Asian-Aust. J. Anim. Sci.
Vol. 21, No. 4 : 561 - 566
April 2008

www.ajas.info

Nitrogen Utilization of Cell Mass from Lysine Production in Goats

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ABSTRACT : Two experiments were conducted to evaluate nutritive value of cell mass from lysine production (CMLP) as a protein supplement for ruminants. In each experiment, animals were fed a diet containing 40% of forages and 60% of concentrates, mainly composed of rice straw and ground corn, respectively, to meet the maintenance requirements, and the diets were formulated to supply equal amounts of energy and nitrogen among treatments. In order to investigate the effect of CMLP on ruminal fermentation (Experiment 1), three Korean native goats weighing 26.1±1.4 kg were allotted into individual cages with a 3×3 Latin square design. Each animal was fed one of three protein sources (CMLP, soybean meal (SBM), and urea). Rumen pH, bacterial and fungal counts, volatile fatty acid concentrations and acetate to propionate ratio were not significantly different among treatments. Concentration of propionate, however, was higher in SBM treatment (14.1 mM) than in CMLP (8.7 mM) or urea (9.3 mM) treatments. There was significantly more branch-chain volatile fatty acid production in CMLP (1.9 mM) and SBM (1.8 mM) treatments than in urea (1.3 mM) treatment. The number of protozoa was the highest in urea treatment, followed by CMLP and SBM treatment with significant differences. A metabolic trial (Experiment 2) was conducted to measure *in vivo* nutrient digestibility and nitrogen retention in Korean native goats fed CMLP and SBM. Two heavy (35.0±1.2 kg) and two light (25.0±0.9 kg) Korean native goats, caged individually, were used in this experiment. A heavy and a light animal were paired and supplemented with either CMLP or SBM. The animals fed CMLP showed a trend of lower total tract digestibility in all the nutrients measured; however, there was no statistical significance except for digestibility of ether extract. Nitrogen digestibility of CMLP was estimated to be about 7% units lower than that of SBM. There was a tendency for lower nitrogen retention in CMLP treatment (35.9%) compared to SBM treatment (42.3%). In summary, CMLP can be a good protein source for ruminant animals from nutritional and economic perspectives and may replace some, if not all, of SBM in a diet without losing nitrogen utilization efficiency. Further research is warranted for investigating the effect of CMLP fed with easily fermentable forage and the effective level of CMLP for replacing SBM. (**Key Words :** Cell Mass from Lysine Production, Nitrogen Retention, Rumen Fermentation, Goat)

INTRODUCTION

Feed cost accounts for about 50 to 70% of livestock production, with protein source being one of the most expensive ingredients (Piao et al., 1998). Moreover, the price of feed ingredients keeps increasing (Kondo et al., 2007). There have been many attempts to find alternative protein supplements that can replace classical protein feeds, such as soybean meal (SBM) and fishmeal. One of the approaches is the use of single-cell proteins produced by recycling of industrial wastes (Moo-Young and Chahal, 1979; Moo-Young et al., 1980; Kellems et al., 1981; Johnson and Remillard, 1983; Hsu et al., 1984;; Perera et al., 1995; Bohnert et al., 1999) and other types of by-products (Wanapat et al., 2006; Kumar et al., 2007).

In this context cell mass from lysine production (CMLP), a by-product produced during the lysine manufacturing process and commercially available, is a good candidate for an alternative protein source in animal feeding. Comprehensive *in vitro* studies (Seo et al., 2008) indicated that CMLP might supply a large amount of metabolizable protein to the ruminant animal by providing both non-protein nitrogen (ammonia) to rumen microbes and rumen undegraded protein, mainly due to its unique nitrogen composition (half soluble and half indigestible in the rumen).

The objective of this study was thus to investigate the digestion and utilization of CMLP in ruminants. For this, the effect of CMLP on microbial fermentation in the rumen was determined and a digestibility trial was also conducted

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Received September 28, 2007; Accepted November 22, 2007

Table 1. Ingredients and nutrient composition in the experimental diets (Exp. 2)

Items	Control (SBM)	Treatment (CMLP)
Ingredients (% as fed)		
Rice straw	40.0	40.0
Corn	46.6	46.6
SBM	8.4	
CMLP		5.7
Beet pulp	4.9	7.7
Vitamin mixture[1]	0.1	0.1
Nutrient composition (% DM)*		
ME (Mcal/kg)	2.28	2.25
Dry matter	86.9	87.0
Crude protein	11.7	11.9
Ether extract	1.5	1.7
Crude fiber	18.1	18.2
Ash	6.5	6.6
NDF	56.7	56.9
ADF	27.3	29.3

[1] Vitamin premix contained 5,512 IU/g of vitamin A, 1,101 IU/g of vitamin D, and 2.2 IU/g of vitamin E.
* ME: calculated value.

to evaluate the protein utilization of CMLP in ruminant animals.

