

## Effects of Supplement Type on Fermentation Parameters and Amino Acid Profile in the Rumen of Sheep

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**Abstract:** This experiment was designed to determine the influence of processed or raw six feed ingredients and environment temperature on ruminal degradability and amino acid profile in twelve male sheep. A total of twelve castrated male sheep (average BW 60 kg) were used in this 9 days trial. The experiment was arranged in a 3x2x2 factorial design, in which sheep (3 replicates), diet type (processed or raw), and environment temperature (20 and 30°C) were the main effects to investigate. The animals were given restrict access to a Totally Mixed Ration (TMR) and infused daily with: Raw corn meal (T<sub>1</sub>), Processed corn meal (T<sub>2</sub>), Raw soybean meal (T<sub>3</sub>), Processed soybean meal (T<sub>4</sub>), Raw distillers dried grains with solubles (T<sub>5</sub>), and Processed distillers dried grains with solubles (T<sub>6</sub>). Environmental temperatures (20 and 30°C) did not affect rumen temperature but had significant effect on ruminal pH and NH<sub>4</sub><sup>+</sup> concentrations (p<0.05). There was significant effect (p<0.001) of dietary treatment on ruminal fermentation parameters pH, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, individual volatile fatty acid concentrations and their ratios (C<sub>2</sub>/C<sub>3</sub>; C<sub>2</sub>/C<sub>3</sub>+C<sub>4</sub>). Amino acid (AA) profile after 6 and 24 h exposure varied between temperature levels and ingredients. Except for Ser, Val, and Pro (p<0.05, p<0.05, and p<0.01) respectively, none of the AA measured showed any significant difference after 24 h of ruminal incubation. In general, all protein supplements, except soybean meal, were high in rumen undegradable protein and had the potential to provide good quality AA to complement microbial AA for production.

**Key words:** Amino acid, environment temperature, feed supplements, ruminal degradation, sheep

### INTRODUCTION

Since the emergence (December, 2000) of the regulation banning the use of animal protein supplements in ruminant diets, more research into the use of diets from plant origins has correspondingly intensified from nutritionists (Borucki Castro *et al.*, 2007). In this search for alternative feed resources, soybean meal, corn meal and DDGs, are among the most commonly employed feed supplements in the animal industry. Soybean meal, with its superior content of essential AA among oilseed meals has received the most extensive investigation and has thus continues to be extensively studied as an excellent source of AA (Borucki Castro *et al.*, 2007). However, due to its extensive degradability in the rumen (Awawdeh *et al.*, 2007) its utilization as sources of rumen undegradable protein for ruminants is limited. Furthermore, results from both live animals and laboratory based experiments continue to show varying degradation of these diets with differences in degradation between individual AA (Tamminga, 1979). Although ruminal degradation depends on the form of nitrogen in the diet as well as its solubility, ruminal fermentation and its products, represent a balance between several factors such as

structure and composition, pH, ammonia concentration, composition of microflora, ruminal motorics, passage rate of feed particles, and intake (Stern *et al.*, 2006; Helgi *et al.*, 2006). Similarly manipulation of dietary factors such as grain processing, forage-to-concentrate ratio, and particle length can alter the chemical composition of bacteria (Boguhn *et al.*, 2006). However, published results have been inconsistent. Several authors (Marshall *et al.*, 1992; Yang *et al.*, 2000; Beauchemin *et al.*, 2001) reported that processing often decreases rumen pH while increased processing can increase rumen ammonia concentration. Yang *et al.* (2000) and Koenig *et al.* (2003) found that feeding highly processed grain increased rumen ammonia levels. Beauchemin *et al.* (2001) and Bengochea *et al.* (2005) found no effect of processing on rumen ammonia levels. A large amount of data exist on the composition of ruminal bacterial AA leading to the conclusion that microbial proteins synthesized in the rumen serve as the most important sources of AA for ruminants. Despite the general awareness of this scenario, few quantitative data exist on the effect of environment temperature on ruminal fermentation characteristic and the subsequent AA composition within the rumen. Any attempt allowing the evaluation of individual ruminal AA

composition in dependence of dietary factors may help improve the accuracy of yield prediction and diet formulation for sheep. Diet and environment temperature are major factors in an animal's environment. Therefore, information concerning the interactions of diet and environment should also be useful in the feeding management of sheep industry in the tropics. The objective of this study was to evaluate the ruminal fermentation characteristics and AA profile of six feed supplement types from sheep exposed to different environmental temperatures.

