixth month (Sunil Chandra and Mahalingam, 1994). Newborn buffalo calves, like bovine calves, can succumb in large numbers to viruses, bacteria, and poor nutrition. This is largely due to poor management during the calf's first two months of life, especially with regard to males. For example, in some countries, breeders often sell the valuable buffalo milk, thus depriving the calves. Apart from poor management, among other causes of calves' mortality, an important problem is represented by neonatal diarrhoea. It heavily affects both buffalo and cattle herds, causing extensive economic losses due to mortality and cost of treatment. 23.7 percent of neonatal buffalo deaths are due to enteric disease (Sunil Chandra and Mahalingam, 1994). Diarrhoea should be studied as a syndrome since it is related to a complex etiology. During outbreaks of buffalo neonatal diarrhoea, different pathogens have been detected: several bacteria, viruses and fungi. Viral agents such as Rotavirus, *Coronavirus* and *Calicivirus* are spread worldwide and have been serologically evidenced in buffaloes (Giglio et al., 1994). E. coli and Cryptosporidium have also been detected (Cavirani et al. 1997) but in other cases the main causes have turned out to be colibacillosis and salmonellosis. E. coli incidence appeared higher in the first week and salmonellosis in the third, but E. coli seems to be the major cause of diarrhoea with an incidence of 54 to 58 percent compared to 13 to 14 percent for salmonellosis. Haemorrhagic diarrhoea has also been reported in buffalo calves due to Clostridium perfringens types A, C and D. Pasteurella, Klebsiella, Proteus vulgaris and Citrobacter have also been recorded (Sharma and Kumar, 2003). Parasitic agents such as Eimeria spp., Strongyloides papillosus, Toxocara vitulorum and Cryptosporidium parvum are commonly found in both diarrhoeic and non-diarrhoeic calves. This demonstrates the significance of all calves as potential sources of infection. E. coli, E coli ETEC, E. cloacae, K. Pneumoniae and Citrobacter spp. show a higher rate of occurrence in diarrhoeic animals but, except for E. coli ETEC and Citrobacter, they are also present in the enteric microflora of the buffalo calves (Ribeiro et al. 2000). Multiple infections due to enteropathogenic viruses and bacteria are more common than single ones.

Epidemiology- The incidence and severity of the disease depends on several factors: colostral immunity, overcrowding, parity of dam, age, sex, birth weight, quality of diet, meteorological conditions and the general care provided to calves. Studies have shown that mortality among buffalo calves is also related to the season; higher mortality, in India, was registered during the months from July to September. Another major factor is colostrum: inadequate feeding on the first day of life makes the calf more susceptible because of the associated low level of serum immunoglobulins (Yadav, 2003). Calves delivered from heifers were more susceptible being weak at birth because of dystokia and the enteric infections were higher in very young calves (El-Garhi et al. 1994). Overall mortality in buffalo calves registered by different authors ranges from 7 to 33.97 percent and among these deaths the major cause is enteritis. Mortality from salmonellosis ranges from 40 to 72 percent, that from colibacillosis is 47 percent. The newborn buffaloes usually present diarrhoea during the first four weeks of life (Ribeiro et al. 2000).

Clinical findings- Generally calves show acute, profuse diarrhoea, sometimes with dysentery, cyanotic mucous membranes, depression, weakness, lack of coordination, severe dehydration and acidosis leading to death within a few days. Sometimes calves also develop pneumoenteritis.

Diagnosis- It can be confirmed by isolation and identification of the organisms.

Therapy- Usually this is based on diet, administration of fluids, electrolytes, antibiotics and intestinal protectants.

Prophylaxis- This is based on cage comfort and hygiene, adequate colostrum administration and increasing non-specific and specific resistance with dams' or calves' vaccination. Colostrum

should be provided within the first two hours of the calves' life, in a quantity of 50 ml/kg, corresponding to 1/20th of the body weight. It has also been proved that calf mortality decreases when feeding is always regularly performed by the same person and that proper housing and ventilation are important as they contribute to reducing stress (Yadav, 2003).

Rotavirus

Etiology- Rotaviruses belong to the Reoviridae family and are characterized by a genome consisting of 11 segments of double-stranded RNA (dsRNA) enclosed in a triple-layered capsid. Its structure accounts for a high resistance in the ambient, seven to nine months at 18 to 20°C, and to various disinfectants. Rotaviruses include seven different serotypes identified by the letters from A to G. Group A rotaviruses are the main cause of acute viral gastroenteritis in humans and animals throughout the world, particularly in newborn animals and among those, in buffaloes. The inner capsid protein VP6 bears the subgroup (SG) specificities that allow the classification of group A rotaviruses into SGI, SGII, both SGI and SGII, or neither SG based on the reactivity with SG-specific monoclonal antibodies. The serotyping and genotyping of rotaviruses within the group A is determined by cross neutralization and by sequence comparison respectively. There is also a classification system based on glycoprotein VP7 and protease sensitive virus protein that recognizes 14 G types and 19 P types in group A rotaviruses (Sunil Chandra, 2000).

Epidemiology- The epidemiology of buffalo rotaviruses is still widely unknown. Rotaviruses are transmitted by the ingestion of viral particles due to oral-faecal contamination. It affects the buffalo calf when it is about one to three weeks old. Rotavirus environmental infecting power is generally high due to the viral excretion from the infected calves, although healthy looking, viral elimination from mothers just before calving, and high viral resistance in the environment. After ingestion, rotaviruses infect the small intestine, multiply in the mature epithelial cells at the tips of the villi, causing the microvilli to degenerate and the release of a large amount of viral particles. The destroyed cells are replaced by underlying ones that are lacking in digestive and absorption functions. So cell death and desquamation cause reduced digestion, malabsorption and villous atrophy. The consequent reactive crypt cell hyperplasia and the increased secretion contributes to the severity of the diarrhoea. Rotavirus antigens or antibodies have been detected in buffaloes faecal or serum samples in Iraq, India, Italy, Sri Lanka and Bulgaria. Buffalo calves showed a greater number of positives compared to calves from cattle. Infection rates were higher in winter than in summer and the highest rates were found in two-week old calves (Adlakha and Sharma, 1992). BRV is pathogenic for calves for the first three weeks of life, then the infection becomes clinically irrelevant. The morbidity for buffalo calves is 43 to 45 percent; mortality is 25 percent. BRV is not highly virulent but proves to be serious in the case of mixed infections. 91 percent of mixed infections comprehend BRV. The viral antigen was detected in 36.6 percent of diarrhoeic calves aged from 10 to 51 days, and only in 11.9 percent of non diarrhoeic calves (Sunil Chandra and Mahalingam, 1994). The incubation period is assumed to range from one to three days.

Clinical findings- The onset of anorexia, depression, sometimes fever, and most of all watery or pasty diarrhoea that can show traces of blood, and dehydration, is rapid. The peak of the diarrhoea is at seven to eight days, afterwards BRV shedding may occur but the clinical disease is not evident. Many pathogens of low pathogenicity can, together with BRV, produce a severe syndrome of diarrhoea, dehydration and acidosis, often resulting in death in young calves. Lesions are essentially intestinal, including dilatation, yellowish content and thinned sections.

Diagnosis- It is based upon virus identification in the faeces, which should be performed at the beginning of the diarrhoea and prior to any antibiotic therapy in order to verify the presence of bacteria. Necroscopy should be carried out within 24 hours. Polyacrilamide gel electrophoresis (PAGE) allows the detection of group A, B and C. Antigenic types are identified with a monoclonal antibody, screening and blocking ELISA and DNA probes. The BRV positives can be confirmed by dot-ELISA and immunofluorescence. Immunoperoxidase focus neutralization

assay can determine the serotype specificity of the rotavirus specific colostral antibody. Rotavirus isolation can be performed using cell lines of a foetal Rhesus monkey kidney and foetal calf kidney (Adlakha and Sharma, 1992).

Therapy- No specific drug has shown to be efficient. It is essential to provide calves with liquids during diarrhoea in order to replace the lost fluids and restore the right hydrosaline balance by saline or lactated Ringer's solution intravenous, subcutaneous or by oral solutions. The use of antibiotics allows the control of secondary bacterial infection in a damaged intestine.

Prophylaxis- Direct prophylaxis is aimed at the maintenance of a high environment hygiene. Buffalo calves should be placed in single disinfected cages which have not been used for 15 to 20 days. High quality colostrum is essential for the prevention of neonatal disease. Various preparations with more antigen valences are sold for passive immunization of calves through vaccination of pregnant female buffaloes. The first two feeding supervisions should permit the attainment of the maximum number of calves with maternal antibodies as maternal vaccinations are not effective if colostrum is not provided in sufficient quantities during the first six hours of life. The amount should be equal to five to ten percent of the calf's body weight or to two litres on the first day (Galiero, 2000).

Salmonellosis

Etiology- Salmonella is a Gram negative bacteria comprehending more than 2 000 species. In the buffalo species several isolations have been reported, but no salmonella serotype seems to be host-adapted. Among the most disseminated serotypes there are S. typhimurium, S. dublin, S. newport, S. bovis morbificans, S. weltevreden, S. abortusbovis, S. java, S. paratyphi B, S. panama, S. enteritidis, S. reading, S tafo, S. kottbus, S. weybridge, S. muenster, S. abortus ovis, S. cholerae suis, S. pullorum (Galiero G., 1999; Sharma and Kumar, 2003; Bahiraie and Moghaddam, 2004). Infection becomes possible due to the presence of adhesines and the different invasive ability in the various strains. Some strains produce enterotoxins and cytotoxic toxins. Salmonella has a good resistance, it can survive for one year in the ground. It is sensible to one percent formaldehyde solution, one percent glutaraldheyde solution and formalin. Salmonellosis is an important zoonoses deriving from contact with infected animals or contaminated equipment.

Epidemiology- The most affected animals are those ranging from one to twelve weeks of age. Older animals generally show no clinical signs but are very significant for the spread of the infection in the herd since one gram of faeces may contain more than ten thousand million of Salmonella bacteria. Other sources of infection are contaminated forages and water as well as rodents, wild winged animals, insects and man. In a study of 250 diarrhoeic calves, bacteriological examination revealed the Salmonella species in 0.8 percent of them (Hegazy et al. 1991).

Clinical findings- Symptoms are anorexia, fever, diarrhoea and dehydration. Diarrhoea, mucous at first, becomes bloody and fibrinous with shreds of mucosa. It sometimes causes sudden death without symptoms. During septicaemia articulations, the lungs and meninges are also involved. The inflammatory lesions are markedly present in the small intestine, but they could also be found in the abomasum and colon. There is congestion, whitish mucus, pseudo-membranes easily removable or not, and blackish or spotted mucosa. The diagnosis should differentiate salmonellosis from colibacillosis, rotavirosis, kriptosporidiosis, coccidiosis, mucous membrane disease, colisepticaemia and clostridiosis.

Diagnosis- It should be performed on fresh faeces from not treated animals, the liver, the liquid of the joints, intestine or whole carcass. The samples should be refrigerated but preferably not frozen. The bacteriological examination is performed through the use of different media: a pre-enrichment, a selective enrichment, solid selective; solid and non selective and media for confirm tests. Biochemical reactions and other tests are also usually employed.

Serotyping of isolated salmonellae is carried out by a test of Fast agglutination on a slide.

Therapy- It is aimed at restoring the correct hydro-saline balance and fighting the infection. Rehydrating therapy is always necessary, while antibiotics are indicated only in cases of severe diarrhoea with a rise in body temperature. Antibiotic resistance is increasing in anti salmonellosis therapy, and for this reason it is preferable to make the choice of the molecule on the basis of the literature reports, farm experiences and antibiogram results. It is important to provide a mass treatment calculating the adequate posology and administrating for at least four to five days.

Prophylaxis- Antibiotic treatment to all neonates prevents serious symptoms and spread of the infection. Direct prophylaxis is always important. It consists in the control of animal introduction with a quarantine period allowing for the execution of three bacterial examinations at a distance of two weeks one from the other, since excretion can be intermittent. Contact with adult and synantrhopical animals, should be avoided, even indirectly. The water should also be analysed. Indirect prophylaxis could be provided on the farm with vaccines prepared from the insulated strain but this implicates a long preparation (Galiero, 1999).

Colibacillosis

Etiology- Escherichia coli is a Gram negative bacillus, asporigenous, facultative aerobe, motile or not, fermenter, characterized by different antigenic structures of the cell wall (antigen O), capsule (antigen K), flagels (antigen H) and fimbriae (antigen F). It includes about 50 000 serotypes of which only a few strains are pathogens. In fact it is a usual species of the intestinal aerobic flora in humans and animals. The pathogenic action relies on the production of virulent structural or secreted factors like adhesines, endotoxins, enterotoxins, cytotoxic toxins, haemolysin. Cytotoxic toxins are responsible for all enterotoxigenic Escherichia coli (ETEC) strains symptoms, heat labile producer strains are rarely isolated from buffalo calves less than one month old; heat-stable toxins producer strains are not very widespread either and affect few day-old buffalo calves. The production of cytotoxic and necrotic factors (CNF) was predominant (21.5 23.2 percent) in comparison with other toxins; VT producing resulted to be 6.5-7.7 percent, Hly 22 percent. Vero cytotoxins 1 (VT1) are those mainly isolated in buffaloes. The buffalo calf is not considered to be a reservoir of serovar O:157, which is an important zoonosic agent (Galiero et al. 1997; Galiero et al. 1999).

Epidemiology- The disease derives from a combined action of different factors: environment, microclima, management, nutrition and sanitary status. It is in fact considered to be a typical conditioned pathology (Galiero, 1998). Morbidity in buffalo calves may be high and mortality may be higher than 50 percent (Galiero et al. 1997). In a study on diarrhoeic calves E. coli was isolated in 4 percent of the animals (Hegazy, 1991).

Clinical findings- Diarrhoea, dehydration, hypothermia and sometimes hypovolemic shock in the presence of enterotoxins. Endotoxins cause fever, diarrhoea, fall in pressure, haemorrhages and vascular thrombosis. The action of cytotoxic toxins as Vero cytotoxins (VT) and cytotoxic and necrotic factors (CNF), results in bloody diarrhoea, weakness, emaciation and anaemia for the first ones and systemic pathologies associated to haemorragic colitis and pulmonary disease. The presence of haemolisin seems to enhance the pathogenic action of the other factors.

Diagnosis- E. coli strains can be isolated from various organic districts (intestine, mesenteric lymph nodes, liver, gall bladder, lungs). Thermolabile toxins (LT), VT and CNF production can be tested on Vero cell cultures; -hemolysin (Hly) on sheep's blood agar; thermostable toxins (ST) on blocking ELISA.

Therapy- It is aimed at re-establishing the electrolitic balance and counteracting the bacteria with antibiotics. The antibiotic choice should be made on the basis of an antibiogram.

Prophylaxis- It is hygienic for calving structures and calf stalls. As for all neonatal pathologies a correct colostrum administration is essential. Vaccines for buffalo species are available.

Cryptosporidiosis

Etiology- Cryptosporidium parvum is a coccidian protozoan parasite of mammals, birds and reptiles. It affects man and all domestic species. The life cycle lasts four to seven days and takes place in the microvillous borders of the enterocytes. It is considered a single-species genus lacking host specificity. Oocysts are extremely resistant to commonly used disinfectants. Their viability is reduced only by five percent ammonia, ten percent formaldehyde and steam under pressure.

Epidemiology- Parasites were evidenced in buffalo in all the countries where it is bred. Interfarm prevalence resulted to be 23-38.8 percent (Galiero, 1999). The ingested oocysts release four infectious sporozoites, after bile salts and trypsin action; they penetrate and reproduce in epithelial cells, then they sporulate within the intestine lumen re-infecting the host or other animals through elimination in the faeces. The transmission is faecal-oral as the hosts start shedding sporulated oocysts at the beginning of the diarrhoea and it persists for 3 to 13 days generally depending on the diarrhoea duration, but it can also happen in asymptomatic carriers. The most affected are one to four week old calves. The incubation period is about two to seven days. The infection has zoonotic implications.

Clinical findings- Enterocolitis is characterized by increased defecation of yellow and watery stools, tenesmus, weight loss, anorexia, depression, weakness and dehydration. Symptoms last more than ten days. Macroscopic lesions are not pathognomonic being represented by an enteritis in particular of the ileus.

Diagnosis- Giemsa stain and modified Baxby or Ziehl-Neelsen techniques can detect cryptosporidial oocysts in faecal samples and preparations of the intestinal mucosa. The oocysts may be concentrated from the faecal matter by centrifugal flotation in a high specific-gravity salt solution (Abd El-Rahim, 1997). Considering that autolytic phenomena take place six hours after death and that freezing destroys the parasite, it is important to preserve samples, for a maximum of 120 days, mixing a part of the faeces with two parts of a 2.5 percent solution of bichromate potassium.

Therapy- No specific drugs are indicated for cryptosporidiosis therapy in the buffalo calf. As in all diarrhoeic pathologies all interventions that restore the hydrosaline balance are useful.

Prophylaxis- Direct prophylaxis aimed at improving management and sanitary levels is useful. Immediate isolation from mothers, division into homogeneous groups by age, isolation of infected animals, sanitation of rooms and equipment are useful measures in every neonatal pathology. Steam jets should be preferred to common disinfectants (Galiero, 1999).

BACTERIAL DISEASES

Bovine brucellosis

Etiology- This disease, generally known as brucellosis, is also called Bang's disease, malta fever and undulent fever (in man), contagious abortion and infectious abortion. It is caused by Brucella abortus (B. abortus), a small gram negative, non-motile, non-sporulating coccobacillus organism that can be easily killed by heat, direct sunlight or common disinfectants and pasteurization. In dry conditions they survive only if embedded in protein, while in tap water they resist for several months at 4-8°C, 2.5 years at 0°C, and several years in frozen tissues or medium. Brucellae can also survive up to 60 days in damp soil, and up to 144 days at 20°C and 40 percent relative humidity. Brucellae can survive 30 days in urine, 75 days in aborted

foetuses and more than 200 days in uterine exudate. In bedding contaminated with infected faecal material Brucella will be destroyed at 56°C-61°C within 4.5 hours. It has been found that Brucella can survive in faeces, slurry, or liquid manure up to 85 to 103 days in the winter, 120 to 210 days in spring, 30 to 180 days in summer, and 50 to 120 days in autumn, indicating that the survival of Brucella is subject to seasonal influences.

