Livestock Science 144 (2012) 197–204

Contents lists available at SciVerse ScienceDirect

Livestock Science

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

Level of crude protein in concentrate supplements influenced rumen characteristics, microbial protein synthesis and digestibility in swamp buffaloes (*Bubalus bubalis*)

Vongpasith Chanthakhoun [a,c,1], Metha Wanapat [a,*], Jan Berg [b]

[a] *Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand*

[b] *Department of Animal and Aquacultural Sciences, Norwegian University of Life Science (UMB), P.O. Box: 5025, N-1432 AAS, Norway*

[c] *Department of Animal Science, Faculty of Agriculture and Forest Resource, Souphanouvong University, Luangprabang, Lao People's Democratic Republic*

ARTICLE INFO

Article history:
Received 4 October 2011
Received in revised form 19 November 2011
Accepted 21 November 2011

Keywords:
Crude protein
Rumen fermentation
Microorganism
Digestibility
Swamp buffalo
Real-time PCR

ABSTRACT

The objective of this research was to evaluate the effect of level of CP in concentrate on rumen fermentation, microbial population (bacteria, protozoa, and fungi), microbial protein synthesis, feed intake and feed digestibility in swamp buffaloes (*Bubalus bubalis*) fed on rice straw based diet. Four, rumen-fistulated 4-year old swamp buffaloes with 381 ± 10 kg liveweight were randomly assigned to receive four dietary treatments according to a 4 × 4 Latin square design. Four levels of crude protein (92, 124, 181 and 219 g/kg CP) in concentrate mixture were fed at 1 g/kg body weight (BW) and rice straw was fed ad libitum. The study revealed that dry matter intake, apparent digestibility of DM, OM, CP and NDF, were significantly higher in buffaloes fed with higher CP level especially at the 124–181 g/kg CP level, while, ADF digestibilities were not affected. Level of CP supplementation had affected on rumen pH, NH_3–N and blood urea N ($P<0.01$). Meanwhile, rumen propionic acid production was significantly higher at 181 g/kg CP level, while total fatty acids (VFA), acetic acid, butylic acid and C_2:C_3 ratio, were similar among treatments. However, protozoal and fungal zoospore populations were not changed while bacterial populations were significantly different among treatments and were higher with high level of CP. Furthermore, application of quantitative PCR to quantify predominant cellulolytic bacteria (16S rRNA) targets revealed that treatments did not change population of *Ruminococcus flavefaciens* bacteria ($P>0.05$) and methanogenic bacteria ($P>0.05$). Meanwhile, total bacteria ($P<0.05$), *Fibrobacter succinogenes* ($P<0.05$) and *Ruminococcus albus* ($P<0.01$) population were significantly increased when CP was at a higher level. Moreover, PD excretion, PD absorption, N absorption, N retention, MNS and EMPS were significantly increased. Therefore, based on this study, it could be concluded that level of CP between 124 and 181 g/kg CP in the concentrate supplement revealed the highest rumen fermentation efficiency in swamp buffaloes fed on rice straw.

1. Introduction

The prevailing long dry season has been becoming more critical in terms of both quantity and quality of feeds especially for the productive ruminants. Traditionally rice straw has been an important roughage for the ruminants in the tropics, although it contains low level of crude protein, energy and minerals. It is essential to improve the nutritional value by either using physical and/or chemical

Abbreviations: ADF, acid detergent fiber; DM, dry matter; NDF, neutral detergent fiber; OM, organic matter; BW, body weight; CP, crude protein; C2, acetic acid; C3, propionic acid; C4, butyric acid; C2/C3, acetic to propionic acid; BUN, blood urea N; RS, rice straw; VFA, volatile fatty acid.

* Corresponding author. Tel./fax: +66 43 202 368.
E-mail addresses: vngpsth@yahoo.com (V. Chanthakhoun), metha@kku.ac.th (M. Wanapat), jan.berg@umb.no (J. Berg).
[1] Tel./fax: +856 74 212 127.

1871-1413/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.livsci.2011.11.011

treatments with or without concentrate supplementation. Home-made-concentrate (HMC) has been shown to improve rumen ecology and ruminant productivity depends on the quality of rice straw and level of HMC supplementation (Wanapat, 1999, 2009).

However, the availability and extensive use of nutrients in the rumen are essential for its efficient use by the animal host (Franzolin and Alves, 2010). Therefore, an important way to reduce nitrogen losses is to promote adequate efficiency of nitrogen assimilation by the animal through a balanced diet for the optimization of protein and amino acids to reconcile with the exact physiological requirement of the animal (Steinfeld and Wassenaar, 2007). In addition, if energy is available, amino acids will be transaminated or used directly for microbial protein synthesis, otherwise, if energy is limiting, amino acids will be de-aminated and their carbon skeleton will be fermented (Bach et al., 2005). The rumen degradable protein (RDP) level below the requirements of the microorganisms may compromise microbial growth, the production of microbial protein, rumen digestion and the availability of nitrogen and energy (Reynal and Broderick, 2005).

However, high levels of nitrogen can induce a case of toxicity due to excess release of ammonia, with high blood concentrations, promoting severe neurological symptoms that can lead to animal death. In fact, the diet for 80 to 110 g/kg of rumen degradable protein enhanced the dry matter intake, digestibility of hemicellulose (Griswold et al., 2003) and rumen fermentation (Davidson et al., 2003). Currently, very few studies have been conducted to evaluate different level of crude protein in concentrate in swamp buffaloes. Therefore, the objective of this study was to evaluate the effects of various level of CP in concentrate on rumen fermentation, microorganisms, microbial protein synthesis, feed intake and feed digestibility in swamp buffaloes fed on rice straw.

2. Materials and methods

2.1. Animals, feeds and management

Four, 4 year old Thai swamp buffaloes (*Bubalus bubalis*), 389 ± 10 kg body weight (BW), were randomly allocated to one at four levels of CP in concentrate mixture (Table 1) in a 4×4 Latin square design experiment. The buffaloes were kept in individual pens, where water and mineral blocks were provided freely. All buffaloes were fed rice straw ad libitum while additional concentrate was fed at 1 g/kg of BW. The experiment consisted of four periods, with each period lasting for 21 d. During the first 14 d, all buffaloes were fed their assigned diets, while concentrate was fed to the buffaloes in two equal portions at 07:00 a.m. and at 16:30 p.m. Refusals of rice straw were weighed daily prior to the morning feeding to determine daily DM intake. The BW of each buffalo was measured at the start and at the end of each period.