## MATERIALS AND METHODS

**Animals and management:** The study was conducted in 2009 at the National Pingtung University of Science and Technology's Innovation and Practical Training Centre (IPTC) farm. Twelve male sheep fitted with ruminal cannulas were used as experimental animals for a period of 9 days. The sheep were blocked by weight and size in a factorial design to twelve treatments with 3 replicates per treatment. The sheep were housed in environment control chambers with temperature set at two different levels 20 and 30°C. A 2 week of adaptation to the experimental diets and housing condition was observed before the commencement of data collection. Animals were dewormed and vaccinated. The feedstuff used were Raw corn meal (T<sub>1</sub>), Processed corn meal (T<sub>2</sub>), Raw soybean meal (T<sub>3</sub>), Processed soybean meal (T<sub>4</sub>), Raw distillers dried grains with solubles (T<sub>5</sub>), and Processed distillers dried grains with solubles (T<sub>6</sub>). Each day 20g of the diets above are prepared in Dacron bags and infused in the cannula of each sheep. The sheep was restrict fed once every morning with 1.5 kg of TMR and had free access to water at all times. Prior to incubation, feeds were prepared in the feed mill and approximately 20 g of each test feed was accurately weighed into Dacron bags with a pore size of 50 µm. The bags plus sample were then inserted into the rumen few minutes (~30 min) after the morning feeding and retrieved after 24 h. Following incubation, the bags and contents were washed through the cold rinse cycle of a domestic washing machine.

**Experimental design:** The experiment was a 3x2x2 factorial design with 3 ingredients (Corn meal, soybean meal, and distillers dried grains with soluble), 2 temperature levels, and 2 diet types arranged on 3 blocks of sheep.

**Rumen measurement:** Following adjustment, ruminal fluid was collected every 2 h for 9 consecutive days using a suction pump during each sampling period. All evacuated ruminal contents were measured immediately for pH (Accumet pH meter; Fisher Scientific, Montreal, Quebec, Canada), temperature, ammonium, and nitrate. After these measurements ruminal fluid was filtered

through three layers of cheesecloth and samples were acidified to pH 2.0 with 50% H<sub>2</sub>SO<sub>4</sub>, and frozen at -20°C for later determination of VFA. Prior to VFA analysis each sample was centrifuged at 30×100 rpm gravity for 20 min. The clear supernatant was collected and analyzed for Volatile fatty acids on a column in a gas Chromatograph (*hp*) HEWLETT PACKAD 5890.

**Volatile fatty acids analysis:** A total of 432 samples were analyzed for VFA. Before analysis and prior to injection unto column, the acidified ruminal samples were thawed at room temperature and centrifuged (30×100 rpm gravity for 20 min) in order to obtain a clear supernatant. VFA content of rumen fluid samples were measured via gas chromatography. Individual rumen VFA was separated using a fused silica capillary column (30 m × 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.

**Ruminal degradation:** The Dacron bag (concentrate bag) was used to determine the extent of ruminal protein degradation of the 12 concentrates. The samples were weighed (20 g) and placed into Dacron bags measuring 5×10 cm with 50 µm pore size (No. R510; Ankom Technology) and incubated in the rumen of cannulated sheep fed TMR for 24 h × 9 d for indigestible degradation determination. Prior to incubation the bags were hermetically heat sealed using hot electric poly sealer (Impulse sealer, Model MWH-300, Electric Heating Equipment, Taipei, Taiwan); each bag was numbered with a permanent waterproof marking pen for sample identification. Each bag was tied to a drop line for easy removal. The bags were removed from the rumen after 24 h incubation. After removal from the rumen, bags were soaked in a water-bath at 20°C for 15 min, washed with water using a conventional domestic washing machine and washings were repeated, squeezed until the rinsing water was clear. Dacron bags were then dried for approximately 3 h at 105°C and weighed after cooling at room temperature in a glass desiccator. Nitrogen in the remaining contents of each bag was determined by the Kjeldahl procedure.