MATERIALS AND METHODS

More complete chemical composition including nitrogen fractions and amino acid composition of CMLP was reported elsewhere (Seo et al., 2008).

In vivo ruminal fermentation

Three fistulated Korean native goats weighing 26.1±1.4 kg were allocated in a 3×3 Latin square design. Each period lasted for 10 days, and samples were collected on the last day of each period. Each of the experimental diets, formulated to supply iso-nitrogen (12.8% CP on dry matter basis) and iso-calorie (2.26 Mcal/kg of metabolisable energy) rations, contained either SBM, CMLP or urea as a primary nitrogen source. The remaining ingredients were 40% rice straw, 45% corn, 0.1% vitamin mixture and beet pulp and wheat bran with different ratios. CMLP was donated by BASF, Korea and SBM and urea were purchased from a local feed company. Experimental animals were fed twice daily at 08:30 and 17:30 h in equal amounts and had free access to water and mineral block.

On the last day of each period, the rumen fluid of each animal was collected three times: before (0 h) and after 4 and 9 h morning feeding. The obtained rumen fluid was strained through two layers of cheesecloth and transferred into 4 different screw capped tubes under strictly anaerobic condition for further analysis. Five ml of sample was combined with 20 ml of methyl green-formalin-saline solution (35% formaldehyde solution, 100 ml; distilled water, 900 ml; methyl green, 0.6 g; NaCl, 8.0 g) and stored in a dark place for protozoa counting with a haematocytometer (Ogimoto and Imai, 1981). Another 5 ml of rumen fluid was mixed with 0.5 ml of saturated $HgCl_2$ and 1 ml of 25% meta-HPO_3 and centrifuged at 3,000 rpm for 15 minutes. The supernatants were transferred into different tubes and stored at -30°C until further analysis for volatile fatty acids (VFA). For ammonium nitrogen determination, 10 ml rumen fluid was centrifuged under the same conditions. The concentration of VFA in samples was determined by gas chromatography as described by Erwin et al. (1961) and ammonium nitrogen concentration was assayed by the method of Chaney and Marbach (1962) using a spectrophotometer (Spectronic 20D, Milton Roy, USA). The number of bacteria and fungi in the sample was measured by the roll tube method as described by Hungate (1966). The composition of media used for the bacterial count was based on RGCA medium (Bryant and Burkey, 1953) with a small modification of adding 0.05 g of soluble starch and trypticase peptone. The Lowe's media (Lowe et al., 1985) was used for enumeration of fungi. Data were statistically analyzed using the GLM procedure of SAS Institute (2002) with period, sample collection time and treatment as fixed variables and animal as a random effect. Since there was no interaction between time and treatment effect, the data are presented as daily averages. Differences between least square means of daily average were tested by the LSD method.

In vivo nitrogen balance metabolism trial

Two large (35±1.2 kg) and two small (25±0.9 kg) Korean native goats were used in this trial. One of the heavy goats was paired with one of the light ones and they were allotted to two different diets (control vs. treatment) with a 2×2 cross-over design. Only three feed ingredients (corn, beet pulp and SBM or CMLP) were used to formulate concentrates in each diet in order to minimize any associate effect of feeds. SBM was completely replaced by CMLP and beet pulp in the treatment diet. Rice straw (DM, 88.31%; CP, 5.60%; ether extract, 1.00%; crude fiber, 33.19%; ash, 10.97; NDF, 75.27% and ADF, 48.82% on DM basis) chopped into 5 cm was used as a forage source and the ratio of forage to concentrate was 4 to 6. Experimental diets were formulated to supply the same nitrogen and energy contents and to meet the maintenance requirement based on NRC (1981). The amount of ingredients and chemical composition of the diets are shown in Table 1. Forage and concentrates were mixed just before feeding and given to the animals twice daily at 8:00 and 17:00 h in equal amounts. The animals had free access to water and mineral block. Each period lasted for 10 days and fecal and urinary samples were collected on the last three days of each period.