Epidemiology- It affects many animal species on every continent and it has been described in all the countries where this species is bred. It is a major pathology concerning buffalo, and it is a zoonose of great economic importance, as well as a public health hazard. Mediterranean buffaloes, not having a specific-species strain, present higher possibilities of contracting this disease especially when it is present in the other receptive species, such as bovine and ovine, and there is promiscuous breeding or uncontrolled livestock movement (Fraulo and Galiero, 1999). Buffaloes are very susceptible both to Brucella abortus, that is the main cause of this disease in bovines, and to Brucella melitensis, more frequent among the ovine species. Frequently in buffaloes biotypes 1, 3 and 6, and less the 7, have been isolated for B. abortus, only 1 and 2 for the B. melitensis (Fraulo and Galiero, 1999). B. abortus appears to be the main species in buffaloes and biotype 1 is that most frequently isolated (Costa, 2002). Although B. abortus is relatively resistant and may survive for a considerable time, the environment is not considered to be an important source of infection. Age, sex, stage of pregnancy and natural resistance to Brucella may influence the progression of infection. Heifers born of infected dams usually test seronegative for Brucella for a long period as the stage of pregnancy at the time of infection determines the incubation period. Abortions in cattle caused by B. abortus seldom occur before the fourth or fifth month of pregnancy. Pregnant females are more likely to become infected than non-pregnant cattle or males. This is because a gravid uterus sustains growth of the organism. Furthermore, the course and incidence of the disease is also influenced by natural resistance to Brucella infection. Finally, the success of the Brucella infection depends on exposure dose, virulence of the organism and natural resistance of the animal, based on its ability to prevent the establishment of a mucosal infection by the destruction of the invading organism. Transmission of B. abortus is very likely to occur via the oral route as cattle tend to lick aborted foetuses and the genital discharge of an aborting cow. So in the animals the infection usually occurs through ingestion of feed and water contaminated with the uterine discharges of an aborted animal or its foetus. Contamination of a cowshed or pasture takes place when infected cattle abort or have a full-term parturition. Although it is generally accepted that B. abortus is not excreted for any considerable time before abortion occurs, excretion in the vaginal discharge of infected cattle may occur as early as 39 days after exposure. A massive excretion of Brucellae starts after abortion and may continue for 15 days. Once the foetal membranes are expelled the uterine discharge diminishes and the number of Brucella organisms excreted decreases rapidly. Although the infectious material from the genital tract usually clears after two to three months, some infected cattle become carriers of Brucella and excrete it intermittently for many years. Congenital infection can be seen in calves as exposure to Brucella organisms is also likely to occur in utero, or when calves born of healthy dams are fed on colostrum or milk from infected dams. Other sources of spread are represented by semen from infected bulls or contaminated udder during milking. The organism can also penetrate intact skin or mucous membranes. Invading Brucella usually localize in the lymph nodes, draining the invasion site, resulting in hyperplasia of lymphoid and reticuloendothelial tissue, and infiltration of inflammatory cells. Survival of the first-line of defence by the bacteria, results in a local infection and the escape of brucellae from the lymph nodes into the blood. During the bacteraemic phase (which may last two to eight weeks) the bones, joints, eyes and brain can be infected, but the bacteria are most frequently isolated from supramammary lymph nodes, mammary lymph nodes, milk, iliac lymph nodes, spleen and uterus. The tropism of Brucella to the male or female reproductive tract was thought to be by erythritol, which stimulates the growth of the organism, but Brucella has also been found in the reproductive tract of animals with no detectable levels of erythritol. In the acute stage of infection, abortion occurs at four or five months of pregnancy, and cattle usually abort only once. Proliferation of Brucella in the uterus induces necrosis and destruction of the foetal and maternal placental membranes resulting in death and then expulsion of the foetus. Excretion

of Brucella after parturition may re-occur after any consecutive normal parturition. Infected cattle excrete Brucellae in the colostrum or milk although it cannot always be detected. Humans usually acquire brucellosis by consumption of raw milk or milk products, but it is also an occupational hazard for farmers, veterinarians and workers in the meat industry within areas with enzootic B. abortus. Farmers and workers in the meat industry may contract brucellosis by percutaneous, conjunctival or by nasal mucous membrane infection. Veterinarians may become infected when handling aborted foetuses or apparently healthy calves born to infected cows and by performing gynaecological and obstetric manipulations or rectal examination of infected cattle. There are definite host preferences. Secondary hosts play a small part if any in the maintenance or spread of a particular Brucella species. Brucella abortus mainly infects cattle and is the main cause of contagious abortion in cattle, however, sheep, goats, dogs, camels, buffaloes as well as feral animals may also contract B. abortus infections. Although sheep do not easily become infected with B. abortus they may become carriers and excrete Brucellae for up to 40 months once they have acquired the infection. The low prevalence of naturally acquired B. abortus infections reported in goats makes this animal species irrelevant as a host for B. abortus. Swine, horses and camels may acquire infection with B. abortus, however, their significance as a host for B. abortus is doubtful while dogs with naturally acquired B. abortus infections play an important role in the epidemiology of cattle and buffaloes brucellosis. Moreover, while indirect exposure to Brucella organisms could be mediated by wildlife, birds and waterways (contaminated with urine, uterine discharge, or slurry from aborting cattle) it seems that only dogs carry pieces of placenta or aborted foetuses from one place to another causing direct exposure. Also feral animals such as buffalo, swine, deer, fox, hare and rodents are susceptible to Brucellae . The significance of fowl as a reservoir of Brucella is unclear. Flies, arthropods and other parasites may be susceptible to Brucella infection. The disease is prevalent in buffaloes throughout the world: in India the incidence has been reported to be 3-5 percent, in Egypt 20-25 percent, in Iraq 4-5 percent, it has also been reported in buffaloes in Vietnam (Sharma and Kumar, 2003), USSR, Turkey, Philippines, Pakistan, Indonesia (Adlakha and Sharma, 1992), Venezuela, where it is increasing more rapidly among buffaloes than among cattle with as many as 57 percent of Venezuelan herds being infected; in Italy, in 1995, up to 3 percent of the tested animals were infected (D'Apice et al., 1997), in Sri Lanka, 4.2 percent not substantially different from that of cattle (4.7 percent) (Silva et al., 2000) as was also the case in India. The seroprevalence is twice as high in mature buffaloes (over three years) as infection occurs in animals of all ages but persists only in sexually mature animals. B. melitensis has also been isolated from the genital tract of female buffaloes (Adlakha and Sharma, 1992).

Clinical findings- Brucellosis in animals is primarily a reproductive disease characterized by abortion, retained placenta and impaired fertility in the principal animal host. The clinical findings mostly depend on immune status and the physiological status of buffaloes: susceptible pregnant females suffer from abortions after six months, retained placenta and catarrhal metritis. After the first abortion the animal can give birth to full term calves. In bulls epididymitis and orchitis may occur involving one or both scrotal sacs with painful swellings and infection of the accessory sex glands. It has been established that brucellosis in bulls does not always result in infertility, although semen quality may be affected. Bulls that remain fertile and functionally active will shed Brucella organisms with the semen during the acute phase of the disease. Shedding, however, may cease or become intermittent. In contrast to artificial insemination, bulls used in natural service may fail to spread the infection, as the infected semen is not deposited in the uterus. Mild cases are characterized by sinovitis and painful swelling of affected joints. The economic loss from brucellosis in developed countries arises from the slaughter of cattle herds that are infected with Brucella. The economic loss from brucellosis in developing countries arises from the actual abortion of calves and resulting decreased milk yield, birth of weak calves that die soon after birth, retention of the placenta, impaired fertility and sometimes arthritis or bursitis. It is difficult to estimate the financial loss caused by brucellosis, as it depends on the type of cattle farming, herd size, and whether it is an intensive or extensive cattle farm. Furthermore, although it is very difficult to estimate the financial loss incurred by human brucellosis there is no doubt that it is substantial.

Symptoms of acute brucellosis in man, caused by Brucella abortus, are 'flu-like' and highly non-specific, while chronic brucellosis is insidious as the vague symptoms might be confused with other diseases.

Diagnosis- Demonstration of characteristic clumps of Brucella organisms in stained smears of hygroma fluid, chorionic epithelium, or the use of fluorescent antibody techniques to examine foetal stomach content and uterine, or vaginal exudate may provide a tentative diagnosis. Bacteriological examination of lochia of aborting cattle is the method of choice for early infections but it is laborious, time consuming and costly. Moreover, negative culture results do not exclude infection.

Several serological tests are used to detect brucellosis in body fluids such as serum, uterine discharge, vaginal mucus, milk, or semen plasma, as infected cattle may or may not produce all antibody types (M, G 1, G 2, and A types) in detectable quantities.

The commonly used tests are the milk ring test (MRT), serum agglutination test (SAT), Rose Bengal (RB) plate test, complement fixation test (CFT), anti globulin (Coombs) test, 2-mercaptoethanol, rivanol and the enzyme-linked immunosorbent assay (ELISA). The use of several tests to reliably detect brucellosis suggests shortcomings in each of the tests. The ELISA has proven to be specific and as sensitive as the MRT and SAT in detecting Brucella antibodies in milk and serum. ELISA results are usually also in agreement with CFT results.

The test can be used for screening and confirmation of brucellosis in both milk and serum. However, it may test false positive or false negative. It seems less sensitive than the CFT. The main advantage of the ELISA when compared with the CFT lies in its relatively simple test procedure. The assay is very costly when only a few samples are tested, therefore, it is unsuitable for testing individual animals but it is the ideal test for screening suspected herds. The reliability of serological tests to detect brucellosis depends on the antibodies present at the time of examination, inevitably some infected animals may elude detection. The skin delayed-type-hypersensitivity (SDTH) test is independent of circulating antibodies, it should be used to confirm serologic test results. Other tests have been developed and tested, for example flow cytometry (D'Apice et al., 1997); Mathias et al (1998) has compared competitive enzyme immunoassay (CEIA) with the complement fixation test (CFT) and the Rose Bengal test (RBT) obtaining a sensitivity of 100 percent and a specificity of 98.55 percent, suggesting that it could be a useful tool for diagnosis of brucellosis in buffaloes. Other serological test evaluations (competitive ELISA, indirect ELISA, agglutination) have been performed by Molnar et al. (2002).

Therapy- Treatment should be prolonged and with a high dosage of antibiotics, with risks for the human food chain and the possibility of relapses.

Prophylaxis- Efforts are directed at prevention or eradication of brucellosis. Suspect herds must be tested at regular intervals until all the animals test negative. Animals that test positive should be removed from the herd. In areas with endemic brucellosis only vaccination will control brucellosis. Vaccination reduces the number of infected animals and eventually permits disease control. It does not prevent buffaloes from becoming infected with B. abortus, but it prevents abortions protecting 65-75 percent of the herd from infection. Brucella vaccines in use are the live B. abortus Strain-19 vaccine and to a lesser extent the whole cell killed adjuvant B. abortus 45/20 vaccine, and the recently introduced B. abortus vaccine RB51. During vaccination trials, the animals vaccinated with B19 showed a lower percentage of seropositives after 18 months, while the ones vaccinated with 45/20 still had a high amount of seropositive animals (Fenizia, 1991). No scientific tests exist, showing the administration methods and the possible protection given by the RB51, for buffaloes. So its use is not authorized in buffaloes. The use of a reduced dosage strain 19 vaccine has proved to provide comparable protection to the original one (Costa, 2002).

Tuberculosis

Etiology- Bovine and buffalo tuberculosis is caused by Mycobacterium bovis. In Australia Mavium, M. fortui and M. flavescens have also been isolated from buffaloes' tubercles. M. bovis is a long, slender, rod-shaped organism, belonging to the acid-fast group of bacilli; it may be slightly curved and occur in small groups. It is moderately resistant to heat, desiccation and many disinfectants, but not to direct sunlight. In warm and moist places it can survive for long periods: in stagnant drinking water up to 18 days (Adlakha and Sharma, 1992); and in the soil for up to two years. It is considered a disease of socio-economic and public-health importance as M. bovis is a zoonotic bacteria and it is the major cause of human infection in developing countries (Tariq Javed et al. 2004).

Epidemiology- An infected animal is the main source of transmission. The organisms are excreted in the exhaled air and in all secretions and excretions (sputum, faeces, milk, urine, vaginal and uterine discharges and open peripheral lymph nodes). Mycobacteria invade cattle by respiratory (90-95 percent) and oral routes (5-10 percent). Inhalation is the chief mode of entry (it accounts for 97 percent of infections in Australia) and for calves infected colostrum/milk is an important source of infection. Other identified sources of infection are represented by infected flies and birds (Tariq Javed et al. 2004). When infection has occurred, tuberculosis may spread by primary complex (lesion at point of entry and the local lymph node) and by dissemination from primary complex. Tuberculosis lesions can be classified as acute miliary, nodular lesions and chronic organ tuberculosis. Some risk factors influence the development of a clinical or non-clinical form, including calving place, calves' group size, types of concentrates, breed, presence of cattle, herd size, exposure to water supplies, housing system, summer mountain pasture and possibility of contact with wild animals (Tariq Javed et al. 2004). Tuberculosis in small ruminants is rare. In pigs the disease may be caused by the bovine and avian types. Superinfection is specific in cattle. The disease occurs in every country of the world. It has been described for buffaloes in Pakistan, India, Egypt, Australia and Thailand, sometimes as being more severe and with higher incidence than in cattle; in other circumstances as lower compared to cattle and Zebu cattle (Sharma and Kumar, 2003). Despite some claims to the contrary, the water buffalo is susceptible to the bovine strain of tuberculosis (Mycobacterium bovis). Previously it was thought that buffaloes were less susceptible or more resistant to the disease. However, a high incidence of TB has been observed in wild buffaloes. In Pakistan the prevalence in two experiment stations resulted to be 8.48 percent and 2.45 percent. Its incidence is generally higher in buffaloes of around six years of age (Tariq Javed et al. 2004).

Mycobacterium bovis has been isolated in buffalo (*Bubalus bubalis*) in Thailand and Australia (Kanameda and Ekgatat, 1995; Hein and Tomasovic, 1981). Morbidity rates are 20 to 50 percent, but mortality rates are 60 to 80 percent. It is a significant zoonotic disease as man is susceptible to the bovine type and infected buffaloes represent a source of infection for human beings. The incidence of human tuberculosis caused by Mycobacterium bovis has markedly dropped with the pasteurization of milk. It has also dropped in areas where tuberculosis eradication programmes have been developed.

Clinical findings- It is a chronic disease running a course of a few months to years. The first appreciable sign in buffaloes is a general malaise. In the pulmonary form a low grade fever, loss of appetite, emaciation, chronic intermittent hacking cough and associated pneumonia, difficult breathing, weakness, dryness of skin and swelling superficial body lymph nodes are observable, especially the supramammary one when mammary glands are affected and painlessly enlarged. In these cases there are signs of mastitis and the milk becomes watery. The intestinal form is characterized by persistent diarrhoea. More indicative are the lesions: tuberculous granuloma in the lymph nodes of the head, lungs, intestine and carcass. These usually have a well-defined capsule enclosing a caseous mass with a calcified centre. They are usually yellow in colour in cattle, white in buffaloes and greyish white in other animals. Active lesions may have a reddened periphery and caseous mass in the centre of a lymph node; inactive lesions may be

calcified and encapsulated. There can be nodules on the pleura and peritoneum, lesions in the lungs, liver, spleen and kidney, a firmer and enlarged udder, particularly hindquarters, bronchopneumonia, lesions in the meninges, bone marrow and joints. The lesions in buffalo are paler and less calcified than those in cattle, exhibiting a lardaceous consistency. Localizations are mostly thoracic, in mediastinal and bronchial lymph nodes, then abdominal, in the liver, and in the head, retropharyngeal lymph node, and deep inguinal lymph node.
The carcass of an animal affected with tuberculosis requires additional postmortem examination of the lymph nodes, joints, bones and meninges. Carcasses are condemned when the natural prevalence is low (in the final stages of eradication) or in high prevalence areas (early stages of eradication). The carcass of a reactor animal without lesions may be approved for limited distribution, but if there is a good economic situation, this carcass should be condemned. Heat treatment of meat is suggested during the early and final stages of an eradication programme: in low and high prevalence areas where one or more organs are affected, and where miliary lesions, signs of generalization or recent haematogenous spread are not observed. If the economic situation permits, then the carcass is condemned. In some countries, the carcass is approved if inactive lesions (calcified and/or encapsulated) are observed in organs and without generalization in the lymph nodes of the carcass.

Diagnosis- Cases are generally detected in vivo by an intradermal tuberculin skin test and clinical findings and, post-mortem, by lesions. Delayed hypersensitivity reactions can be used as a single intradermal test or comparative test with tuberculins of various origin; also short thermal test and Stormont test are used. The buffalo reaction is more pronounced than that in cattle and can last for more than ten days (Sharma and Kumar, 2003; Tariq Javed et al. 2004). The diagnosis may be confirmed by making a smear of the lesions or excretions; coloured with Ziehl-Neelsen, the TB bacterium appears as a very small red staining bacillus. Inoculation in laboratory animals, like the Guinea pig, can also be used. For a differential diagnosis all these diseases should be considered: lung and lymph node abscess, pleurisy, pericarditis, chronic contagious pleuropneumonia, actinobacillosis, mycotic and parasitic lesions, tumours, caseous lymphadenitis, Johne's disease, adrenal gland tumour and lymphomatosis.

In cattle, lesions of tuberculosis caused by the avian type are commonly found in the mesenteric lymph nodes. For the in vivo diagnosis, apart from the intradermal tuberculin skin test, the indirect hemagglutination test is sensitive for early and advanced cases (Sharma and Kumar, 2003). The use of a dot blot immunoassay has been suggested for the detection and quantification of circulating Ag85. A positive relationship was found between comparative intradermal tuberculin positive tests and serum total proteins and globulins. An inverse relationship was found with the monocyte percentage (Tariq Javed et al. 2004).

Therapy- No treatment is performed since it would be too long and expensive. In animals of high genetic value some therapeutic protocols can be suggested: streptomycin and para-amino salycilic acid; isonicotinic acid and dihydrostreptomycin; isoniazid; streptomycin 2.5 g IM associated with rifampicin 1.5 g and isoniazid 2.0 g PO (Sharma and Kumar, 2003).

Prophylaxis- It is based on eradication: prevention of any introduction or spread of the disease, removing infected animals by slaughter of any positive animal, both clinical and tuberculin. Physical separation for the rearing of offspring.

Paratuberculosis

Etiology- Paratubercolosis is a serious bacterial disease of ruminants caused by Mycobacterium avium subspecies paratuberculosis, developing as chronic granulomatous enteritis and clinically manifested by emaciation and diarrhoea (Chiodini et al. 1984). Cattle become infected at an early age and clinical signs develop after a long incubation period lasting years. In an affected herd most animals are clinically healthy and only occasionally the causative agent is demonstrable in faecal samples (Whitlock et al., 2000).

M. paratuberculosis is an aerobic, non-spore forming, Gram, non motile, acid fast bacillus that is a slow growing intracellular parasite. It is closely related to M. avium and the wood pigeon bacillus M. silvaticum from which it can be separated by DNA techniques such as restriction endonuclease analysis, restriction fragment length polymorphism (RFLP) analysis, pulsed field gel electrophoresis and field inversion gel electrophoresis.

Epidemiology- Johne's disease affects livestock welfare and productivity by way of direct effects on growth and production and indirectly through restrictions on trade. It occurs in a range of animal species, especially ruminants. The most important source of infection is faeces from animals with M. paratuberculosis infection. Early in the disease shedding in faeces may be intermittent. The number of organisms in faeces increases as the disease progresses and may increase when infected animals are subject to stress. Most animals become infected by ingesting the organism in contaminated feed or water. Cattle are usually infected as young calves and develop resistance to infection with age. The survival of M. paratuberculosis in the environment is favoured by low temperatures, moisture and protection from solar radiation. Some animals may become infected in utero and the chance of this occurring increases as the disease progresses in the dam.

Clinical findings- Clinical Johne's disease in cattle typically presents syndromes of chronic and progressive emaciation and persistent diarrhoea. The faeces are usually green and bubbly and do not contain blood or mucus. Faecal consistence may improve over short periods and then diarrhoea may return with increased severity. Affected animals are bright and alert and eat well throughout the course of the disease but in advanced cases, submandibular oedema may be observed. On rectal examination the mucosa may feel thickened or corrugated. The age of onset of clinical signs can be quite variable. In most cases, clinical signs do not appear until animals are more than three or four years of age, but in some herds the onset of disease has been seen in two year old animals.

