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Effect of Dietary Crude Protein Level on UT-B Expression and Nitrogen Efficiency in Growing Baluchi Male Lambs Fed Low or High Concentrate Diets

Research Article

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ABSTRACT

An experiment was carried out to evaluate how interactions between forage to concentrate ratio and dietary crude protein level may alter nitrogen efficiency and UT-B expression in growing Baluchi male lambs. Four Baluchi male lambs [30 ± 2 kg BW] were used in a 4 × 4 latin square design with 28-d periods and a 2 × 2 factorial arrangement of dietary treatments. The treatments fed forage: concentrate [FC; DM basis] ratios of 45:55 low concentrate (LC) or 25:75 high concentrate (HC) with dietary levels of CP of 14 low protein (LP) or 18% high protein (HP) [CP, DM basis]. Dry matter intake increased as dietary concentrate level increased. Treatments, dietary CP content and F: C ratio had significant effect on CP intake. Increasing dietary N content of the diet increased dry matter (DM), crude protein (CP) and organic matter (OM) digestibility. Forage to concentrate ratio had a significant effect on neutral detergent fiber (NDF) and OM digestibility. Treatments had significant effect on the CP, NDF and OM digestibility. There were an interaction between dietary CP content and F: C ratio on the ruminal pH, NH_3-N concentration, individual volatile fatty acids (VFA) concentration, acetate: propionate ratio and BUN concentration. Except ruminal pH, all ruminal fermentation and blood metabolite factors were affected by both dietary CP content and F: C ratio in trial. Treatments had significant influence on the NI (g/d) and urinary N excretion (g/d) (% of N intake). Also, both dietary CP content and F: C ratio had significant effect on NI. The lambs consume high crude protein treatments tended to have greater urinary N excretion (g/d) than those consume low crude protein treatments. The F: C ratio had a significant effect on urinary N excretion (g/d). Approximately 6.56 times more UT-B was expressed by the rumen ventral sac for lambs on the treatments contain 18% crude protein relative to those on the treatments contain 14% crude protein. In conclusion this study shows that changes in characteristic of the diet produce significant changes in UT-B urea transporter expression within the ovine rumen. Changing urea entry into the GIT via dietary regulation of UT-B could serve as important mechanism to maintenance of nitrogen balance and increase nitrogen efficiency in Baluchi growing lambs. Our findings suggest that the dietary regulation of urea transporters plays a major role in altering urea entry into the gastrointestinal tract.

KEY WORDS crude protein level, forage to concentrate ratio, sheep, urea transporter B expression.

INTRODUCTION

The nitrogen supply of the ruminal microorganisms in the host is essential for amino acids synthesis. During the process of urea nitrogen salvaging (UNS), urea entry into the ruminant gastrointestinal tract, (Marini and Van Amburg, 2003). It is thought that urea enters into the ruminant gastrointestinal tract via facilitative urea transporters (Stewart

and Smith, 2005). The passage of urea across cell membranes is facilitated by such transporters then the urea descends a concentration gradient (Smith and Rousselet, 2001).

The Urea Transporter-A (Slc14a2) and Urea Transporter-B (Slc14a1) genes release them to play a crucial role in the mechanism of the urinary concentration (Fenton *et al.* 2004).

Such urea transporters have been spotted in the gastrointestinal track (GIT) of many species, such as cattle (Marini and Van Amburgh, 2003; Stewart *et al.* 2005) and sheep (Ritzhaupt *et al.* 1998; Marini *et al.* 2004; Ludden *et al.* 2008). Decreasing forage to concentrate ratio in feedlot system increased dietary energy content, which would significantly provide ruminal available energy, thus increasing the utilization of NH_3-N for microbial protein synthesis. Providing higher amounts of dietary Rumen fermentable carbohydrate (RFC) is associated with increased urea-N transfer to the rumen (Kennedy and Milligan, 1980; Huntington, 1989) and increases sequestration of NH_3-N into microbial protein. Furthermore high producing ruminants are normally given high portions of dietary N so that protein requirements are sufficiently maintained (25.6 to 32.0 g of N/kg of DM; NRC, 2001). According to Marini and Van Amburgh (2003), despite high levels of N intake (25.0 to 34.0 g of N/kg of DM), about 29 to 42% of hepatic urea N output was recycled to the GIT.

Obviously there is probability for usage of urea N recycling to the GIT, even in the ruminants which are consuming high-N diets, to enhance N efficiency of ruminants. Ruminal NH_3-N concentration is negatively correlated with the rate of urea N transfer into the rumen because increased NH_3-N concentration decreases the permeability of the ruminal epithelium to urea-N (Kennedy and Milligan, 1980). Hence, the Level of the N fed, is important because it determines how much N is directed toward ruminal NH_3-N (Lapierre and Lobley, 2001; Ibrahimi *et al.* 2013). Anyway, except a few studies, there is no more information about the effect of concurrent alters in dietary forage to concentrate ratio and crude protein (CP) on urea N kinetics in ruminants which on high N diets.

Our hypothesis was that changes in the proportion of dietary N that is digested in the rumen (by varying dietary CP level) would alter urea N recycling to the rumen and that this effect would be more pronounced with decreasing forage to concentrate ratio, which could cause the high presence of ruminal starch degradation leading to the urea N recycling to the rumen. This study aimed to show how concurrent alters in dietary forage to concentrate ratio and crude protein change nutrients intake and their digestibility, ruminal fermentation, nitrogen balance, ruminal UT-B expression in Baluchi lambs.

MATERIALS AND METHODS

Animals and experimental design

Four Baluchi wether lambs [30 ± 2 kg BW] equipped with ruminal cannulas were used in a 4 × 4 Latin square design with 28-d periods (adaptation: 21 d and sampling: 7 d) and a 2 × 2 factorial arrangement. Lambs had free access to clean water over the experimental period. Due to high N requirements, it was considered to select fast growing Baluchi lambs, in current study.

Experimental treatments and feeding management

Four total mixed treatments were formulated according to two ratios of forage: concentrate [FC; DM basis] (45:55 (LC) or 25:75 (HC)) with two dietary levels of CP (14 (LP) or 18% (HP)) [CP, DM basis]. The ingredients for 4 concentrate mixtures used to formulate the experimental treatments are presented in Table 1. Treatments were offered to the animals twice daily for *ad libitum* intake (09:00 and 16:00 h). Chemical composition of the treatments, based on the determined total feed intake which measured as described in AOAC (1990), is presented in T 2.