Table 2. Fermentation profiles from *in vivo* rumen fermentation of Korean native goats fed with different diets (Exp. 1)

Item[1]	SBM	CMLP	Urea	PSE[2]
pH	6.4	6.6	6.6	0.1
Ammonia nitrogen (mg/dl)**	8.2 [ab]	10.9 [a]	5.6[b]	1.1
Volatile fatty acid concentration (mM)				
Total VFA[3]	55.4	49.5	46.2	3.6
Acetate	35.2	33.2	30.4	2.1
Propionate*	14.1[a]	8.7[b]	9.3[b]	1.4
Butyrate	3.7	5.1	4.8	0.8
Isobutyrate*	0.7[a]	0.8[a]	0.6[b]	0.1
Valerate	0.6	0.5	0.4	0.0
Isovalerate*	1.1[ab]	1.1[a]	0.8[b]	0.1
Branch-chained VFA[4]	1.8 [a]	1.9 [a]	1.3 [b]	0.1
Acetate to propionate ratio	3.0	3.8	3.4	0.3
Microbial counts (cfu/ml)[5]				
Bacteria ($\times 10^{12}$)	3.2	2.0	2.3	1.1
Fungi ($\times 10^{3}$)	4.3	1.2	1.0	1.4
Protozoa ($\times 10^{4}$)**	0.8[c]	2.5[b]	4.7[a]	0.4

[1] Measurements before feeding and 4 and 9 h after feeding were averaged.
[2] Pooled standard error of least square means. * $p<0.05$. ** $p<0.01$.
[3] Sum of all the volatile fatty acids listed in the table. [4] Sum of isobutyrate and isovalerate. [5] Colony forming unit.
[a, b, c] Means in the same row with different superscripts are significantly different.

Total feces and urine from each animal were collected, weighed and sub-sampled daily prior to feeding in the morning. Urine was collected in a glass bottle containing 200 ml of 2.8% HCl. Sub-sampled (100 g) feces was dried at 60°C for 96 h, ground through a 2 mm screen and analyzed for DM, ether extract and crude fiber according to AOAC (1984). Sub-sampled (30 ml) urine was stored at -20°C until nitrogen was measured. The amount of nitrogen in feed, feces and urine was determined using a Kjeltec auto 1035/1038 system (Tecator, Sweden). Data were statistically analyzed using the GLM procedure of SAS Institute (2002) with period, animal pair, size of the animals and treatment as fixed effects. Differences between least square means were tested by the LSD method.

RESULTS AND DISCUSSION

In vivo fermentation characteristics

During *in vivo* fermentation, pH was not significantly different among the treatments (Table 2). However, ammonia concentration in the rumen was highest in the animals supplemented by CMLP (10.9 mg/dl), followed by SBM (8.2) and urea (5.6). Similar or higher ammonia concentration in the urea treatment was expected since urea is instantaneously converted into ammonia, mediated by urease, in the rumen (Sniffen et al., 1992; Puga et al., 2001); however, a significantly higher ($p<0.01$) ammonia concentration was observed in CMLP compared to urea treatments. No explanation can be given for this unexpected result. Ammonia is used by structural carbohydrate (SC) fermenting bacteria for a nitrogen source (Russell et al., 1992; Van Soest, 1994). Although a large amount of ammonia was supplied by CMLP right after feeding due to the high level of ammonium sulfate in CMLP (Seo et al., 2008), ammonia uptake by SC fermenting bacteria might be low because digestion of rice straw is slower than that of most forage sources (NRC, 2001). If more ammonia is supplied than needed, it may accumulate in the rumen (Mahadevan et al., 1976). Rice straw was used in this study because it is the major forage source in Korea (Seo, 2005). A decrease in ammonia nitrogen accumulation is expected if more rapidly fermentable forage is fed instead of rice straw.

