

Effect of Six Feed Supplements on Ruminal Degradation Characteristics and Amino Acid Profile of Sheep

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Abstract: The objective of this study was to determine the influence of degradation characteristics of six feed supplements on ruminal volatile fatty acid and amino acid production and profile using three ruminally cannulated male sheep. The sheep were fed a concentrate to forage ratio of 35:65. The supplements came from Soybean Meal (SBM), Corn Meal (CM), Meat and Bone Meal (MBM), Feather Meal (FtM), Blood Meal (BM), and distillers Dried Gain with Soluble (DDGs). Sheep were fed at 6 h interval for 42 days. Ruminal parameters such as pH, NH_4^+ , temperature, NO_3^- , and Volatile Fatty Acids (VFA) were measured over a 12 h period after feeding. Concentrations of pH, NH_4^+ , and NO_3^- showed significant difference ($p < 0.001$) across all dietary treatments. Sheep fed BM had higher pH (6.64 vs. 5.81), NH_4^+ (262.31 vs. 1.95 ppm), and NO_3^- (559.71 vs. 77.40 ppm) values than did those fed CM whereas FtM showed intermediate values. The $\text{C}_2/(\text{C}_3+\text{C}_4)$ ratio of the major VFA showed a significant difference ($p < 0.001$). Ruminal profile (%) of the sum of amino acids measured was similar among feeds, except Asp, isoleucine, lysine, and proline, respectively. Specific first limiting AA was methionine for SBM while lysine was co-limiting for FtM and DDGs. In general, ruminal parameters were clearly influenced by dietary treatments, with a lower ruminal pH and a higher NH_4^+ in BM than CM. Overall, the concentration of both total EAA and NEAA were similar among feeds, but numerically SBM had more EAA concentrations followed by CM, MBM, DDGs, BM, and FtM.

Key words: Amino acid, feed supplements, ruminal degradation, sheep, volatile fatty acids

INTRODUCTION

Ruminal feed metabolism is both a fascinating and complicated phenomenon. Ruminants live in symbiosis with microorganisms where the host animal supplies the microorganisms in the rumen with plant materials, which often are of low nutritional quality, and in return gets access to high-quality microbial protein from microbes. Rumen microorganisms, mainly bacteria, degrade feed proteins to peptides, amino acids, and ammonia. Approximately 70 to 80% of ruminal microorganisms attach to undigested feed particles in the rumen and 30 to 50% of those have proteolytic activity (Clark *et al.*, 1992). The supply of amino acids to the small intestine of ruminants is the sum of amino acids resulting from the synthesis of rumen microbial protein, non-degraded dietary protein and a small amount of endogenous amino acids. However, if energy (fermentation products such as VFA) supply in the diet is adequate, the degree to which the amino acid requirements of ruminants are met will depend primarily on the amount of dietary protein, its source and the extent

of its degradation in the rumen (Urbaniak, 1995; Taghizadeh *et al.*, 2005). Therefore, ruminal metabolic changes associated with a particular feed may have an important influence on the quantities (amount and composition) of amino acids reaching the small intestine and its subsequent availability to tissues and organs of ruminant animals and consequently affect their overall performance (Van der Aar *et al.*, 1984). The objective of this experiment was to determine the degree of rumen degradation of diets from Soybean Meal (SBM), Corn Meal (CM), Meat and Bone Meal (MBM), Feather Meal (FtM), Blood Meal (BM), and Distillers Dried Gain with soluble (DDGs) and their effects on metabolic changes in the rumen, and individual amino acids profile of sheep.