Sample collection

During 7-d collection periods, individual lamb feed intake was recorded daily. Samples of experimental total mixed ration (TMR) and orts were collected daily, weighed, stored at -20 ˚C and composited per lamb for each experimental period before chemical analysis. Two days before the beginning of feces sampling to let adaptation, lambs equipped with special bags. A preweighed plastic container was used for collection of total daily output of urine and feces. Each day for digestibility detection a subsample (10%) of total fecal output was grabbed and dried at 55 ˚C. Urine was collected in a solution of 3.6 *M* H_2SO_4 to keep the pH < 3 to prevent bacterial growth and the loss of ammonia. To detect urine N content, at each collection, urine volume was quantified for each lamb; an aliquot (20%) was seized daily, and stored at -20 ˚C. At 0900, 1100, 1300, 1500 and 1700 h, on d 26, approximately 200 mL of ruminal contents were sampled. Ruminal pH was detected via a portable pH meter right away.

Two aliquots (10-mL) of ruminal fluid were mixed with 2 mL of metaphosphoric acid (25% wt/vol), and 10 mL of HCl 0.2 N, respectively and frozen for next analyses. Blood samples were done concurrent ruminal fluid sampling via syringe from jugular vein. Resulted plasma from centrifuged Blood samples at (1500×g for 15 min at 4 ˚C), frozen for next analysis. Two h after the morning feeding, on d 27, approximately 1 cm ruminal epithelial tissue from the ventral sac was clipped, freezed right away in liquid N, and stored at -80 ˚C.

Sample analysis

Thawed feed, refusals, and feces were dried in a forced-air oven at 60 ˚C for 48 h and ground through a 1-mm sieve before being analyzed. The DM, OM, and N contents were determined according to the AOAC (1990). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analyzed according to Van Soest *et al.* (1991). Volatile fatty acids were separated and quantified by gas chromatography. Ruminal NH_3-N was determined using distillation method. Total N in pooled urine was determined using the macro-Kjeldahl procedure (AOAC, 1990). For UT-B gene expression analysis, total RNA was extracted from a 20 to 30 mg tissue sample using a High Pure RNA Isolation Kit, followed by digestion with RNasefree DNase (High Pure RNA Isolation Kit). About, 1 µg of RNA was used to generate first-strand cDNA using cDNA Synthesis Kits. The cDNA obtained was stored at -20 ˚C until analyzed. Gene transcript abundance was quantified using real-time quantitative PCR using SYBR Green fluorescence detection. The primers used for urea transporter-B (UT-B) and ovine glyceraldehyde 3-phosphate dehydrogenase (ovine GAPDH; NCBI Accession No. BC102589) were previously reported (Stewart *et al.* 2005; Ludden *et al.* 2009). Ovine GAPDH was used as an internal reference to normalize UT-B mRNA expression. Briefly, the PCR primers were UT-B (forward, 5′-ggacctgcctgtcttcactc-3′; reverse, 5′-gatcaaggtgcttgggaaaa-3′) and ovine GAPDH (forward, 5′-gattgtcagcaatgcctcct-3′; reverse, 5′-ggtcataagtccctccacga-3′) with amplicon size of 97 and 94 bp, respectively. Amplification conditions for ovine GAPDH and UT-B included a predwell for 3 min at 95 ˚C and 35 cycles of denaturing for 30 s at 95 ˚C and annealing for 30 s at 58 ˚C. The real-time qPCR reaction mixture used for each gene consisted of 12.5 µL of Maxima SYBR Green qPCR Master Mixes, 0.5 µL of each primer (25 µ*M*), and 1.0 µL of template cDNA, made up to 25 µL. The amplification efficiency was 100.1%.

Statistical analysis

Data were applied to the mixed model (SAS, 2004) of with the following statistical model of:

$$Y_{ijklm} = \mu + P_i + L_j + A_k + B_l + (AB)_{il} + \varepsilon_{ijklm}$$

Where:

Y_{ijklm}: dependent variable.

μ: overall mean.

P_i: period effect.

L_j: lamb effect.

A_k: effect of forage to concentrate ratio (fixed effect).

B_l: effect of CP level (fixed effect).

$(AB)_{kl}$: interaction between effect of forage to concentrate ratio and effect of CP level.

ε_{ijklm}: residual error.

Treatments were considered as a fixed effect and period and animal were considered as random effects. Differences between least squares means were considered significant at ($P<0.05$), using PDIFF in the LSMEANS statement.

RESULTS AND DISCUSSION

Diet characteristics

Table 2 presents the chemical compositions of experimental treatments. Experimental TMR contained 14 or 18% crude protein (as % of DM; Table 2). Slight deviation between dietary CP and actual CP levels, data were shown in Table 2. According to the NRC (1985), normal growth rate lambs CP requirement drop from 16.7% to 14.7% for lambs 20-40 kg. Because Baluchi lambs have the potential for acceptable growth rates the, optimum dietary CP for fattening must be maintained. Drouillard *et al.* (1991) observed a 7% increase in dry matter intake (DMI) when lambs were fed a 14.5% CP diet compared with an 8.9% CP diet. Fluharty and McClure (1997) also observed an increase in DMI when lambs were fed a high protein diet (18.9% CP) compared with a lower CP diet (14.5% CP). According to the NRC (1985) recommendations, in this experiment, the CP level of experimental treatments considered as 14 or 18% to obtain optimum dietary CP for fattening.

Nutrient intake and total tract nutrient digestibilities

As shown in Table 3, dry matter intake was not affected by interaction between crude protein content and forage to concentrate ratio (P=0.15). The forage to concentrate ratio had a significant effect on dry matter intake (P=0.01). Treatments had Significant effect on crude protein intake (P=0.001). Also, both crude protein content (P<0.01) and forage to concentrate ratio (P=0.01) had Significant effect on crude protein intake. No significant differences between treatments were found in either dry matter (P=0.14) or ADF digestibility (P=0.44).

However crude protein (P=0.05) NDF (P=0.002) and organic matter (P=0.001) digestibility were affected by interaction between crude protein content and forage to concentrate ratio. Besides, forage to concentrate ratio had a significant effect on NDF (P<0.01) and organic matter (P=0.006) digestibility. Significant differences were observed for dry matter (P=0.001), crude protein (P=0.007) and organic matter (P<0.001) digestibility in lambs fed treatments contain 18% crude protein compared to the lambs fed treatments contain 14% crude protein.

