Research Article
Ruminal Degradation Pattern and Amino Acid Production in Sheep Fed Six Feed Ingredients as Influenced by Time and Day

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Abstract: This study was designed to investigate hourly ruminal degradation pattern and daily Amino Acid (AA) production of sheep fed six feed ingredients during a nine consecutive days trial. Twelve sheep (av. BW 60 kg), with ruminal cannulas were assigned to six dietary treatments according to a 6×2 factorial arrangement in which dietary treatment were: Raw corn meal (T_1), Processed corn meal (T_2), Raw soybean meal (T_3), Processed soybean meal (T_4), Raw distillers dried grains with solubles (T_5) and Processed distillers dried grains with solubles (T_6) and 2 environment temperature (20 and 30°C). Animals were given restrict access to Totally Mixed Rations (TMR) and ruminally infused daily with each of the above ingredients. Digestive characteristics showed two hourly degradation of pH and NH^+_4 differed (p<0.001) between sampling periods. Six hourly ruminal fermentation for acetate (C2) were affected (p<0.001) with greater production at noon sampling period. Propionic, butyric acids and acetate: propionate ratio (C2/C3) decreased (p<0.05) post noon sampling period. Environment temperature affected AA degradability with greater degradability achieved at 30°C for Val, Ser and Pro (p<0.05, p<0.05 and p<0.01, respectively) during 6 h of ruminal incubation. Except Try, Cys and Val the entire AA detected showed daily increment (p<0.001) after 6 h of ruminal incubation. All other AA, but Val differed (p<0.001) during the 12 h daily sampling period. Concentrations of AA remaining in the bag after 6 and 12 h of incubation peaked at 6 and 4 h daily. In conclusion, degradability and AA output of feed ingredients differed between hours and days.

Keywords: Amino acids, feed ingredients, ruminal degradation, sheep

INTRODUCTION

Rumen feed degradation is one of the most important quantitative and qualitative factors determining the value of a feed when ruminants' feeding is concerned. The net result of this degradation and metabolism of the feed components going on in the rumen is responsible for the bioconversion of feed into such form that is required by the ruminant host as a source of Amino Acids (AA) and vitamins (Kamra, 2005) to the intestines and tissues and energy available for use by the ruminal microorganism. Volatile Fatty Acids (VFA) arising from ruminal fermentation are the primary metabolizable energy source (account for 60-70%) for the animal (Seymour *et al*., 2005). Rumen degradation and the subsequent AA production by the rumen microbes occurs at variable rates during the day and is influenced by the nature of the diet and feeding patterns of the animal (Van Soest, 1982). Little research has been conducted to determine the magnitude of the differences in degradation characteristics and the resulting quantities of AA output in sheep under tropical conditions. For the past decade or so ruminant nutritionist developed a keen interest on rumen ecology and manipulating its fermentation patterns. However, most of these papers have dealt with ruminants raised in temperate areas and fed on good quality roughages with high levels of concentrate supplementation. Until now, except (Narvaez *et al*., 2011), most data from literature (Zebrowska *et al*., 1997; Taghizadeh *et al*., 2005) commonly refer to single, 8, 12 or 16 h, respectively incubation times in experiments to investigate the influence of rumen exposed feed ingredients on the AA profile. Our assumption is that the degradation profile for AA in the residues after a single rumen incubation time might not only differ between treatments but possibly between hours and days. Hence, caution must be observed when the AA profile in the residue of a single incubation point is used as a predictor for the AA profile reaching the small intestine. Therefore, information on rumen degradability of individual AA as influenced by time and day is necessary.

Understanding the influence of hourly ruminal degradation pattern and daily AA production by the rumen microbial population may help in discovery of how to decrease ruminal feed degradation and/or dependably predict assimilation. The objective of the study was to investigate possible relationships between

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rumen fermentation pattern and AA production as influenced by time and day.

MATERIALS AND METHODS

Animals, housing and feeding: 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 six treatments with three replicates per treatment. The feedstuffs used were Raw corn meal (T_1), Processed corn meal (T_2), Raw soybean meal (T_3), Processed soybean meal (T_4), Raw distillers dried grains with solubles (T_5) and Processed distillers dried grains with solubles (T_6). Sheep were restrict fed once every morning with 1.5 kg of TMR and had free access to water at all times. The sheep were housed in climate-controlled chambers with temperature set at two different levels 20 and 30°C. Each day 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. Bags were hermetically heat-sealed using hot electric poly sealer (Impulse sealer, Model MWH-300, Electric Heating Equipment, Taipei, Taiwan), numbered with a permanent waterproof marking pen for sample identification and tied to a drop line for easy removal. The bags plus samples were then inserted into the rumen few minutes (≈30 min) after the morning feeding and retrieved after 24 h daily. Following incubation, the bags and contents were washed through the cold rinse cycle of a domestic washing machine. Animals were dewormed and vaccinated. A 2 week of adaptation to the experimental diets and housing condition was observed before the commencement of data collection.

Experimental design: The experiment was a 6×2 factorial design. Factors in the design were 6 ingredients: Raw corn meal (T_1), Processed corn meal (T_2), Raw soybean meal (T_3), Processed soybean meal (T_4), Raw distillers dried grains with solubles (T_5) and Processed distillers dried grains with solubles (T_6) and 2 temperature (20 and 30°C) levels arranged on 3 blocks of sheep.