2.2. Sampling method and samples' chemical analyses

After the first 14 d, all buffaloes were well adapted to respective feeds then were on metabolism crates during the last 7 d of each period. Feces, urine, rice straw and concentrate were collected and sampled by total collection and were analyzed for chemical compositions of DM, Ash, and CP by the methods of AOAC (1990) and of NDF and ADF by (Van Soest et al., 1991).

Table 1
Ingredients and chemical composition of the concentrates and rice straw.

Items	Treatments, g/kg CP				RS
	92	124	181	219	
Cassava chip	60	60	60	70	–
Rice bran	15	15	17	2	–
Coconut meal	15	10	4	6	–
Cassava hay	5	5	5	5	–
Urea	0	2	4	6	–
Molasses	1	3	4	5	–
Tallow	1	2	3	3	–
Salt	1	1	1	1	–
Sulfur	1	1	1	1	–
Mineral mixture	1	1	1	1	–
Total	100	100	100	100	–
Chemical composition, g/kg of DM					
TDN[1]	787	764	736	743	402
DM	925	924	906	907	942
OM	900.5	894	889	885	896
CP	92	124	181	219	28
NDF	510	455	420	271	779
ADF	221	196	178	163	452

T1 = 92, T2 = 124, T3 = 181, T4 = 219 g/kg CP, RS = rice straw, [1]TDN = total digestible nutrient (calculated value), DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral-detergent fiber, ADF = acid-detergent fiber.

On day 21 of each period, rumen fluid samples were collected 0, 2, 4 and 6 h after the morning feeding through the rumen fistula and measured immediately for pH and temperature (HANNA Instrument HI 8424 microcomputer, Singapore). Rumen fluid was separated into three parts, one part was immediately fixed with 5 ml of sulfuric acid (H_2SO_4) solution (1 M) to determine the action of volatile fatty acids using High Pressure Liquid Chromatography (HPLC, Instruments by controller water model 600E; water model 484 UV detector; column novapak C18; column size 3.9 mm × 300 mm; mobile phase 10 mM H_2PO_4 pH 2.5) according to Samuel et al. (1997) and NH_3–N analysis, using the micro Kjeldahl method (AOAC, 1990). The second part was immediately fixed with 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin) to measure microbial populations by total direct counts of bacteria, protozoa and fungal zoospores (Galyean, 1989). The third part was cultured for groups of bacteria using the roll-tube technique Hungate (1969), to identify bacterial groups (i.e., cellulolytic, proteolytic, amylolytic, total viable bacterial counts).

A blood sample (about 10 mL) was collected on day 21 of each period from the jugular vein at 0, 2, 4 and 6 h after the morning feeding. Blood was sampled into tubes containing 12 mg of EDTA, and plasma was separated by centrifugation at 500 × *g* for 10 min at 4 °C and stored at −20 °C until analysis of plasma urea N according to (Crocker, 1967). Urine samples were analyzed for total N (AOAC, 1990) and allantoin in urine was determined by HPLC as described by (Chen and Gomes, 1995). The amount of microbial purines absorbed was calculated from purine derivative excretion

based on the relationship derived by (Chen and Gomes, 1995).

Rumen fluids were collected at 4 h post-feeding, and were immediately prepared for DNA extraction for methanogens, total bacteria and predominant cellulolytic bacteria populations using real-time PCR technique. Community DNA was extracted from 0.5 g of rumen content by the RBB + C method (Yu and Morrison, 2004). In brief, the RBB + C method employs two rounds of bead beating in the presence of NaCl and SDS, followed by sequential ammonium acetate and isopropanal precipitations. The precipitated nucleic acids were then treated with RNase A and proteinase K, and the DNA was purified using columns from QIAgen DNA Mini Stool Kit (QIAGEN, Valencia, CA), according to manufacturer's recommendations.

2.3. Primers and real-time polymerase chain reaction (real-time PCR)

The targeted populations were total bacteria, total anaerobic fungi, cellulolytic bacteria as *Ruminococcus albus*, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*. The *mcrA* primer set design is used for the real-time PCR to detect total methanogens. All primer set sequences are shown in Table 2. Six sample-derived standards (total bacteria, total methanogens, *R. albus, F. succinogenes* and *R. flavefaciens*) were prepared from treatment pool set of community DNA. The regular PCR was used to generate sample-derived DNA standards for each real-time PCR assay. Then the PCR product was purified using a QIA quick PCR purification kit (QIAGEN, Inc., Valencia, CA) and quantified using a spectrophotometer. For each sample-derived standard, copy number concentration was calculated based on the length of the PCR product and the mass concentration (Yu et al., 2005). The target DNA was quantified by using serial 10-fold dilutions from 10^1 to 10^8 DNA copies of the previously quantified DNA standards. (Yu et al., 2005). In total, 5 real-time PCR standards were prepared. The conditions of the real-time PCR assays of target genes were the following cycle conditions: 1 cycle of 50 °C for 2 min and 95 °C for 2 min for initial denaturation, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min for primer annealing and product elongation. Fluorescence detection was performed at the end of each denaturation and extension step. Amplicon specificity was performed via dissociation curve analysis of PCR end products by increasing the temperature at a rate of 1 °C every 30 s from 60 to 95 °C. For *R. albus* condition was as follows 30 s at 94 °C for denaturing, 30 s at 55 °C for annealing and 30 s at 72 °C for extension (48 cycles), except for 9 min denaturation in the first cycle and 10 min extension in the last cycle. Real-time PCR amplification and detection were performed in a Chromo 4™ system (Bio-Rad, USA). In brief, Biotools QuantiMix EASY SYG KIT (B and M Labs, S. A., Spain) was used for real-time PCR amplification and samples were assayed in duplicate in a 20 μl reaction mixture containing 4–6 mM $MgCl_2$, 10 μl of Mastermix (including; *Taq* DNA polymerase, reaction buffer, dNTP mixture, $MgCl_2$ and SybrGreen), 2 μl of DNA template, and 0.8 μl of each primer (10 μM/μl).

Table 2
PCR primer sets for real-time PCR and PCR-DGGE assays.