Table 1 Ingredient of total mix rations with F: C (45:55 and 25:75) containing low (14, % of DM) or high (18, % of DM) crude protein

Items	14% CP		18% CP	
	Low concentrate	High concentrate	Low concentrate	High concentrate
Ingredients, % DM				
Alfalfa hay	45	24.99	45	25
Barley grain	25	24.99	25	25
Canola meal	-	-	19	19
Wheat barn	29	48.22	10	30
Limestone	0.2	1	0.2	0.2
Mineral, vitamin supplement	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3

CP: crude protein and DM: dry matter.

Table 2 Chemical composition of total mix rations with F: C (45:55 and 25:75) containing low (14, % of DM) or high (18, % of DM) crude protein

Items	14% CP		18% CP	
	Low concentrate	High concentrate	Low concentrate	High concentrate
Chemical composition				
DM, %	90.3	90.5	90	90.6
CP, % DM	14	13.9	17.8	17.8
NDF, % DM	39.1	40.1	34.6	36
ADF, % DM	19.34	14.5	19.9	14.6
NFC, % DM	38.9	37.3	39.8	38.5
Fat, % DM	7.7	8	7.7	7.3
Ash, % DM	3.3	3.5	3.1	3.3
ME, Mcal/kg	2.35	2.38	2.39	2.40

CP: crude protein; DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber and NFC: nonfiber carbohydrates.

Table 3 Value of nutrient intake and total track nutrient digestibilities in growing Baluchi male lambs fed treatments with forage: concentrate ratio with 45:55 (LC) or 25:75 (HC) containing 14% or 18% crude protein (CP, % DM)

Item	CP level (%)					Main effects				P-value		
	14		18			F:C ratio		CP level (%)		F:C	P	E × P
	LC	HC	LC	HC	SEM	LC	HC	14	18			
Intake, (g/d)												
DM	743.48	789.14	720.48	789.64	19.60	731.9	789.3	766.3	755.0	0.01	0.58	0.15
CP	103.27[a]	111.20[a]	133.18[b]	144.28[b]	03.10	118.2	127.7	107.2	138.7	0.01	< 0.001	0.001
Digestibility, %												
DM	65.95	66.60	66.68	70.71	1.03	66.3	68.6	66.2	69.6	0.22	0.001	0.14
CP	67.99[a]	70.20[a]	74.53[b]	77.78[b]	1.32	71.2	73.9	69.0	76.1	0.07	0.007	0.05
NDF	38.11[a]	34.03[b]	38.75[a]	34.51[b]	0.34	38.4	34.2	36.0	36.6	< 0.001	0.1	0.002
ADF	32.86	27.19	26.78	25.36	2.55	29.8	26.2	30.0	26.0	0.2	0.1	0.44
OM	69.01[a]	69.75[a]	71.60[b]	73.46[c]	0.23	70.3	71.6	69.3	72.5	0.006	< 0.001	0.001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
LC: low concentrate; HC: high concentrate; CP: crude protein; DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber and OM: organic matter.
SEM: standard error of the means.

High concentrate treatments usually have lower cell wall content, higher ruminal degradability and faster digestion and passage rates compared to grasses (Mertens, 1997). Thus, grater dietary NDF and ADF content, reduce dry matter intake in lambs on low concentrate diets. The grater crude protein intake in lambs received the treatments contain 18% crude protein have been attributed to greater dietary protein level.

In the high concentrate treatments, higher level of soluble substrates could be the reason for improving NDF and organic matter digestibility. In present study, NDF and organic matter digestibility trended to the highest level at 75% concentrate in treatments, which indicated that, increase in concentrate up to 75% in diet could improve nutrient digestibility.

Dry matter, organic matter and crude protein digestibility were higher (P<0.05) for lambs fed treatments contain 18% crude protein. These data are similar to the findings of Manso *et al.* (1998) who observed an increase in dry matter, organic matter and crude protein digestibility when lambs were fed two treatments differing in their crude protein content (168 g per day *vs.* 86 g per day). Bunting *et al.* (1987) and Sultan and Loerch (1992) obtained similar results. This result implies that the lambs have high nutrient uptake when fed treatments contain 18% crude protein. If crude protein supply is lower than the minimum required for microbial growth, intake may be restricted because of depressed ruminal digestion. Feeding more crude protein increases the deamination of amino acid in the rumen and the supply of branched-chain volatile fatty acids (VFA),

which may improve nutrient digestion (Misra and Thakur, 2001).

Ruminal fermentation and blood metabolite

The ruminal pH and NH_3-N, BUN, VFA concentration and acetate: propionate ratio data are shown in Table 4. There were an interaction between crude protein content and forage to concentrate ratio on the ruminal pH (P=0.001), NH_3-N concentration (P<0.001), acetate (P=0.02), propionate (P=0.02), butyrate concentration (P=0.002), acetate: propionate ratio (P=0.04) and BUN concentration (P<0.001). The ruminal pH (P=0.05), NH_3-N concentration (P<0.001), acetate (P=0.001), propionate (P=0.003), butyrate concentration (P=0.001), acetate: propionate ratio (P<0.001) and BUN concentration (P<0.05) were affected either by forage: concentrate ratio through the trial. Significant differences were recorded for NH_3-N concentration (P<0.001), acetate (P=0.01), propionate (P=0.002), butyrate concentration (P=0.001), acetate: propionate ratio (P=0.005) and BUN concentration (P<0.003) in lambs fed treatments contain 18% crude protein compared to the lambs fed treatments contain 14% crude protein.

The pH value decreased as the amount of concentrate in diet increased, which was similar to findings from Kumar *et al*. (2013). However, the pH value was not affected by crude protein content. This results is in agreement with previous *in vivo* studies (Promkot and Wanapat, 2005; Chantiratikul *et al*. 2009; Chen *et al*. 2010), which reported that pH was not significantly affected by increasing crude protein level. Except 2 h after feeding (P=0.004), time intervals had no significant effect on ruminal pH (Figure 1). A general increase in rumen pH was observed before the morning feeding at 09:00 as can be seen in Figure 1. This may be due to increased rumination during night time, roughage intake in the early mornings (Bae *et al*. 1979) and N influx into the rumen when nitrogen concentrations were low. Rumination stimulates saliva production and therefore, an increase in pH may occur due to the buffering capacity of saliva (Maekawa *et al*. 2002).

A decline in rumen pH was observed after 11:00 (Figure 1). A combination of decreased forage intake and the high availability of fermentable energy from starch degradation and consequently a decrease in rumen NH_3-N due to utilization of rumen NH_3-N by rumen microbes for microbial growth and microbial protein synthesis, could be the reasons for the decline in rumen pH after 11:00. This greater change in pH was also accompanied by a much greater change in total concentration of VFA. Because Roughage tends to have a more stable pH as a result of a slow fermentation and digestion rate (Chapaval *et al*. 2008; Ribeiro *et al*. 2011). The finding of current study is same with pervious result (Lindberg, 1983; Suarez *et al*. 2007) which noticed that raising forage: concentrate ratio, caused an increase in ruminal pH. A slight increase in pH was observed from 15:00 until 17:00 (Figure 1). The increase in pH followed after 15:00 could be due to the afternoon feeding taking place at 16:00, resulting in increased saliva production during intake.