MATERIALS AND METHODS

Animals, housing, diets, and feeding: The experiment was carried out in 2009 at the National Pingtung University of Science and Technology's Innovation and Practical Training Centre (IPTC) farm. Three ruminally cannulated male sheep (3.5 cm Center Diameter) were

Table 1: The effect of dietary treatments on rumen fermentation parameters

| Item | Treatment | | | | | | | | Interaction | | |
|-----------------------------------|--------------------|----------------------|---------------------|---------------------|---------------------|---------------------|--------|------|-------------|-----|-----|
| | SBM | CM | MBM | FtM | BM | DDGs | SEM | Sig. | TXD | TXS | DXS |
| Ph | 6.42 ^{bc} | 5.81 ^d | 6.43 ^{abc} | 6.55 ^{ab} | 6.64 ^a | 6.33 ^c | 0.07 | *** | NS | NS | NS |
| Temp | 39.63 ^a | 39.56 ^{ab3} | 9.02 ^{c3} | 9.02 ^c | 39.00 ^c | 39.17 ^{bc} | 0.15 | ** | NS | NS | NS |
| ORP/ppm | -228.8 | -203.2 | -226.67 | -187.58 | -278.67 | -218.42 | 21.15 | NS | NS | NS | NS |
| NH ₄ ⁺ /ppm | 2.68 ^c | 1.95 ^c | 2.07 ^c | 96.04 ^{b2} | 62.31 ^a | 117.7 ^b | 13.32 | *** | NS | ** | NS |
| NO ₃ ⁻ /ppm | 83.84 ^c | 159.08 ^c | 77.40 ^c | 413.72 ^b | 559.71 ^a | 362.33 ^b | S39.23 | *** | NS | *** | NS |

^{a, b, c, d}: Means in the same row with different superscripts are significantly different; ***, p<0.001; **, p<0.01; NS: No Significance; SBM: Soybean meal; CM: corn meal; MBM: Meal and bone meal; FtM: Feather meal; BM: Blood meal; DDGs: Distillers dried grain with soluble; TXD: Treatment x Day; TXS: Treatment x Sheep; DXS: Day x Sheep

used in this 6 weeks experiment. Sheep were prepared surgically under general anesthesia by a clinical veterinarian at the National Pingtung University of Science and Technology. The surgical procedures and justification were approved by National Pingtung University of Science and Technology. The sheep were monitored for a minimum of 4 weeks of recovery during which analgesics were administered as deemed necessary to reduce the sheep's discomfort. Three individual pens of size (1 m × 1.5 m) with steel slatted floors were used to house the sheep in the experiment. Each pen was equipped with over head aluminum feeders and plastic waterer. Prior to the commencement of the experiment sheep were subjected to a 2 weeks adaptation period (preconditioning). Each of the three Sheep received each of the six dietary treatments over 6 week's periods. Sheep were individually fed throughout the length of the experiment. The roughage and the concentrate were all given at the same time for each of the sheep. Sheep were fed three times a day with water and mineral lick provided *ad libitum*. Each animal, at each feeding time received 2 kg of fresh Napier grass and 215 g of concentrates. Animals were dewormed and vaccinated. Both cleaning and watering were carried out twice daily prior to the morning and evening feeding. Plastic buckets and locally improvised brooms were employed in these processes. Since the floor is slatted type the debris is washed down the drain to the main waste collection system. Maximum health surveillance systems was in place and were any animals shows a sign of a disease is treated with immediate effect.

Experimental design: The experiment was a 6x3 design in which 3 sheep were randomly arranged in 3 replicates of 6 treatments. The dietary treatments examined were SBM, CM, MBM, FtM, BM, and DDGs. Each treatment was replicated 3 times and every treatment used 1 sheep for 7 day. Each week's composite samples were collected at 1st, 3rd, 5th, and 7th day. Each of the three sheep received each of the six dietary treatments over 6 week's periods.

Analysis:

Rumen measurement: Following adjustment, ruminal fluid was collected every 6 h post feeding using a suction

pump. All evacuated ruminal contents were measured immediately for pH and temperature with a portable pH meter. Following pH and temperature measurements, rumen contents were also measured for ammonium, nitrate and ORP. Ruminal fluid was filtered through three layers of cheesecloth and samples were acidified to pH 2.0 with 50% H₂SO₄, centrifuged at 30×100 rpm for 20 min. The clear supernatant was collected and frozen at -20°C until analyzed for Volatile fatty acids.