Although animals with advanced Johne's disease may have bacteraemia, the only specific lesions are found in the intestine and associated lymph nodes. Early in the course of M. paratuberculosis infection, gross lesions may not be evident but, in clinical cases, the mesentheric lymph nodes are enlarged, pale and oedematous. In all host species, specific intestinal lesions are usually more developed in the lower jejunum and ileum. The ileocaecal valve may be enlarged, but the presence of specific lesions in the valve and immediately adjacent tissues is not constant and a wider range of specimens must be examined to ensure a reliable diagnosis. The classical intestinal lesion is diffuse thickening of the intestinal mucosa with development of transverse folds or corrugations. The crests of the rugae may be congested and the mucosal surface is velvety. Necrosis rarely occurs in cattle, and unlike sheep and goats, there is no calcification or caseation.

Diagnosis- Diagnostic test results should be interpreted in the light of epidemiological, clinical and pathological findings. The tests comprehend:

- histopathological techniques (Ziehl.Neelsen staining method).
- bacteriological method (Herrold'egg yolk agar).
- BACTECO (Radiometric culture: growth in liquid medium is identified by the detection of radiolabelled metabolites).
- DNA detection (PCR).
- Intradermal test (no longer widely used because of its poor sensitivity and specificity in individual mammals).
- Interferon test (it is marketed for bovine tuberculosis detection and includes M.bovis
- PPD (purified protein derivative) and M.avium PPD).
- AGID (agarose immunodiffusion test)
- CFT (no longer recommended for diagnosis or certification).
- ELISA (the most sensitive and specific test for serum antibodies).

Therapy- M. paratuberculosis is naturally resistant to many commonly used antimicrobial

drugs. Information about the susceptibility of M. paratuberculosis to antimicrobial drugs is minimal. This is largely due to the fact that treatment of animals with Johne's disease is considered to be too costly. In fact, it has not been considered economically prudent to treat animals with Johne's disease. The chances of curing the animal are low, the cost of the drugs is high and the meat and milk derived from animals treated with the kind of potent drugs required are not suitable for human consumption. Hence, very little research has been done to establish a profile of drug susceptibility based on laboratory tests (i.e. in vitro drug susceptibility testing). In a trial on bovine calves, rifampicin, streptomycin and pyrazinamide were used at doses respectively of 30-25-50 mg/kg/day for a period of seven months and proved successful (Arrigoni et al. 1995).

Prophylaxis- Vaccinating against Mycobacterium paratuberculosis reduces the average economic loss and, therefore, turns out to be profitable. For cows with clinical paratuberculosis the decrease in milk production in the vaccinated group was 13 percent, while it was 21 percent in the non-vaccinated group (Kalis et al. 1995).

Haemorrhagic septicaemia

Etiology- Haemorrhagic septicaemia (HS) is a contagious bacterial disease, also known as shipping fever, caused by two serotypes of Pasteurella multocida, B2, E2. It is a small gram negative, bipolar coccobacillus, not resistant to heat, which in favourable surroundings can survive as long as one week.

Epidemiology- It is regarded as one of the most serious diseases of large ruminants in south east Asia. It affects cattle (*Bos taurus* and *B. indicus*) and water buffaloes (*Bubalus bubalis*) which is the most susceptible species, with a high mortality rate in infected animals. In buffaloes it is mainly caused by type B2 (Sharma and Kumar, 2003). Carriers are the source of microorganism and can include, apart from cattle and buffalo, pigs, sheep, goats and horses. The nasopharynx is the main route of entry by aerosol, vectors are not considered significant but indirect transmission is possible.

HS is principally a disease of animals under stress. In endemic areas about 2 percent of healthy cattle and buffalo carry the organism in the lymphatic tissue of the upper respiratory tract. Intermittently, even in the presence of a circulating antibody, the organisms invade the nasopharynx and are excreted in nasal secretions. These episodes may be triggered by stress. Infection is transmitted by: direct contact between animals or contaminated feedstuffs or water. The bacterium does not survive in the environment for more than a few days. The disease occurs in South and South East Asia, the Middle East and most of Africa. It has also been reported to occur occasionally in Southern Europe.

Clinical findings- Some studies indicate that endotoxin plays a role in the pathogenesis of HS (Horadagoda et al. 1997). Hot, hard, painful swelling of the ventral neck region from throat to dewlap are the most conspicuous signs of HS. The tongue is swollen and mucous membranes are hyperaemic. Breathing is laboured and painful. HS is an acute febrile disease causing heavy mortality in younger animals. In the febrile stage hepatic damage causes bilirubin and other bile salts in blood to increase and cholesterol to decrease. Hot humid conditions favour the spread of the disease. Most cases are acute or peracute, showing: high fever (42°C), depression, reluctance to move, salivation and nasal discharge, painful, oedematous swelling of the throat, extending to the brisket, congested mucous membranes, respiratory distress; calves may have haemorrhagic gastro enteritis. Death occurs in 6 to 48 hours after onset of clinical signs. Recovery is rare. Lesions are mainly represented by oedematous swellings of the throat, brisket containing a clear, straw-coloured serous fluid, blood-tinged fluid in body cavities, pharyngeal and cervical lymph nodes are swollen and congested, subserosal petechial haemorrhages, generalized congestion of the lungs, variable congestion of the abomasum and intestinal tract (calves may have haemorrhagic gastro enteritis). In the case of a quick death the findings could be minimal.

Diagnosis- The organisms disappear from dead animals after some time. Smears should therefore be done immediately after death. In any case they sometimes reveal to be negative in buffaloes. In these cases cultural and biological tests can be performed.
The diagnosis is based on the isolation of Pasteurella multocida from heparinized blood or affected tissues. Samples should be collected aseptically and kept cool. Tissue samples can also be used for histology and immunohistochemistry (Horadagoda et al. 1991).

A differential diagnosis is necessary for blackleg, rinderpest and anthrax.

Therapy- Prompt veterinary care is effective and helpful but treatment is meaningful only in the preliminary stage. Early cases can be treated with sulphonamides coupled with antibiotics like oxytetracycline (Adlakha and Sharma, 1992).

Prophylaxis- In free areas, restriction of imports of live animals from endemically infected countries would keep the risk of introduction at a low level as the micoorganism hardly survives outside the host, but it would not exclude it completely; in fact healthy animals are liable to carry the organism. For the control of HS, three types of vaccines are commonly in use: broth bacterin, alum precipitated and oil adjuvant vaccines. Annual immunization using adjuvant vaccines gives good control in endemically infected areas. Some studies indicate that oil adjuvant vaccines induce a higher response than alum precipitated and lyophilized ones (Farrag et al. 1991), providing immunity for six months to a year, instead of five months (Sharma and Kumar, 2003). Preventative vaccination is usually undertaken annually, one month prior to the monsoon.

Chlamydiosis

Etiology- Chlamydia psittaci is a small bacterium that does not replicate in traditional in vitro media but only in living organisms. It resists up to four months in dung and litter, 17 days in surface water and 10 days in carcasses.

Epidemiology- It mainly affects calves aged three to ten months. The disease has a seasonal trend increasing in autumn. The morbidity is up to 10 to 15 percent and the mortality can reach 100 percent (Adlakha and Sharma, 1992; Galiero and Sica, 1997). Immunosuppression may aggravate the extension and severity of lesions (Gupta et al. 1991). It has been diagnosed in Bulgaria, Brazil, India and Italy. The seroprevalence is high: 13.9 percent in Campania with high detected titres (Baldi et al. 1997).

Clinical findings- Reproductive disorders, abortion, respiratory diseases, kerato conjunctivitis (Baldi et al., 1997). In the buffalo calf, encephalomyelitis at the beginning of the disease causes depression, prestomaches atony followed by the suspension of rumination and constipation without fever. The signs progress from anorexia, sialorrhoea, hard swallowing, pupil dilatation and blindness to evident nervous symptoms: shaky gait, head stretched on the neck (opisthotonos), turning around, paralysis of back limbs, lying down on their back and death after 10 to 15 days, sometimes death occurs in two to three days. At necropsy only hyperemia of the meninx and necrotic foci on the cerebral parenchyma are seen. Cerebral lesions consist of meningoencephalitis lymph-plasmacellular associated with necrosis foci with areas of malacia on the hippocampus, bridge and cerebral cortex (Galiero and Sica, 1997).

Diagnosis- It is based on germ isolation, microscopic lesions, serum conversion. Only laboratory tests can differentiate it from a lack of thiamine (vitamin B1) or an excess of sulphur in the ration.

Therapy- Chlamydia psittaci is sensitive to tetracycline and oxytetracycline (Fenizia et al., 1991). 15 to 20 mg/kg every 12 hours for five days should be administered early, within the first 24 hours from the appearance of the nervous signs. A supportive therapy including cortisone, re-hydration and detoxicants allows a quicker resolution.

Prophylaxis- It is actually based on environmental hygiene using 2 percent formaldehyde, hypochlorite calcium or caustic soda, and destruction of infected animals carcasses, dung and litter (Galiero and Sica, 1997).

Leptospirosis

Etiology- Leptospira spp. are the causative agents that induce infection with various serovars of bacteria. All leptospire are now classified into one species: Leptospira interrogans. It includes over 180 serovars which are divided into 16 different serogroups.

In Brazil, Langoni et al. (1995) found mainly wolffi serovar in 16 farms in Sao Paulo State. Recently the most frequent serovar to which feral buffaloes reacted is pomona (Girio et al. 2004), while from urine samples collected from female adult buffaloes located in a farm, a leptospira strain was isolated belonging to serogroup sejroe which is closely related to serovar guaricura. In a previous study carried out in different buffalo premises in central Italy, serovar tarassovi and hardjo were documented (Autorino et al. 1991). Clinical disease in buffalo seems to result most frequently from Pomona and hardjo infections (Costa, 2002). Leptospiras can survive for months in moist and humid environments, particularly in swamps, ponds and streams or poorly drained pastures.

Epidemiology- Leptospirosis is an important and relatively common disease of domestic and wild animals and humans. It is also considered one of the most important pathologies concerning buffaloes for public health reasons. In fact it is a zoonosis and also represents an occupational hazard for farmers, veterinarians and butchers. Human infection may occur by contamination with infected urine and urine contents. The bacteria may also be found in milk in acute cases, however, it does not survive for an extended period of time in milk. Pasteurization will also kill leptospiras.

Animals contract the disease by eating and drinking leptospira-contaminated urine, water, or by direct contact of broken skin or mucous membranes with mud, vegetation or aborted foetuses of infected or carrier animals. Recovered animals and animals with unapparent (subclinical) leptospirosis frequently excrete billions of leptospiras in their urine for several months or years.

The wallowing habit of buffaloes makes them prone to leptospiral infections since water sources are often contaminated by rodents and wildlife, that are natural carriers of the organism. Buffaloes are important carriers and shedders especially in rice-growing countries. The disease has been reported in India, the USSR, Bulgaria, Romania, Brazil and Egypt.

Clinical findings- In cattle it is manifested by interstitial nephritis, anaemia and mastitis and abortion in most species. The symptoms in the acute and subacute forms are: transient fever, loss of appetite, mastitis, lactating cows may stop milking and milk may be yellow, clotted and frequently blood stained. If animals are severely affected there could be jaundice and anaemia, pneumonia, abortion with frequent retention of the placenta (afterbirth). In young calves the severe illness may be associated with yellowish discoloration of mucous membranes and reddish-brown urine before death. The most indicative symptoms are represented by haemorrhages of mucosa, haemoglobinuria and icterus. In the chronic form there are mild clinical signs and only abortion may be observed. If meningitis occurs, the animal may show lack of coordination, salivation and muscular rigidity.

Lesions are commonly: anaemia and jaundice, subserosal and submucosal haemorrhage, ulcers and haemorrhages in the abomasal mucosa, rarely pulmonary edema or emphysema, interstitial nephritis and septicaemia The carcass of an animal affected with acute leptospirosis is condemned. A chronic and localized condition may warrant an approval of the carcass.

Diagnosis- Direct microscopic examination can be performed on body fluids. Other diagnostic methods are bacteriological culture, biologic test (animal inoculation) and serological tests (dark-field microagglutination test: MAT).

Acute and subacute forms are to be differentiated from babesiosis, anaplasmosis, rape and kale poisoning, bacillary haemoglobinuria, post parturient haemoglobinuria and acute haemolytic anaemia in calves. The presence of blood in the milk is a characteristic clinical sign which will differentiate leptospirosis from other infectious diseases.

Therapy- Antibiotic therapy: Streptomycin: 12 to 15 mg/kg BW, twice daily for three days, or 25 mg/kg BW in a single dose to eliminate infection in carriers; also Dihydrostreptomycin at a dose level of 10 g/1 000 pound cow, has been reported to be effective for termination of the carrier or shedder state; other antibiotics used are chlortetracycline or oxytetracycline. The therapy should be given early, before kidney or liver damage occurs. A supportive therapy for an early recovery should consist of liver tonics and haematinics (Sharma and Kumar, 2003).

Prophylaxis- Livestock herds can be protected against leptospirosis by a combination of proper management and vaccination procedures. Prevention and control is substantially based on periodic testing in endemic areas, elimination or treatment of carrier and clinically infected animals, hygienic measures, and vaccination of susceptible animals.Vaccination should be performed in animals over four months of age and with a booster dose to be given every six months thereafter, as it is not unusual to diagnose abortions caused by pomona in dairy cows vaccinated 8 to 12 months previously. Vaccination programmes can help to control this disease. Usually this is undertaken with formalin inactivated bacterin with either aluminum hydroxide or Freund's complete adjuvant. The latter gives a better serological response. The protocol starts at four to six months of age, followed by annual revaccinations (Sharma and Kumar, 2003). The vaccine should be given to all susceptible livestock on the premises where infection has been identified and the vaccine used in infected herds should be identical with the serotype causing the diseases, as there is little or no cross-protection between vaccine serotypes. In endemic areas a bivalent vaccine should be used. Hardjo is poorly antigenic and does not prevent infection, leptospiruria (shedding), abortions and neonatal weakness for six months. In the case of hardjo infected herds booster vaccination should be performed at three month intervals (Costa, 2002). The future breeding efficiency of herds that have experienced leptospirosis is usually unaltered. Animals should not be culled because they have had the disease. In fact, their value may be enhanced because they are solidly immune against re-infection with the same serotype.

Contagious bovine pleuropneumonia

Etiology- The causative agent is Mycoplasma mycoides subsp. mycoides SC (small colony type) (bovine biotype); there is only one antigenic type.

Mycoplasmas are microorganisms deprived of cell walls that are, therefore, pleomorphic and resistant to antibiotics of the beta-lactamine group, such as penicillin. They cannot survive for more than three to four hours outside the host and are easily killed by heat treatment or by common disinfectants.

Epidemiology- Not being resistant in the environment, the transmission requires close contact and it is aerial, due to droplets emitted by coughing animals, saliva, and urine. Transmission up to several kilometres has been suspected under favourable climatic conditions. Also transplacental infection can occur. Water buffalo (*Bubalus bubalis*) is present among hosts of the disease, while wild bovids and camels are resistant. Buffaloes of all age groups are equally susceptible but once infected, they become immune for subsequent infections. It is of little significance in buffaloes as they are more resistant than cattle, show milder clinical findings and have a higher rate of recovery than cattle (Sharma and Kumar, 2003). However, since international buffalo exports are becoming more common, and since buffaloes may

transmit the infection to cattle, the disease should be taken into account.

CBPP is widespread in Africa and it is also present in other regions of the world, including southern Europe, the Middle East and parts of Asia. Periodically, CBPP occurs in Europe, and outbreaks have occurred in Spain, Portugal, and Italy. Contagious bovine pleuropneumonia was eradicated from the United States in the Nineteenth century. Currently, CBPP is not present in the Western hemisphere.

During an outbreak of natural disease, only 33 percent of animals present symptoms (hyperacute or acute forms), 46 percent are infected but have no symptoms (subclinical forms) and 21 percent seem to be resistent. The incubation period is one to three months (sometimes longer).

Clinical findings- Symptoms are represented by moderate fever with polypnoea, cough (at first dry, slight, and not fitful, becoming moist), characteristic attitude: elbows turned out, arched back, head extended. After exercise breathing becomes laboured and grunting can be heard; at percussion, dull sounds can be noticed in the low areas of the thorax. Infected calves generally present arthritis with swelling of the joints. The disease is difficult to produce experimentally in the buffalo species.

Characteristic lesions are: important amount of yellow or turbid exudate in the pleural cavity (up to 30 litres) that coagulates to form large fibrinous clots; fibrinous pleurisy; interlobular oedema, marbled appearance due to hepatisation and consolidation at different stages of evolution usually confined to one lung; sequestrae with fibrous capsule surrounding grey necrotic tissue in recovered animals.

Diagnosis- Laboratory diagnosis consists of: Identification of the agent: Isolation of pathogen and identification by metabolic and growth inhibition tests; MF-dot; Polymerase chain reaction (PCR).

Serological tests: Complement fixation that should be used only at herd level and never for individual diagnosis; Competitive ELISA (under validation by the International Atomic Energy Agency and several reference laboratories), and haemagglutination; and agglutination test can be used as penside test in active outbreaks at the herd level.

Samples: Lung lesions, pleural fluids, lymph nodes, lung tissue exudate - frozen for isolation of the organism; acute and convalescent sera.

Therapy- There is no efficient treatment. Mycoplasma mycoides mycoides (SC type) is susceptible to a variety of antimicrobials, including streptomycin, oxytetracycline, and chloramphenicol, but antimicrobial therapy may only serve to slow the progression of the disease or may even in some cases favour the formation of sequestra. In the case of chronically affected animals or subclinically affected carriers, the organisms may be in an inaccessible location within an area of coagulative necrosis, which by definition is not served by a blood supply. That is why antibiotic treatment should be prohibited.

Prophylaxis- Sanitary prophylaxis in disease-free areas should consist in quarantine, serological tests (complement fixation) and slaughtering of all animals of the herd in which positive animals have been found. Control of cattle movements is the most efficient way of limiting the spread of CBPP.

In infected areas a CBPP vaccine containing T1 strain is widely used, while a CBPP-rinderpest combined vaccine is sometimes used. Immunity subsequent to vaccination is generally good and lasts at least 12 months. It is advisable to vaccinate animals for export to CBPP free areas.

Anthrax

Etiology- The causative agent is Bacillus anthracis, a gram positive spore forming rod. Anthrax bacilli spores contaminate soil for many years, in fact it can survive from 15 to 20 years in soil. The organisms possess a capsule producing a toxin.

Epidemiology- The disease occurs sporadically. Cattle are generally more susceptible than buffaloes. This is an acute bacterial infection of humans and animals which may be rapidly fatal. The disease occurs worldwide and is an occupational hazard for persons such as wool-sorters, farm workers and veterinarians in contact with infected animals or their by-products. All domestic, zoo and wild animals are potentially at risk of infection. It can be transmitted to humans through blood, meat, hides, etc. The infection in man usually occurs by inoculation from direct contact with infected animals, carcasses or animal products and contaminated soil. Inhalation or ingestion of spores may occur. Animals are infected from contaminated feed, forage, water or carcasses. Insects like biting flies have been shown to be capable of transmission. The disease has a worldwide distribution. Outbreaks are more common in warm and humid conditions like rains after droughts (Sharma and Kumar, 2003).