Crude protein concentration is the primary reason for observed difference in NH_3-N concentration among treatments with different crude protein content. Increase in deamination of amino acid in response to rise of CP content in treatments may cause elevate concentrations of NH_3-N. Treatments with lower forage to concentrate ratio differed numerically from other treatments with higher forage to concentrate ratio before start feeding (Figure 2). The lower NH_3-N concentration observed for treatments with lower forage to concentrate ratio before feeding could have been due to better availability of nitrogen to the rumen bacteria and improved NH_3- N utilization by rumen bacteria in the rumen (Wahrmund *et al*. 2007). The rumen NH_3-N concentration at 2 hours after feeding was significantly higher than other sampling intervals after feeding for all treatments (P=0.001) (Figure 2). Therefore, indicating that the rumen NH_3-N concentration only started to increase significantly at 2 hours after feeding for all treatments. This may be due to higher crude protein intake and higher digestibility of crude protein especially in the treatments contain 18% crude protein (Table 3). The NH_3-N concentrations of the treatments containing lower levels of crude protein increased at a slower rate than those containing higher percentage of crude protein. A considerable decrease in NH_3-N concentration was observed for all treatments from 4-8 hour after feeding (P=0.01). The NH_3-N reduction monitored might have been due to synchronization between soluble carbohydrate and NH_3-N which increase microbial protein synthesis. This data is agreement with result were found by (Santoso *et al*. 2004; Hristov *et al*. 2005; Lee *et al*. 2006; Suarez *et al*. 2007).

Treatments with lower forage to concentrate ratio had a significant impact on in VFA concentration due to more digestible organic matter content. The molar proportion of acetate and acetate to propionate ratio decreased with increasing concentrate level in treatments with lower forage to concentrate ratio, whereas the proportions of propionate and butyrate increased as dietary roughage was replaced by concentrate (Table 4) (Archimède *et al*. 1996). This is consistent with the results of Miettinen and Huhtanen (1996), who reported that a higher proportion of dietary concentrate would result in higher propionic acid in rumen and a reduced ratio of acetic acid and propionic acid. According to Currier *et al*. (2004), high concentrations of acetate are characteristic of high forage diets. Acetate production increased with increasing levels of fiber in the diet.

Table 4 Value of pH, concentration of NH_3-N and blood urea nitrogen (BUN), molar proportion of individual volatile fatty acids (VFA) and acetate: propionate ratio in growing Baluchi male lambs fed treatments with forage: concentrate ratio with 45:55 (LC) or 25:75 (HC) containing 14% or 18% crude protein (CP, % DM)

Item	CP level (%)					Main effects				P-value		
	14		18			F:C ratio		CP level (%)		F:C	P	E × P
	LC	HC	LC	HC	SEM	LC	HC	14	18			
pH	6.68[a]	6.36[b]	6.62[a]	6.35[b]	0.08	6.65	6.35	6.52	6.48	0.05	0.18	0.001
NH_3-N, (mg/dL)	21.44[c]	16.05[a]	25.18[d]	20.10[b]	0.25	23.3	18.0	18.7	22.6	< 0.001	< 0.001	< 0.001
VFA, (mol/100 mol)												
Acetate	67.55[a]	62.25[b]	69.72[a]	65.45[b]	0.82	68.6	63.8	64.9	67.5	0.001	0.01	0.02
Propionate	16.82[a]	19.05[b]	17.80[a]	22.85[b]	0.47	17.3	20.9	17.9	20.3	0.003	0.002	0.02
Butyrate	11.37[a]	12.35[b]	11.95[a]	13.40[b]	0.13	11.6	12.8	11.8	12.6	0.001	0.001	0.002
Acetate / propionate	04.01[a]	03.26[b]	03.91[a]	02.87[b]	0.05	3.96	3.06	3.63	3.39	< 0.001	0.005	0.04
BUN, (mg/dL)	28.00[a]	23.25[a]	38.25[b]	35.75[b]	1.48	33.1	29.5	25.6	37.0	0.05	0.003	< 0.001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
LC: low concentrate; HC: high concentrate; CP: crude protein and DM: dry matter.
SEM: standard error of the means.

Other studies also indicated that butyric acid concentrations increased significantly in the rumen with increasing proportions of dietary concentrate (Wang *et al*. 2005; Sun *et al*. 2008).

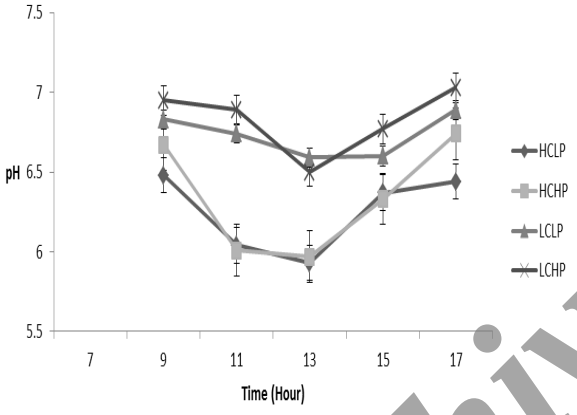

Figure 1 Trend of rumianl pH in growing Baluchi male lambs fed treatments with forage: concentrate ratio with 45:55 (LC) or 25:75 (HC) containing 14% or 18% crude protein (CP, % DM)

Blood urea nitrogen is highly correlated with ruminal ammonia (Thornton, 1970; Hammond, 1983; Hennessi and Nolan, 1988). Therefore, increasing dietary crude protein leads to an increase in BUN. It was expected that an Increase in dietary energy intake while holding protein intake constant would decrease BUN (Chase *et al*. 1993). The impact of increased level of intake on BUN concentration seems not to be different from the effect associated with increased energy intake. In this study lambs received treatments contain 18% crude protein consumed more crude protein compared to other lambs.

Nitrogen balance

There were an interaction between crude protein content and forage to concentrate ratio on the nitrogen intake (g/d) (P<0.001) and urinary N excretion (g/d) (% of N intake) (P<0.001) (Table 5). However fecal N excretion (g/d) and retained N were not affected by interaction between crude protein content and forage to concentrate ratio in trial. However, all experimental animals were in positive N balance. Also, both crude protein content (P<0.01) and forage to concentrate ratio (P=0.01) had Significant effect on nitrogen intake (Table 5).