Target species	Primer sequence	Size (bp)
General bacteria[a]	5′-CGG CAA CGA GCG CAA CCC-3′ 5′-CCA TTG TAG CAC GTG TGT AGC C-3′	130
General methanogens[a]	5′-TTC GGT GGA TCD CAR AGR GC-3′ 5′-GBA RGT CGW AWC CGT AGA ATC C-3′	160
F. succinogenes[a]	5′-GTT CGG AAT TAC TGG GCG TAA A-3′ 5′-CGC CTG CCC CTG AAC TAT C-3′	121
R. flavefaciens[a]	5′-CGA ACG GAG ATA ATT TGA GTT TAC TTA GG-3′ 5′-CGG TCT CTG TAT GTT ATG AGG TAT TAC C-3′	132
R. albus[b]	5′-CCC TAA AAG CAG TCT TAG TTC G-3′ (Ra1281f) 5′-CCT CCT TGC GGT TAG AAC A-3′ (Ra1439r)	175

[a] Makkar and McSweeney (2005).
[b] Koike and Kobayashi (2001).

2.4. Statistical analysis

All data were statistically analyzed for a Latin square design using the GLM procedure SAS (1996) in which buffalo, period and level of CP supplementation were main factors. Treatment means were compared by polynomial comparison according to the model:

$$Y_{ijk} = \mu + T_i + C_j + R_k + e_{ijk}$$

where: Y_{ijk} = the criteria under study, in treatment i; column j; row k; μ = over all sample mean; T_i = effect of treatment i; C_j = effect of treatment i at column j; R_k = effect of treatment i at row k; e_{ijk} = error.

3. Results and discussion

3.1. Chemical composition of feeds

The composition of the diets and rice straw is shown in Table 1. Rice straw contained 402 g/kg TDN and 28 g/kg CP. Rice straw was fed as basal forage. Rice straw has been largely available as a crop-residue during the harvesting season and used as a roughage source for ruminant feeding. As rice straw contained high fibrous fractions (NDF, ADF) with low rumen degradation, therefore chemical treatment methods especially urea (50 g/kg) or urea-lime (25 + 25 g/kg) have been used to improve digestibilities and overall feed intakes (Wanapat et al., 2009). However, Leng (1993) reported that the ruminants fed on low-quality roughages required additional fermentable N, required to increase rumen microbial protein synthesis. Therefore, different substrates required different concentrations of ammonia to achieve optimal microbial yield (Ørskov, 1999). While, treatments were the supplement as concentrate mixture and were readily consumed by the buffaloes throughout the entire experimental period. Concentrate diets contained 92, 124, 181, and 219 g/kg CP and 787, 764, 736 and 743 g/kg TDN on a DM basis for the four treatments, respectively.

3.2. Effect on feed intake and digestibility

Effects of various CP level and treated rice straw based diet on feed intake and digestibility of nutrients in swamp

Table 3
Influence of level of crude protein in concentrates on voluntary dry matter feed intake and nutrient digestibility in swamp buffaloes.

Items	Treatments, g/kg CP				SEM	Contrasts	
	92	124	181	219		L	Q
DMI, kg/d of BW daily							
Rice straw	3.00[a]	4.02[b]	4.04[b]	4.60[c]	0.11	**	**
Concentrate	2.43[a]	2.70[b]	2.70[b]	2.43[a]	0.02	**	**
Total	5.45[a]	6.43[b]	6.70[b]	7.32[c]	0.10	**	**
Apparent digestibility, %							
DM	53.6[a]	64.7[b]	62.7[b]	60.6[b]	0.95	**	**
OM	59.2[a]	68.7[c]	67.2[c]	64.2[b]	0.66	**	**
CP	54.9[a]	69.4[b]	74.1[c]	75.5[c]	0.87	**	**
NDF	57.0[a]	67.0[b]	65.4[b]	53.7[a]	2.72	NS	*
ADF	31.0	47.9	45.7	34.1	4.80	NS	NS

[a,b,c]Means in the same row with different superscripts differ (P<0.05), NS = not significance. T1 = 92, T2 = 124, T3 = 181, T4 = 219 g/kg CP, DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral-detergent fiber, ADF = acid-detergent fiber, SEM = standard error of the mean.
** P<0.01.
* P<0.05.

buffalo are presented in Table 3. Apparent digestibility of DM, and CP were significantly increased (P<0.01) at 124, 181 and 219 g/kg CP levels of supplementation, while those of OM (P<0.01) and NDF were significantly increased (P<0.05) at 124 and 181 g/kg CP levels of supplementation, while ADF digestibilities were similar among treatments. The result suggests that level of CP in concentrate mixture supplementation improved rumen degradability and, as a consequence improved DM intakes (P<0.01) at 124, 181 and 219 g/kg CP levels of supplementation (Table 3).

The apparent digestibility and DM intake were affected by adding increased level of dietary protein. Under this finding, it was higher than in the earlier works, which were similarly influenced by the diet of 80 to 110 g/kg of RDP that enhanced the dry matter intake, digestibility of hemicellulose Griswold et al. (2003) and rumen fermentation (Davidson et al., 2003). In dairy cows, Reynal and Broderick (2003) observed lower DMI (1.8 kg/d) in cows that received a diet containing 160 g/kg CP when compared to animals fed a 190 g/kg CP diet, with the extra CP provided by soybean meal. Broderick (2003) observed a crescent linear effect on DMI when soybean meal partially replaced high moisture corn in increasing amounts of 44 g/kg of total DM, with increasing CP contents of the diets (151, 167 and 184 g/kg CP). Dry matter intake increased 0.9 and 1.4 kg/d for diets containing 167 and 184 g/kg CP, respectively, as compared to the 15.1 g/kg CP diet. Gleghorn et al. (2004) who evaluated effects of CP concentration (115, 130, and 140 g/kg of dietary CP) and degradability on the performance of beef steers fed 900 g/kg concentrate diets, and had found no differences in ADG as influenced by various level of CP.

Nevertheless, apparent digestibilities of DM, OM, CP and NDF were affected by the increased dietary protein treatments. These were in agreement with Paengkoum and Tatsapong (2009) who found that DM and OM digestibilities increased, while NDF and ADF digestibilities were similar, when increasing the levels of dietary protein content, while increasing the dietary rumen degradable protein level increased the DM digestibility (Javaid et al., 2008). On the other hand, Renno et al. (2005) reported no differences among levels of dietary urea (up to 460 g/kg of total N as NPN) on total tract digestibility of several nutrients in steers fed bermudagrass hay. However, Infascelli et al. (1995) reported higher protein degradation rates in the buffalo rumen than ovines.