Clinical findings- It is a febrile disease with high temperatures and swelling of the neck, thorax and lumbar region. Cutaneous anthrax causes localized ulceration (sores) and scabs with fever and headache which may be followed within a few days by severe illness such as septicemia and meningitis. Inhaled anthrax causes fulminating pneumonia. Intestinal anthrax is associated with acute gastroenteritis (nausea, vomiting, and bloody diarrhoea). It is characterized by an abnormal enlargement of the spleen. Blood discharge from natural orifices is common and mortality is very high. In buffaloes there are acute and peracute forms. The first one has a course of about 48 hours with body temperature of 42°C, depression, deep and rapid respiration, congestion of mucous membranes with haemorrhagic spots. Milk can be blood tinged, there can be diarrhoea and edema of tongue, throat, sternum and perineum. In the peracute form death is sudden after convulsions and collapse, without signs except for loss of blood from nostrils, anus and mouth (Sharma and Kumar, 2003).

Diagnosis- This is easy based on records and clinical signs. Peripheral blood or edema fluid smears can reveal the organism, a precipitation test (Ascoli test) from small pieces of ear or muzzle, can be performed. It should be differentiated from peracute black quarter, lead poisoning, acute leptospirosis and bacillary haemoglobinuria. All possible precautions should be observed when handling the carcass.

Therapy- There is not enough time to permit treatment in both forms. In animals of great value an anti-anthrax serum at 100-150ml intravenous associated to antibiotics (Streptomycin 8-10 g/day, oxytetracycline 5mg/kg BW for a minimum duration of five days) can be administered.

Prophylaxis- Spore vaccine works well and provides immunity for one year. The application of anthrax vaccine in risk situations can be helpful. After an outbreak, annual vaccination should be performed for at least three years.

PARASITIC DISEASES

Trypanosomiasis

Etiology- Trypanosoma protozoa are a large family belonging to the class of Mastigophora with a worldwide distribution. In the tropical regions some species are pathogen for animals and man and cause high mortality and morbidity rates. All Trypanosoma, but one (*T. equiperdum*), are transmitted by arthropodes as Glossina. In the water buffalo *T. evansi*, long and narrow (8-39 m), is the agent of Surra disease. It is widely prevalent in the Indian sub-continent and in a number of countries of south-east Asia.

Epidemiology- It is influenced by three factors: arthropodes distribution, protozoa virulence and host immune response. Surra disease is a chronic infection in water buffaloes, characterized by weight loss, infertility and abortion (Luckins, 1988; Davison et al. 1999; Lohr et al. 1986; Thu et al., 1998). It hardly shows clinical signs therefore these animals are often considered as reservoirs. Inapparent infections in buffaloes may develop into clinical conditions if they are stressed by inclement weather or by other infections including liver fluke, rinderpest, foot-and-mouth disease or piroplasm; they may also appear after vaccination. It is present in the blood and within vertebrates' tissues. In the water buffalo *T. evansi* has a high mortality rate as evidenced from different countries in Asia (Luckins, 1988; Lun et al. 1993). It is also widespread in North Africa, South America and throughout most of the livestock-producing areas of Indonesia. In a study carried out on water buffalo in Indonesia, Payne et al. (1991) have observed a prevalence rate of infections higher than for cattle. Furthermore an age-dependent prevalence rate was seen in buffalo and cattle with the highest rates seen in animals older than two years. *T. evansi*, in the water buffalo is incriminated for immunosuppression and may be the cause of vaccination failure against Pasteurella (Stephen, 1986; Holland et al., 2001). To survive in an immunocompetent host, *T. evansi* is able to regularly change the variable surface glycoproteins (VSGc) of the cell surface, a mechanism called antigenic variation. The appearance of new variable antigens types (VATs) results in sequential peaks of parasitaemia intermitted by periods during which the parasites are hardly detectable in the blood (Jones and McKinnell, 1985).

T. vivax is also known to parasitize river buffalo in Central and South America. The disease syndrome has a mortality of 22 percent in buffalo calves.

Clinical findings- There are three basic aspects of trypanosomiasis: Linphoadenopathy and spleen enlargement, haemolytic anaemia, as a main feature of the disease, mainly in cattle, and cell degeneration and inflammatory infiltrates in several organs and tissues such as muscles and the CNS. In buffaloes the disease is characterized by enlargement of the lymph nodes, bilateral mucous discharge from the eyes, emaciation, rough coat, weakness of the hindquarters and recumbency. Acute forms can be fatal (Bhatia, 1992).

Diagnosis- In the case of trypanosomiasis, in order to confirm a clinical suspect, parasites have to be detected in the blood by observing stained blood films.

The diagnosis of Surra can be problematic, particularly in chronic infections. Payne et al. (1991) have evidenced the infection with *T.evansi* by the microhaematocrit centrifugation technique (MHCT) and ELISA test for detection of antibodies to *T.evansi*. Several antibody detection assays based on a predominant VAT have been developed (Verloo et al. 2000). These tests include the CATT/*T.evansi*, which is a simple card agglutination test appropriate for antibody detection in blood or serum applicable under field conditions (Bajyana Songa and Hamers 1988; Davison et al., 1999; Holland et al. 2002).

Therapy- In sheep, cattle and goats diminazene (Berenil) and bromide (Ethidium and Novidium) are usually used. The first one has been used in buffaloes at a dose rate of 10 to 15mg/kg BW in intravenous injection (IV). Chemotherapy for trypanosomiasis in both cattle and buffaloes can be performed with suramin and antrycide methyl sulphate. The drug of choice for treating surra in buffaloes has been indicated to be a 10 percent solution of quinapyramine sulphate, 5mg/kg BW, subcutaneous injection (Bhatia, 1992).

Prophylaxis- In order to control the disease, two strategies are adopted: fighting the flies and a rational use of drugs (isometamidium-Samorin).

An important way to control Trypanosomiasis is through the protection of animals bound for endemic areas and coming from areas where Glossina is absent. A further suggestion involves the introduction, in endemic areas, of trypanotolerant breeds and a simultaneous use of drugs. Antrycide chloride is useful in prophylaxis. Finally, through genetic selection, it would be

possible to obtain trypanotolerant breeds, as the only possible solution to the disease.

Ascaridiosis

Etiology- Toxocara vitulorum is the larger worm of the small intestine of ruminants and it is prevalent in the buffalo population in a number of countries. It is considered a highly prevalent parasite of water buffalo calves between 15 and 120 days of age (Starke et al. 1983). Furthermore it is responsible for high morbidity and mortality rates resulting in serious economic losses.

Epidemiology- The severity of infestation varies from place to place, depending upon many factors such as management and nutrition. Buffalo calves are more susceptible to *T. vitulorum* than cattle calves under conditions of natural infection when they are raised together. This may have been due to differences in the natural immunity of each species (Lau, 2002). Griffiths quoted reports on high incidences of *T. vitulorum* infection in buffalo calves in India, the Amazon valley of Brazil, Malaysia, Sri Lanka and Pakistan. The usual routes of infection are transplacentally and transmammary. In the first route, during pregnancy, larvae become active and the foetus can be infected by ingestion of larvae present in the amniotic fluid. In the second route, the parasite is acquired by calves when they suckle colostrum/milk contaminated with infective larvae from infected cows. It is common to find buffalo calves highly infected between 15 and 90 days of age with the peak egg output occurring 31 to 45 days post-infection (Starke et al. 1983; Roberts, 1990).

After reaching the infection peaks, the parasites begin to be rejected by the hosts and, 120 days post-birth, eggs of *T.vitulorum* are no longer found in the faeces of the calves, suggesting a process of self-cure and immune protection against intestinal infection (Starke et al. 1983; Roberts, 1990). In addition to this, buffalo cows are also able to mount a significant specific antibody response against *T. vitulorum* and the antibodies are transferred through the colostrum to the young buffalo calves after the birth (Rajapakse et al., 1994; Starke-Buzetti et al. 2001).

These passively acquired antibodies do not protect the calves against the acquisition of *T. vitulorum* infection, but may have an important role in worm rejection by calves. However, the rejection is a complex process that involves not only humoral but also cellular immune response and little is known about the immune mechanism of *T. vitulorum* rejection in buffalo calves.

Clinical findings- Main clinical symptoms are due to the presence of adult parasites in the gut of six month old calves. Serious infestations cause growth reduction and diarrhoea in young buffaloes.

Diagnosis- The diagnosis is confirmed by checking faecal samples. Furthermore it is possible to detect serum and colostrum antibodies by indirect ELISA procedure (Starke-Buzetti et al. 2001).

Therapy- Adult worms are sensitive to a wide range of antihelminthics such as piperazine, levamisole and ivermectine.

While the adult parasites are relatively easy to remove from the intestines by anti helminthics, the larvae are difficult to kill, particularly larvae that can be hypobiotic in the musculature and the brain (Abo-Shehada and Herbert, 1984).

Prophylaxis- The diffusion of infestation can be successfully reduced by treating three to six week old calves in order to stop parasite development.

Fasciolosis

Etiology- Fasciolosis is a common disease of domestic ruminants worldwide. Infection by Fasciola hepatica, Fasciola gigantica and Paramphistomum spp. are important parasitic diseases of water buffalo and other livestock both in temperate and tropical climates.

Epidemiology- In some countries the disease has a huge economic importance since water buffalo is the main labour animal, used for work in rice fields and for meat and milk production. It is a serious disease of the liver measured in terms of lowered production and mortality. Young calves acquire infection readily during early winter and may suffer from an acute condition leading to death. It has been observed in many countries including India, Pakistan, Egypt, Turkey, Iraq and Europe. F. hepatica is widespread in Europe and in the higher altitude districts of India. Lymnea truncatula, a mud snail, is involved as the intermediate host for this species in these areas. F. gigantica is widely prevalent in the Indian sub-continent and south-east Asia. Lymnea rufescens, an aquatic snail, acts as an intermediate host in the Indian sub continent. L. rubiginosa is the intermediate host in south east Asia and L. natalensis in Egypt. Buffaloes also carry Dicrocoelium dendriticum infections in their livers in the hill districts of India, Italy and Turkey, and Eurytrema pancreaticum infections of pancreatic ducts in South-East Asia and Brazil (Griffith, 1974; FAO, 1977).

Clinical findings- Fasciolosis, in buffaloes, usually appears as a chronic infection, causing anorexia, weight loss, reducing labour and production capacities, similar symptoms to those in cattle.

Diagnosis- The diagnosis relies upon egg detection in faecal samples, clinical signs and two laboratory tests. The first one evaluates the serum level of hepatic enzymes GLDH (glutamate dehydrogenase), GGT and AST as a result of haepatic cell damage; the other one detects the presence of serum antibodies against some components of the parasite by ELISA method or passive haemoagglutination. Finally, nowadays, biotechnologies (PCR) allow a different and safer approach to the diagnosis.

Therapy- In cattle the antihelminthic treatment aims to reduce the parasite number during winter time when Fasciola is sensitive to drugs for adult parasites.

Prophylaxis- The control of fascioliasis can be dealt with in two ways: by reducing the number of intermediate hosts and by administering drugs. The most correct way to reduce mud population is the reclamation of land by elimination of water ponds in order to remove the intermediate host habitat. Otherwise it is possible to treat muddy areas with copper sulphate.

Babesiosis

Etiology- Babesiosis in cattle is a tick-borne haemoparasitic disease, which is the cause of livestock morbidity and mortality in all semi-tropical and tropical areas of the world. It is an acute and often fatal disease resulting in heavy economic losses. The aetiological agents belong to the genera Babesia and Theileria (Kjemtrup and Conrad, 2000). Babesia bovis is the main pathogen, killing more than half the susceptible cattle that it infects whereas Babesia bigemina, although it infects up to 40 percent of red cells, causes less severe infections (Brown, 2001).

Epidemiology- Griffith reviewed the status of this disease in buffalo in India, West Malaysia and Italy and concluded that babesiosis in buffaloes has rarely been reported.

Clinical findings- Usually B. bovis and B. bigemina invade and replicate exclusively within bovine erythrocytes, causing anaemia and in the case of B. bovis, a fatal cerebral disease associated with the adherence of infected erythrocytes to brain microcapillary endothelial cells (Wright, 1988; Clark et al. 1998).

The disease has a typical biphasic trend: an acute hemolitic crisis followed by a long convalescence in those animals that recover from the infection.

Diagnosis- For diagnosis, anamnesis and clinical signs are useful indications of infection. In order to confirm the diagnosis, it is necessary to examine a blood film stained by the Giemsa method.

Therapy- It depends upon the Babesia species and upon the availability of drugs in the different countries. The most known drugs are imidocarb, pentamidine and amicarbilide.

Prophylaxis-. The adoption of specific treatments for animals born in endemic areas is usually not necessary since the acquired immunity by colostrum is gradually strengthened following repeated babesia infections. On the contrary the main problem of babesiosis is due to difficulties in introducing new animals in endemic areas for genetic improvement. In Australia, selection and breeding of cattle resistant to tick infestations is practised. Control of babesiosis through the eradication of vector ticks is difficult because of the high prevalence rates of vector ticks, the high cost of modern acaricides and the development of resistance to acaricides in ticks. The use of vaccines against babesiosis is practised in tropical countries. In India the inoculation of B. bigemina exoantigens induced a protective immune response. Recently in Australia a live vaccine has been used. In addition a recombinant protein (Bm86 from intestinal cells of the tick Boophilus microplus) has been widely utilized as an anti-tick vaccine (Sharma et al. 2001).

Theileriosis

Etiology- Buffaloes are highly susceptible to the Theileria parva infection which occurs in East African cattle as East Coast Fever. This infection still represents a constraint to cattle breeding because of the large diffusion of the transmitting Rhipicephalus ticks, and the high mortality in animals introduced in endemic areas. In some regions of Africa, where cattle and buffaloes share the same pasture, the epidemiology is complicated by the presence of T. parva lawrenci, buffalo parasite and the natural reservoir of infection.

Epidemiology- Transmitted by Rhiphicephalus appendiculatus theileriosis, it causes illness and high mortality in cattle. Ticks can survive for more than two years on pastures and it is not necessary for buffaloes and cattle to be present together on the pasture for infection to occur. In India, Egypt and Asia, T. annulata infection affects both buffaloes and cattle, although less frequently in river buffaloes than in cattle; the disease is widely spread in tropical and subtropical areas including Portugal, East Europe, the Mediterranean countries (Mediterranean theileriosis), the Middle East, India and China. The infection is transmitted by ticks genera Hyalomma and contrary to T. parva the disease is not always lethal. T. mutans has been reported in swamp buffaloes from Indonesia, West Malaysia and a number of countries in south-east Asia (Griffith, 1974).

Clinical findings- The pathogenesis of the disease shows a first phase without clinical signs (incubation); a second phase characterized by a prominent limphoadenopathy starting from the lymph node involved in the tick bite; and a final phase with lymphoid depletion and lymphopoiesis depression. Usually death occurs after three weeks of infection.

Diagnosis- It is an endemic disease only in those areas where ticks are living. It is possible to observe parasites from spleen and lymph nodes film prepared with needle suction. Parasites may also be detected inside erythrocytes in blood films stained by the Giemsa method. IFAT (indirect immunofluorescence test) can be used to detect those animals that have overcome the infection.

Therapy- The first choice in Theileria treatment is represented by the group of naphthoquinone. Furthermore, tetracyclines can be effective but only when administered at the

beginning of the infection.

Prophylaxis- Traditionally the control of Theileriosis is based on a limitation of animal movements, enclosure of pastures in order to avoid contact with cattle and buffaloes bred extensively; ultimately, periodical use of acaricides is the practice most recommended. The use of an effective vaccine is not useful due to the immune mechanisms of Theileria and the presence of some T.parva strains immunologically different. Purified protein, recombinant protein and cell culture vaccines have been studied. The latter was evaluated on calves during extensive immunization trials and proved non-pathogenic, immunogenic and protective from the fifteenth day following administration up to six months, without passive transferrance from dams to offspring (Sharma et al. 2001).

Strongilosis

Etiology- In weaned buffaloes the main gastrointestinal parasites are the Trichostrongyles: Haemonchus, Cooperia, Ostertagia, Trichostrongylus, Oesophagostomum, Bunostomum, and Nematodirus. The most important gastrointestinal (GI) nematode responsible for considerable production losses in cattle is Ostertagia ostertagi and to a lesser extent, Cooperia oncophora, Nematodirus spp. and Trichostrongylus spp. (Armour, 1989).

Epidemiology- It is generally agreed that mainly first grazing season (FGS) calves may be heavily infected (Ostertagiosis type I) by larvae ingested three to four months previously, whereas, in yearling cattle infection by the Ostertagiosis type II is more common when the animals are turned out for their second grazing season. This is due to the maturation of ingested larvae during the previous autumn. In many cases yearling buffaloes may become immune. In general this is true for genera such as Cooperia and Nematodirus, which induce a rapid build up of protective immunity in their host (Armour, 1989). In contrast, during the FGS calves only develop a partial resistance to the highly pathogenic abomasal nematode O. ostertagi.

Clinical findings- In less developed agricultural systems the severity of the disease caused by these parasites may present the classical clinical signs of stunted growth, tissue oedema, and severe diarrhoea. In more affluent agricultural systems the extensive use of highly efficacious broad spectrum anthelmintics has resulted in a situation where clinical disease is not commonly encountered. But even in these intensively managed herds, the parasites hinder optimal growth and productivity of their hosts. Furthermore, numerous studies have shown that even in well-managed herds, with no signs of clinical parasitism, the presence of the parasites in the herds results in decreased growth in young animals, and decreased milk production in adult cows. Gastrointestinal nematode infections of cattle remain a constraint on the efficient raising of cattle on pasture throughout the world.

Diagnosis- Although sufficient morphological differences exist among adult and larval cattle GI nematodes to allow their accurate identification, the availability of similar techniques for nematode eggs remains an obstacle to reliable diagnosis. In some cases the structure and size of the egg can be diagnostic; however, in many instances, similarities among eggs from different species and even distinct genera require alternative methods for their differentiation. Presently, the method commonly utilized for GI nematodes of cattle involves in vitro cultivation of eggs up to infective, third stage larvae (L3), followed by recovery and morphological identification (Keith, 1953). This procedure is labour intensive, time-consuming and prone to errors due to the variation in egg viability and parasite development in culture. Other methods utilizing microscopic examination require measurements on as many as twenty different parameters per egg, followed by computer analysis and assimilation of the data (Georgi et al. 1989; Sommer, 1996). Clearly, these procedures are equally labour intensive, requiring expensive equipment and the creation of a considerably large data system to decrease the error rate. Christensen et al. (1994) identified and cloned, genus-specific repetitive DNA fragments from a number of important genera of strongylid nematodes infecting cattle and used these as

probes to screen parasite genomic DNA. While this method is sensitive, specific and adaptable to screening egg-derived DNA, as designed, the technique employs radioisotopes and requires multiple assays to distinguish mixtures of eggs. Zarlenga et al., (1998), utilizing a unique internal repeat within the first internal transcribed spacer (ITS 1) of O. ostertagi and Ostertagia lyrata, the most pathogenic of nematode species infecting cattle, developed, a polymerase chain reaction (PCR)-based technique that collectively differentiates and quantifies these species from other common bovine GI nematodes. This method, however, fails to discern one specific species among the other nematode species in the midst of a mixed population. A plethora of parasite-specific PCR primers have been generated for identifying individual nematode species some of which work at the level of a single egg. These and other similar tests require that each parasite DNA be analyzed through a matrix of primer sets and PCR reactions for identification.

Therapy- Ostertagia is sensitive to benzimidazole, levamisole and avermectins both in the adult and developing phase. Following treatment it would be advisable to move animals onto non-exploited pastures. The same therapy is applied for Hemonchus, Trichostrongylus and Cooperia.