**Amino acids analysis:** A total of 432 samples were subjected to AA analysis in this experiment. Rumen content samples (100 mL) were stone rock frozen in (JUST-A-TILT™ Shell freezer FTSS SYSTEMS) at -40°C

Table 1: Effects of environment temperature on ruminal degradability of sheep fed the six supplements

Item	Env Temp						Interaction	
	20 C	30 C	SEM	Sig.	E*Ing	E*	Ding*D	Ing*T
RT	38.61	39	0.39	NS	NS	NS	NS	NS
pH	6.88 <sup>a</sup>	6.84 <sup>b</sup>	0.01	*	**	***	***	***
NH <sub>4</sub> <sup>+</sup> /ppm	1.75 <sup>a</sup>	1.56 <sup>b</sup>	0.07	*	*	NS	NS	***
NO <sub>3</sub> <sup>-</sup> /ppm	289.78	256.19	8.54	NS	***	*	*	*

<sup>a,b</sup>: Means in the same row with different superscripts are significantly different; \*\*\*: p<0.001; \*\*: p<0.01; \*: p<0.05; NS: No Significance (p>0.05); E\*Ing: Environment Temperature x Ingredient; E\*D: Environment Temperature x Day; Ing\*D: Ingredient x Day; Ing\*T: Ingredient x Time

Table 2: Ruminal degradation parameter of the six supplements used in the experiment

Item(ppm)	Ing						Interaction					
	1	2	3	4	5	6	SEM	Sig.	E*Ing	E*D	Ing*D	Ing*T
RT	40.31	38.75	38.31	38.36	38.65	38.45	0.67	NS	NS	NS	NS	NS
Ph	6.93 <sup>a</sup>	6.77 <sup>b</sup>	6.98 <sup>a</sup>	6.69 <sup>c</sup>	6.96 <sup>a</sup>	6.82 <sup>b</sup>	0.02	***	***	***	***	**
NH <sub>4</sub> <sup>+</sup> /ppm	2.47 <sup>a</sup>	1.69 <sup>b</sup>	1.28 <sup>c</sup>	1.47 <sup>bc</sup>	1.48 <sup>bc</sup>	1.52 <sup>bc</sup>	0.12	***	*	NS	NS	***
NO <sub>3</sub> <sup>-</sup> /ppm	263.30 <sup>b</sup>	197.18 <sup>c</sup>	384.67 <sup>a</sup>	284.18 <sup>b</sup>	145.88 <sup>c</sup>	59.90 <sup>d</sup>	23.76	***	***	*	*	*

<sup>a,b,c</sup>: Means in the same row with different superscripts are significantly different; \*\*\*: p<0.001; \*\*: p<0.01; \*: p<0.05; NS: No Significance (p>0.05); 1: Raw corn meal; 2: Processed corn meal; 3: Raw soybean meal; 4: Processed soybean meal; 5: Raw DDGs; 6: Processed DDGs; E\*Ing: Environment Temperature x Ingredient; E\*D: Environment Temperature x Day; Ing\*D: Ingredient x Day; Ing\*T: Ingredient x Time