Volatile fatty acids analysis: Before analysis and prior to injection unto column, the acidified ruminal samples were thawed at room temperature and centrifuged (30×100 rpm for 20 min) in order to obtain a clear supernatant. The supernatant was analyzed for Volatile Fatty Acid (VFA) concentrations. VFA content of rumen fluid samples were measured via gas chromatography. Individual rumen VFA was separated using a fused silica capillary column (30 m x 0.53 mm ID, 1 µm film thickness). An internal standard of 1 mL 30 mM 4-methylvaleric was prepared prior to the preservation of the sample. A split injection (51:1) of 0.5 µL was used. Injector and detector temperatures were 250°C. Initial oven temperature of 125°C was held for 5 min and increased to 180°C at a rate of 15°C/min., and held for 6 min. The total run time was 16 min per sample. High purity methanol was the carrier gas with a flow rate of 4.2 mL/min. Inlet pressure was held constant. A Chem Data Station was used for integration and quantification of individual VFA.

Amino acid analysis: Rumen content samples (100 mL) were stone rock frozen in (JUST-A-TILT™ Shell freezer FTS SYSTEMS) at -40°C for at least two consecutive days before being transferred to a freeze dryer (EYELA FDU- 1200 Bennett®) at -50°C for extraction of the liquid content. The dried residues of the composite sample of each dietary treatment were separated on a High Performance Liquid Chromatography (HPLC) machine. A wet digester was used for extraction and neutralization of acid fumes and reaction gasses. Prior to acid analysis the preparations above were neutralized and standardized to pH 2.2±0.1 by adding about 30-40 mL of NaOH (4N) and sodium citrate buffer. Cooled hydrolysates were filtered through filter paper. About 1.5 mL of the clarified hydrolysates was injected using glass stringe in HPLC auto sampler for the separation of the various AA.

Table 2: Influence of acetic, propionic and butyric acids and their ratios in the rumen fluid of sheep

| Item (ppm) | Treatment | | | | | | | | Interaction | | |
|---------------------------------------------------|---------------------|---------------------|---------------------|--------------------|---------------------|---------------------|-------|------|-------------|-----|-----|
| | SBM | CM | MBM | FtM | BM | DDGs | SEM | Sig. | TXD | TXS | DXS |
| C ₂ | 4724.3 | 5605.0 | 4770.2 | 4113.7 | 4808.4 | 4345.0 | 588.4 | NS | NS | NS | * |
| C ₃ | 2601.3 ^a | 3107.6 ^a | 2329.4 ^a | 846.3 ^b | 870.3 ^b | 1328.2 ^b | 333.0 | *** | NS | NS | NS |
| C ₄ | 1379.9 ^b | 2215.2 ^a | 1276.9 ^b | 159.2 ^c | 288.7 ^c | 534.6 ^c | 194.8 | *** | NS | ** | NS |
| C ₅ | 163.1 ^b | 337.4 ^{ab} | 187.2 ^b | 716.5 ^a | 648.8 ^a | 478.0 ^{ab} | 129.0 | * | NS | *** | NS |
| iC ₄ | 1425.3 ^a | 1374.8 ^a | 611.8 ^{ab} | 425.1 ^b | 274.0 ^b | 298.0 ^b | 277.3 | * | NS | * | NS |
| iC ₅ 3 | 95.9 | 369.9 | 212.0 | 138.7 | 166.8 | 129.6 | 97.9 | NS | NS | * | NS |
| C ₂ /C ₃ | 2.33 ^b | 3.28 ^b | 2.88 ^b | 7.06 ^{ab} | 15.39 ^{ab} | 35.77 ^a | 9.6 | * | NS | * | NS |
| C ₂ /(C ₃ +C ₄) | 1.48 ^c | 1.33 ^c | 1.79 ^c | 5.40 ^a | 8.12 ^{ab} | 3.28 ^{bc} | 1.0 | *** | NS | ** | NS |

^{a,b,c}: Means in the same row with different superscripts are significantly different; *: p<0.05; **: p<0.01; ***: p<0.001; NS: No Significance (p>0.05); SBM: Soybean meal; CM: corn meal; MBM: Meal and bone meal; FtM: Feather meal; BM: Blood meal; DDGs: Distillers dried grain with soluble; TXD: Treatment x Day; TXS: Treatment x Sheep; DXS: Day x Sheep8

Statistical analysis: The effects of feed sources on ruminal degradation parameters and AA concentrations were evaluated by analysis of variance using the GLM Procedure of SAS (2004). The differences among feed sources on mean concentration of the rumen variables were compared using the Duncan's Multiple Range test.