Prophylaxis- Historically, control of GI nematodes was accomplished by complicated management programmes which kept stocking rates low and minimized the exposure of susceptible animals to heavily infected pastures. The development of broad spectrum, highly efficacious anthelmintic drugs changed the nature of parasite control programmes, and has resulted in a situation where parasite control now relies almost exclusively upon the repeated administration of drugs to a large percentage of herd members (Zarlenga et al. 2001). For livestock producers, it is important that they identify both the animals harbouring high numbers of parasites, as well as those individuals carrying the more pathogenic parasite species, such as Ostertagia ostertagi in the more temperate regions. Rapid parasite identification would greatly assist the development of control programmes and reduce the number of unnecessary drug treatments (Zarlenga et al., 2001). With regard to Ostertagia, it is kept under control through routine treatments in calves when the larvae number increases. Although animals are treated, they still remain exposed to re-infestations, affecting their productivity. Nowadays the risk of development of helminthic strains resistant to drugs recommends avoiding frequent treatments. The control of other strongylosis is quite similar to what has already been mentioned for Ostertagia.

An increasingly attractive adjunct or alternative for the control of GI nematodes in cattle is the identification of host genes that influence acquired or innate resistance to the parasites and the use of the vast potential of the host genome to reduce parasite transmission in cattle populations. On account of the type of disease caused by these parasites, the control of disease does not require absolute protection from infection. An optimal control programme should instead minimize both the impact of these parasites on productivity and the level of economic input into the production system, while maximizing utilization of renewable resources such as pastures.

Coccidiosis

Etiology- Coccidia are intracellular parasites of the epithelial cells of the intestine. They present a single host in which they undergo both asexual and sexual multiplication.

Epidemiology- Coccidiosis in buffaloes, as in cattle, is widely prevalent and usually affects calves below one year of age, but it may occasionally also occur in yearling calves. Older buffaloes are more resistant to clinical coccidiosis due to either age resistance or acquired immunity. Thirteen Eimeria species are common to both the hosts parasitizing their intestines. In 13 pathogenic species cattle have been detected. Main pathogens are E. zuernii and E. bovis. The first one is particularly severe, affecting the coecum and colon thus causing haemorragic diarrhoea. E. bovis affects the same part of the gut causing enteritis. The prevalence of the

disease may vary from place to place, depending on climatic conditions. The trend of the disease varies according to particular conditions such as overcrowding and lack of hygiene, that are liable to influence the number of oocistis ingested.

Clinical findings- The disease develops in acute, subacute and chronic forms and is responsible for exceedingly heavy economic losses to the dairy industry as its main adverse effect strikes young calves below six months of age. An acute form of coccidiosis in buffalo calves is characterized by bloody diarrhoea. The infected animals may show other clinical signs, such as anorexia, weakness, loss of body weight, anaemia, emaciation and dehydration. Most lesions are found in the small intestine while the large intestine manifests severe catarrhal enteritis and is full of fluids, with blood and fibrinous clots.

Diagnosis- Diagnosis is based on anamnesis, clinical signs and the presence of pathogen oocistis in faecal samples.

Therapy- The preferred drug is sulphamidine and amprolium.

Prophylaxis- Overcrowding and lack of hygiene present optimal conditions for the diffusion of Eimeria. As a result, disease control is based on good farm management, especially with regard to food and water recipients which must be clean as well as litters which should be dry.

Echinococcosis/hydatidosis

Etiology- The genus Echinococcus is very important in the Taeniidae family and is one of the smallest cestodes.

Epidemiology- E. granulosus and E. multilocularis play a significant role in veterinary medicine since their larvae, the hydatids, affect several intermediate hosts including man. E. granulosus is known to exist as biologically distinct subspecific variants or strains which may vary in their infectivity to domestic animals and man.

Clinical findings- Unilocular hydatid disease, caused by the metacestode of E. granulosus is widely recognized as an increasingly important disease in domestic animals in the developing countries. Shamsul (1994) carried out a study regarding lesions of the disease in Bangladeshi buffaloes and reported the higher prevalence of infection in the liver and lungs. Other authors (Munir, 1982; Prasad, 1980) reported the incidence of hydatid infection in different organs of buffaloes.

Diagnosis- Diagnosing hydatidosis is possible through scanning, radiology, serology and postmortem examination. The postmortem examination is usually an important component in monitoring the efficiency of control programmes.

Therapy- Several benzimidazole compounds have been shown to have efficacy against the hydatid cyst in the intermediate host. Long-term treatment with albendazole has a particularly marked effect on the cysts, while long-term treatment with praziquantel only has a limited effect with few changes in the germinal layer of the cyst.

Prophylaxis- Echinococcosis can be controlled through preventive measures that break the cycle between the definitive and the intermediate host. These measures include controlling dogs, inspecting meat, and educating the public regarding the risk to humans and avoiding feeding offal to dogs, as well as introducing legislation. However, none of these measures will work if not applied on a wide scale. Recently, a recombinant vaccine has been developed to be used on sheep.

Mange

Etiology- A serious skin disease in buffaloes, it is caused by Sarcoptes scabiei var. bubalis which may often become fatal in calves. Psoroptic mange in buffaloes is due to an unknown variety of Psoroptes communis.

Epidemiology- It is very common in Swamp buffaloes. The incidence of the disease is likely to increase during periods of drought when opportunities for wallowing become restricted. The disease has been reported in India and Thailand (Dissamaran, 1960). A wide prevalence of sarcoptic mange in buffalo in India has been reported in other works (Chakrabarti et al., 1981). Nowadays such pathology is often reported in cattle of some Northern European countries such as the UK even though the introduction of cattle from areas where this mange is particularly disseminated (Canada and the USA) is forbidden (Urquhart et al., 1996). Griffith (1974) suggested that the incidence of psoroptic mange in South East Asia was much lower than that of sarcoptic mange, from which it should be differentiated by identification of the mite involved. It frequently occurs in Egypt, Pakistan, India, Burma, Indonesia, the Philippines and Thailand (FAO, 1977). It is reported to be most prevalent in Egypt. Maske and Ruprah (1981) reported a high incidence of Psoroptic mange in buffaloes in India from June to September (rainy season) with the highest level of 71 percent being in July in Northern India.

Clinical findings- Early lesions are usually observed where the skin is thin, in particular parts of the body such as the neck and tail. Hair falls out and the skin becomes folding and scaly. Later, wrinkled crusts are formed containing numerous sarcoptic mites in their immature stages. In the beginning, small papules are formed which may turn into scabs. The affected animals try to relieve the irritation or itching by rubbing the lesions against various solid objects. As far as other kinds of sarcoptic mange are concerned, itching is very intense and the economic consequences are weight loss, reduced labour, and milk and meat production capacities, as well as the quality of the leather. In general, little effect is noticed regarding health status. Progressive emaciation, restlessness, weakness, and even death can be observed in heavy infestations. Psoroptic mange mainly affects the shoulder region and the root of the tail (Kassem and Soliman, 1966).

Diagnosis- In order to confirm a diagnosis a skin scraping is usually performed and the parasites are evidenced by microscopic examination, in the case of positivity.

Therapy- A 0.1 percent coumaphos water suspension is effective when applied four times at weekly intervals; a 10 percent sulphur suspension in liquid paraffin should be applied every one to two days (Sukhapesna, 1992). In addition, 0.03 percent water suspension of gamma-BHC, dieldrin, triclorphon, lindane, chlorpyriphos (0.012-0.025 percent), diazinon (0.025-0.05 percent), malathion (1 percent), carbaryl (1 percent) proved to be effective. The first choice is represented by avermectins/milbemycin. A further alternative is pyrethroids application (flumethrin) and amitraz.

Prophylaxis- Most control practices involve the use of insecticides or acaricides, but in some instances it may be necessary to replace chemical applications with accurate management and environmental manipulations.

FUNGAL DISEASES

Buffalo fungal diseases are mainly represented by mycotic mastitis, mycosis of the female reproductive system, mycotic abortion, pulmonary mycosis, mycotic gastroenteritis, cutaneous aspergillosis, keratomycosys, rhinosporidiosis and ringworm. Trichophyton verrucosum is the principal etiological agent of dermatophytosis in buffaloes (Refai, 1991; Adlakha and Sharma, 1992).

Deg Nala disease

Etiology- It is a mycotoxicosis most frequently caused by Aspergillus niger, Alternaria alternata, Fusarium avenaceum, Mucor heimalis, Fusarium oxysporum, Fusarium fusarioides, Cladosporium cladosporoides, Aspergillus flavus and Penicillium notatum (Maqbool et al.,1994).

Epidemiology- The disease affects cattle and buffaloes fed mouldy paddy straw (Sikdar et al., 2000). In buffaloes the disease is more severe than in cattle, due to the higher susceptibility of this species. Secondary bacterial infection of the lesions are partly responsible for the severity of the disease. Rice straw containing multiple dark specks is the main cause. In fact, the disease has been reported from rice-growing areas of India, Pakistan and Nepal and has been responsible for causing considerable economic losses.

Clinical findings- Also called gangrenous syndrome, affected buffaloes show lameness, edema, gangrenous ulceration of limbs, hooves, ears or tail that are cold to the touch. Sometimes the muzzle and tip of the tongue become gangrenous; there is emaciation, recumbency and eventually death. Sometimes gangrenous portions of the body drop off; in the case of hooves, bones can be exposed (Maqbool et al. 1994; Hokonohara et al. 2003). Usually lesions heal within a few weeks, but severe cases can last 1 to 32 months.

Diagnosis- Different fungi species can be isolated from rice straw in agar or liquid media.

Therapy- Lesions should be washed and dressed with nitroglycerine 2 percent ointment. In order to obtain a higher recovery rate, a therapeutic regimen can consist in oxytetracycline at 20 mg/kg BW in a single intramuscular injection or, better, in oral administration of penta-sulphate at 30 g daily for ten days (Maqbool et al., 1994).

Prophylaxis- It is certainly based on the control of straw quality. Maqbool et al. (1994) suggested the use of hydrated sodium calcium alumosilicate (HSCAS) or other sorbents to bind aflatoxins in the gastrointestinal tract.

References

Abd El-Rahim, I.H.A. 1997. Bovine cryptosporidiosis in newborn buffalo calves. Proc. Fifth World Buffalo Congress, October 13-16 Caserta, Italy: 582-587.

Abo-Shehada, M.N. and Herbert, I.V. 1984. Antihelminthic effect of levamizole, ivermectin, albendazole and fenbendazole on larval Toxocara canis infection in mice. Res.Vet.Sci. 36: 87-91.

Ackermann, M., Belak, S., Bitsch, V., Edwards, S., Moussa, A., Rockborn, G. and Thiry, E. 1990. Round table on infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus infection diagnosis and control. Vet. Microbiol., Jun 23 (1-4): 361-3.

Adlakha, S.C., and Sharma, S.N. 1992. Infectious diseases. Worlds Animal Science C6. Buffalo Production. Elsevier Tulloh N.M. and Holmes J.H.G. Ed.: 292-298.

Armour, J. 1989. The influence of host immunity on the epidemiology of trichostrongyle infections in cattle. Vet. Parasitol. 32: 5-19.

Arrigoni. N., Belloli, A, Belletti, G.L., Proverbio, D., Greppi, G. and Vacirca, G. 1995. First results of paratuberculosis therapy in calves experimentally infected by intravenous route. Proc. Fourth Int. Coll. Paratuberculosis, July 17-21, Cambridge, UK.

Autorino, G., Masotti, G., Amaddeo, D. and Korver, H. 1991. Isolation of leptospira interrogans serovar hardjo and tarassovi in buffaloes. Proc. Third World Buffalo Congress, May 13-17, Varna, Vol IX: 8-14.

Bahiraie, A. and Moghaddam, A. 2004. Isolation and identification of salmonellae serotypes from buffalo and bovine meat which were slaughtered in the Ahvaz slaughterhouse. Proc. Seventh World Buffalo Congress, 20-23 October, Manila: 10-11.

Baiiana Songa, E. and Hamers, R. 1988. A card agglutination test (CATT) for veterinary use based on an early VAT RoTaT ½ of Trypanosoma evansi. Ann. Soc. Belg. Med. Trop. 68: 233-240.

Baldi, L., Autiro, N., Pazzanese, L., Mizzoni, Casupola, V., Sorice, G. and Romano, R. 1997. Chlamydiosis in buffaloes of Campania, Italy (Preliminary findings). Proc. Fifth World Buffalo Congress, 13-16 Oct., Caserta, Italy: 692.

Bathia, B.B. 1992. Parasites of River Buffaloes. Worlds Animal Science C6. Buffalo Production. Elsevier Tulloh N.M. and Holmes J.H.G. Ed.: 305-327.

Bolin, S.R. 1995. Control of bovine diarrhoea infection by use of vaccination. Vet. Clin. North Am. 11, 616-625.

Booth, P.J., Stevens, D.A., Collins, M.E. and Brownlie, J. 1995. Detection of bovine viral diarrhoea virus antigen and RNA in oviduct and granulose cells of persistently infected cattle. J. Reprod. Fert. 105: 17-24.

Brown, W.C. 2001. Molecular approaches to elucidating innate and acquired immune responses to Babesia bovis, a protozoan parasite that causes persistent infection. Vet. Parasitol. 101: 233-248.

Brownlie, J., Clarke, M.C. and Howard, C.J. 1989. Experimental infection of cattle in early pregnancy with a cytopathic strain of bovine virus diarrhoea virus. Res. Vet. Sci. 46: 307-311.

Brownlie, J. 1991. The pathways for bovine virus diarrhoea virus biotypes in the pathogenesis

of disease. Arch. Virol. 3: 79-96.

Capezzuto, A.R. and Galiero, G. 2001. Blue-tongue: a problem regarding also the buffaloes. Bubalus bubalis II: 31-33.

Cavirani, S., Consalvo, F., D'Onofrio, G., Capucci, L., Cabassi, C.S., Allegri, G., Bigliardi, E., Civardi, A. and Flammini, C.F. 1997. A serological survey of different bovine herpesviruses (BHV-1, BHV-2, BHV-4) in dairy buffaloes of southern and northern Italy. Proc. Fifth World Buffalo Congress, October 13-16, Caserta, Italy: 626-630.

Cavirani, S., Pozzi, P., Cabassi, C.S., Bigliardi, E. and Consalvo, F. 1997. A field trial to control neonatal diarrhoea in a buffalo (Bubalus bubalis) dairy herd. Proc. Fifth World Buffalo Congress, October 13-16, Caserta, Italy: 588-592.

Chakrabarti, A., Chatterji, A., Chakrabarti, K. and Sen Gupta, D.N. 1981. Human scabies from contact with water buffaloes infested with Sarcoptes scabiei var. bubalis. Annals Trop. Med. Hyg. 75 : 353-357.

Chiodini, R.J., Van Kruiningen, H.J. and Merkal, R.S. 1984. Ruminant paratubercolosis (Johne's disease). The current status and future prospects. Cornell Vet. 74: 218-262.

Christensen, C.M., Zarlenga, D.S. and Gasbarre, L.C. 1994. Ostertagi. Haemonchus, Cooperia, Oesophagostomum: construction and chacterization of genus-specific DNA probes to differentiate important parasites of cattle. Exp. Parasitol. 78: 93-100.

Clark, I.A. and Jacobsen, L.S. 1998. Do babesiosis and malaria share a common disease process? Ann. Trop. Med. Parasitol. 92: 483-488.

Costa, E.O. 2002. Influence of infections and infectious disease on buffaloes reproduction. Proc. First Buffalo Symposium of Americas, 1-8 September, Belem, Para, Brazil: 5-14.

D'Apice, L., Russo, A., Di Somma, G., Serpe, L., Gallo, P., De Franciscis, G. and Fenizia, D. 1997. Identification of antibodies anti-brucella abortus in the serum and in the milk of buffalo by flow cytometry. Proc. Fifth World Buffalo Congress, October 13-16, Caserta, Italy: 616-621.

Davison, H.C., Thrusfield, M.V., Muharsini, S., Husein, A., Partoutomo, S., Rae, P.F., Masake, R. and Luckins, A.G. 1999. Evaluation of antigen detection and antibody tests for Trypanosoma evansi infections of buffaloes in Indonesia. Epidemiol. Infect. 123: 149-155.

Dissamaran, R. 1960. The use of dieldrin as a control agent of sarcoptic mange in buffalo and cattle in Thailand. Proc. Ninth Pacific Congr. Pacific Sci. Ass. 2: 106.

El-Garhi, M.M., El-Rashidy, A.A., Mettias, K.N., Hassen, E.R. and Hassen, H.M. 1994. Studies on Neonatal Diarrhoea in Buffalo Calves. Proc. Fourth World Buffalo Congress, 27-30 June, Sao Paulo: 361.

FAO. 1977. Statistical data.

Fagiolo, A., Scherm, B., Scicluna, M.T., Condoleo, R.U. e Saralli, G. 2001. Controlli sanitari in 21 allevamenti bufalini nella Regione Lazio. Proc. I° Congresso Nazionale sull'Allevamento del Bufalo: 409-412.

Fagiolo, A. and Roncoroni, C. 2003. Patologia nell'allevamento bufalino italiano - Italian buffalo breeding pathology. Proc. Secondo Congresso Nazionale sull'Allevamento del Bufalo, Monterotondo (Roma) 28-30 agosto 2003: 99-105.

Farina, R. e Scatozza, F. 1998. Malattie infettive degli animali. UTET: 720-728.

Farrag, I., Salem, A.A., Ghanem, F., Gergis, S.M., Ebeid, M.H. and Hussein, A.Z. 1991. Immune response of buffalo calves for combined blackquarter and haemorrhagic septicaemia vaccines. Proc. Third World Buffalo Congress, Varna May 13-18, Vol IV: 1035-1041.

Fenizia, D., Guarino, A., Russo, A., De Franciscis, G. and Migliaccio, G. 1991. Vaccination trials with strains B19 and 45/20 in both infected and Brucella-free buffalo herds of Campania (Italy). Proc. Third World Buffalo Congress, Varna, May 13-18, Vol IV: 1076-1084.

Fenizia, D., Guarino, A., Russo, A., De Franciscis, G. and Migliaccio, G. 1991. Encephalomyelitis in buffalo calves. Proc. Third World Buffalo Congress, Varna, May 13-18, Vol I: 218.

Fraulo, P. and Galiero, G. 1999. Buffalo Brucellosis: epidemiologic survey in Salerno province (Italy). Bubalus bubalis II: 59-64.

Galiero, G., De Carlo, E., D'Alessandro, G., Lai, O. and Durante, G. 1997. Detection of verocytotoxin (VT), cytotoxic necrotizing factor (CNF) and hemolisin (HLY) producing strains of E. coli isolated from buffalo calves. Proc. Fifth World Buffalo Congress, October 13-16 Caserta, Italy: 593-597.

Galiero, G. and Sica, G. 1997. Encephalomyelitis (so called "male feruto") aetiology in buffalo calves: latest findings. Bubalus bubalis IV: 17-20.

Galiero, G. 1998. Infectious Bovine Rhinotracheitis (IBR), a complex of diseases concerning also buffaloes. Bubalus bubalis II: 29-31.

Galiero, G. 1998. Buffalo neonatal pathologies: description of the Escherichia coli main virulence factors. Bubalus bubalis IV: 19-23.

Galiero, G. 1999. Neonatal pathologies: salmonellosis (1st part). Bubalus bubalis II: 23-25.

Galiero, G. 1999. Neonatal pathologies: salmonellosis (2nd part). Bubalus bubalis III: 29-32.

