Livestock Science 122 (2009) 130–133

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effect of varying ruminally degradable to ruminally undegradable protein ratio on nutrient intake, digestibility and N metabolism in Nili Ravi buffalo calves (*Bubalus bubalis*)

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ARTICLE INFO

Article history:
Received 23 February 2008
Received in revised form 22 July 2008
Accepted 5 August 2008

Keywords:
RDP
RUP
Digestibility
N metabolism
Buffalo calves

ABSTRACT

Four Nili-Ravi buffalo calves (100 ± 4 kg) were used in 4 × 4 Latin Square Design to evaluate the influence of varying ruminally degradable protein (RDP) to ruminally undegradable protein (RUP) ratio on dry matter intake (DMI), digestibility and nitrogen (N) metabolism. Four experimental diets A, B, C and D were formulated to contain RDP:RUP of 70:30, 65:35, 60:40 and 55:45, respectively. The calves were fed ad libitum. Dry matter intake by calves fed C diet was higher ($P<0.05$) than those fed D diet and lower ($P<0.05$) than calves fed A diet, however, it was similar to those fed B diet. There was a linear decrease ($P<0.01$) in DMI with decreasing the RDP to RUP ratio. Similar trend was noticed in crude protein (CP) intake. Neutral detergent fiber (NDF) intake was significantly different across all treatment. The decrease in CP and NDF intake was due to decreasing trend of DMI. Dry matter (DM) digestibility in calves fed A and B diets was higher ($P<0.05$) than those fed C and D diets. A linear decrease ($P<0.01$) in DM digestibility was observed with decreasing the RDP to RUP ratio. Crude protein digestibility remained unaltered across all treatments. Neutral detergent fiber digestibility was higher in calves fed A and B diets than those fed C and D diets. Higher NDF digestibility in calves fed A and B diets was due to higher level of dietary RDP that might resulted in higher ruminal ammonia concentration which stimulate activity of cellulytic bacteria and ultimately increased NDF digestibility. The N retention (g/d) was similar among the calves fed B, C and D diets, however, it was higher ($P<0.05$) than those fed A diet. Decreasing the RDP to RUP ratio resulted in linear increase ($P<0.01$) in N retention. The N retention, as percent of N intake was significantly different across all treatments. Decreasing RDP to RUP ratio resulted in linear increase ($P<0.01$) in N retention, as percent of N intake. A similar trend was noticed in N retention, as percentage of N digestion. Blood urea nitrogen (BUN) concentration in calves fed B diet was higher ($P<0.05$) than those fed D diet and was lower ($P<0.05$) than those fed A diet, however, it was not different from calves fed C diet. Decreasing dietary RDP to RUP ratio resulted in linear decrease ($P<0.05$) in BUN concentrations. The decrease in BUN concentration was because of decreasing level of dietary RDP. The N retention can be increased by decreasing RDP to RUP ratio in the diet of growing buffalo calves and diet containing RDP to RUP ratio 55:45 is considered optimum regarding N retention in buffalo calves.

1. Introduction

Protein is one of the limiting nutrients in the diet of ruminants. Growing ruminants require metabolizable protein for tissue synthesis. The metabolizable protein is supplied

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doi:10.1016/j.livsci.2008.08.004

primarily by combinations of ruminally degradable protein (RDP) and ruminally undegradable protein (RUP). Ruminally degradable protein is required for ruminal microbial growth. This not only improves the ruminal fermentation but it also ensures an adequate supply of microbial protein to the host animal, however, microbial protein is unable to meet metabolizable protein requirement of rapidly growing calves and supplementation of RUP has been shown to enhance the performance. The RUP supplementation to ruminant results to improve protein utilization (Titgemeyer et al., 1989; Sultan and Loerch, 1992; Goedeken et al., 1990). Increasing the RUP content in the diet increased N retention and weight gain in calves (Wankhede and Kalbande, 2001; Pattanaik et al., 2003) and goats (Paengkoum et al., 2004).