3.3. Characteristics of ruminal fermentation

Rumen fluid pH was lower in high crude protein level, while NH_3–N and blood urea N were found higher (P<0.05) with a high level of crude protein supplementation, while rumen temperature was not altered among treatments. In addition, propionic acid (C_3) was increased (P<0.05) at 181 and 219 g/kg CP, while total volatile fatty acid, acetic acid (C_2), butyric acid (C_4), ratio of acetic acid to propionic ratio (C_2/C_3) were not influenced by level of CP in the concentrate (Table 4).

The overall mean ruminal pH was 6.2 to 6.5, and was greater than the 5.0 to 5.5 range suggested by Hoover (1986) which the ruminal digestibility of fiber was negatively affected. While, Wanapat and Pimpa (1999) reported that ruminal NH_3–N concentrations increased from 7.1 to 34.4 mg% could improve rumen fermentation and digestibility in swamp buffaloes. However, ruminal NH_3–N concentration was a predictor of efficiency of dietary N conversion into microbial N (Firkins et al., 2007). Furthermore, Chanthakhoun et al. (2011) found that ruminal NH_3–N concentrations increased from 8.8 to 9.2 mg%, increased DM intake, digestibility, increased N retention, reduced protozoal and methane gas production in swamp buffaloes with 600 g/hd/d of legume hay as a protein source supplementation. As obtained in this study, ruminal NH_3–N concentrations were increased with higher levels of crude protein in concentrate of 124, 181, and 219 g/kg CP and were stable throughout the sampling periods, except at 92 g/kg CP.

Blood-urea nitrogen concentrations were increased with the addition of crude protein in the diets. According to Javaid et al. (2008), increasing dietary RDP resulted in reduced ruminal pH and increased ruminal NH_3–N in *Niliravi* buffalo. During periods of excessive N availability in the rumen, ruminal NH_3 is absorbed and appears as urea in the plasma urea pool (Cocimano and Leng, 1967). Accordingly, results were in agreement with previous observations especially by Renno (2003) and Magalhaes et al. (2005), who

Table 4
Ruminal pH, temperature, ammonia–nitrogen (NH_3-N), blood-urea nitrogen (BUN) and VFA concentrations in swamp buffaloes fed with different levels crude protein supplementation.

Items	Treatments, g/kg CP				SEM	Contrasts	
	92	124	181	219		L	Q
Rumen parameters							
pH	6.5[a]	6.5[a]	6.2[b]	6.3[b]	0.05	**	0.07
Temperature, °C	38.2	38.5	38.6	38.3	0.2	NS	NS
NH_3-N, mg/dL	5.5[a]	9.3[b]	12.9[c]	16.5[c]	0.69	**	NS
Blood urea N, mg/dL	7.3[a]	8.4[a]	10.0[a]	18.1[b]	1.27	**	*
VFA, mol/100 mol	91.6	110.2	110.4	116.1	5.7	NS	NS
Acetic acid (C_2)	62.4	75.8	77.7	71.6	4.7	NS	0.08
Propionic acid (C_3)	18.8[a]	24.0[a]	28.1[b]	26.9[b]	1.9	**	NS
Butyric acid (C_4)	10.4	10.4	10.2	11.8	0.6	NS	NS
C_2:C_3	3.71	3.78	3.00	3.1	0.38	NS	NS

[a,b,c]Means in the same row with different superscripts differ ($P<0.05$), NS = non-significance, L = linear, Q = quadratic.
VFA = volatile fatty acid, acetic acid to propionic acid ratio, T1 = 9.2, T2 = 12.4, T3 = 18.1, T4 = 21.9% CP, SEM = standard error of the mean.
** $P<0.01$.
* $P<0.05$.

reported that plasma urea was increased when NPN (urea) added to the diets. However, blood urea N concentration could result in nitrogen losses from the rumen fermentation (Xin et al., 2010). In the present study, urea accumulation in the blood was lower in buffaloes receiving high CP levels. This indicates that, feed grade urea in concentrate, provides more nitrogen available for ruminal protein synthesis and relatively less NH_3 absorption for further urea synthesis in the liver in these swamp buffaloes. On the other hand, Thanh and Ørskov (2006) clearly showed that difference in purine derivative excretion in buffalo was due to lower glomerular filtration rate (GFR) spending more time in the blood thus recycling more to the rumen and metabolized by bacteria or the permeability from the blood to rumen was greater in buffalo than in cattle.

Generally, rate and extent of volatile fatty acid production were influenced by carbohydrate fraction and degradability of carbohydrate (Mcdonald et al., 1995). Under the in vitro study of Oliveira et al. (2011) who reported that although propionic acid was not used as energy source by ruminal microorganims, different from lactic acid, both affect bacterial metabolism, propionic acid, and especially lactic acid, at high concentrations, inhibiting growth of some ruminal bacterial species. According to the study of Russel (1992), at very low pH concentration it has demonstrated that accumulation of fermentation acids particularly those of acetic acid and lactic acid could dramatically lower rumen pH and is ultimately toxic to rumen microbes especially to the cellulolytic bacteria. Furthermore, Bello and Escobar (1997) verified that the increase of propionate in the medium promotes a selective effect on the group of bacteria. However, under this in vivo study rumen pH could be maintained at a higher level (>6.2), which could improve microbe growth and propionic acids. Total VFA concentrations in all treatments ranged from 91.6 to 116.1 mM and were similar to those reported by Wanapat et al. (2008) in swamp buffaloes fed on low-quality roughage such as rice straw.

3.4. Rumen microorganism populations

The use of total direct count technique of rumen microorganism populations, revealed that total bacterial counts were enhanced ($P<0.05$) by increasing CP level from 124 to 219 g/kg CP, while fungi zoospores, and protozoa populations were not altered among treatments. In addition, by roll tube technique to determine rumen microorganism populations of total viable bacteria, amylolytic, proteolytic and cellulolytic bacteria, the results showed no significant difference among treatments ($P>0.05$).