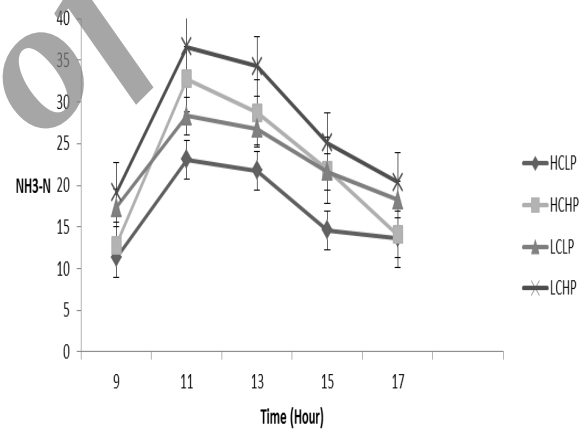

Figure 2 Trend of rumianl NH_3-N in growing Baluchi male lambs fed treatments with forage: concentrate ratio with 45:55 (LC) or 25:75 (HC) containing 14% or 18% crude protein (CP, % DM)

The lambs fed treatments contain 18% crude protein and lambs fed treatments with lower forage to concentrate ratio had significantly greater nitrogen intake compared to those fed treatments contain 14% crude protein and lambs fed treatments with higher forage to concentrate ratio, respectively. The lambs consume high crude protein treatments tended to have greater urinary N excretion (g/d) than those consume low crude protein treatments (P<0.001; Table 5). The forage to concentrate ratio had a significant effect on urinary N excretion (g/d) (P<0.001; Table 5). Higher nitrogen intake by lambs fed treatments contains 18% crude protein and treatments with lower forage to concentrate ratio might be due to higher crude protein intake.

Table 5 Value of N intake, fecal N excretion, urinary N excretion and N retention in growing Baluchi male lambs fed treatments with forage: concentrate ratio with 45:55 (LC) or 25:75 (HC) containing 14% or 18% crude protein (CP, % DM)

Item	CP level (%)					Main effects				P-value		
	14		18			F:C ratio		CP level (%)		F:C	P	E × P
	LC	HC	LC	HC	SEM	LC	HC	14	18			
N intake												
(g/d)	16.48[a]	17.77[a]	21.32[b]	23.04[b]	0.25	18.9	20.4	17.1	22.1	0.01	< 0.001	< 0.001
Fecal N excretion												
(g/d)	5.27	5.32	5.42	5.12	0.29	5.35	5.22	5.29	5.27	0.68	0.94	0.91
% of NI	31.94[a]	29.91[a]	25.94[b]	22.24[b]	1.34	28.7	26.0	30.9	23.8	0.08	0.008	0.05
Urinary N excretion												
(g/d)	8.23[a]	9.27[b]	11.95[c]	13.80[d]	0.05	10.0	11.5	8.75	12.8	< 0.001	< 0.001	< 0.001
% of NI	50.37[a]	52.33[a]	56.24[ab]	60.06[b]	1.58	53.3	56.1	51.3	58.1	0.1	0.002	0.05
N retention												
(g/d)	2.96	3.17	3.93	4.11	0.47	3.44	3.64	3.06	4.02	0.69	0.07	0.42
% of NI	17.69	17.74	18.21	17.69	2.01	17.9	17.7	17.7	17.9	0.91	0.90	0.64

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
LC: low concentrate; HC: high concentrate; CP: crude protein and DM: dry matter.
SEM: standard error of the means.

Usually, if feed intake is not depressed, fecal N remains similar regardless of the N content of the diet (Siddons *et al*. 1985; Marini and Van Amburgh, 2003; marini *et al*. 2004). In current study, treatments were offered to the lambs for *ad libitum* intake and no difference (P=0.15) was observed in the dry matter intake between treatments. In current study, increasing the crude protein content from 14 to 18% increased urinary N excretion from 8.75 to 12.8 (g/d).

This finding is in agreement with Sannes *et al*. (2002) which reported increasing in urinary N excretion when dietary crude protein content increased. The treatments with lower forage to concentrate ratio had grater nitrogen digestibility compared to treatments with higher forage to concentrate ratio (Table 3). The greater nitrogen intake and its digestibility in lambs on treatments contain 18% crude protein could have caused numerically higher N balance (Legleiter *et al*. 2005). Numerically lower N balance in lambs fed treatments contain 14% crude protein may possibly because of reduced dry matter and crude protein digestibility (Woods *et al*. 1962).

Expression of UT-B

As shown in Table 6, expression of urea transporter-B mRNA (expressed as copies/copy of ovine GAPDH) was not affected by interaction between crude protein content and forage to concentrate ratio (P=0.63). Also, forage to concentrate ratio could not impact on the expression of urea transporter-B mRNA (P=0.2; Table 6). However, the results of expression of urea transporter-B mRNA in ruminal mucosa biopsies demonstrated that UT-B were expressed more when lambs were fed HP treatments (P=0.002). Approximately 6.56 times more UT-B was expressed by the rumen ventral sac for lambs on the treatments contain 18% crude protein relative to those on the treatments contain 14% crude protein.

Ritzhaupt *et al*. (1998) by using RT-PCR techniques confirm the presence of UT-B in the sheep rumen. Stewart *et al*. (2005) also verified the presence of UT-B in the rumen by locating a 43- to 54-kDa UT-B protein band, describing the bovine UT-B gene and utilizing RT-PCR to detect UT-B mRNA.

Regulation of ruminal UT-B expression by diet current study confirm pervious results were found by (Marini and Van Amburgh, 2003) who noticed expression of ruminal UT-B was changed by nitrogen intake levels. Marini and Van Amburgh (2003) observed greater expression of UT-B (based upon visual evaluation) in ruminal papillae collected from the ventral sac of the rumen in dairy heifers fed high-N diets (2.97 to 3.4% N) compared with low-N diets (1.45 to 1.89% N). Marini and Van Amburgh (2003) suggested that when a high-N diet is fed and ruminal ammonia is high, urea diffuses into the gastrointestinal tract via the paracellular space. Urease activity is known to be reduced by high ammonia concentrations (Bunting *et al*. 1989), a condition that arises when high-N diets are fed. Therefore, it is possible that the amount of crude protein fed to lambs receiving the treatments contain 18% crude protein daily was sufficient to increase UT-B abundance in the ventral rumen (Ludden *et al*. 2009). Moreover, expression of UT-B may influenced by other dietary elements, such as VFA and pH (Simmons *et al*. 2009; Abdoun *et al*. 2010). Engelhardt *et al*. (1978) reported that ruminal VFA, particularly butyrate, has a stimulatory effect on urea-N transfer across the ruminal epithelium. Simmons *et al*. (2009) reported a higher bUT-B2 mRNA and protein expression in steers fed concentrate-based diet compared to those fed silage-based diet. In that study, ruminal butyrate concentration was numerically higher (9.3 *vs*. 11.7 as % of total VFA) in steers fed concentrate-based compared to silage-based diet and, may play a role in expression of bUT-B2 thus, increasing urea-N transfer into the rumen.