RESULTS AND DISCUSSION

Analyses of several ruminal variables and their interactions are presented in (Table 1). Ruminal pH, NH₄⁺, NO₃⁻, and temperature were significantly affected by source of diet, indicating that the diets were different in fermentability. Mean pH of rumen contents, was generally affected by diet (p<0.001). Normal ruminal pH range from 5.07 to 7.52, although in this study means for individual animals exhibited a much narrower range (5.81 to 6.64) and were relatively within the normal value range from 5.5 to 7.5. BM had the highest pH mean value (p<0.001) followed by FtM, MBM, SBM, DDGs and lowest with CM (6.64, 6.55, 6.43, 6.42, 6.33 and 5.81) respectively. Increased ruminal pH in the current study may be the result of decreased starch levels in DDGs compared with corn. Starch from corn is degraded rapidly in the rumen; thus, it may decrease ruminal pH (Leupp *et al.*, 2009; Monteils *et al.*, 2002; Hakan *et al.*, 2006). In their experiment, Hakan *et al.* (2006) reported a similar situation in which rumen pH and acetate: propionates were higher in diets containing slowly degradable starch than in diets rapidly degradable starch. Ham *et al.* (1994) fed steers 40% WDGs, partially replacing dry-rolled corn in 90% concentrate diets, and reported no differences in pH or total VFA concentration when compared with steers consuming dry-rolled corn diets. Peter *et al.* (2000) reported no differences in ruminal pH when 20% DDGs (DM basis) was included in finishing diets. Decreased rumination and salivation normally associated with high concentrate diets and the consequent lowering of the buffering capacity, coupled with rapid microbial degradation of soluble carbohydrates, results in a decline of ruminal pH (Tripathi *et al.*, 2007). This is in agreement

with Hsia *et al.* (2007) who reported that cattle offered high concentrates (0:100) once a day not only reduced feed intake but also caused lowest pH value and highest rumen temperature compared to those fed three or six time a day. According to Hsia *et al.* (2007), under this condition of low pH the animal is exposed to a lot of metabolic disorders. Under these conditions, increased competition occurs for essential nutrients and the cellulolytic micro-organisms, which appear to be less competitive, are unable to metabolize and reproduce at a rate fast enough to maintain their population in the rumen (Tripathi *et al.*, 2004). Results of the present study are strengthened by the findings of Kobayashi *et al.* (2008) who reported that the rumen pH has to be maintained above 6.0 in order to maintain rumen fibrolytic activities when forages are supplemented with rapidly fermentable carbohydrate, because fibrolysis in the rumen is pH dependent. Mean values for rumen temperature showed significant difference (p<0.01) between all the dietary treatments. On the contrary, dietary treatments with seemingly lower pH values tended to show higher rumen temperature (39.63 vs. 39.00°C) for SBM and BM, respectively. Differences (p<0.001) were observed in the level of NH₄⁺ in the rumen liquid. The lowest level was found in lambs fed CM and MBM. Inclusion of FtM, DDGs, and BM diets resulted in increase (p<0.01) NH₄⁺ concentration. Ruminal NH₄⁺ comparatively throughout the trial was higher (262.31 ppm) in BM followed by DDGs (117.70 ppm) and lowest (1.95 ppm) in CM (p<0.001). This is due to the fact that corn, a high starch source is less degradable than all the other protein sources employed in this experiment. In light of the results obtained by (Satter and Slyter, 1974; McCarthy *et al.*, 1989), who reported that 5 to 6 mg/dL of ammonia is adequate to stimulate microbial protein synthesis, the level of ammonia noted in this study exceeded amount in all diets, leading to the assumption that the requirements of microorganisms for ammonia had been met and therefore adequate enough to allow maximum microbial protein synthesis.