Galiero, G. 1999. Buffalo neonatal diseases: Cryptosporidiosis. Bubalus bubalis IV: 23-25.

Galiero, G. De Carlo E. and Giordanelli M.P., 1999. Detection of verocytotoxin, thermolabile enterotoxin, thermostable enterotoxin, cytotoxic necrotizing factor and haemolysin producing strains of E. coli isolated from buffalo calves. Bubalus bubalis IV: 75-78.

Galiero, G. 2000. Buffalo neonatal disease: rotavirus infections. Bubalus bubalis I: 23-25.

Georgi, J.R. and Mc Culloch, C.E. 1989. Diagnostic morphometry: identification of helminth eggs by discriminant analysis of morphometric data. Proc. Helminthol. Soc. Wash. 56: 44-57.

Giglio, M., Guarino, A., Nobile, G., Fusco, G. and Landolfi, M.C. 1994. Coronavirus as a cause of enteropathy in buffalo calves. Proc. Fourth World Buffalo Congress, 27-30 June, Sao Paulo: 113.

Girio, R.J.S., Pereira, F.L.G., Marchiori Filho, M. et al. 2004. Investigation of antibodies to Leptospira spp. in wild and feral animals from the region of Nhecolandia, Mato Grosso do Sul, Brasil. Use of the immunohistochemistry technique for agent detection. Cienc Rural, Jan/Feb, vol. 34, no1, 165-169. ISSN 0103 8478.

Gomes, I., Ramalho, A.K. and De Mello, P.A. 1997. Infectivity assays of foot-and-mouth disease

virus: contact transmission between cattle and buffalo (*Bubalus bubalis*) in the early stages of infection. Vet.Rec. Jan 11, 140 (2): 43-7.

Griffith, R.B. 1974. Parasites and parasitic diseases. In: W.R. Cockrill (Editor). The Husbandry and Health of the Domestic Buffalo. Food and Agriculture Organization of the United Nations, Rome: 236-275.

Gupta, P.P., Sandhu, D.S. and Banga, H.S. 1991. Effect of immunosuppression on experimental chlamidial pneumonia in buffalo calves (Bubalus bubalis). Proc. Third World Buffalo Congress, 13-18 May, Varna, Bulgaria: 210.

Hajela, S.K. and Sharma, S.K. 1978. Indian Vet. Med. J. 2: 103-104.

Hegazy, A.A., Nabina Ramadan Alt, Ismail, I.M., Afaf Saad El Dean Fahmy, Abd El-Ghany M. and Lamia Abd El-Hamid El-Frasn. 1991. Studies on enteritis in newly born calves. Proc. Third World Buffalo Congress, 13-18 May, Varna, Bulgaria, Vol I: 215.

Hegazy, A.A., Shelaby, M.A., Bagouri, G.E., Sami, A.M., Rhawia Omran, El Begawi, M.E. and Reda, I.M. 1991. Studies on buffalo calves experimentally infected with bovine viral diarrhoea virus. Proc. Third World Buffalo Congress, 13-17 May, Varna, Bulgaria, Vol IX: 105-115.

Hein, W.R. and Tomasovic, A.A. 1981. An abattoir survey of tuberculosis in feral buffaloes. Aust. Vet. J. Dec. 57 (12): 543-7.

Hoffmann, D., Soeripto, S., Sobironingsih, S., Campbell, R.S. and Clarke, B.C. 1984. The clinico-pathology of a malignant catarrhal fever syndrome in the Indonesian swamp buffalo (*Bubalus bubalis*). Aust. Vet. J., Apr. 61 (4): 108-12.

Hokonohara, S., Singh, U.M., Jha, V.C., Pradhan, A., Dev, S. and Mandar, R.K. 2003. Clinical and hematological findings on "Degnala", a disease of buffalo in Eastern Nepal. J.Vet.Med.Sci. Jun. 65: 719-722.

Holland, W.G., My, L.N., Dung, T.V., Thanh, N.G., Tam, P.T., Vercruysse, J. and Goddeeris, B.M. 2001. The influence of T.evansi infection on the immuno responsiveness of experimentally infected water buffaloes. Vet. Parasitol. 102: 225-234.

Holland, W.G., Thanh, N.G., My, L.N., Magnus, E., Verloo, D., Buscher, P., Goddeeris, B.M and Vercruysse, J. 2002. Evaluation of whole fresh blood and dried blood on filter paper discs in serological tests for T. evansi in experimentally infected water buffaloes. Acta Trop. 81: 159-165.

Horadagoda, N.U., Belak, K., De Alwis, M.C.L., Comis, A.I.U. and Vipulasiri, A.A. 1991. Immunoperoxidase evaluation of tissues following experimental haemorrhagic septicaemia in buffalo calves. Proc. Third World Buffalo Congress, 13-17 May, Varna, Bulgaria, Vol I: 212-213.

Horadagoda, N.U., Eckersall, P.D., Wijewardana, T.G., Packiarajah, P. and Thalagoda, S.A. 1997. Clinical and haematological responses of buffaloes following intravenous administration of endotoxin extracted from Pasteurella multocida serotype B:2. Proc. Fifth World Buffalo Congress, 13-16 October, Caserta, Italy: 611-615.

Houe, H. 1999. Epidemiological features and economical importance of bovine viral diarrhoea (BVDV) infections. Vet. Microbiol. 64: 89-107.

Jones, T.W. and Mckinnell, C.D. 1985. Antigenic variation in Trypanosoma evansi: variable antigen type (VAT) composition of first relapse populations in mice. Trop. Med. Parasitol. 36: 127-130.

Kalis, C.H.J., Van Schaik, G., Dijkhuizen, A.A. and Benedictus, G. 1995. Economic significance of vaccination against paratuberculosis. Proc. Fourth Int. Coll. Paratuberculosis, July 17-21, Cambridge, UK.

Kanameda, M. and Ekgatat, M. 1995. Isolation of Mycobacterium bovis from the water buffalo (Bubalus bubalis). Trop. Anim. Health. Prod. Nov. 27(4): 227-8.

Kassem, M.H and Soliman, K.N. 1966. Diseases of the buffalo. In: International Encyclopedia of Veterinary Medicine, Sir T. Dalling, Edinburgh, Green: 527-533.

Keith, R.K. 1953. The differentiation of the infective larvae of some common nematode parasites of cattle. Aust. J. Zool. 1: 223-235.

Kjemtrup, A.M. and Conrad, P.A. 2000. Human babesiosis: an emerging tick borne disease. Int. J. Parasitol. 30: 1323-1337.

Langoni, H., Del Fava, C., Cabral, K.G., Da Silva, A.V. and Chagas, S.A.P. 1997. Epidemiological survey on anti-leptospira agglutinins on buffaloes from Vale Do Ribeira, Sao Paulo State (Brazil). Proc. Fifth World Buffalo Congress, 13 16 October, Caserta, Italy: 622-625.

Lau, H.D. 2002. Economic impact of gastrointestinal parasitism in an Amazon buffalo farm, Brazil. Proc. First Symp. of Americas, 1-8 Sept., Belem Parà, Brazil: 21-25.

Lohr, K.F., Pholpark, S., Siriwan, P., Leesirikul, N., Srikitjakarn, L. and Staak, C. 1986. Trypanosoma evansi infection in north east Thailand. Trop. Anim. Health Prod. May 17 (2): 121-125.

Lohr, K.F., Pholpark, S., Siriwan, P., Leesirikul, N., Srikitjakarn, L. and Staak, C. 1986. Trypanosoma evansi infection in north east Thailand. Trop. Anim. Health Prod. May 18 (2): 103-108.

Luckins, A.G. 1988. Trypanosoma evansi in Asia. Parasitol. Today 4: 137-142.

Lun, Z.R., Fang, Y., Wang, C.J. and Brun, R. 1993. Trypanosomiasis of domestic animals in China. Parasitol. Today 9: 41-45.

Maqbool, A., Mohammad, G., Irfan, M. and Mahmood, F. 1994. Prevalence, aetiology, chemotherapy and control of Deg Nala disease in buffaloes and cattle. Proc. Fourth World Buffalo Congress, 27-30 June, Sao Paulo, Brazil, Vol II: 313 315.

Maske, D.K. and Ruprah, N.S. 1981. Note on the bionomics of psoroptic mange in buffaloes. Indian J. Anim.Sci. 51: 494-497.

Mathias, L.A., Chaves, L.F., Girio, R.J.S., et al. 1998. Evaluation of a competitive enzyme immunoassay for the serological diagnosis of brucellosis in buffaloes (Bubalus bubalis). Pesq Vet Bras, July, vol 18, no 3-4, 111-114. ISSN0100-736X.

Meyers, G. and Thiel, H.J. 1996. Molecular characterization of pestiviruses. Adv. Virus Res. 47: 53-118.

Molna,r L., Molnar, E., Lima, E.S.C., et al. 2002. Evaluation of six serological tests for the diagnosis of brucellosis in water buffaloes. Pesc Vet Bras, Apr/June, vol 22, no 2, 41-44. ISSN 0100-736X.

Munchow, G. and Pisarz, M. 1994. Epidemiological studies of some economical significant

infectious diseases in water buffaloes in the central Amazon region, Brazil. Proc. Fourth World Buffalo Congress, 27-30 June, Sao Paulo, Brazil, Vol II: 353-355.

Munir, M.A. Anwar, A.H. and Chowdhury, A.H. 1982. The nature and organ specificity of hydatid disease in buffaloes. Pakistan Vet. J. 2: 12-15.

Nandi, S. and Negi, B.S. 1999. Bovine ephemeral fever: a review. Comp Immunol Microbiol Infect Dis. Apr 22 (2): 81-91.

Payne, R.C., Sukanto, I.P., Djauhari, D., Partoutomo, S., Wilson, A.J., Jones, T.W., Boid, R. and Luckins, A.G. 1991. Trypanosoma evansi infection in cattle, buffaloes and horses in Indonesia. Vet. Parasitol. 38: 109-119.

Prasad, B.N. and Mondal, L.N. 1980. Incidence of hydatid cysts in buffaloes in Bihar, India. Kerala J. Vet. Sci. 10: 220-225.

Pringle, C.R. 1999. The universal system of virus taxonomy, updated to include the new proposal ratified by the International Committee on Taxonomy of Viruses during 1998. Arch. Virol. 144: 421-429.

Rajapakse, R.P.V.J., Lloyd, S. and Fernando, S.T. 1994. Toxocara vitulorum: maternal transfer of antibodies from buffalo cows (Bubalus bubalis) to calves and levels of infection with T. vitulorum in the calves. Res.Vet.Sci. 57: 81-87.

Refai, M. 1991. Bacterial and fungal diseases of buffaloes in Egypt. Third World Buffalo Congress, 13-17 May, Varna, Bulgaria, Vol I: 202.

Ribeiro, M.G., Langoni, H., Jewrez, J.A., Leite, D., Fereira, F. and Gennari, S.M. 2000. Identification of enteropathogens from buffalo calves with and without diarrhoea in the Ribeira Valley, State of Sao Paulo, Brazil. Braz. J. Vet. Res. Anim. Sci., Vol. 37 (2).

Roberts, J.A. 1990. The egg production of T. vitulorum in Asian buffalo (Bubalus bubalis). Int. J. Parasitol. 37: 113-120.

Shamsul Islam, A.W.M. 1994. Hydatidosis in buffaloes in Bangladesh. Proc. Fourth World Buffalo Congress, 27-30 June, San Paolo, Brazil: 310-312.

Sharma, R.D., Rakha, N.K., Nichani, A.K. and Malhotra, D.V. 2001. Modern strategies for the control of important livestock diseases. Sustainable Animal Production. Eds: Singh and Khanna SSARM, CCS HAU, Hisar: 109-118.

Sharma, M.C. and Mahesh Kumar. 2003. Infectious diseases of buffaloes. Proc. Fourth Asian Buffalo Congress, 25-28 Febr., New Delhi, India: 152-168.

Silva, I.D, Dangolla, A. and Kulachelvy, K. 2000. Mastitis, Brucellosis and Rinderpest in buffaloes in Sri Lanka: a comparison with cattle. Proc. Third Asian Buffalo Congress, 27-31 March, Kandy, Sri Lanka: 277-282.

Sikdar, A., Chakraborty, G.C., Bhattacharya, D., Bakshi, S., Basak, D.K., Chatterjee, A. and Halder, S.K. 2000. An outbreak of gangrenous syndrome among buffaloes and cattle in west Bengal: clinicopathological studies. Trop.Anim.Health Prod., Jun., 32: 165-171.

Sommer, C. 1996. Digital image analyses and identification of eggs from bovine parasitic nematodes. J.Helminthol. 70: 143-151.

Starke, W.A., Machado, R.Z., Honer, M.R. and Zocoller, M.C. 1983. Natural course of

gastrointestinal helminthic infections in buffaloes in Andradina County (SP), Brazil. Arq.bras.Med.Vet.Zoot. 40: 758-762.

Starke-Buzetti, W.A., Machado, R.Z. and Zocoller-Seno, M.C. 2001. An enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against T. vitulorum in water buffaloes. Vet.Parasitol. 97: 55-64.

Stephen, L.E. 1986. Trypanosomiasis: A Veterinary Perspective. Pergamon Press, Oxford (Ed.): 551.

Sukhapesna, V. 1992. Parasites of Swamp Buffaloes. Worlds Animal Science C6. Buffalo Production. Elsevier Tulloh N.M. and Holmes J.H.G. Ed., 329-354.

Sunil Chandra, N.P. and Mahalingam, S. 1994. Rotavirus associated diarrhoeas in buffalo calves in Sri Lanka. Proc. Fourth World Buffalo Congress, 27-30 June, Sao Paulo, Brazil, Vol II: 322-324.

Sunil-Chandra, N.P. 2000. Viral infections of buffaloes. Rotaviruses and herpes viruses. Proc. of the Third Asian Buffalo Congress on the Changing Role of the Buffalo in the New Millennium in Asia, Sri Lanka, 27-31 Mar.: 255-261.

Tariq Javed, M., Usman, M., Cagiola, M., Ahmad ,I. and Irfan, M. 2004. A study on tuberculosis in buffaloes: some epidemiological aspects along with haematological and serum protein changes. Proc. Seventh World Buffalo Congress, 20-23 October, Manila: 73-83.

Thu, L.T., My, L.R., Lan, P.D., Lang, P.S., Phuc, D.V. and Doanh, P.Q. 1998. Epidemiological survey on T. evansi infection in Vietnam. J. Protozool. Res. 8: 177-181.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. and Jennings, F.W. 1996. Veterinary Parasitology. II Edition (UTET).

Vasconcellos, S.A., Oliveira, J.C.F., Morais, Z.M., et al. 2001. Isolation of Leptospira santarosai, serovar guaricura from buffaloes (Bubalus bubalis) in Vale do Ribeira, Sao Paulo, Brazil. Braz J Microbiol, Oct/Dec, vol32 (4), ISSN 1517 8382: 298-300.

Verloo, D., Holland, W.G., My, L.N., Thanh, N.G., Tam, P.T., Goddeeris, B.M and Vercruysse, J. 2000. Comparison of serological tests for T. evansi natural infections in water buffaloes from North Vietnam. Vet. Parasitol. 92: 87-96.

Withlock, R.H., Weels, S.J., Sweeney, R.W. and Van Tiem, J. 2000. ELISA and faecal culture for paratuberculois (Johne's disease): sensitivity and specificity for each method. Vet. Microbiol. 77: 387-398.

Wright, I.G. and Goodger, B.V. 1988. Pathogenesis of babesiosis. Ristic, M. (Ed), Babesiosis of Domestic animals and Man. CRC Press, Boca Raton, FL: 99-118.

Yadav, M.P. 2003. Health barriers to buffalo productivity and their management. Proc. Fourth Asian Buffalo Congress, 25-28 Febr., New Delhi, India: Lead Papers: 142-147.

Zarlenga, D.S., Gasbarre, L.C., Boyd, P., Leighton, E. and Lichtenfels, J.R. 1998. Identification and semi-quantification of O. ostertagi eggs by enzymatic amplification of ITS1 sequences. Vet. Parasitol. 77: 245-257.

Chapter XIV

BUFFALO INTERNATIONAL ORGANIZATIONS

Antonio Borghese

Istituto Sperimentale per la Zootecnia
(Animal Production Research Institute),
Via Salaria 31, 00016 Monterotondo (Rome), Italy

THE FAO INTER-REGIONAL RESEARCH NETWORK ON BUFFALO FOR EUROPE AND THE NEAR EAST (BUFFALO NETWORK)

The Italian Animal Production Research Institute in Monterotondo (Rome) is the coordination centre for the FAO Buffalo Research Network. This Network is part of the FAO European System of Cooperative Research Networks for Agriculture which includes 13 networks, six of which include other regions in addition to Europe. In 1992 FAO decided to assign some funds in order to establish a Buffalo Research Network for countries where buffalo research occupied a secondary role compared to research on cattle.

OBJECTIVES OF THE NETWORK

The main objective of the Network is to develop a system of cooperation among research institutions from buffalo-producing countries of Europe and the Near East with a view to providing scientific and professional support to the buffalo production sector in general, and to small subsistence farmers in particular. The Network collects and analyses data on production systems, buffalo reproduction and marketing of buffalo products, and disseminates this information through meetings, workshops and the "Buffalo Newsletter". Short to medium-term objectives include the collection of data on animal genetic diversity, reproduction and the establishment of performance recording systems.

PRESENT STRUCTURE OF THE NETWORK

A. Structure

The Network consists of four working groups: with coordinators and vice-coordinators.
1. Reproduction and Biotechnology - A.H. Barkawi (Egypt) - G.M. Terzano (Italy)
2. Farming Systems - Y. Rouzbehan (Iran) - O. Sekerden (Turkey)
3. Products - D. Matassino (Italy) - A. Georgoudis (Greece)
4. Genetic resources - B. Moioli (Italy) - T. Peeva (Bulgaria)

The general coordinator is A. Borghese (Italy).

B. Objectives of the working groups

1. Reproduction and Biotechnology Working Group
The Group acts as a forum for the exchange of research results and as a coordinating body for research on buffalo reproduction and biotechnology. The Group has identified major issues affecting buffalo reproduction and the efficiency of buffalo production and adopted a programme of cooperative research in the following fields:
- puberty and ovarian activity maintenance;
- post partum anoestrus and interpartum period reduction;
- ovulation detection and improvements in artificial insemination efficiency;
- seasonality and oestrus induction;

- follicular dynamics;
- superovulation and embryo transfer;
- *in vitro* maturation and fertilization.

The main objective of the Group is to contribute to the efficiency of buffalo production through the attainment of better reproduction rates, early maturity and development and improvement of insemination efficiency.

The Group organized an International Symposium on Buffalo Reproduction (Sofia, Bulgaria, 6-8 October 1995) the proceedings of which were published in the Bulgarian Journal of Agricultural Sciences (1996), and a Satellite Symposium during the 54th Annual Meeting of the European Association for Animal Production (EAAP) "Recent Progress in Buffalo Reproduction" (Rome, Italy, 30 August 2003), the report of which was published in the Proceedings of the II° Congresso Nazionale sull'Allevamento del Bufalo (Monterotondo, Roma, 28-30 Agosto 2003).

2. Farming Systems Working Group
The objective of the Group is to improve the efficiency of buffalo production through enhanced research in the area of production systems, in particular for smallholders. The following two areas were considered as priorities for research and extension: (i) to ascertain and calculate buffalo nutritional requirements for growth, pregnancy and milk production; (ii) to improve the quality of crop residues as a base for buffalo nutrition in extensive production systems. The Group was responsible for organizing a Symposium on Buffalo Resources (Cairo, Egypt, October 1996) the proceedings of which were published by the Animal Production Research Institute of Cairo.