Imbalance of RDP and RUP in ruminant diet can compromise the microbial protein synthesis, ruminal digestion and protein availability to the animals (Santos et al., 1998; Reynal and Broderick, 2005). Increasing the dietary RDP level increased the DM digestibility (Javaid et al., 2008), however, high level of dietary RDP causes excessive ammonia production in rumen which ultimately results in increased blood urea nitrogen (BUN) concentration (Butler, 1998; Dhali et al., 2006; Javaid et al., 2008). As there is little information on the literature regarding the optimal ratio of RDP and RUP in the diet for optimal growth in buffalo calves. Therefore, the present study was planned to determine the optimum ratio of RDP to RUP and its effect on nutrient intake, digestibility and N metabolism in buffalo calves.

2. Materials and methods

2.1. Animal, housing and diet

Four Nili Ravi buffalo calves (100 ± 4 kg) were used in 4 × 4 Latin Square Design. Four isonitrogenous and isocaloric A, B, C and D diets were formulated (Table 1) having RDP to RUP ratio 70:30, 65:35, 60:40 and 55:45 (2.33, 1.86, 1.50 and 1.22), respectively, according to NRC (2001). The calves were fed ad libitum. The calves were individually housed on concrete floor in separate pens and fed twice a day (8.00 am and 8.00 pm). Fresh water was ensured round the clock during the experimental period. The calves were fed for four periods and each period was of three weeks. The first two weeks of each period served as adaptation time while the third week was a collection period.

Table 1
Ingredients and chemical composition of experimental diets.

Ingredients	Diets			
	A	B	C	D
Wheat straw	20.00	20.00	20.00	20.57
Wheat bran	11.00	14.00	12.00	11.00
Corn grains	26.00	25.00	31.25	35.00
Fish meal	0.45	2.10	5.50	7.46
Cotton seed meal	9.00	9.00	6.60	6.10
Sunflower meal	19.80	14.40	8.10	3.00
Vegetable oil	4.53	4.00	1.70	0.35
Molasses	7.00	9.34	13.00	15.00
Urea	0.11	0.05	0.05	0.02
Dicalcium phosphate	1.61	1.61	1.3	1.0
Vitamin–mineral mixture	0.50	0.50	0.50	0.50
Chemical composition				
Crude protein (CP)	15.98	16.10	16.07	15.97
Metabolizable energy (Mcal/kg)	2.6	2.6	2.6	2.6
[a]RDP % of CP	70	65	60	55
[b]RUP % of CP	30	35	40	45
RDP:RUP	2.33	1.86	1.5	1.22
Neutral detergent fiber	33.50	31.45	28.98	27.12

[a] Ruminally degradable protein.
[b] Ruminal undegradable protein.

2.2. Digestibility, N metabolism and blood sampling

Feed offered and orts were weighed and recorded daily during each collection period. Digestibility was determined by using total collection method. During collection periods, complete collections of urine and feces were made according to the procedure described by Javaid et al. (2008). The feces of each animal were collected daily, weighed, mixed thoroughly and 20% of it was sampled and dried at 55 °C. In the end of each collection period, dried fecal samples were composited and 10% of the composited samples were taken for analysis. For urine collection, special leather collection bag fitted with plastic pipe were made to surround the prepuce. The plastic pipe discharged into a large container (30 L). The urine excreted by each animal was acidified with 50% H_2SO_4 and 20% of it was sampled and preserved at minus 20 °C. In the end of each collection period, the preserved urine samples, after thawing, were composited by animal and 10% of the composited sample was used for analysis. Blood samples were collected from the jugular vein of each animal at 0, 3, 6 and 9 h post feeding to harvest blood serum which was stored in aliquots at minus 20 °C awaiting analysis to determine BUN. The N retention was calculated by the difference of N consumed and the sum of fecal N plus urinary N excreted (Devant et al., 2000).