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 product is now been analyzed for AA content. The dried residues of the composite sample of each dietary treatment were separated on a high performance liquid chromatography (HPLC) machine (Agilent Technologies 1200 series, serial No. JP 73061109) following acid hydrolysis. Before placing the mixture in the digester a vacuum pump (model: multipose® 700 mm Hg Vac) was used to suck the air out each taking 3 min. Acid hydrolysis involved placing 0.1 g of sample and 1.5 mL of NaOH (0.2N) and 1.0 mL of HCl in a ceramic crucibles and heating (infrared heater plate) in a wet digester (BUCHI wet Digester B- 440, SUNTEX® ISO 9001 certified) for 24 h at 110°C. The wet digester was connected to a BUCHI Scrubber B-414 (Switzerland) for extraction and neutralization of acid fumes and reaction gasses. Prior Acid hydrolysis the preparation above was neutralized and standardized to pH 2.2±0.1 by adding about 30-40 mL of NaOH (4N) and sodium citrate buffer before amino acid analysis. Cooled hydrolysates were filtered through filter paper (AVANTEC LOT No. 902100050, 110 mm, Toyo Roshi Kaisha, Ltd. Made in Japan). About 1.5 mL of the clarified hydrolysates were injected using glass string in Agilent auto sampler vials (LOT No. manufactured from first hydrolytical class, glass type 1 under Iso 9001 certified environment) tightly fitted with blue vial screw caps and septa (Part No. 5182-0717). These vials were then placed in the HPLC auto sampler for the separation of the various AA.

**Statistical analysis:** The effects of protein sources, day, time, rumen temperature, and protein sources x day, time, environmental temperature, on ruminal degradation parameters and AA concentrations were evaluated by analysis of variance using the GLM Procedure of SAS (2004). The differences among protein sources on the

mean concentration of the rumen variables were compared using the Duncan's Multiple Range test.

## RESULTS AND DISCUSSION

**Ruminal metabolites:** The effect of environment temperature, ruminal parameters and their interactions are shown in Table 1. Rumen pH and NH<sub>4</sub><sup>+</sup> concentrations were affected by environment temperature (p<0.05). The cool (20°C) treatment tended to elevate the mean pH and NH<sub>4</sub><sup>+</sup> concentrations while the hot (30°C) treatment resulted in pH and NH<sub>4</sub><sup>+</sup> deduction. This result agrees with that of Mishra *et al.* (1970) even though these authors used lower temperature levels (18.3°C and 29.4°C). Rumen temperature and NO<sub>3</sub><sup>-</sup> did not differ between temperature levels (Table 1) but were significantly different (p<0.001) between dietary treatments. Mean ruminal pH, NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup> were highly significant (p<0.001).

Average ruminal pH was greatest (p<0.001) for the DDGs treatment and numerically least for the SBM treatment, but maximum pH, minimum pH, and pH change were not different among treatments (Table 2). Ingredient x time interactions existed for all variables measured except for rumen temperature. Environment x ingredient or x day showed significant interactions for rumen pH, NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup> (p<0.05) but there were no interactions between day x time and environment temperature x time. The reason behind this could not be explained in this study. Ruminal pH plays an important role in regulating the microbial ecosystem, especially for low-pH sensitive microorganisms such as cellulolytic bacteria (Russell and Wilson, 1996; Marden *et al.*, 2008). Mean Ruminal pH for the 6 experimental diets remained above 6.0 at all time indicating that all sheep had a satisfactory ruminal environment for nutrient fermentation, including fiber digestion (Olmos Colmenero and Broderick, 2006). The mean ruminal pH measured in

Table 3: Individual ruminal volatile fatty acids concentrations of sheep exposed to the six supplement types

Item(ppm)	Ing						SEM	Sig.
	1	2	3	4	5	6		
C <sub>2</sub>	125.34 <sup>a</sup>	103.38 <sup>b</sup>	46.18 <sup>d</sup>	62.99 <sup>c</sup>	41.70 <sup>d</sup>	43.28 <sup>d</sup>	5.09	***
C <sub>3</sub>	74.13 <sup>a</sup>	69.48 <sup>a</sup>	14.15 <sup>c</sup>	38.53 <sup>b</sup>	7.08 <sup>c</sup>	6.74 <sup>c</sup>	6.92	***
C <sub>4</sub>	46.09	49.02	30.76	60.02	16.5	3.5	13.19	NS
C <sub>5</sub> 1	4.38	14.06	8.76	17.4	10.55	10.72	2.25	NS
iC <sub>4</sub> 2	3.89 <sup>ab</sup>	32.03 <sup>a</sup>	11.97 <sup>b</sup>	25.51 <sup>ab</sup>	8.88 <sup>b</sup>	14.36 <sup>ab</sup>	6.3	*
iC <sub>5</sub> 3	8.56	33.94	11.99	35.25	29	7.67	15.41	NS
C <sub>2</sub> /C <sub>3</sub>	2.31 <sup>c</sup>	2.42 <sup>c</sup>	1.90 <sup>ab</sup>	1.43 <sup>b</sup>	1.54 <sup>ab</sup>	1.60 <sup>a</sup>	0.3	***
C <sub>2</sub> /(C <sub>3</sub> +C <sub>4</sub> )	1.31 <sup>b</sup>	1.05 <sup>b</sup>	1.72 <sup>b</sup>	1.04 <sup>b</sup>	3.64 <sup>a</sup>	4.73 <sup>a</sup>	0.45	***