In addition, the accuracy of reach real-time PCR was validated by quantifying known numbers of target species templates (total bacteria, methanogen, *F. succinogenes, R. flavefaciens* and *R. albus*) and these templates were used for generating standard curve. In this study, those standard curves showed a highly linear relationship between ct value and known number template dilution by giving high R^2 values of standard curve in each species such as; total bacteria ($Y=-0.3311x+11.61$; $r^2=0.992$), methanogen ($Y=-0.3311x+11.84$; $r^2=0.997$), *F. succinogenes*($Y=-0.3084x+12.65$; $r^2=0.987$), *R. flavefaciens* ($Y=-0.4427x+12.08$; $r^2=0.994$) and *R. albus* ($Y=-0.3483x+11.91$; $r^2=0.977$). All supplemented treatments affected total bacteria ($P<0.05$), *F. succinogenes* ($P<0.05$) and *R. albus* population ($P<0.01$), and were lowest when buffaloes consumed treated rice straw with 9.2%CP in concentrate supplement, whereas methanogens and *R. flavefaciens* were unaffected. Quantifying the predominant cellulolytic bacteria in in vivo provided an interesting data. It was shown in Table 5, that population of total bacteria ranged from 4.76 to 9.40×10^9 copies/ml of buffalo rumen, while it was surprising to find that *F. succinogenes* were 0.4×10^5 to 6.62×10^7 and *R. albus* were 6.00×10^6 to 2.00×10^{10} copies/ml of buffalo rumen. On the contrary, it was found that *R. flavefaciens* were 2.00 to 15.21×10^5 and methanogens were 6.04 to 18.03×10^8 copies/ml of buffalo rumen. Therefore, different levels of crude protein clearly showed under this experiment that there was a shift of microorganism populations and it could lead to a reduced swamp buffalo performance.

There is asynchronous carbohydrate and protein degradation in the rumen, large amounts of ammonia–N are absorbed across the rumen wall into the blood or energy use for microbial protein synthesis will decrease (Bach et al., 2005). Under this study, it was obvious in the treatment

Table 5
Influence of level of CP in concentrate mixtures on ruminal microbial population in swamp buffaloes.

Items	Treatments, g/kg CP of concentrate				SEM	P-value
	92	124	181	219		
Rumen microbes, cells/ml by total direct counts						
Bacteria, $\times 10^{11}$	1.8[a]	2.5[b]	2.5[b]	3.4[b]	0.31	0.04
Protozoa, $\times 10^{5}$	8.1	10.1	12.4	15.3	2.74	0.36
Fungal zoospores $\times 10^{6}$	5.7	6.1	7.5	7.2	0.61	0.27
Viable bacteria, CFU/ml by roll-tube technique						
Total viable bacteria $\times 10^{9}$	9.0	3.7	7.9	11.6	4.20	0.64
Amylolytic, $\times 10^{7}$	6.4	6.5	9.4	7.4	2.02	0.71
Proteolytic, $\times 10^{8}$	8.8	9.3	10.8	11.2	6.04	0.99
Cellulolytic, $\times 10^{7}$	1.5	1.2	7.6	1.5	3.01	0.42
Total predominant cellulolytic bacteria, $\times 10^{7}$ copies/ml of rumen fluid by real-time PCR						
Total bacteria, $\times 10^{9}$ copies/ml	4.76[a]	6.29[ab]	8.17[b]	9.40[b]	1.03	0.04
F. succinogenes, $\times 10^{5-7}$ copies/ml	$0.4^{a}\times 10^{5}$	$4.88^{b}\times 10^{6}$	$5.64^{b}\times 10^{6}$	$6.62^{c}\times 10^{7}$	69.48	0.05
R. flavefaciens, $\times 10^{5}$ copies/ml	2.81	2.00	15.21	7.72	5.81	0.31
R. albus, $\times 10^{6-10}$ copies/ml	$6.00^{a}\times 10^{6}$	$9.54^{ab}\times 10^{8}$	$2.00^{b}\times 10^{10}$	$1.29^{b}\times 10^{10}$	3275.10	0.006
Methanogens, $\times 10^{8}$ copies/ml	8.81	18.03	11.39	6.04	4.26	0.22

[a,b,c]Means in the same row with different superscripts differ at $P<0.05$, and $P<0.01$ T1 = 92, T2 = 124, T3 = 181, T4 = 219 g/kg CP, CFU = colony-forming units, SEM = standard error of the mean, *F = Fibrobacter, R = Ruminococcus*.

of 92 g/kg CP. However, ruminal total bacteria were highest with high dietary CP in concentrate mixture supplementation. Vinh et al. (2011) reported that total rumen bacteria, *F. succinogenes*, *R. albus* cellulolytic bacteria were increased by dietary treatment with urea–lime treated rice straw and with 4% urea in concentrate mixture while *R. flavefaciens*, protozoal population were significantly reduced ($P<0.05$), while amylolytic, proteolytic bacterial groups, total bacteria were not changed among treatments. Meanwhile, Chanthakhoun and Wanapat (2010) found legume (*Phaseolus calcaratus*) hay as protein source supplementation, using real-time PCR technique for quantification of cellulolytic bacterial species (*F. succinogenes*, *R. albus* and *R. flavefaciens*) and reported that the rumen microorganisms were improved in both the rumen fluid and digesta in swamp buffalo. Whereas, in this study *R. flavefaciens* were not significantly different by treatments, while Koike et al. (2003) suggested that the increase in attached cell numbers observed could be mostly attributed to cell proliferation on the straw after 6 h, the numbers of attached cells of the three species gradually increased and peaked at 24 h (10^{9} g^{-1} DM for *F. succinogenes* and 10^{7} g^{-1} DM for *R. flavefaciens*) or 48 h (10^{6} g^{-1} DM for *R. albus*). On the contrary, McSweeny et al. (1999) observed that in the animals fed on tannin rich *Calliandra calothyrsus*, the population of *Ruminococcus spp*. and *Fibrobacter spp*. was reduced considerably. Currently, Singh et al. (2011) found that real time PCR data revealed a decrease in *R. flavefaciens*, an increase in methanogens and no change in the *F. succinogenes* population by feeding of pakar leaves as tannin and protein rich leaves.