3. Buffalo products Working Group
The major objective of the Group is to provide a scientific base and guidelines for the production, control and protection of the quality of typical buffalo products in order to enhance consumption and thus contribute to the efficiency of the sector and to improving the standards of buffalo producers.

The Working Group coordinates an interchange of experience and information regarding ongoing research on buffalo products. This research covers consumption and marketing of buffalo products. The results of pertinent comparative studies on buffalo products: i.e. milk, meat and skin, were presented at the International Symposium on Buffalo Products held at Paestum, Italy (1 - 4 December, 1994) and published in the EAAP publication.

This Group will work towards regulating the different products from the developing countries that are very important for the economy of the Mediterranean area.

4. Genetic Resources Working Group
The Group has various objectives:
- The introduction of milk performance recording of buffalo in developing countries through the preparation of simplified guidelines in accordance with the standards of the International Committee for Animal Recording (ICAR), which promoted a Meeting in Slovenia (Bled, 16-17 May 2000).
- Comparative studies concerning genetic diversity of buffalo breeds. A research project aiming to evaluate the genetic diversity of three populations of buffalo has been carried out. This is the first research project which has been initiated as a result of the international links created by the Network. Participating countries are: Italy, Greece and Egypt. The Animal Genetics laboratory of the Animal Production Research Institute, Rome, provided the facilities, the researchers and the materials for the research, including DNA samples of the Italian buffalo population. The Greek and Egyptian partners have also provided DNA samples of the buffalo populations of their respective countries. This study revealed a genetic differentiation between the Italian and Greek buffalo of 0.031 ± 0.015; the differentiation between the Egyptian buffalo and each of the other two is 0.070 ± 0.020.

- The extension of the buffalo progeny testing trial to other countries since, at present, the Progeny testing trial is only beginning in Turkey.

ACTIVITIES OF THE NETWORK (1997-2004)

A. Joint programmes with other organizations

1. With the International Buffalo Federation (IBF)

The Coordination Centre of the Buffalo Network (the Animal Production Research Institute, Rome) actively participated in the organization of the Fifth World Buffalo Congress (Caserta, Italy, 13-16 October 1997) together with the International Buffalo Federation, through: (i) revision of the papers to be presented at the Congress; (ii) editing of the Proceedings; (iii) convening of a round table during the Congress regarding the status of buffalo research and future priorities in Europe and the Near East.

At the Sixth World Buffalo Congress (Maracaibo, Venezuela, 20-23 May 2001) the International Buffalo Federation nominated Professor Antonio Borghese as General Secretary: and this nomination is very important for the continuity of the relationship between the FAO Network and the IBF.

During the Seventh World Buffalo Congress (Manila, Philippines, 20-23 October, 2004) the IBF Constitution and By-laws, registered in Italy, were approved by the General Assembly of the meeting and Professor Luigi Zicarelli was elected as the new President of the IBF for the period 2004 to 2007. The Buffalo Newsletter reports on the activities of the IBF.

2. With animal nutrition scientists from Italian universities

The Coordination Centre of the Buffalo Network (the Animal Production Research Institute, Rome), with the participation of Italian experts on animal feeding and nutrition, convened a meeting in Turkey with buffalo farmers from several countries in order to discuss feeding strategies in intensive and extensive production systems.

3. With INTERBULL (the International Committee for the Standardization of Genetic Evaluation of Bulls)

The major accomplishment of the INTERBULL meeting (1997) was the decision to involve a few countries in a pilot multi-country project on bull evaluation, which began in 2002 as a Progeny testing trial in Turkey.

4. With the International Committee for Animal Recording (ICAR)

The joint goal of this cooperation activity is to promote buffalo recording in developing countries through the preparation of simplified standardized guidelines.
The major achievement of the cooperation with the International Committee for Animal Recording (ICAR) was the organization of the Joint FAO/ICAR Workshop on Animal Recording for Improved Buffalo Management Strategies in 2000, which was attended by 30 participants from 17 countries. As a result of this Workshop, simplified guidelines for milk recording in buffaloes in developing countries were prepared which are in agreement with the ICAR standards. These guidelines were drafted in order to promote animal recording at the country level in addition to encouraging the exchange of information on buffalo productivity in the world.
The Coordination Centre has conducted a survey on the extent of milk recording of buffalo and has published the results of this survey which includes data on production and reproductive parameters from 15 countries.

B. Other outcomes

The scientifical/technical journal of the Buffalo Network (the Buffalo Newsletter) is published regularly twice a year and has a circulation of 1 200 copies. This is an important and unique means of communication among developing and developed countries for the exchange of knowledge and research results.

A major achievement of the round table on the status/future of buffalo research was that it assembled not only participants from the traditional countries (Egypt, Bulgaria, Romania, Iraq, Syria, Greece, Italy, Turkey) but also from Azerbaijan and Iran. The round table therefore proved to be a first step in cooperation with these two more isolated countries.

Conclusions

1. The Buffalo Network is unique in the world.
2. The Buffalo Newsletter is a valuable instrument for communication and for the transfer of technical and scientific news.
3. The Network is the recognized centre for international research projects.

INTERNATIONAL BUFFALO FEDERATION

History

The International Buffalo Federation (IBF) was created during the First World Buffalo Congress, that took place from 27 to 31 December 1985, in Cairo, Egypt.

The initiators were the eminent scientists Professor Dr. M. R. Shalash, President of the Egyptian Veterinarian Buffalo Association and the American scientist Professor W. Cripe from the University of Florida, Gainsville.

Participants at this Congress also approved the organizational structure of the International Buffalo Federation and elected its managing body - the IBF Standing Committee.

The distinguished buffalo expert Dr. W. Ross Cockrill (England) was elected as Honorary President.

Professor Dr. M.R. Shalash was elected as President with three Vice-presidents and fourteen members of the Standing Committee, including scientists and experts from Australia, Brazil, Bulgaria, India, Italy, China, Pakistan, the USA, Singapore, Thailand, Trinidad and the Philippines.

The activities of the IBF to date have been undertaken in accordance with the Statutes and Rules, developed and approved by the Standing Committee.

The Second World Buffalo Congress was held from 12 to 16 December 1988, in New Delhi, India, under the Presidency of Dr. R.M. Acharya and with Professor V.D. Mudgal as Secretary-General.

The Third World Buffalo Congress was held from 13 to 17 May 1991, in Varna, Bulgaria, under the Presidency of Professor Dr. Tzeno Hinkovski and with Professor Dr. Aleko Alexiev as Secretary-General: ten volumes of Proceedings were published in addition to a special report on the FAO Workshop on the Biotechnology of Reproduction, which was the first link between FAO and the IBF, and which would prove to be the foundation of the FAO Inter-Regional Cooperative Research Network on Buffalo.

The Fourth World Buffalo Congress was held from 27 to 30 June 1994, in Sao Paolo, Brazil, under the Presidency of Professor Manoel Osorio Luzardo de Almeida and with Joao Ghasper de Almeida as Secretary-General. Three volumes of Proceedings were published.

During this Congress, the Italian scientist Professor Giovanni de Franciscis was elected President of the IBF. President de Franciscis went on to organize the Eighth Standing Committee Meeting of the IBF in Rome on 2 April 1996, where the following issues were on the Agenda: 1. A commemoration for Professor Shalash; 2. The organizational arrangements and the scientific programme for the Fifth World Buffalo Congress to be held in the Royal Palace in Caserta, Italy, were established; 3. A decision was taken regarding the preparation of an official letter to request the Breeders Associations to contribute US$100 each towards the cost of the organization of the Congress; 4. The transfer of the Secretariat to the Istituto Sperimentale per la Zootecnia (Animal Production Research Institute) was effected; 5. Professor Sayed Gharieb Hassan from Egypt and Dr. Hugh Popenoe from the USA were nominated to the Standing Committee.

The Fifth World Buffalo Congress was held from 13 to 18 October 1997, in Caserta, Italy; under the Presidency of Professor Giovanni de Franciscis and with Professor Antonio Borghese acting as Scientific Secretary. For the first time each paper to be communicated to the Congress was submitted for revision to two referee scientists from the specific field of competence. 189 papers

were published in the Proceedings, a book of 990 pages, that was distributed prior to the Congress and represented the State of the Art in buffalo sciences for many years.

During this Congress, the renowned Venezuelan buffalo breeder Mr. Pablo Moser Guera was elected the new President of the IBF.

It was decided that the Sixth World Buffalo Congress would take place in Venezuela in the year 2000 and the Seventh World Buffalo Congress in the Philippines, changing Continent each time (every three years) and appointing a new president to organize each Congress.

The Sixth World Buffalo Congress was held in Maracaibo (Venezuela) from 21 to 23 May 2001 and resulted in the first electronic version of the Proceedings on CD. During the business meeting of the IBF, Professor Borghese reported on the previous Congress, that had realized a profit of US$4 300, which had subsequently been transferred to the next Congress, and suggested establishing closer relations with the FAO Buffalo Network. The Standing Committee agreed to establish the Secretariat in Rome at the Istituto Sperimentale per la Zootecnia and Professor Antonio Borghese was appointed as General Secretary to be assisted by two Executive Officers: Aleko Alexiev and Hugh Popenoe. The next meeting was to be organized in the Philippines with Libertado Cruz as President assisted by two Vice-Presidents: S.K. Ranjhan and Jesus Reggeti. Libertado Cruz proposed that the changes to the Constitution could be presented to the General Assembly and put to a vote at the next Congress. The sub-committee, consisting of Libertado Cruz, Joao Gaspar, Antonio Borghese and Hugh Popenoe, would proceed with deliberations regarding the revised Constitution. In particular: 1. The Constitution needed to be more precise; 2. The membership could consist of two members from each country; country representatives could not miss more than two congresses or they would be dropped; the official language of the Congress would be English and that of the host country; the profit from any one Congress should go to the Secretariat to cover expenses and for the costs of future congresses. The Standing Committee approved the deliberations of the sub-committee.

There were several changes in the membership of the IBF Standing Committee members during the period 1984 to 2001.

The managing body of the IBF had the following membership at the Sixth World Buffalo Congress, in the period 1997 to 2001:
Honorary President: Dr. W. Ross Cockrill (England)
President: Mr Pablo Moser Guera (Venezuela)
Vice-President for Asia: Prof. Libertado C. Cruz (the Philippines)
For Europe : Prof. Dr. Tzeno Hinkovski (Bulgaria)
For America: Dr. Joao Gaspar de Almeida (Brazil)
Standing Committee members:
Eng. Marco Zava (Argentina)
Dr. Manoel Osorio de Almeida (Brazil)
Prof. Aleko Alexiev (Bulgaria)
Prof. K. H. Lu (China)
Prof. Wangzhen Quan (China)
Dr. L. Ricardo Bolero Jaramillo (Colombia)
Dr. L. Garcia Lopas (Cuba)
Prof. S. G. Hassan (Egypt)
Prof. Kamal Fouad (Egypt)
Prof. P. N. Bhat (India)
Prof. V. D. Mudgal (India)
Prof. Giovanni de Franciscis (Italy)
Mrs. Ingrid Caproni (Italy)
Prof. Antonio Borghese (Italy)
Dr. Abdul Rahman Khan (Pakistan)

Prof. Oswin Perera (Sri Lanka)
Dr.C. Devendra (Singapore)
Prof. Maneevan Kamonpatana (Thailand)
Prof. Charan Chantalakhana (Thailand)
Dr. Stephen P. Bennet (Trinidad)
Prof. Hugh Popenoe (USA)
Dr. Thomas J. Olson (USA)
Mr. Jesus Reggeti (Venezuela)

In 1992 the Asian Buffalo Association (ABA) was established under the Presidency of Dr. P.N. Bhat (India).

An IBF Council Meeting took place at the Congress Palace in Rome on 30 August 2003, organized by the General Secretary, Professor Antonio Borghese. The President Libertado Cruz, M. Zava from Argentina, W. Vale and J.G. de Almeida from Brazil, L. Zicarelli and R. Garofalo from Italy, H. Popenoe from USA, M. Larbier from FAO, A. Barkawi from Egypt, O. Sekerden from Turkey, Ruzbehean from Iran attended the meeting. The President Libertado Cruz thanked the organizer and recalled the Scientist Aleko Alexiev who had passed away. He then distributed the programme of the next Congress, to be held in Manila, from 20 to 23 October 2004. Gaspar de Almeida underlined the difficulties for Cuba to organize the next Congress following that to be held in Manila. Professor Borghese stressed the necessity of receiving funds from the Breeders Associations in different countries in order to run the Secretariat and to contribute to the Congress organization and he proposed the creation of a continuous link with the FAO Buffalo Network in order to locate researchers, exchange information, organize the congresses, and publish the Buffalo Newsletter with FAO and IBF sponsors and logos, distributing 1 200 copies free of charge worldwide and to promote TCP (Technical Cooperation Projects) in developing countries. In order to arrange for the legal Registration of the IBF in Rome and to create a website for the IBF, a contribution of US$100 was suggested from each country. A request was made for the financial report of the previous Congress from Mr Pablo Moser. Professor Zicarelli was elected Executive Officer and Representative for Europe in place of Dr Alexiev.

The President thanked the participants and asked for assistance in the organization of the Congress in Manila.

The organization of the IBF during the period 2001 to 2004 was the following:

International Buffalo Federation
Libertado Cruz, President (Philippines)
Antonio Borghese, General Secretary (Italy)
Hugh Popenoe, Executive Officer (USA)
Luigi Zicarelli, Executive Officer (Italy)

Honorary Committee
Pablo Moser G. (Venezuela)
Steve Bennet (USA)
Giovanni de Franciscis (Italy)

Vice Presidents
Jesus Reggeti, America (Venezuela)
S. Ranjhan, Asia (India)
Luigi Zicarelli, Europe (Italy)
Barry Lemcke, (Australia)
S.G. Hassan, Africa (Egypt)

Standing Committee

Brazil: Joao Gaspar de Almeida, William Vale.
Argentina: Marco Zava, Armando Rozenblum.
Colombia: Ricardo Botero, Berdugo J. A. Gutierrez, Alfonso Bernal.
Venezuela: Hector Scannone.
Italy: Raffaele Garofalo.
Trinidad: Leela Rastogi, Floyd Necles.
Cuba: Alina Mitat.
USA: Tom Olson.
Bulgaria: T. Hinkovski, T. Peeva.
India: Siran Uddin Qureshi.
Thailand: C. Chantalakana, M. Kamonpatana.
Sri Lanka: Oswin Perera, Abeygunawardena.
Vietnam: Julio Ly, Zao.
China: Yang Bing Zhuang, Xu Dianxin.
Philippines: Patricio Faylon.
Pakistan: R. Usmani.
England: Robert Palmer.
Egypt: A.H. Barkawi.
Germany: Henzi Heneton.
Australia: Barry Lemcke.
Turkey: O. Sekerden.
Iran: Y. Ruzbehean

The Seventh World Buffalo Congress took place in Manila, the Philippines, from 20 to 23 October 2004 and produced three volumes of Proceedings: Vol.I Invited Papers, Vol. II Contributed Papers, Vol. III Recent Developments in Animal Production, and one volume of Proceedings' Abstracts, plus one volume of "Abstracts of Researches on the Philippine Water Buffalo".

During the Congress the IBF Assembly Meeting took place at 19:00 hours on 21 October 2004 at the Makati Shangri-la Hotel in Manila.

The President of the IBF, Dr. Libertado C. Cruz opened the meeting, and thanked the delegates from sixteen countries.

The President recalled Professor Aleko Alexiev, an eminent scientist in the buffalo field, who had been Director of the Buffalo Research Institute in Shumen, Bulgaria, and President of the Bulgarian Buffalo Breeders Association. He had been involved with the IBF since its establishment in 1985 and had been elected Vice-President in 2001. He had passed away in 2002. The President also remembered Professor Giovanni de Franciscis, Professor at Naples University (Italy), Faculty of Veterinary Medicine, who had founded the School of Buffalo Sciences in Italy and had been the first President of the Italian Buffalo Breeders Association; he had also been involved with the IBF since its establishment and had been elected President in Sao Paolo, Brazil, (1994) and organized the Fifth World Buffalo Congress in Caserta, Italy, from 13-16 October 1997. He had passed away a few months earlier.

Following this all the delegates from the sixteen countries introduced themselves: A. Borghese, General Secretary, L. Zicarelli, A. Coletta, F. Infascelli, G.M. Terzano, V. L. Barile, from Italy; S. Ranhjan and O.P. Dhanda from India; H. Popenoe and T. Olson, from the USA; M. Zava, from Argentina, I. Soliman from Egypt; B. Lemcke, from Australia; T. Peeva and M. Alexieva, from Bulgaria; G. de Almeida, W. Vale, M. Almeida from Brazil; M. Eslami, from Iran; N. Ahmad, from Pakistan; L.C. Cruz, the President and A. del Barrio, from the Philippines; T. Seresinhe, from Sri Lanka; M. Wanapat, from Thailand; O. Sekerden, from Turkey; J. Reggeti, from Venezuela and Mai Van Sanh, from Vietnam.

Point 1. IBF Constitution.
The General Secretary of the IBF, Professor Antonio Borghese, submitted, for confirmation by the Assembly, the Legal Act of the IBF Constitution, registered in Monterotondo, Rome, on 11 October 2004, by the legal notary Dr. Francesco Di Pietro together with Professor A. Borghese, avv Raffaele Garofalo, Professor Luigi Zicarelli, Dr. Giuseppina Maria Terzano and Dr. Vittoria Lucia Barile; the legal address is the same as that of the General Secretariat: Istituto Sperimentale per la Zootecnia, Via Salaria 31, 00016 Monterotondo, Rome, Italy. The Constitution and By-laws are the same as those approved in Caserta on 16 October 1997, with the formal modifications proposed by President L. Cruz, and published in the Buffalo Newsletter, Number 20, dated September 2004, printed in 1 200 copies and distributed worldwide. The organization contained in the Legal Act is the same as that approved in Maracaibo, Venezuela from 21 to 23 May 2001, and published in the same issue of the Buffalo Newsletter. The IBF subscription is set at a minimum of US$100 for the years 2004 to 2007, and the IBF founder members will be the signers of the Application Form, which involves adherence to the IBF Constitution and By-laws, and payment of the minimum subscription.

The General Secretary emphasized the three priority goals for the efficient functioning of the IBF:
A. The legal constitution of the IBF, that was officially founded in 1985 but had not been bound by any legal documents in the preceding years;
B. The official publication of the IBF Constitution, By-laws, Organization, Representatives, Meetings and Activities in the Buffalo Newsletter, that originally was the bulletin of the FAO Inter-Regional Cooperative Research Network on buffalo and has now become also the bulletin of the IBF;
C. An economic foundation for the IBF activities, that clearly will be supported in part by subscriptions, and will allow participation in the IBF.

The Assembly approved points A and B, but there was some discussion regarding point C, concerning the subscription. Dr. Ranjhan proposed that the subscription be only made obligatory for Associations, and that there be free subscription for memberships. Many participants (Reggeti, Soliman, De Almeida, Zava, Popenoe, Cruz) commented on this point, and some proposed a referendum. The General Secretary replied that a referendum would be impossible since up until the present time it is difficult to know who really are the IBF members. Every year membership changes. At each congress some countries, who in the past were really interested in the IBF, have no representatives, or representatives change because there is no formal act of adhesion to the IBF. For the functionality of the IBF, there was a need for a Secretariat office, an economic foundation, but specifically a real act of adhesion to the IBF by members. Many participants agreed with the General Secretary's position.