Individual and total VFA production: Individual volatile fatty concentrations, their ratios, and mean values are presented in (Table 2). Ruminal propionic and butyric acids concentrations were significant across all dietary treatments ($p < 0.001$) however, there was no significance with regard to acetic acid production. The diet differences in the mean concentrations of acetic, propionic, and butyric acids broadly reflect the differences in net production rates. The VFA and total ammonia concentrations were largely determined by pH and increased as pH decreased. Lana *et al.* (1998) a reduction in *in vitro* pH (6.50 to 5.70) also decreased ($p < 0.001$) the rates of ammonia production. It is important to realize that *in vivo*, the VFA concentration is the driving force for reducing ruminal pH, and results presented herein may seem supportive. Calsamiglia *et al.* (2008) explained that under *in vitro* conditions the reduction in pH and accompanying reduction in VFA production may be part of the self-regulating mechanism of the rumen ecosystem against ruminal acidosis. Our experiments supported the idea that the ratio of ruminal acetate: propionate was highly influenced by the capacity of the dietary treatment to produce pH, NH_4^+ and NO_3^- ($p < 0.001$) since sheep with lower acetate: propionate ratios also had lower ruminal pH values, and *in vitro* experiments corroborated the idea that pH was having a major impact on acetate: propionate ratio. Significant relations were demonstrated between rumen ammonia levels and C_2/C_3 and $\text{C}_2/(\text{C}_3+\text{C}_4)$ volatile acid ratios.

The SBM and CM based concentrates produced lowest ratios while the MBM and FtM diets exhibited moderate C_2/C_3 and $\text{C}_2/(\text{C}_3+\text{C}_4)$ ratios. This could be explained by the fact that even though DDGs is a corn co-product, it has higher fiber content (4 times) than corn. From this it was concluded that, as rumen ammonia levels increased, there was a modest shift from propionate to higher proportions of butyrate and acetate. Propionic acid was higher in diets containing rapidly degradable starch than in diets containing slowly degradable starch (Hakan *et al.*, 2006). The higher propionate and lower acetate production in the CM diet is consistent with increased ruminal fermentation of starch and decreased degradation of fiber. In such a situation, the microbial species in the rumen most likely to be affected by a shortage of ammonia are those that grow slowly, such as the cellulolytic bacterial. The ratio for acetic: Propionic+ butyric acids were significantly different ($p < 0.001$) between treatments. The pattern of rumen fermentation changed from high acetate in CM to high propionate in FtM and BM with large and significant reductions in the concentrations and proportions of propionic and butyric acids. Propionate was the only VFA affected by the carbohydrate source of the supplement, with higher levels

in CM than the rest of the diets ($p < 0.001$). The dietary supplement DDGS had the highest ratios for C_2/C_3 and $\text{C}_2/(\text{C}_3+\text{C}_4)$. The FtM and CM diets resulted in the numerically lowest ratio of acetate to propionate and very high proportion of propionate. Bateman *et al.* (2002) related such situations to more dietary energy captured in the VFA. However, the CM diet which had the overall highest mean concentration of total VFA (Data not shown) did not also have the highest mean concentration of NH_4^+ . The reason for this phenomenon is not clear and requires further studies.

This study clearly indicates that supplements with the highest VFA concentrations produced the lowest proportion of ratios. In contrast to the major fatty acids, the branched chain acids showed numerically lower concentrations. Meng *et al.* (1999) noted that the production of limited Branched-chain Fatty Acids (BCFA) or the non detection of Isobutyrate in particular, probably is a reflection of a greater demand for cellulolytic bacterial growth than the production of these acids. Isoacids or BCFA are required by cellulolytic bacteria for the synthesis of branched-chain amino acids and longer BCFA. The starch based concentrate produced moderate ratios, indicating the greater neoglucogenic potential of the ration. By contrast, C_2/C_3 and $\text{C}_2+\text{C}_4/\text{C}_3$ ratios were higher in diets containing slowly degradable starch than in diets rapidly degradable starch which mean that there was more degradation activity in the rumen, and probably a more effective synthesis of microbial protein. Though not significant, ruminal isobutyrate and isovalerate differed by diet; isovalerate was highest in the diet containing SBM and CM, probably because of the high Leu content of CM. The individual VFA fermentation pattern of the six feeds used in the study further revealed high level of interactions between the major and minor energy supplier from all diets within individual sheep. Propionic and valeric acids showed significant ($p < 0.001$) levels of interactions. In general, ruminal parameters were clearly influenced by the feed source, with a lower ruminal pH (5.81 vs 6.64; $p < 0.001$) and a higher NH_4^+ (262.3 vs 1.95 ppm; $p < 0.001$) in BM than CM.