However, in the present study, cellulolytic bacteria numbers were dependent on the diet fed, particularly the imbalance of energy and nitrogen, which could be a reflection of the ability of both *R. albus* and *F. succinogenes* to use glucose as substrate for growth (Shi et al., 1997). On the other hand, the highest levels of total bacteria, *F. succinogenes* and anaerobic fungi were found when using slow-release compounds (Urea–$CaCl_2$, Urea-$CaSO_4$) (Cherdthong et al., 2011). Kongmun et al. (2011) also found that *F. succinogenes* population was highest, while, *R. flavefaciens* and *R. albus* were lowest in swamp buffalo influenced by supplementation of 7% coconut oil plus 100 g/d of garlic powder. Therefore, it

Table 6
Effect level of crude protein in concentrates on nitrogen balance, purine derivatives (PD) and microbial nitrogen supply in swamp buffaloes.

Items	Treatments, g/kg CP				SEM	Contrasts	
	92	124	181	219		L	Q
PD excretion, mml/d	76.1[a]	105.1[b]	101.7[b]	106.8[b]	4.42	**	*
PD absorption	50.6[a]	84.8[b]	80.2[b]	86.3[b]	5.13	**	*
Absorbed N	30.6[a]	50.6[a]	76.3[b]	79.0[b]	0.60	**	*
Retained N	19.2[a]	34.6[b]	58.8[c]	59.3[c]	1.90	***	**
MNS[1] (g N d^{-1})	36.8[a]	61.6[b]	58.3[b]	62.7[b]	3.70	**	*
EMPS (g N kg^{-1} of OMDR[2])	10.5[a]	12.2[ab]	11.0[a]	14.2[b]	0.91	*	NS

[a,b,c]Means in the same row with different superscripts differ ($P<0.05$), NS = not significant. T1 = 92, T2 = 124, T3 = 181, T4 = 219 g/kg CP, [1]Microbial nitrogen supply (MNS g N/d) = 0.727 × mmol of purine derivatives absorbtion Chen et al. (1993), [2]Efficiency of microbial N synthesis (EMNS, g/kg) of OM digested in the rumen (OMDR) = [(MCP (g/d) × 1000)/DOMR (g)], assuming that rumen digestion = 65% of digestion in total tract, EMPS = Efficiency of Microbial Protein Synthesis, OMDR = Organic Matter Digestible in the Rumen (65% of organic matter digestible in total tract) according to Agricultural Research Council (1984), SEM = standard error of the mean.

** $P<0.01$.
* $P<0.05$.
*** $P<0.001$.

could be explained for the increase in total bacteria, *F. succinogenes* and *R. albus* population in high crude protein in concentrate fed to buffaloes with rice straw as roughage while the availability of the substrate did not affect on *R. flavefaciens*.

3.5. N balance and efficiency of microbial protein synthesis

As shown in Table 6, N intake, excretion of N, nitrogen retention and N absorption were influenced ($P<0.05$) by increasing the level from 124 to 219 g/kg CP in concentrate mixtures. Nevertheless, CP level of concentrate mixture increased, level of allantoin absorption, efficiency of microbial N synthesis and microbial N synthesis increased from 124 to 219 g/kg CP as shown in Table 6. Based on the results of this experiment, supplementation of 92, 124, 181 and 219 g/kg CP resulted in improved DM digestibility, rumen microbial population, and microbial protein supply.

This study revealed that allantoin excretion, allantoin absorption, microbial nitrogen supply (MNS), and efficiency of microbial protein synthesis (EMPS) in concentrates containing 124, 181, and 219 g/kg CP were significantly higher than that of 92 g/kg CP and agreed with other reports showing that synthesis of microbial protein was affected by dietary protein (Cecava et al., 1991; Ludden and Cecava, 1995). Ipharraguerre and Clark (2005) suggested that the degradability of dietary protein might modulate the microbial crude protein outflow, which resulted in the change of animal performance. However, higher efficiency of microbial nitrogen supply with soybean meal as a protein source versus urea diet might be due to availability of peptide or amino acid N, which can subsequently enhance microbial growth (Galo et al., 2003; Xin et al., 2010). On the contrary, the increase in the RUP/RDP ratio may reduce CPB and improve protein efficiency, because at a high RDP, more N would be absorbed as ammonia or more amino acids would be deaminated, that might increase N excretion in urine (Castillo et al., 2001), while, reducing dietary protein concentration from 190 g/kg of DM to approximately 160 g/kg of DM could reduce ammonia emissions by 200 g/kg and reducing the degradability of protein to match the microbial requirement by 190 g/kg (Kebreab et al., 2004). The positive effect of RUP on protein efficiency is also reported by Flis and Wattiaux (2005) and Kalscheur et al. (2006). Moreover, Castillo et al. (2001) and Reynal and Broderick (2005) found that an increase in dietary CP degradability results in more urinary N excretion. However, post-ruminal digestibility of RUP and amino acid balance could be considered as an important factor for increasing metabolizable protein flow to the intestine (Noftsger and St-Pierre 2003) and, subsequently, optimizing dietary RUP to improve protein efficiency. Therefore, the potentially improved synchronization between ruminal NH_3 from respective crude protein in the diet and carbohydrate availability (i.e., cassava chips) was improved, consequently resulting in higher microbial CP synthesis when rice straw was fed.

4. Conclusions

Higher level of crude protein (124–181 g/kg CP) in concentrate mixtures improved DMI, DM, OM, CP, and NDF digestibilities, rumen fermentation, and microbial CP synthesis in swamp buffalo. In addition, there were increases in rumen total bacteria, *F. succinogenes* and *R. albus* population. Based on this study it could be concluded that the level between 124 and 181 g/kg CP in concentrate mixtures improved rumen fermentation efficiency and it is recommended for use in swamp buffaloes fed on rice straw.

Conflict of interest statement

There is no conflict of interest statement at all.

Acknowledgments

The authors would like to express their most sincere thanks to the Tropical Feed Resources Research and Development Center (TROFEC), for their kind financial support of this experiment research and the Norwegian Program for Development Research and Education project (NUFU) and The Khon Kaen University Higher Education Research Promotion and National Research University Project of Thailand Scholarship for Research in providing Ph.D. Scholarship for the first author.