VFA profiles from *in vivo* rumen fermentation of the animals fed CMLP were more or less similar to those of animals fed urea except for branch-chain VFA. Total VFA, acetate, butyrate and valerate concentrations and acetate to propionate (A/P) ratio were not significantly ($p>0.05$) different among treatments. Concentrations of total VFA, acetate and valerate in CMLP fed animals were similar to those on other diets, but CMLP feeding resulted in the lowest propionate concentration, and the highest butyrate, isobutyrate and isovalerate concentrations and A/P ratio among the treatments. Abundant ammonia concentration might lead to relatively higher production of butyrate (Wohlt et al., 1976). A significantly higher concentration of propionate was observed in SBM treatment (14.1 mM) than CMLP (8.7 mM) or urea (9.3 mM) treatments. Isobutyrate concentration was significantly lower in urea (0.6 mM) than SBM (0.7 mM) or CMLP (0.8 mM) treatments. Concentration of isovalerate differed significantly between CMLP (1.1 mM) and urea (0.8 mM) treatments; however, there was no difference either between CMLP and SBM (1.1 mM) or between SBM and urea.

In vivo VFA profiles obtained from the present study

Table 3. *In vivo* total tract digestibility of nutrients and nitrogen utilization by Korean native goats fed with different diets (Exp.2)

Item[1]	Control (SBM)	Treatment (CMLP)	PSE[2]
Nutrients digestibility (%)			
Dry matter	66.7	64.8	1.3
Crude protein	71.4	67.6	1.2
Ether extract*	63.1	53.8	1.8
Crude fiber	57.3	53.5	1.9
Nitrogen utilization (g/d)			
Nitrogen intake*	9.7	9.9	0.0
Nitrogen excretion	5.6	6.3	0.5
Fecal nitrogen	2.8	3.2	0.1
Urinary nitrogen	2.8	3.1	0.3
Nitrogen retention	4.1	3.6	0.5
Nitrogen retention rate (%/d)	42.3	35.9	4.5

[1] Samples collected for the last three days in each period were pooled before analysis.
[2] Pooled standard error of least square means. * $p<0.05$.

imply that SC bacterial fermentation was dominant in the rumen of animals fed CMLP. SC bacteria use ammonia as the major nitrogen source and produce more acetate than propionate, whereas bacteria that ferment non-structural carbohydrate (NSC) are able to utilize both ammonia and peptides or amino acids as a nitrogen source and produce more propionate (Russell et al., 1992; Van Soest, 1994). Some NSC bacteria have an absolute requirement for preformed amino acids and grow only in the presence of peptides (Russell and Martin, 1984). Additional non-ammonia nitrogen in the form of free amino acids (Argyle and Baldwin, 1989) or peptides (Griswold et al., 1996) enhanced fiber digestion and microbial protein production during *in vitro* fermentation. Van Kessel and Russell (1997) showed that mixed ruminal bacteria grew more rapidly and efficiently *in vitro* when provided enzymatic hydrolysates of casein or soy protein in addition to ammonia. The Cornell net carbohydrate and protein system assumes the presence of peptides in a diet increases protein yield from NSC bacteria by as much as 18% (Fox et al., 2004). Although CMLP contains a large amount of amino acids (40.6% DM), a relatively large portion of these amino acids were undegradable in the rumen (Seo et al., 2008). Instead, CMLP may supply a large amount of ammonia that is readily available to SC bacteria in the rumen, and thus more acetate than propionate was produced by fermentation in the rumen of animals fed CMLP, compared to the animals fed SBM which also provides amino acids and peptides.

Unlike urea, CMLP seemed to supply branch-chain amino acids into the rumen, and this was indicated by significantly higher concentration of branch-chain VFA (1.9 mM), including isobutyrate and isovalerate, than urea treatment (1.3 mM). Some SC bacteria require branch-chain amino acids in order to maximize their growth and fiber digestion (Russell, 2002). Some, although not much, true protein of CMLP might be degraded in the rumen, supply branch-chain amino acids to SC bacteria and enhance fiber digestion. However, significant difference in bacterial population in the rumen was not observed between CMLP- and urea-fed animals.

Total numbers of bacteria and fungi were not significantly different among the treatments. The number of protozoa in the rumen, however, was the highest in urea treatment (4.7×10^4 cfu/ml), followed by CMLP (2.5×10^4 cfu/ml) and SBM treatment (0.8×10^4 cfu/ml), and the differences were statistically significant ($p<0.05$). Significantly ($p<0.05$) higher numbers of protozoa in the rumen of animals fed urea than animals fed SBM were also observed in cattle (Dennis et al., 1983). The environmental change in rumen fluid caused by non-protein nitrogen supplementation may lead to increased protozoan population. The number of bacteria and fungi were numerically but not significantly ($p>0.05$) lower in CMLP and in urea than SBM treatment (Table 2). Orpin and Joblin (1988) reported that not only bacterial growth but also fungal growth was stimulated by amino acid supply. Therefore, lowered bacteria and fungi populations indicated that peptides and/or amino acids were insufficient in the rumen of animals fed CMLP and urea, compared to the animals fed SBM.