Table 6 The expression of urea transporter-B mRNA (fold change) in the ventral rumen in growing Baluchi male lambs fed treatments with forage: concentrate ratio with 45:55 (LC) or 25:75 (HC) containing 14% or 18% crude protein (CP, % DM)

Item	CP level (%)					Main effects				P-value		
	14		18			F:C ratio		CP level (%)		F:C	P	E × P
	LC	HC	LC	HC	SEM	LC	HC	14	18			
Ventral ruman												
Fold change	1	3.73	5.91	7.21	1.42	3.45	5.47	2.36	6.56	0.2	0.02	0.63

LC: low concentrate; HC: high concentrate; CP: crude protein and DM: dry matter.
SEM: standard error of the means.

These results are in agreement with the finding of current study, because the butyrate concentration in lambs on the treatments contains 18% crude protein significantly higher than those fed the treatments contain 18% crude protein. On the other hand, earlier work of Rémond *et al.* (1993) showed that shifts in the ruminal pH relative to fed and fasting state of an animal may play a role in urea-N transfer across the ruminal epithelium.

Recently, Abdoun *et al.* (2010) demonstrated *in vitro* using isolated ruminal epithelium in Using chambers that in presence of short-chain fatty acids, reducing ruminal mucosal buffer pH from 7.4 to 5.4 showed a bell-shaped curve for urea transport from serosal to mucosal direction with highest rate of urea transport between pH 6.0 to 6.4. If the ruminal pH is approximately in the range of 6.0 to 6.4, the range which is typically observed under *in vivo* physiological conditions in the rumen, changing the ruminal factors (e.g., VFA) may have a positive impact on urea-N recycling to the rumen (Abdoun *et al.* 2010). The ruminal pH in lower forage to concentrate ratio was 6.35 and because it falls in the optimum range of pH for urea transporting, reduce the ruminal concentration of NH_4^+ (less lipid soluble). This effect combined with the effect of high crude protein content on the urease activity and butyrate concentration and thus increase urea-N transfer into the rumen in lambs specially fed lower forage to concentrate ratio treatment contain 18% crude protein.

CONCLUSION

Briefly, lambs on lower forage to concentrate ratio treatment contain 18% crude protein, consume more N (g/d) compared to the other diets. However, no significant differences were observed in fecal N excretion between treatments. Thus, lambs on lower forage to concentrate ratio treatment contain 18% crude protein, conserved more N (g/d) numerically compared to the other diets. In addition, UT-B were expressed numerically more when lambs were fed lambs on lower forage to concentrate ratio treatment contain 18% crude protein. Changing urea entry into the GIT via dietary regulation of UT-B could serve as important mechanism to maintenance of nitrogen balance and increase nitrogen efficiency in Baluchi growing lambs.

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REFERENCES

Abdoun K., Stumpff F., Rabbani I. and Martens H. (2010). Modulation of urea transport across sheep rumen epithelium *in vitro* by SCFA and CO_2. *Am. J. Physiol. Gastrointest. Liver Physiol*. **298,** 190-202.

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Archimède H., Sauvant D., Hervieu J., Ternois F. and Poncet C. (1996). Effects of the nature of roughage and concentrate and their proportion on ruminal characteristics of non-lactating goats, consequences on digestive interactions. *Anim. Feed Sci. Technol*. **58,** 267-282.

Bae D.H., Welch J.G. and Smith A.M. (1979). Forage intake and rumination by sheep. *J. Anim. Sci*. **49,** 1292-1299.

Bunting L.D., Boiling J.A., Mackown C.T. and Muntifering R.B. (1987). Effect of dietary protein level on nitrogen metabolism in lambs: studies using 15 N nitrogen. *J. Anim. Sci*. **64,** 855-867.

Bunting L.D., Boling J.A., MacKown C.T. and Davenport G.M. (1989). Effect of dietary protein level on nitrogen metabolism in the growing bovine: II. Diffusion into and utilization of endogenous urea nitrogen in the rumen. *J. Anim. Sci*. **67,** 820-826.

Chantiratikul A., Chumpawadee S., Kanchanamayoon W. and Chantiratikul P. (2009). Effect of dietary protein on nutrient digestibility and nitrogen metabolism in thai-indigenous heifers. *J. Anim. Vet. Adv*. **8,** 297-300.

Chapaval L., Melotti L., Junior P.R., Olivindo C.S. and Rego J.P.A. (2008). Roughage / concentrate ratio on ruminal ammonia concentration, pH and volatile fatty acids in crossbred dairy cows. *Revista Bras Saúde Prod. Anim*. **9,** 18-28.

Chase C.C., Larsen J.R., Hammond R.E. and Randel R.D. (1993). Effect of dietary energy on growth and reproductive characteristics of Angus and Senepol bulls during summer in Florida. *Theriogenology*. **40,** 43-67.

Chen S., Paengkoum P., Xia X. and Na-Lumpang P. (2010). Effects of dietary protein on ruminal fermentation, nitrogen utili

zation and crude protein maintenance in growing Thaiindigenous beef cattle fed rice straw as roughage. *J. Anim. Vet. Adv*. **9,** 2396-2400.

Currier T.A., Bohnert D.W., Falck S.J., Schauer C.S. and Bartle S.J. (2004). Daily and alternate-day supplementation of urea and biuret to ruminants consuming low-quality forage: II. Effects on site of digestion and microbial efficiency in steers. *J. Anim. Sci*. **82,** 1518-1527.

Drouillard J.S., Klopfenstein T.J., Britton R.A., Bauer M.L., Gramlich S.M., Wester T.J. and Ferrell C.L. (1991). Growth, body composition, and visceral organ mass and metabolism in lambs during and after metabolizable protein or net energy restriction. *J. Anim. Sci*. **69,** 3357-3375.