<sup>a,b,c,d</sup>: Means in the same row with different superscripts are significantly different; \*\*\*: p<0.001; \*: p<0.05; NS: No Significance; 1: Raw corn meal; 2: Processed corn meal; 3: Raw soybean Meal; 4: Processed soybean meal; 5: Raw DDGs; 6: Processed DDGs; C<sub>2</sub>: Acetic acid; C<sub>3</sub>: Propionic acid; C<sub>4</sub>: Butyric acid; C<sub>5</sub>: Valeric acid; IC<sub>4</sub>: Isobutyric acid; IC<sub>5</sub>: Isovaleric acid

Table 4: Profiles of AA of the feedstuffs remaining in the bags after 6 h of incubation in the rumen.

Item (%)	Ing						SEM	Sig.	Interaction Ing*D
	1	2	3	4	5	6			
Asp	1.20	1.40	1.42	1.16	1.33	1.21	0.07	NS	**
Glu	1.41	1.76	1.80	1.47	2.01	1.55	0.14	NS	**
Ser	0.61	0.67	0.73	0.59	0.62	0.63	0.04	NS	*
Gly	0.89	1.03	1.00	0.86	0.94	0.89	0.05	NS	**
Thr	0.63	0.64	0.68	0.60	0.68	0.64	0.05	NS	*
Agr	0.80	0.91	0.98	0.82	0.91	0.86	0.06	NS	*
Ala	1.38 <sup>bc</sup>	1.59 <sup>a</sup>	1.55 <sup>ab</sup>	1.31 <sup>c</sup>	1.40 <sup>bc</sup>	1.34 <sup>c</sup>	0.06	**	*
Tyr	0.60	0.63	0.71	0.61	0.98	0.66	0.12	NS	NS
Cys	0.430	.32	0.32	0.32	0.30	0.31	0.04	NS	NS
Val	0.66 <sup>c</sup>	0.83 <sup>ab</sup>	0.74 <sup>abc</sup>	0.79 <sup>abc</sup>	0.86 <sup>a</sup>	0.68 <sup>bc</sup>	0.05	*	*
Met	0.42	0.44	0.46	0.38	0.40	0.41	0.04	NS	NS
Phe	0.65	0.68	0.66	0.63	0.68	0.61	0.04	NS	NS
Ile	0.68	0.71	0.66	0.67	0.65	0.58	0.05	NS	NS
Leu	0.07	1.28	1.26	1.13	1.23	1.09	0.07	NS	**
Lys	0.77	0.88	0.87	0.82	0.89	0.81	0.05	NS	*
Pro	0.75	0.62	0.66	0.57	0.75	0.71	0.09	NS	NS

<sup>a,b</sup>: Means in the same row with different superscripts are significantly different; \*\*: p<0.01; \*: p<0.05; NS: No significance (p>0.05); 1: Raw corn meal; 2: Processed corn meal; 3: Raw soybean meal; 4: Processed soybean meal; 5: Raw DDGs; 6: Processed DDGs; 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; Ing\*D: Ingredient x Day

our study is in agreement with the data obtained by Marden *et al.* (2005, 2008). With regards to interactions, the results in Table 2 followed a similar pattern with that of Table 1 with pH, NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup> exhibiting significant interaction.