References

AOAC, 1990. Official Methods of Analysis, 15th ed. AOAC International, Arlington, UA, USA.
Bach, A., Calsamiglia, S., Stern, M.D., 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88, E9–E21.
Bello, M.G.D., Escobar, A., 1997. Rumen manipulation for improved utilization of tropical forages. Anim. Feed. Sci. Technol. 69, 91–102.
Broderick, G.A., 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86, 1370–1381.
Castillo, A.R., Kebreab, E., Beever, D.E., Barbi, J.H., Sutton, J.D., Kirby, H.C., France, J., 2001. The effect of energy supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. J. Anim. Sci. 79, 240–246.
Cecava, M.J., Merchen, N.R., Berger, L.L., Mackie, R.I.J., Fahey, G.C., 1991. Effects of dietary energy level and protein sources on nutrient digestion and ruminal nitrogen metabolism in steers. J. Anim. Sci. 69, 2230–2244.
Chanthakhoun, V., Wanapat, M., 2010. Effect of legume (*Phaseolus calcaratus*) hay supplementation on rumen cellulolytic bacterial populations in swamp buffaloes investigated by the real-time PCR technique. J. Anim. Vet. Adv. 9 (11), 1654–1659.
Chanthakhoun, V., Wanapat, M., Wachirapakorn, C., Wanapat, S., 2011. Effect of legume (*Phaseolus calcaratus*) hay supplementation on rumen microorganisms, fermentation and nutrient digestibility in swamp buffalo. Livest. Sci. 140, 17–23.
Chen, X.B., Gomes, M.J., 1995. Estimation of Microbial Protein Supply to Sheep and Cattle based on Urinary Excretion of Purine Derivative—An Overview of the Technique Details. Occasional Publication 1992. International Feed Resources Unit, Rowett Research Institute, Aberdeen, UK.
Cherdthong, A., Wanapat, M., Wachirapakorn, C., 2011. Influence of urea–calcium mixtures as rumen slow-release feed on in vitro fermentation using a gas production technique. Arch. Anim. Nutr. 65 (3), 242–254.
Cocimano, M.R., Leng, R.A., 1967. Metabolism of urea in sheep. Br. J. Nutr. 21, 353–371.
Crocker, C.L., 1967. Rapid determination of urea nitrogen in serum or blood without deproteinization. Anim. J. Med. Technol. 33, 361–365.
Davidson, S., Hopkins, B.A., Diaz, D.E., Bolt, S.M., Brownie, C., Fellner, V., Whitlow, L.W., 2003. Effects of amount and degradability of dietary protein on lactation, nitrogen utilization, and excretion in early lactation Holstein cows. J. Dairy Sci. 86, 1681–1689.
Firkins, J.L., Yu, Z., Morrison, M., 2007. Ruminal nitrogen metabolism: perspectives for integration of microbiology and nutrition for dairy. J. Dairy Sci. 90 (E. Suppl.), E1–E16.
Flis, S.A., Wattiaux, M.A., 2005. Effects of parity and supply of rumen degraded and undegraded protein on production and nitrogen balance in Holstein dairy cows. J. Dairy Sci. 88, 2096–2106.