Amino acid composition and profile: The AA profile of the feed sources in this study remaining after 12 h of post feeding are presented in (Table 3). Ruminal AA profile of all essential amino acids except Lys and Ile ($p < 0.05$) and of all nonessential amino acids except pro ($p < 0.05$) and Asp ($p < 0.001$) were not significantly different in all diets. Thr, Cys, Met, and Lys are the essential amino acids that were produced to a certain degree by the inclusion of SBM in the diet. Klemesrud *et al.* (2000) reported that these amino acids have been shown to limit the growth of

Table 3: Ruminal amino acid profile of the dietary treatments used in the experiment

| Item (%) | Treatment | | | | | | | Interaction | |
|----------|--------------------|--------------------|---------------------|-------------------|--------------------|---------------------|------|-------------|-----|
| | SBM | CM | MBM | FtM | BM | DDGs | SEM | Sig. | TXD |
| Asp | 1.83 ^a | 1.73 ^a | 1.59 ^{ab} | 0.91 ^c | 1.18 ^{bc} | 1.08 ^c | 0.14 | *** | NS |
| Glu | 1.65 | 1.44 | 1.42 | 0.85 | 1.36 | 1.32 | 7.08 | NS | NS |
| Ser | 0.67 | 0.69 | 0.44 | 0.33 | 0.48 | 0.34 | 0.14 | NS | NS |
| His | 0.71 | 0.17 | 0.13 | 0.06 | 0.12 | 0.08 | 0.19 | NS | NS |
| Gly | 0.76 | 0.72 | 0.79 | 0.61 | 0.72 | 0.69 | 0.09 | NS | NS |
| Thr | 0.97 | 0.75 | 0.69 | 0.50 | 0.52 | 0.66 | 0.13 | NS | NS |
| Arg | 0.71 | 0.58 | 0.51 | 0.42 | 0.47 | 0.49 | 0.08 | NS | NS |
| Ala | 0.84 | 0.88 | 0.92 | 0.64 | 0.86 | 0.82 | 0.09 | NS | NS |
| Tyr | 0.36 | 0.31 | 0.30 | 0.27 | 0.37 | 0.32 | 0.05 | NS | NS |
| Cys | 0.30 | 0.18 | 0.12 | 0.14 | 0.14 | 0.08 | 0.06 | NS | NS |
| Val | 0.79 | 0.58 | 0.77 | 0.63 | 0.70 | 0.68 | 0.10 | NS | NS |
| Met | 0.41 | 0.27 | 0.40 | 0.24 | 0.65 | 0.47 | 0.10 | NS | NS |
| Phe | 0.89 | 0.57 | 0.54 | 0.46 | 0.56 | 0.62 | 0.11 | NS | NS |
| Ile | 0.86 ^{ab} | 0.59 ^{bc} | 0.91 ^a | 0.40 ^c | 0.54 ^c | 0.69 ^{abc} | 0.10 | * | NS |
| Leu | 1.11 | 1.03 | 0.97 | 0.64 | 0.93 | 0.89 | 0.13 | NS | NS |
| Lys | 0.76 ^{ab} | 1.20 ^a | 0.57 ^{ab} | 0.14 ^b | 0.22 ^b | 0.18 ^b | 0.26 | * | NS |
| Pro | 0.94 ^a | 0.87 ^{ab} | 0.76 ^{abc} | 0.42 ^d | 0.56 ^{cd} | 0.57 ^{bcd} | 0.10 | * | NS |