Mean rumen VFA concentrations did not differ between the two temperature but sheep exposed to lower temperature levels had a numerically lower acetic acid: propionic acid (A: P) ratio compared to the other temperature level (Table 3). Generally, in a diet containing a high proportion of readily fermentable carbohydrate, any pH decrease is systematically associated with an increase in VFA and lactate concentrations (Marden *et al.*, 2008). Proportions of acetate were greater (p<0.001) and those of propionate were less (p<0.001) for the CM treatment. However, the proportion of butyrate was not affected by treatment. Decreased acetate and increased propionate proportions for the SBM and DDGs treatment led to the acetate: propionate ratio being less (p<0.001) for both treatments compared with the other treatments. The significantly higher acetate: propionate ratios for the corn based diets

could be an indication that fiber digestion was being promoted. These findings are in accordance with (Vander Pol *et al.*, 2009) who reported increases in acetate: propionate ratio as corn bran inclusion increased from 0 to 45% of DM where as replacing concentrates with high quality fiber sources increased acetate: propionate ratio and subsequent milk fat percentage in lactation diets. In contrast, Vander Pol *et al.* (2009) reported that other works demonstrated a decrease in acetate: propionate ratio as inclusion of corn steep, a liquid byproduct from wet milling plants, increased. In our study, modest declines in pH (6.93 to 6.77 and 6.98 to 6.82) caused small but statistically significant (p<0.001) decreases in the acetate to propionate ratio of corn meal base diets. Although total VFA concentration was similar among all diets, sheep infused with DDGs had a significantly lower (p<0.001) propionate concentration, resulting in a significant increased (p<0.001) acetate: propionate ratio. There was a tendency for the branched chained VFA to be higher with the SBM diet, indicating greater protein degradation with this treatment.

Table 5: Profiles of AA of the feedstuffs remaining in the bags after 12 h of incubation in the rumen

Item/%	Ing						SEM	Sig.	Interaction Ing*D
	1	2	3	4	5	6			
Asp	1.14 <sup>bc</sup>	1.39 <sup>ab</sup>	1.43 <sup>a</sup>	1.49 <sup>a</sup>	1.41 <sup>a</sup>	1.06 <sup>c</sup>	0.09	**	NS
Glu	1.40 <sup>bc</sup>	1.76 <sup>ab</sup>	1.87 <sup>a</sup>	1.90 <sup>a</sup>	1.75 <sup>ab</sup>	1.31 <sup>c</sup>	0.12	**	NS
Ser	0.58	0.68	0.71	0.76	0.69	0.56	0.05	NS	NS
Gly	1.68 <sup>b</sup>	1.98 <sup>ab</sup>	2.14 <sup>a</sup>	2.15 <sup>a</sup>	2.11 <sup>a</sup>	1.75 <sup>b</sup>	0.1	**	NS
Thr	0.56 <sup>b</sup>	0.72 <sup>a</sup>	0.82 <sup>a</sup>	0.78 <sup>a</sup>	0.70 <sup>a</sup>	0.52 <sup>b</sup>	0.04	***	**
Agr	0.66 <sup>b</sup>	0.81 <sup>ab</sup>	0.95 <sup>a</sup>	0.94 <sup>a</sup>	0.92 <sup>a</sup>	0.74 <sup>b</sup>	0.05	**	*
Ala	1.20 <sup>b</sup>	1.51 <sup>ab</sup>	1.45 <sup>ab</sup>	1.61 <sup>a</sup>	1.50 <sup>ab</sup>	1.24 <sup>b</sup>	0.1	*	NS
Tyr	0.64	0.67	0.71	0.74	0.65	0.55	0.05	NS	NS
Cys	0.28 <sup>b</sup>	0.30 <sup>ab</sup>	0.31 <sup>a</sup>	0.33 <sup>a</sup>	0.30 <sup>ab</sup>	0.28 <sup>b</sup>	0.01	***	**
Val	0.56	0.8	3.76	0.89	0.76	0.56	1.24	NS	NS
Met	0.40 <sup>ab</sup>	0.40 <sup>ab</sup>	0.44 <sup>a</sup>	0.42 <sup>ab</sup>	0.42 <sup>ab</sup>	0.36 <sup>b</sup>	0.02	**	NS
Phe	0.52 <sup>b</sup>	0.69 <sup>a</sup>	0.72 <sup>a</sup>	0.74 <sup>a</sup>	0.68 <sup>a</sup>	0.48 <sup>b</sup>	0.04	***	NS
Ile	0.55 <sup>b</sup>	0.70 <sup>a</sup>	0.72 <sup>a</sup>	0.75 <sup>a</sup>	0.75 <sup>a</sup>	0.54 <sup>b</sup>	0.05	**	NS
Leu	0.97 <sup>b</sup>	1.19 <sup>a</sup>	1.29 <sup>a</sup>	1.39 <sup>a</sup>	1.26 <sup>a</sup>	0.90 <sup>b</sup>	0.08	***	NS
Lys	0.61 <sup>b</sup>	0.81 <sup>ab</sup>	0.80 <sup>ab</sup>	0.84 <sup>ab</sup>	1.12 <sup>a</sup>	0.66 <sup>b</sup>	0.12	*	NS
Pro	0.66	0.74	0.71	0.75	0.73	0.72	0.06	NS	NS