Franzolin, R., Alves, T.C., 2010. The Ruminal Physiology in Buffalo Compared with Cattle. 9th world buffalo congress, pp. 104–111.
Galo, E., Emanuele, S.M., Sniffen, C.J., White, J.H., Knapp, J.R., 2003. Effects of a polymer-coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. J. Dairy Sci. 86, 2154–2162.
Galyean, M., 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM, USA.
Gleghorn, J.F., Elam, N.A., Galyean, M.L., Duff, G.C., Cole, N.A., Rivera, J.D., 2004. Effects of crude protein concentration and degradability on performance, carcass characteristics, and serum urea nitrogen concentrations in finishing beef steers. J. Anim. Sci. 82, 2705–2717.
Griswold, K.E., Apgar, G.A., Bouton, J., Firkins, J.L., 2003. Effects of urea infusion and ruminal degradable protein concentration on microbial growth, digestibility, and fermentation in continuous culture. J. Anim. Sci. 81, 329–336.
Hoover, W.H., 1986. Chemical factors involved on ruminal fiber digestion. J. Dairy Sci. 69, 2755–2766.
Hungate, R.E., 1969. A roll tube method for cultivation of strict anaerobes. In: Norris, J.R., Ribbons, D.W. (Eds.), Methods in Microbiology. Academic, New York, pp. 117–313.
Infascelli, F., Di Lella, T., Piccolo, V., 1995. Dry matter, organic matter and crude protein degradability of high protein feeds in buffaloes and sheep. Zoot. Nutr. Anim. 2, 89–94.
Ipharraguerre, I.R., Clark, J.H., 2005. Impacts of the source and amount of crude protein on the intestinal supply of nitrogen fractions and performance of dairy cows. J. Dairy Sci. 88, E22–E37.
Javaid, A., Nisa, Mahr un, Sarwar, M., Shahzad, M.A., 2008. Ruminal characteristics, blood pH, blood urea nitrogen and nitrogen balance in Nili-Ravi buffalo (*Bubalus bubalis*) bulls fed diets containing various level of ruminally degradable protein. Asian-Australas J. Anim. Sci. 21, 51–59.
Kalscheur, K.F., Baldwin, R.L., Glenn, B.P., Kohn, R.A., 2006. Milk production of dairy cows fed differing concentrations of rumen-degraded protein. J. Dairy Sci. 89, 249–259.
Kebreab, E., Mills, J.A.N., Crompton, L.A., Bannink, A., Dijkstra, J., Gerrits, W.J.J., France, J., 2004. An integrated mathematical model to evaluate nutrient partition in dairy cattle between the animal and its environment. Anim. Feed. Sci. Technol. 112, 131–154.
Koike, S., Kobayashi, Y., 2001. Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*. *Ruminococcus albus* and *Ruminococcus flavefaciens*. FEMS Microbiol. Lett. 204, 361–366.
Koike, S., Pan, J., Kobayashi, Y., Tanaka, K., 2003. Kinetics of in sacco fiber-attachment of representative ruminal cellulolytic bacteria monitored by competitive PCR. J. Dairy Sci. 86, 1429–1435.
Kongmun, P., Wanapat, M., Pakdee, P., Navanukraw, C., Yu, Z., 2011. Manipulation of rumen fermentation and ecology of swamp buffalo by coconut oil and garlic powder supplementation. Livest. Sci. 135 (1), 84–92.
Leng, R.A., 1993. Quantitative ruminant nutrition—a green science. Res. Aust. J. Agric. 44, 363.
Ludden, P.A., Cecava, M.J., 1995. Supplemental protein sources for steers fed corn-based diets: I. Ruminal characteristics and intestinal amino acid flows. J. Anim. Sci. 73, 1466–1475.
Magalhaes, K.A., Valadares Filho, S.C., Valadares, R.F.D., Paixao, M.L., Pina, D.S., Paulino, P.V.R., Chizzotti, M.L., Marcondes, M.I., Araujo, A.M., Porto, M.O., 2005. Effects of feeding different levels of urea or cottonseed hulls on microbial protein synthesis, plasma urea concentration and urea excretion in steers. Rev. Bras. Zootecn. 34, 1400–1407.
Makkar, H., McSweeney, C.S., 2005. Methods in Gut Microbial Ecology for Ruminants. Springer, The Netherlands. p. 225.
Mcdonald, P., Edwards, R.A., Greenhalgh, J.E.D., Morgan, C.A., 1995. Animal Nutrition, 5th Edn. Prentice Hall, United States. 693.
McSweeny, C.S., Palmer, B., Krause, D.O., Brooker, J.D., 1999. Rumen microbial ecology and physiology in sheep and goats fed a tannin containing diet. Tannins in Livestock and Human Nutrition. Proc. International Workshop, Adelaide, Australia, pp. 140–145. 31 May-2 June, 1999.
Noftsger, S., St-Pierre, N.R., 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J. Dairy Sci. 86, 958–969.
Oliveira, J.S., Queiroz, A.C., Mantovani, H.C., Melo, M.R., Detmann, E., Santos, E.M., Bayão, G.F.V., 2011. Effect of propionic and lactic acids on in vitro ruminal bacteria growth. Rev. Bras. Zootecn. 40, 1121–1127.
Ørskov, E.R., 1999. Supplement strategies for ruminants and management of feeding to maximize utilization of roughages. Prev. Vet. Med. 38, 179–185.
Paengkoum, P., Tatsapong, P., 2009. Effect of different levels of protein on feed intake, digestibility and growth rate of Thai native beef cattle fed pangola grass as roughages. In: Oshio, S., Otsuka, M., Sommart, K. (Eds.), Establishment of a Feeding Standard of Beef Cattle and a Feed Database for the Indochinese Peninsula. JIRCAS, Tsukuba, Ibaraki, Japan, pp. 76–78.
Renno, L.N., 2003. Consumo, digestibilidade total e parcial, producao microbiana, parametros ruminais e excrecoes de urea e creatinina em novilhos alimentados com dietas contendo quatro nıveis de ureia ou dois de proteına. PhD Diss. Federal University of Vicosa, MG, Brazil.
Renno, L.N., Valadares, Filho S.C., Valadares, R.F.D., Cecon, P.R., Backes, A.A., Renno, F.P., Alves, D.D., Silva, P.A., 2005. Nı'veis de ure'ia na raça o de novilhos de quatro grupos gene'ticos: consumo e digestibilidades totais. Rev. Bras. Zootecn. 24, 363–370.
Reynal, S.M., Broderick, G.A., 2003. Effects of feeding dairy cows protein supplements of varying ruminal degradability. J. Dairy Sci. 86, 835–843.
Reynal, S.M., Broderick, G.A., 2005. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. J. Dairy Sci. 88, 4045–4064.
Russel, J.B., 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus in coupling. J. Appl. Bacteriol. 73, 363–370.
Samuel, M., Sagathewan, S., Thomas, J., Mathen, G., 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Indian J. Anim. Sci. 67, 805–811.
SAS, 1996. SAS/STAT User's Guide: Statistics, Version 6.12. Edition. SAS Inc, Cary, NC, USA.
Shi, Y., Odt, C.L., Weimer, P.J., 1997. Competition for cellulose among three predominant cellulolytic bacteria under substrate-excess and substrate-limiting conditions. Appl. Environ. Microbiol. 63, 734–742.
Singh, B., Chaudhary, L.C., Agarwal, N., Kamra, D.N., 2011. Effect of feeding *Ficus infectoria* leaves on rumen microbial profile and nutrient utilization in goats. Asian Aust. J. Anim. Sci. 24 (6), 810–817.
Steinfeld, H., Wassenaar, T., 2007. The role of livestock production in carbon and nitrogen cycles. Annu. Rev. Environ. Resour. 32, 271–294.
Thanh, K.V.T., Ørskov, E.R., 2006. Causes of differences in urinary excretion of purine derivatives in buffaloes and cattle. Br. Soc. Anim. Sci. 82, 355–358.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Vinh, N.T., Wanapat, M., Khejornsart, P., Kongmun, P., 2011. Studies of diversity of rumen microorganisms and fermentation in swamp buffalo fed different diets. J. Anim. Vet. Adv. 10 (4), 406–414.
Wanapat, M., 1999. Feeding of Ruminants in the Tropics based on Local Feed Resources. Khon Kaen, Public Company Limited, Khon Kaen, Thailand. pp. 236.
Wanapat, M., 2009. Potential uses of local feed resources for ruminants. Trop. Anim. Health Prod. 41, 1035–1049.
Wanapat, M., Pimpa, O., 1999. Effect of ruminal NH3 – N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. Asian Aust. J. Anim. Sci. 12, 904–907.
Wanapat, M., Cherdthong, A., Pakdee, P., Wanapat, S., 2008. Manipulation of rumen ecology by dietary lemongrass (*Cymbopogon citratus Stapf.*) powder supplementation. J. Anim. Sci. 86, 3497–3503.
Wanapat, M., Polyrach, S., Boonnop, K., Mapato, C., Cherdthong, A., 2009. Effect of treating rice straw with urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. Livest. Sci. 125, 238–243.
Xin, H.S., Schaefer, D.M., Liu, Q.P., Axe, D.E., Meng, Q.X., 2010. Effects of polyurethane coated urea supplement on in vitro ruminal fermentation, ammonia release dynamics and lactating performance of Holstein dairy cows fed a steam-flaked corn-based diet. Asian Aust. J. Anim. Sci. 23, 491–500.
Yu, Z., Morrison, M., 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechnol. Tech. 36, 808–812.
Yu, Z., Michel Jr., F.C., Hansen, G., Wittum, T., Morrison, M., 2005. Development and application of real-time PCR assays for quantification of genes encoding tetracycline resistance. Appl. Environ. Microbiol. 71, 6926–6933.