Point 2. Appreciations.
Many participants expressed their appreciation for the reformatted IBF Constitution, as proposed by the President Libertado C. Cruz, and published in the Buffalo Newsletter, and congratulated the President on the excellent Congress organized in the Philippines, which had been an important success for the scientific community and for the buffalo breeders in the world.

Point 3. Next congress and President.
With regard to the next World Buffalo Congress in 2007, many people expressed their views (Cruz, Ranjhan, Vale and Zava) and the past willingness of Cuba and China to organize the Congress was reported. However, this proposed readiness was not confirmed by the presence of the respective representatives at the IBF Assembly, even if clearly invited. Professor Borghese proposed to change the Continent, as traditionally undertaken in the past: in 2001 the Congress had taken place in America, this year in Asia, the next would be in Europe and therefore he proposed Professor Zicarelli as President. Professor Sekerden also proposed Turkey as a host for the next Congress.

All the delegates voted for Italy, appreciating the past experiences in organizing congresses and the link with FAO (Peeva, Dhanda, De Almeida, Vale, Zava, Reggeti) and the economic possibilities. Professor Zicarelli thanked the delegates for the honour and declared his satisfaction to take up the legacy of his teacher Giovanni de Franciscis, requesting the assistance of the Italian Breeders Association, of the Agricultural Ministry, of Professor Borghese's Institute and of the other Italian organizations.

Point 4. Actual IBF organization.
The actual organization of the IBF for the period 2004 to 2007 was voted as follows:
President: Luigi Zicarelli (Italy) zicarell@unina.it
General Secretary: Antonio Borghese (Italy) antonio.borghese@isz.it
Executive Officer: Libertado C. Cruz (Philippines) pcc-oed@mozcom.com
Exexutive Officer: S. Ranjhan (India) sk_ranjhan@hotmail.com
Executive Officer: Hugh Popenoe (USA) hlp@ufl.edu
Vice-Presidents:
America: Marco Zava (Argentina) bufalosmz@fibertel.com.ar
Asia: S. Ranjhan (India) sk_ranjhan@yahoo.com
Africa: Ibrahim Soliman (Egypt) ibsoliman@hotmail.com
Australia: Barry Lemcke barry.lemcke@nt.gov.au
Europe: Tzonka Peeva (Bulgaria) tzonkapeeva@abv.bg

Standing Committee:

Argentina:	Armando Rozenblum cientigicacuatro18@cofasa.com.ar
	John Nelson jnelson@fibertel.com.ar
Australia:	Barry Lemcke barry.lemcke@nt.gov.au
Brazil:	Joao Ghaspar de Almeida ghaspar@terra.com.br
	William G. Vale, wmvale@hotmail.com
	Manoel Almeida, manoelluzardo@terra.com.br
Bulgaria:	Maria Alexieva, abb@techno-link.com
China:	Yang Bingzhuang, gxbri@public.nn.gx.cn
	Xianwei Liang, liangbri@126.com
Colombia:	Alfionso Bernal, asobufalos@cis.net.co
	J.A. Berdugo, jberdugo@quimbaya.udea.edu.co
	Ricardo Botero, rbotero@telesat.com
Cuba:	Alina Mitat
Egypt:	A.H. Barkawi, esap@tedata.net.eg
Germany:	Henzi Heneton
India:	O.P. Dhanda (India) op_dhanda@hotmail.com
	Siran Uddin Qureshi
Iran:	Moossa Eslami, MEslami93@hotmail.com
	Yousef Rouzbehan, faranakuk@yahoo.com
Italy:	Raffaele Garofalo, anasbce@libero.it
	Angelo Coletta, colettangelo@virgilio.it
	Federico Infascelli, infascel@unina.it
	Giuseppina M. Terzano, marinella.terzano@isz.it
	Vittoria L. Barile, vittoria.barile@isz.it
Pakistan:	Nasim Ahmad, d.nasim@yahoo.com
	R. Usmani
Philippines:	Arnel del Barrio, arneldb-25@yahoo.com
	Patricio Faylon
Sri Lanka:	Thakshala Seresinhe, headas@slnet.lk
	Oswin Perera, oswinperera@yahoo.co.ok
	H. Abeygunawardena, indra@vetpre.pdn.ac.lk
Thailand:	Maneewan Kamonpatana, Maneewan.K@Chula.ac.th
	C.Chantalakhana, swkcrc@ku.ac.th
	Metha Wanapat, metha@kku.ac.th

Trinidad:	Leela Rastogi, leela3053@hotmail.com
	Floyd Necles sfc@carib-link.net
Turkey:	Ozel Sekerden, sekerden@mku.edu.tr
UK:	J. Palmer, buffalouk@aol.com
USA:	Thomas Olson, tcwb@valornet.com
Venezuela:	Jesus Reggeti, jarego@cantv.net
	Hector Scannone
Vietnam:	Mai Van Sanh, mvsanh@netnam.vn

Hand over of the IBF Flag from the Past President Libertado C. Cruz to the new elected President Luigi Zicarelli, Manila, the Philippines, 21 October 2004.

Participants of the IBF Assembly Meeting, Manila, the Philippines, 21 October 2004.

INTERNATIONAL BUFFALO FEDERATION
CONSTITUTION AND BY-LAWS

Section I. Name, Address and Nature

1. The International Buffalo Federation (IBF) was founded upon the unanimous recommendation of the General Assembly at the First World Buffalo Congress in Cairo, Egypt in 1985.

2. The Federation is an independent, non-political, non-religious and non-profit international organization.

3. The permanent headquarters of the International Buffalo Federation is Rome.

4. The seat of the Federation is the country which will be hosting the World Buffalo Congress.

Section II. Objectives and Activities

1. The Federation objectives are to promote the advancement of research and development regarding buffaloes worldwide.

2. The Federation organizes world congresses and roundtables; promotes the exchange and dissemination of scientific and technological knowledge; facilitates the spread of information on buffalo production and development; promotes internationally planned research; enhances contacts among scientists and extension personnel concerned with buffalo production; assists in strengthening the linkages between national, regional and international research; establishes and maintains relations with other organizations whose interests are related to the objectives of the Federation.

Section III. The Official Language

1. The official language at the World Congresses of the Federation is English.
2. The working language of the Secretariat is English.

Section IV. World Buffalo Congress

1. The World Congress shall be held every three years. Regional and National Congresses will not be held in conflict with the World Congress.

Section V. Organization and Institution and Election of Officers

1. The International Buffalo Federation is organized on a regional basis. For the purposes of the Federation, each continent shall be a region.

2. The Institutions of the International Buffalo Federation are the General Assembly, the Secretariat and the Executive Council.

3. The Executive Council of the International Buffalo Federation is composed of the elected Chairmen of the Regional Associations, a Secretary appointed by the President, and the President elected in the General Assembly by the representatives of the National Associations.

4. The Executive Council will be vested of its powers after having been elected (at the

end of the World Congress) and its tenure will be for three (3) years.

5. The Executive Council will meet as often as necessary. An absolute majority is the required quorum for the meetings. Resolutions will be approved by a two-thirds vote. The President does not vote except when the vote is a tie. His presence however counts for the quorum.

6. Members of the Regional Associations will elect their own Executive Councils. The Chairman of the Regional Association is by right a member of the International Executive Council.

7. The President of the International Buffalo Federation is elected at the World Congress.

8. Each National Association affiliated with the IBF nominates a representative to the World Congress for the purpose of electing the President.

9. National Associations enjoy equal voting rights, being allowed one vote each.

10. Nominations for the candidacy to the Presidency of the IBF is submitted to the Executive Council six months prior to the World Congress. The Executive Council is responsible for circulating information on the upcoming election and about nominees to all IBF members.

11. The committee organizing the World Congress allocates sufficient time in the official programme to hold the election of the President. The election shall be public. Only the official representatives of the National Association duly appointed by them, are considered electors of the President.

12. The election is to be held by secret ballot. The candidate who obtains the absolute majority, is proclaimed President by the outgoing Executive Council of the International Buffalo Federation.

12.1 If no winner can be proclaimed on the first turn a run-off election is held between the two highest vote getters.

12.2 The President serves for a maximum of two terms.

Section VI. Duties of the President

1. It is the duty of the President to represent the Federation at international meetings, and with International Organizations.

2. To convene the Executive Council as often as necessary or when at least two Regional Chairmen ask for it to be convened.

3 To promote the initiatives which will further the knowledge regarding buffaloes and pursue the objectives of the Federation.

4. To convene the Assembly to discuss the Federation's administration, general programme, policies and priorities.

Section VII. Membership

1. National, institutional and individual membership are recognized and encouraged by the International Buffalo Federation. However representation in the Executive

Council is accorded to Regional Officers who will be appointed according to the procedures set out in Article 6, Section V.

2. Membership of the International Buffalo Federation is renewable by submitting the application form and dues. Deadlines and fees shall be established by the Executive Council. Non payment of the fees, actually US$100/year, implies forfeiture of membership status.

3. Membership in the International Buffalo Federation falls into three categories: Collective, Associate and Individual membership.

3.1 National or Regional associations are Collective members.

3.2 Departments and Research Institutes are Associate members.

Section VIII. General Assembly Meeting

1. The General Assembly meets at the World Congresses every three (3) years.

Section IX. Amendments of the Constitution

1. In order to amend the By-laws of the International Buffalo Federation a written notice of the amendments must be circulated to all members in advance of the meeting at which they are to be considered.

2. If a meeting of the General Assembly cannot at that time be called, the membership is allowed to express its vote through a written ballot.

3. The amendment is approved if the majority of the members voting are in favour of it.

Section X. Transitory and Final Provisions

I. This Constitution and By-Laws adopted by the General Assembly of the Federation, in Caserta on 16 October 1997, promulgated by the President of the International Buffalo Federation, will render the former constitution null and void.

II. Elections of the Executive of the Regional Associations shall be called within one year of the implementation of this Constitution and By-Laws.

III. The first IBF Executive Council, under this Constitution and By-Laws, will be composed of the Presidents of the member National Associations until all the regional associations and their executives have been set up. The rules pertaining to the quorum and voting regulations shall be the same as those prescribed in Article 5 Section V of the Constitution and By-Laws

Finalized in Caserta, Italy on 16 October 1997.

LIST OF ACRONYMS

AACB	Asociacion Argentina de Criadores de Bufalos
ABA	Asian Buffalo Association
ACTH	adrenocorticotropic hormone
ADF	acid detergent fibre
ADL	acid detergent lignin
AETE	Association European Embryo Transfer
AGID	agarose immunodiffusion test
AHV-1	alcelaphine herpes virus-1
AHV-2	alcelaphine herpes virus-2
AI	artificial insemination
AIA	Italian Breeders' Association
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANASB	Italian Buffalo Breeders' Association
AOAC	Association of Official Analytical Chemists
APA	Associazione Provinciale Allevatori (Provincial Breeders' Association)
APRI	Animal Production Research Institute
ASPA	Associazione Scientifica Produzione Animale (Scientific Association of Animal Production)
AST	asparagine aminotransferase
ATP	adenosin triphosphatase
BCS	body condition score
BEF	bovine ephemeral fever
BES	buffalo oestrus serum
BLUP	best linear unbiased prediction
BO	Brackett & Oliphant medium
BOHB	beta-hydroxybutyrate
BRL	buffalo rat liver cells
BRV	bovine rotavirus
BUFF	buffalo follicular fluid
BVD	bovine viral diarrhoea
BW	body weight
CATT	card-type Testryp CATT agglutination test
CBC	cells blood count
CBG	corticosteroid binding globulin
CBPP	contagious bovine pleuropneumonia
CEIA	competitive enzyme immunoassay
CF	crude fibre
CFT	complement fixation test
CIDR	controlled internal drug releasing device
CIRB	Central Institute for Research on Buffaloes (India)
CIEP	counterimmunoelectrophoresis
CISE	Cattle Information System/Egypt
CL	corpus luteum
CNF	cytotoxic and necrotic factors
CNS	central nervous system
COCs	cumulus oocyte complexes
Co-EDTA	cobalt-ethylendiaminetetraacetic acid
COFA	Cooperativa Fecondazione Artificiale, Cremona, Italy
CP	crude protein
CPE	cytopathologic effects
Cr	Cr_2O_3, solid marker
CR	conception rate
CRESTAR	progestagen ear implant

CRF	corticotropin releasing factor
CRL	crown-rump length
CV	variability coefficient
DCP	digestive crude protein
DF	dominant follicles
DIPA	dairy herd improvement programme actions
DM	dry matter
DNA	deoxyribonucleic acid
D.O.P.	Denomination of Protected Origin
DWG	daily weight gain
E2	estradiol
EAAP	European Association for Animal Production
EBW	empty body weight
eCG	equine corionic gonadotrophin
ECM	equivalent correct milk
EDTA	etilendiamminicotetracetic acid
EE	ether extract
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
ESCORENA	European System of Cooperative Research Networks in Agriculture
ET	embryo transfer
ETEC	enterotoxigenic escherichia coli
EU	European Union
F1	first generation cross
F2	interbred of F1
FAO	Food and Agriculture Organization of the United Nations
FAT	fluorescent antibody test
FCM	fat corrected milk
FCS	fetal calf serum
FFA	free fatty acids
FGF	fibroblast growth factor
FGS	first grazing season
FL	femtolitre (10-15)
FMC	fat corrected milk
FMD	Foot and Mouth Disease
FSH	follicle stimulating hormone
FU	feed units
GGT	γ-glutamyltransferase
GI	gastrointestinal
g/l	grams/litre
GLDH	glutamate dehydrogenase
GnRH	gonadotrophin releasing hormone
GR	glutathione-reductase
GSH	glutathione
GSH-Px	glutathione peroxidase
GSH-S-t	GSH-S-transferase
HAU	Haryana Agricultural University
Hb	hemoglobin
hCG	human corionic gonadotrophin
HCT	hematocrit
HDL	high density lipoprotein
HGF	haematopoietic growth factor
Hly	hemolysin
HMG	human menopausal Gonadotrophin
HS	haemorrhagic septicaemia
HSCAS	hydrated sodium calcium alumosilicate

IBF	International Buffalo Federation
IBR/IPV	infectious bovine rhinotracheitis/infectious pustular vulvovaginitis
ICAR	International Committee for Animal Recording
ICAR	Indian Council of Agriculture Research
IETS	International Embryo Transfer Society
IFAT	indirect immunofluorescence test
IGF	insulin-like growth factor
IGFRI	Indian Grassland and Fodder Research Institute (India)
IGP	Indication of Protected Geographic Origin
INRA	Institut National de la Recherche Agronomique - France
INTERBULL	International Committee for Standardization of Genetic Evaluation of Bulls
ISZ	Istituto Sperimentale per la Zootecnia (Animal Production Research Institute)
ITS	internal transcribed spacer
IU	International Units
IV	intravenous
IVC	in vitro culture
IVEP	in vitro embryo production
IVF	in vitro fertilization
IVM	in vitro maturation
Keller	tank for urine and dung
KSOM	potassium simplex optimized medium
L3	third stage larvae
LDH	lactate dehydrogenase
LH	luteinizing hormone
l/l	litre/litre
LT	thermolabile toxins
MII	metaphase II
mAbs	monoclonal antibodies
MALR	Ministry of Agriculture and Land Reclamation (Egypt)
MAT	microagglutination test
MCF	malignant catarrhal fever
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
mcmol/l	micromoles/litre
mcU/l	microunit/litre
MCV	mean corpuscular volume
MD	mucosal disease
MDS	myelodysplastic syndrome
ME/MJ	milliequivalent/megajoule
MFU	milk feed unit
MHCT	microhaematocrit centrifugation technique
MHz	megahertz
ML	modified live
mmol/l	millimoles/litre
MOET	multiple ovulation and embryo transfer
MPUAT	Maharana Pratap University of Agriculture and Technology (India)
MPV	mean platelet volume
MRT	milk ring test
NARC	Nepal Agricultural Research Council
NDDB	National Dairy Development Board
NDF	neutral detergent fibre
NE	net energy
NE_L	net energy milk
NEFA	not esterified fatty acids
NEFDCCO	Nueva Ecija Federation of Dairy Carabao Cooperatives
ng	nanograms (10-9)

ng/lt	nanograms/litre
NH3	ammonia
NK	natural killer
NORs	nucleolus organizer regions
NPN	non proteic nitrogen
NSC	non-structural carbohydrates
NWFP	North West Frontier Provinces (Pakistan)
OHV-2	ovine herpes virus-2
OIE	Organisation Mondiale de la Santé Animale (World Organization for Animal Health)
OM	organic matter
OPU	ovum pick-up
P4	progesterone
PAGE	polyacrilamide gel electrophoresis
PAGs	pregnancy-associated glycoproteins
PCR	polymerase chain reaction
PCV	packed cell volume
PDI	intestinal digestible protein
PDGF	Plateled-derived growth factor
P/E	protein/energy
PFA	Prevention of Food Adulteration
PFDM	protein-free dry matter
PGF2α	prostaglandine F2α
PGFM	15 cheto-diidro PGF2α metabolite
PI	persistent infection
PMN	polymorphonuclear cells
PMSG	pregnant mare serum gonadotrophin
PPD	purified protein derivative
PRID	progesterone-releasing intravaginal device
PRL	prolactin
PSPB	pregnancy specific protein B
Px	peroxidase
RB	Rose Bengal
RBT	Rose Bengal Test
RDW	red cell distribution width
RFLP	restriction fragment length polymorphism
RIA	radioimmunoassay
RMSE	root mean square of error
RNA	ribonucleic acid
RP	rinderpest
SAR	rapid serum agglutination
SAS/NLIN	Statistical Analysis System/non linear regression
SAT	serum agglutination test
Sd	standard deviation
SDS	sodium dodecyl sulphate
SDTH	skin-delayed-type-hypersensitivity
SG	sub group
SO	superovulation
SOD	superoxide dismutase
SOF	synthetic oviductal fluid
S-phase	solid phase
ST	thermostable toxins
ST.E.	standard error
SVD	swine vesicular disease
T_3	triiodothyronine
T_4	thyroxine

TALP	Tyrode's modified medium
TCA	tricarboxylic acid
TCM	tissue culture medium
TCP	Technical Cooperation Project
TDN	total digestible nutrients
TE	transferable embryos
TGF	transforming growth factor
TH1	TH1 Lymphocytes
TH2	TH2 Lymphocytes
TNF	tumour necrosis factor
t.q.	tal quale (as fed)
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone
TTR	total retention time
U car.	Carratelli unity
UFC	meat feed unit
U/L	unit/litre
VAT	variable antigens type
VER	vaginal electrical resistance
VP1	viral capsid protein
VS	vesicular stomatitis
VSGc	variable surface glycoproteins
VT	vero cytotoxin
WBC	white blood count
WCY	warm carcass yield
WC1-N3	leucocytes cluster
WC1-N4	leucocytes cluster
$\bar{x}$	statistical mean

C.R.A.

Consiglio per la ricerca e la sperimentazione in agricoltura

Via Nazionale 82 - 00184 Rome - Italy

ISTITUTO SPERIMENTALE PER LA ZOOTECNIA

Via Salaria 31 - 00016 Monterotondo Scalo, Rome - Italy

FAO INTER-REGIONAL COOPERATIVE RESEARCH NETWORK ON BUFFALO

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

REGIONAL OFFICE FOR EUROPE

Viale delle Terme di Caracalla 1 - 00100 Rome - Italy

www.fao.org