The present results may indicate that supplementation of CMLP can sufficiently support fiber digestion by SC bacteria in the rumen. NSC fermentation, however, may be depressed due to insufficient supply of preformed amino acids from CMLP.

In vivo digestion and nitrogen retention

The animals fed CMLP exhibited lower total tract digestibility of DM (64.8% vs. 66.7%), CP (67.6% vs. 71.4%), ether extract (53.8% vs. 63.1%) and crude fiber (53.5% vs. 57.3%) than those fed SBM (Table 3). Differences, however, were not statistically significant ($p>0.05$) except for ether extract. Lower digestibility of ether extract may be partially due to higher content of ether extract in CMLP (5.8% DM) than SBM (2.0% DM) or due to some structural modification of lipid in CMLP during processing. The numerically lowered digestibility of CMLP nutrients compared to SBM was consistent with rumen fermentation profiles and microbial populations. We thus conclude that differences in nutrient digestibility between CMLP- and SBM-based diets were primarily due to differences in ruminal digestibility of the diets.

Total tract CP digestibility of CMLP and SBM were estimated based on total tract CP digestibility of the diets, assuming that total tract CP digestibility of other ingredients (mainly rice straw and corn) was the same as total tract digestibility of DM in each diet. The estimated CP

digestibility of CMLP and SBM were 73.4 and 80.2%, respectively, and the difference was 6.8% units. In the *in vitro* rumen study (Seo et al., 2008), CP digestibility of CMLP and SBM was 49.4 and 52.8%, respectively. Assuming 81.1 and 94.1% of rumen undegraded CP were digested in the lower gut based on results from pepsin digestibility in the same study, true digestibility of CP was estimated as 90.4 and 97.2% for CMLP and SBM, respectively, and the difference was 6.8% units. Discrepancy between true and total tract digestibility may result from contribution of endogenous CP loss. Endogenous CP loss is often closely related with the amount of indigestible DM (Fox et al., 2004). Since the two experimental diets contained similar indigestible DM (Table 3), the contributions of endogenous CP loss to total tract CP digestibility in the two diets were expected to be similar. Therefore, about 7% unit difference in CP digestibility between CMLP and SBM may be a reasonable estimate.

No significant difference was observed in nitrogen utilization between control and treatment (Table 3). Animals receiving CMLP excreted more nitrogen through urine and feces, but no statistical significance was observed. An excess amount of ammonia in the rumen is likely to be absorbed through rumen wall, converted into urea in the liver and excreted as urinary nitrogen (Van Soest, 1994). A numerical increase in nitrogen excretion as urinary nitrogen on the CMLP diet compared to the control may reflect a decrease in efficiency of nitrogen utilization by microbes in the rumen because a large amount of ammonia was too rapidly supplied by CMLP (Castillo et al., 2001). The lower digestibility of CMLP in both the rumen and lower gut probably caused an increase in nitrogen excretion as fecal nitrogen, compared to the control diet. Due to decreases in digestible and metabolisable nitrogen, nitrogen retention and nitrogen retention rate were lower in CMLP treatment (3.6 g/d and 35.9%) than SBM (4.1 g/d and 42.3%), although the differences were not statistically significant.

Overall, the results from this study present a comprehensive view of protein digestion in the ruminant animal by showing the effect of CMLP supplementation on rumen fermentation, apparent digestibility and nitrogen metabolism. The results of this study may not be conclusive due to the limited number of experimental units; however, they suggest for the first time that CMLP can be a useful protein source for ruminant animals, which is useful information for farmers and the animal science community. Since the initial rate of NPN release from CMLP is high, easily fermentable forage may need to be provided with CMLP in order to maximize the nutritive value of CMLP. Further research is needed to study effects of CMLP on rumen fermentation and nutrient utilization when fed with easily fermentable forages, to investigate metabolic fate of absorbed amino acids originated from CMLP and to determine an adequate amount of CMLP that can replace expensive protein sources such as SBM, with greater nutritional and economic benefits.

ACKNOWLEDGMENT

This work was supported by a grant (20070101-034-011) from Biogreen 21 Program, Rural Development Administration, Republic of Korea and BASF, Korea.

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