<sup>a, b</sup>: Means in the same row with different superscripts are significantly different; \*\*\*: p<0.001; \*\*: p<0.01; \*: p<0.05; NS: No significance (p>0.05); 1: Raw corn meal; 2: Processed corn meal; 3: Raw soybean meal; 4: Processed soybean meal; 5: Raw DDGs; 6: Processed DDGs; 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; Ing\*D: Ingredient x Day

The AA profile in percentages of the feedstuffs remaining in the bags after 6 h of incubation in the rumen are presented in Table 4. Environment temperature had no effect on all other AA measured after 6 h of ruminal incubation (data not shown). No significant difference was detected in AA concentration among the supplements except for Ala and Val (p<0.01 and p<0.05) respectively. In general, the degradability of individual amino acids was feed dependent. Lys seems to be one of the more degradable of the important amino acids. It decreased, or tended to decrease, as a proportion of total amino acids after ruminal incubation with all feedstuffs. Similar observations were found for Phe. Erasmus *et al.* (1994) reported a similar finding for Lys and Phe with 9 out of 12 feedstuffs examined except soybean meal. This is further supported by (Borucki Castro *et al.*, 2007) reported decreased effective degradability of the branch-chain AA and Phe from treated SBM. Met was reported by Tamminga (1979) to be one of the amino acids most resistant to ruminal degradation, which happens to be the case in our own finding. Glu acid was one of the most relatively degradable (p<0.01) amino acids in the feedstuffs. In Table 4 a significant treatment x day interaction were recorded but these authors could not explain the reason behind this.

Table 5 presents the Profiles of AA of the feedstuffs remaining in the bags after 12 h of incubation in the rumen. For Met, the degradation was even lower than that for Lys 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. The results obtained for all supplemental diets showed effects

on the amino acid profile, except for Ser, Try, Val and Pro whose proportion increased and decreased respectively. Nevertheless, these variations were moderate. Amongst the nonessential amino acids, significant effects (p<0.05) were observed for Asp, Glu, Gly, Ala but not Pro and Ser. The result of the present study supports the idea that ruminal microorganisms prefer Met and possibly His and Tyr containing ingredients consequently leading to the complete undetectability of His in this experiment. Higher Glu contents could probably be explained by the presence of high concentrations of this AA in the dietary supplemental protein used in this experiment (Jenkins, 1993).

## CONCLUSION

This study revealed that environmental temperature does have influence on sheep rumen pH as Sheep exposed to the higher ambient temperature (30°C) had higher ruminal pH than those subjected to lower ambient temperature (20°C) but it does not necessarily have effect on rumen temperature. No differences were found between the amino acid composition of the protein sources at 6 and 24 h ruminal incubation however, their individual concentrations were higher with the later. Met, Lys or Cys ranked first, second or third-limiting across all diets and Phe, Glu, Ala, and Leu were greatly produced for all diets. It is interesting to note that while there was no interaction between environmental and diet type, ruminal AA degradation was higher at the higher temperature than the lower temperature for the entire AA tested. The reason behind this phenomenon is not clear and requires further investigation.

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