Engelhardt W.V., Hinderer S. and Wipper E. (1978). Factors influencing the endogenous urea-N secretion and utilization in the gastrointestinal tract. Pp. 401-412 in Ruminant Digestion and Feed Evaluation. D.F. Osbourn, D.E. Beever and D.J. Thomson, Eds. ARC, London.

Fenton R.A., Chou C.L., Stewart G.S., Smith C.P. and Knepper M.A. (2004). Urinary concentrating defect in mice with selective deletion of phloretin-sensitive urea transporters in the renal collecting duct. Pp. 7469-7474 in Proc. National Acad. Sci., USA.

Fluharty F.L. and McClure K.E. (1997). Effect of dietary energy intakes and protein concentration on performance and visceral organ mass in lambs. *J. Anim. Sci*. **75,** 604-610.

Hammond A.C. (1983). Effect of dietary protein level, ruminal protein solubility and time after feeding on plasma urea nitrogen and the relationship of plasma urea nitrogen to other ruminal and plasma parameters. *J. Anim. Sci*. **57(1),** 435.

Hristov A.N., Ropp J.K., Grandeen K.L., Abedi S., Etter R.P., Melgar A. and Foley A.E. (2005). Effect of carbohydrate source on ammonia utilization in lactating dairy cows. *J. Anim. Sci*. **83,** 408-421.

Huntington G.B. (1989). Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. *Can. J. Anim. Sci*. **69,** 215-223.

Ibrahimi Khoram Abadi E., Tahmasbi A.M., Danesh Mesgaran M. and Valizadeh R. (2011). Influence of protein sources with different degradability on performance, ruminal fermentation, blood metabolites and protozoal population in lactating dairy cows. *J. Anim. Vet. Adv*. **10(1),** 43-49.

Kennedy P.M. and Milligan L.P. (1980). The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. *Can. J. Anim. Sci*. **60,** 205-221.

Kumar S., Dagar S.S., Sirohi S.K., Padhyay R.C. and Puniya A.K. (2013). Microbial profiles, *in vitro* gas production and dry matter digestibility based on various ratios of roughage to concentrate. *Ann. Microb*. **63,** 541-545.

Lapierre H. and Lobley G.E. (2001). Nitrogen recycling in the ruminant: a review. *J. Dairy Sci*. **84,** 223-236.

Lee M.R.F., Tweed J.K.S., Dewhurst R.J. and Scollan N.D. (2006). Effect of forage: concentrate ratio on ruminal metabolism and duodenal flow of fatty acids in beef steers. *Anim. Sci*. **82,** 31-40.

Legleiter L.R., Mueller A.M. and Kerley M.S. (2005). Level of supplemental protein does not influence the ruminally unde gradable protein value. *J. Anim. Sci*. **83,** 863-870.

Lindberg J.E. (1983). Factor affecting predictions of rumen degradability using the nylon bag (*in sacco*) technique and a comparison between *in vivo* and in sacco degradability measurements. Ph D. Thisis. Swedish Univ. Agric. Sci. Uppsala, Sweden.

Ludden P.A., Stohrer R.M., Austin K.J., Atkinson R.L., Belden E. and Harlow H.J. (2009). Effect of protein supplementation on expression and distribution of urea transporter B in lambs fed low-quality forage. *J. Anim. Sci*. **87,** 1354-1365.

Maekawa M., Beauchemin K.A. and Christensen D.A. (2002). Effect of concentrate level and feeding management on chewing activities, saliva production and ruminal pH of lactating dairy cows. *J. Dairy Sci*. **85,** 1165-1175.

Manso T., Mantecón A.R., Giráldez F.J., Lavín P. and Castro T. (1998). Animal performance and chemical body composition of lambs fed diets with different protein supplements. *Small Rumin. Res*. **29,** 185-191.

Marini J.C., Klein J.D., Sands J.M. and Van Amburgh M.E. (2004). Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. *J. Anim. Sci*. **82,** 1157-1164.

Marini J.C. and Van Amburgh M.E. (2003). Nitrogen metabolism and recycling in Holstein heifers. *J. Anim. Sci*. **81,** 545-552.

Mertens D.R. (1997). Dietary fiber components: Relationship to the rate and extent of ruminal digestion. *Fed. Pro*. **36,** 87-94.

Miettinen H. and Huhtanen P. (1996). Effects of the ratio of ruminal propionic acid to butyrate on milk yield and blood metabolites in dairy cows. *J. Dairy Sci*. **79(5),** 851-861.

Misra A.K. and Thakur S.S. (2001). Effect of dietary supplementation of sodium salt of isobutyric acid on ruminal fermentation and nutrient utilization in a wheat straw based low protein diet fed to crossbred cattle. *Asian-austral J. Anim. Sci*. **14,** 479-484.

NRC. (1985). Nutrient Requirement of Sheep. 6th Ed. National Academy Press, Washington, DC, USA.

NRC. (2001). Nutrient Requirements of Dairy Cattle. 7th Ed. National Academy Press, Washington, DC, USA.

Promkot C. and Wanapat M. (2005). Effect of level of crude protein and use of cottonseed meal in diets containing cassava chips and rice straw for lactating dairy cows. *Asian-australas J. Anim. Sci*. **18,** 502-511.

Rémond D., Chaise J.P., Delval E. and Poncet C. (1993). Net transfer of urea and ammonia across the ruminal wall of sheep. *J. Anim. Sci*. **71,** 2785-2792.

Ribeiro S.S., Vasconcelos J.T., Morais M.G., Itavo C.B.C.F. and Franco G.L. (2011). Effects of ruminal infusion of a slow-release polymer-coated urea or conventional urea on apparent nutrient digestibility, *in situ* degradability and rumen parameters in cattle fed low quality hay. *Anim. Feed Sci. Technol*. **164,** 53-61.

Ritzhaupt A., Wood I.S., Jackson A.A., Moran B.J. and Shirazi-Beechey S.P. (1998). Isolation of a RT-PCR fragment from human colon and sheep rumen RNA with nucleotide sequence similarity to human and rat urea transporter isoforms. *Biochem. Soc. Trans*. **26,** 40.

Sannes R.A., Messman M.A. and Vagnoni D.B. (2002). Form of

rumen-degradable carbohydrate and nitrogen on microbial protein synthesis and protein efficiency of dairy cows. *J. Dairy Sci*. **85,** 900-908.

Santoso B., Mwenya B., Sar C., Gamo Y., Kobayashi T., Morikawa R. and Takahashi J. (2004). Effect of Yucca schidigera with or without nisin on ruminal fermentation and microbial protein synthesis in sheep fed silage and hay based diets. *Anim. Sci. J*. **6,** 525-531.