2.3. Chemical analysis

Feed offered, orts and fecal samples were dried at 55 °C in a forced air oven until there was no further loss of weight. The samples were ground through a 1 mm screen in a Wiley mill and analyzed for dry matter (DM). The N content was determined by Kjeldahl method (AOAC, 1990) and crude protein (CP) was calculated as N multiplied by 6.25. Neutral detergent fiber (NDF) contents were measured by method described by (Van Soest et al., 1991). Blood urea N was determined photometrically using the analytical kit (Cat # CS 612, Crescent Diagnostics, Saudi Arabia) following the Berthelot method.

2.4. Statistical analyses

The data collected on nutrient intake, digestibility, BUN and N balance were subjected to ANOVA using general linear model procedure of SPSS (SPSS 10.0.1., 1999).

3. Results and discussion

3.1. Nutrients intake

Dry matter intake (DMI) by calves fed C diet was higher ($P<0.05$) than those fed D diet and lower ($P<0.05$) than animals fed A diet, however, it was similar to those fed B diet

(Table 2). There was a linear decrease ($P<0.01$) in DMI with decreasing the RDP to RUP ratio. The higher DMI in calves fed A diet was due to higher level of RDP (70%) in this diet. Kalscheur et al. (2006) reported higher DMI in cows fed higher level of RDP. Ruminally degradable part of dietary protein is of vital significance as far as ruminal microbial activity and proliferation is concerned which can alter the nutrient digestion and thus nutrient intake. Provision of adequate amount of RDP ensures optimum microbial activity and proliferation which increases DM digestibility and its intake (Westwood et al., 2000). Similarly, Erdman and Vandersall (1983) also reported higher DMI (22 kg/d) in cows fed higher RDP (73%) than those (21 kg/d) fed lower RDP (53%). Similar trend was noticed in CP intake. Neutral detergent fiber (NDF) intake was significantly different across all treatment (Table 2). A linear decrease ($P<0.01$) in NDF intake was noticed with decreasing RDP to RUP ratio. The decrease in CP and NDF intake was due to decreasing trend of DMI.

3.2. Digestibility

Dry matter digestibility was higher ($P<0.05$) in animals fed A and B diets than those fed C and D diets (Table 2). A linear decrease ($P<0.01$) in DM digestibility was observed with decreasing the RDP to RUP ratio. Increase in dietary RDP results in increased ruminal ammonia N concentrations (Roffler and Satter, 1975; Baumann et al., 2004). Increased ruminal ammonia N concentration results in increased ruminal microbial population (Suwanlee and Wanapt, 1994; Pimpa et al., 1996; Wanapat and Pimpa, 1999). Ultimately higher microbial activity and growth with higher RDP (Fu et al., 2001) resulted in higher DM digestibility (Griswold et al., 2003). Crude protein digestibility remained unaltered across all treatments. Neutral detergent fiber digestibility was higher in calves fed A and B diets than those fed C and D diets (Table 2). Higher NDF digestibility in calves fed A and B diets might be due to higher level of dietary RDP that had resulted in higher ruminal ammonia N concentration which stimulate activity of cellulytic bacteria and ultimately increased NDF digestibility.

Table 2
Nutrient intake and their digestibilities in buffalo calves fed diets containing different RDP to RUP ratio.

Parameters	Diets[1]				SE	L[2]	Q[3]
	A	B	C	D			
Intake, kg/d							
Dry matter	4.19^a	4.08^{ab}	4.00^b	3.81^c	0.038	0.001	0.149
Crude protein	0.669^a	0.656^{ab}	0.643^b	0.609^c	0.009	0.001	0.151
Neutral detergent fiber	1.40^a	1.28^b	1.16^c	1.03^d	0.038	0.001	0.269
Digestibility, %							
Dry matter	69.03^a	67.45^a	62.62^b	59.70^b	1.13	0.001	0.628
Crude protein	66.44	66.24	65.86	65.6	1.43	0.017	0.109
Neutral detergent fiber	59.0^a	57.92^a	56.3^b	54.22^b	1.64	0.001	0.859

Means within row with different superscripts differ ($P<0.05$).
[1]RDP to RUP ratio in A, B, C and D diet is 2.33, 186, 1.5 and 1.22, respectively.
[2]p value for linear effect.
[3]p value for quadratic effect.