^{a, b, c, d}: Within a row, means without a common superscript letter differ; *: p<0.05; ***: (p<0.001); NS: No significance; SBM: Soybean meal; CM: corn meal; MBM: Meal and bone meal; FtM: Feather meal; BM: Blood meal; DDGs: Distillers dried grain with soluble; TXD: Treatment x Day; His: histidine; Thr: threonine; Arg: arginine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Lys: lysine; Asp: aspartic acid; Glu: glutamic acid; Ser: serine; Gly: glycine; Ala: alanine; Pro: proline; Tyr: tyrosine

steers but were increased with BM supplementation. Surprising, the lowest amino acid production was observed in lambs fed diets containing FtM, BM, and DDGs. Probably, excessive degradation in the rumen accompanied by a high level of ammonia exceeding the assimilative capabilities of microorganisms, could be the reasons that these diets supplied the lowest quantities of AA. Excessive ammonia production in the rumen is a major nutritional inefficiency in ruminant animals. This could possibly explain the low concentration of AA in those diets that accumulated substantial amounts of ruminal ammonia (Eschenlauer *et al.*, 2002). It is important to note that DDGs is high in Met content and low in Lys content but overall, is low compared to other Lys-rich feeds, such as BM, CM, and SBM. The poor EAA profile of DDGs especially for Lys, and a shortage of RDP to support microbial protein synthesis might be the reason for this (Santos *et al.*, 1998). In this experiment Asp was the highest across all the 6 dietary treatments and Cys was the lowest. This is understandable since Cys is dependent on Met production level which also had low production levels. Klemesrud *et al.* (2000) suggested that though a physiological requirement exists for both Met and Cys, a dietary requirement, however, exists only for Met because Cys can be synthesized from Met. Harouna and Schingoethe (1997) reported in their trial that in MBM after 12 h of post feeding, Arg was higher and His was lower. Our results showed that in MBM, Ile was higher and Cys was slightly lower than His. For His, the degradation was even lower than that for Met across all dietary treatments. For both AA, the possibility of a net synthesis in the rumen cannot be dismissed and would, in

part, explain the low values. SBM had the greatest concentrations of Asp, Glu, and Leu and the lowest concentrations of Cys, Try, and Met. Overall, the concentration of both total essential AA (EAA) and none essential AA (NEAA) (data not shown) was similar among feeds, but numerically SBM had more EAA concentration followed by CM, MBM, DDGs, BM, and FtM (Data not shown). This result is in agreement with those observed by Kleinschmit *et al.* (2007) in which SBM had higher TAA concentrations compared to 5 DDGs sources. Lys appears to be the first limiting AA when corn or barley based diets are supplemented with cereal based sources and Met is likely to be the first limiting AA with legume or animal based proteins. However, in the present study His and Cys were the first and second limiting AA for CM and for the animal based protein supplements, Cys was second limiting for both FtM and BM. His was first limiting for MBM, Tyr second while Lys was first limiting for FtM and third for BM. Though they showed fluctuations, at the highest pH detected (i.e., 6.64), Asp, Glu, Leu and Ala registered higher concentration. Overall, most degradation took place at pH 6.0 and above, which lies in the vicinity of the pH optima for most microbial enzymes (and is also close to the physiological minimum). In addition, the AA most extensively degraded are those possessing a hydrophilic character (i.e., Glu, Gly, Asp, Lys, Arg), and which are said to dissolve better in water-based buffers. It is interesting to note that Pro, Val and Leu, all highly hydrophobic AA and Leu in particular been said to be more resistant to microbial degradation in the rumen (Erasmus *et al.*, 1994), were also extensively degraded; the reason for this is unclear in this experiment.

CONCLUSION

Results from this experiment indicate that VFA and total ammonia concentrations were largely determined by pH and increased as pH decreased. Our experiment further revealed that the ratio of ruminal acetate: propionate was highly influenced by the capacity of the dietary treatment to produce pH, NH_4^+ and NO_3^- since sheep with lower acetate: propionate ratios also had lower ruminal pH values. Overall, the concentration of both total EAA and NEAA were similar among feeds, but numerically SBM had more EAA concentrations followed by CM, MBM, DDGs, BM, and FtM. In general, ruminal parameters were clearly influenced by the feed source, with a lower ruminal pH and a higher NH_4^+ in blood meal than corn meal.

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