SAS Institute. (2004). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Siddons R.C., Nolan J.V., Beever D.E. and MacRae J.C. (1985). Nitrogen digestion and metabolism in sheep consuming diets containing contrasting forms and levels of N. *Br. J. Nutr*. **54,** 175-187.

Simmons N.L., Chaudhry A.S., Graham C., Scriven E.S., Thistlethwaite A., Smith C.P. and Stewar G.S. (2009). Dietary regulation of ruminal bovine UT-B urea transporter expression and localization. *J. Anim. Sci*. **87,** 3288-3299.

Smith C.P. and Rousselet G. (2001). Facilitative urea transporters. *J. Membr. Biol*. **183,** 1-14.

Stewart G.S. and Smith C.P. (2005). Urea nitrogen salvage mechanisms and their relevance to ruminants, non-ruminants and man. *Nutr. Res. Rev*. **18,** 49-62.

Suarez B.J., Van Reenen C.G., Stockhofe N., Dijkstra J. and Gerrits W.J.J. (2007). Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. *J. Dairy Sci*. **90,** 2390-2403.

Sultan I.S. and Loerch C. (1992). Effects of protein and energy supplementation of wheat starw-based treatments on site and nutrient digestion and nitrogen metabolism of lambs. *J. Anim. Sci*. **70,** 2228-2234.

Sun D., Wei Z.M., Bao L. and Masahiro O. (2008). Effects of total mixed ration with different forage to concentrate ratios on rumen index of dairy cows. *Feed Res*. **10,** 47-50.

Thornton R.F. (1970). Factors affecting the urinary excretion of urea nitrogen in cattle. II. The plasma urea nitrogen concentration. *Asian-australas J. Agric. Res*. **21,** 145-152.

Van Soest P.J., Robertson J.B. and 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.

Wahrmund J., de Araujo D.V., Hersom M. and Arthington J. (2007). Evaluation of optigen II as a source of rumen degradable protein for mature beef cows. *J. Anim. Sci*. **85,** 28.

Wang J., Wang Li J.S., Wang S., Yao M. and Liu S. (2005). Effects of forage to concentrate ratio on pattern of rumen fermentation and performance of lactating dairy cows. *Acta Vet. Zootech. Sin*. **36(6),** 569-573.

Woods W.R., Richardson H., Kruse K., Gallup W.D. and Tillman A.D. (1962). Further studies on the nutritive value of cotton seed meal for ruminants. *J. Anim. Sci*. **21,** 284-289.

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اثر سطوح مختلف پروتئین خام بر بیان ژن ناقل اوره (نوع ب) و بازده نیتروژن در بره‌های در حال رشد بلوچی تغذیه شده با نسبت‌های مختلف علوفه به کنسانتره

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چکیده

به منظور بررسی اثرات همزمان نسبت‌های مختلف علوفه به کنسانتره و مقادیر مختلف پروتئین‌خام خوراک بر کنترل نیتروژن اوره‌ای بازگردانده شده به شکمبه و تنظیم بیان ژن ناقل اوره (نوع ب)، از چهار رأس بره نر بلوچی (۲±۳۰ کیلوگرم) در قالب طرح مربع لاتین ۴ × ۴، استفاده شد. مدت هر دوره آزمایشی ۲۸ روز (۲۱ روز عادت پذیری و ۶ روز جمع‌آوری اطلاعات و نمونه برداری) بود. تیمارهای آزمایشی از ترکیب دو نسبت کنسانتره به علوفه (بر اساس ماده خشک) ۴۵ به ۵۵ درصد و ۲۵ به ۷۵ درصد و دو مقدار پروتئین خام (بر اساس ماده خشک) ۱۴ درصد و ۱۸ درصد تشکیل شده بودند. با افزایش نسبت کنسانتره به علوفه، مصرف ماده خشک به طور معنی داری افزایش یافت. تیمارها، مقدار پروتئین خام و نسبت کنسانتره به علوفه تأثیر معنی داری بر روی مقدار مصرف پروتئین خام داشتند. با افزایش مقدار نیتروژن خوراک، قابلیت هضم ماده خشک، پروتئین خام و ماده آلی به طور معنی داری افزایش یافت. نسبت کنسانتره به علوفه تأثیر معنی داری بر روی قابلیت هضم فیبر نامحلول در شوینده خنثی و ماده آلی داشت. تیمارها نیز تأثیر معنی داری بر روی قابلیت هضم پروتئین خام، شوینده خنثی و ماده آلی داشتند. اثر متقابل بین سطح پروتئین‌خام و نسبت کنسانتره به علوفه تأثیر معنی داری بر روی pH، غلظت نیتروژن آمونیاکی، غلظت اسیدهای چرب فرار، نسبت استات به پروپیونات و غلظت نیتروژن اوره‌ای خون داشت. به جز pH، تمامی فاکتورهای تخمیر شکمبه‌ای و متابولیت‌های خونی تحت تأثیر سطح پروتئین‌خام و نسبت کنسانتره به علوفه قرار گرفتند. تیمارها تأثیر معنی داری بر روی میزان دریافت نیتروژن (گرم در روز) و میزان دفع نیتروژن از طریق ادرار (گرم در روز، درصدی از نیتروژن خوراک) داشتند. همچنین هر دو عامل سطح پروتئین‌خام و نسبت کنسانتره به علوفه تأثیر معنی داری بر روی میزان دریافت نیتروژن داشتند. میزان دفع نیتروژن از طریق ادرار (گرم در روز) در بره‌های مصرف کننده تیمارهای دارای ۱۸ درصد پروتئین خام، بیشتر بود. نسبت کنسانتره به علوفه تأثیر معنی داری بر روی میزان دفع نیتروژن از طریق ادرار (گرم در روز) داشت. میزان بیان ژن ناقل اوره (نوع ب) در بره‌های مصرف کننده تیمارهای دارای ۱۸ درصد پروتئین خام، ۶/۵۶ بار بیشتر از بره‌های مصرف کننده تیمارهای دارای ۱۴ درصد پروتئین خام بود. این نتایج نشان می‌دهد که می‌توان از طریق تغییر نسبت علوفه به کنسانتره و سطح پروتئین خام خوراک، میزان بیان ژن ناقل اوره و در نهایت میزان نیتروژن اوره‌ای بازگردانده شده به شکمبه را کنترل کرد. لذا تنظیم ناقل‌های اوره در دیواره شکمبه از طریق خوراک می‌تواند نقش مهمی در کنترل نیتروژن اوره‌ای وارد شده به مسیر هضمی ایفا کند.

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