Table 3
The N metabolism in buffalo calves fed diets containing different RDP to RUP ratio.

Parameters	Diets[1]				SE	L[2]	Q[3]
	A	B	C	D			
N intake, g/d	106.96^a	104.86^{ab}	102.82^b	97.38^c	1.04	0.001	0.148
Fecal N, g/d	35.9^a	35.4^a	35.1^a	33.5^b	0.317	0.005	0.276
Urinary N, g/d	34.0^a	30.5^b	27.89^c	24.89^d	0.94	0.001	0.938
N retention, g/d	37.06^b	38.96^a	39.83^a	39.39^a	0.33	0.001	0.017
N retention, % of N intake	34.65^d	37.15^c	38.74^b	40.45^a	0.58	0.002	0.334
N retention, % of N digestion	52.16^d	56.09^c	58.82^b	61.66^a	0.93	0.001	0.306
BUN[4], mg/dL	37.0^a	32.25^b	27.75^b	21.25^c	1.64	0.001	0.567

Means within row with different superscripts differ ($P<0.05$).
[1]RDP to RUP ratio in A, B, C and D diet is 2.33, 186, 1.5 and 1.22, respectively.
[2]p value for linear effect.
[3]p value for quadratic effect.
[4]Blood urea nitrogen.

3.3. N metabolism

The N intake by calves fed C diet was higher ($P<0.05$) than those fed D diet and lower ($P<0.05$) than those fed A diet, however, it was similar with those fed B diet (Table 3). A linear decrease ($P<0.01$) in N intake was observed with decreasing the RDP to RUP ratio. Fecal N in animals fed A, B and C diets was higher ($P<0.05$) than those fed C and D diets. A linear increase ($P<0.01$) in fecal N and linear decrease ($P<0.01$) in urinary N was noticed with decreasing RDP to RUP ratio. Similarly, Paengkoum et al. (2004) reported linear increase in fecal N (g/d) in Saanen goat when RDP to RUP ratio was decreased. Kalscheur et al. (2006) also reported linear increase in urinary N excretion with increasing dietary RDP level in dairy cows. Decreasing the RDP to RUP ratio resulted in linear increase ($P<0.01$) in N retention (g/d). Similar findings were reported by Pattanaik et al. (2003) in crossbred calves. The N retention, as percentage of N intake was significantly different across all treatments. Decreasing RDP to RUP ratio resulted in linear increase ($P<0.01$) in N retention, as percentage of N intake. The N retention as percent of intake in calves fed diet D was 16.74, 11.8 and 7.22% higher than the calves fed diet A, B and C, respectively. A similar trend was noticed in N retention percent of N digestion. The N retention as percent of digestion in calves fed diet D was 18.21, 12.77 and 7.53% higher than the calves fed diet A, B and C, respectively.

Decreasing RDP to RUP ratio resulted in linear decrease ($P<0.01$) in BUN concentrations (Table 3). The BUN values were higher in calves fed diets containing higher level of dietary RDP. The higher BUN concentration observed with increased dietary RDP level probably can be explained by increased absorption of ruminal ammonia N, resulting in greater quantities of ammonia N being detoxified in the liver to form urea N. Wanapat and Pimpa (1999) reported linear increase in BUN concentration with increased ruminal ammonia N concentration in swamp buffaloes. Similar findings were reported by Chumpawadee et al. (2006) and Vongsamphan and Wandapat (2004). Higginbotham et al. (1989) found an increase of 2.6 mg/dL in BUN in cows fed isonitrogenous and isocaloric diets by increasing RDP (0.2 kg/d) from 58 to 65% of dietary CP.

4. Conclusion

The results of present study reveal that N retention can be enhanced by decreasing RDP to RUP ratio in the diet of growing buffalo calves and diet containing RDP to RUP ratio 55:45 (1.22) is optimum regarding N retention in buffalo calves.

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