Ruminal fluid collection: Following adjustment, ruminal fluid was collected every 2 h for 9 consecutive days using a suction pump during each sampling period. The suction pump was introduced through a ruminal trocar to reach different locations within the rumen and obtain a representative sample. All evacuated ruminal contents were measured immediately for pH (Accumet pH meter; Fisher Scientific, Montreal, Quebec, Canada), temperature, ammonium and nitrate. Rumen samples for VFA analysis were collected every other day at 6 h intervals i.e., at 06:00, 12:00, 18:00 and 00:00 h. After the above-mentioned measurements, ruminal fluid was filtered through three layers of cheesecloth and acidified to pH 2.0 with 50% H_2SO_4

and frozen at -20°C for later determination of VFA and AA. Prior to VFA analysis, each sample was centrifuged at 30×100 rpm gravity for 20 min. The clear supernatant was collected and analyzed for VFA on a column in a gas Chromatograph (hp) HEWLETT PACKAD 5890.

VFA 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 contents of rumen fluid samples were measured via gas chromatography. Individual rumen VFA were 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/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. After the preparation for VFA, the remaining samples were kept and frozen at -20°C for subsequent amino acids analysis.

AA 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 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 (Agilent Technologies 1200 series, serial No. JP 73061109) following acid hydrolysis. Acid hydrolysis involved placing 0.1 g of sample and 1.5 mL of NaOH (0.2 N) 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 digester connects to a BUCHI Scrubber B-414 (Switzerland) for extraction and neutralization of acid fumes and reaction gasses. Prior Acid hydrolysis the preparations above were neutralized and standardized to pH 2.2±0.1 by adding about 30-40 m/L of NaOH (4 N) and sodium citrate buffer. 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

Table 1: Influence of environmental temperature on ruminal AA composition after 6 h in the rumen

| Item/% | Environment temperature | | | |
	20°C	30°C	S.E.M.	Sig.
Asp	1.23	1.35	0.05	NS
Glu	1.65	1.69	0.08	NS
Ser	0.61[b]	0.68[a]	0.02	*
Gly	0.92	0.95	0.03	NS
Thr	0.62	0.66	0.03	NS
Arg	0.89	0.88	0.03	NS
Ala	1.41	1.45	0.03	NS
Tyr	0.73	0.68	0.07	NS
Cys	0.31	0.35	0.02	NS
Val	0.77[a]	0.74[b]	0.03	*
Met	0.39	0.45	0.03	NS
Phe	0.64	0.66	0.02	NS
Ile	0.67	0.65	0.03	NS
Leu	1.15	1.21	0.04	NS
Lys	0.82	0.86	0.03	NS
Pro	0.58[b]	0.77[a]	0.05	**

[a,b]: Means within the same row having different superscripts differ significantly ($p<0.05$); **: $p<0.01$; *: $p<0.05$; NS: No significance ($p>0.05$); 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

stringe in Agilent auto sampler vials (glass type 1-Iso 9001) 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 methods: The effects of feed ingredient, day, time and ingredient x day, time, environmental temperature, on ruminal degradation parameters and AA production were evaluated by analysis of variance using the GLM Procedure of SAS (2004). The differences among feed ingredients on the mean concentration of the rumen variables were compared using the Duncan's Multiple Range test.

RESULTS AND DISCUSSION

The influence of environmental temperature on ruminal AA composition after 6 h of ruminal exposure is presented in Table 1. The higher environment temperature achieved greater degradation compared to the lower environment temperature rigime. Among the EAA Val was the only AA affected ($p<0.05$) whereas Ser and Pro were the only affected NEAA ($p<0.05$; $p<0.01$ respectively). Nearly all AA exhibited no significant interactions between feed ingredient and ambient temperature. The absence of interaction between the feed ingredient and environmental temperature on the performance of the sheep's rumen indicates that the protein level to be adopted is independent of environmental temperature. Environmental temperature had no significant effect on degradation characteristics (VFA production) as well as AA degradation after 12 h of exposure in the sheep rumen (data not shown).

Table 2 presents the results for hourly ruminal fermentation parameters. Ruminal parameters (pH and NH^+_4) were clearly influenced by period. Sampling period had no effect on rumen temperature or nitrate concentrations ($p>0.05$). Ruminal pH and NH^+_4 concentrations differed ($p<0.001$) during the sampling periods. Maximum concentrations of fermentation parameters detected few hours post feeding were probably a response to rapid degradation of all sources of dietary treatments. At 6 h post feeding, both ruminal production of pH and NH^+_4 peaked and NH^+_4 remained greater than 1 ppm throughout the collection period. These findings are in concordance with Yang *et al*. (2001) and Beauchemin *et al*. (2003) who observed highest pH values in the afternoon and lowest pH values at night with cows fed TMR but differed with that of McMurphy *et al*. (2011). The lower concentration of NH^+_4 may be an indication of the greater use of energy and ammonia by ruminal microorganisms. Grigsby *et al*. (1992) reported that fiber degradation is reduced when ruminal pH is decreased below 6.2 and 6.0 respectively, whereas other studies showed that decreased ammonia may indicate lowered proteolysis, degradation of peptides and deamination of AA in the rumen (Koenig *et al*., 2003; Soltan, 2009). However, ruminal pH in this experiment remained above 6.0 at all 12-time points throughout the sampling cycle, indicating that all sheep had a satisfactory ruminal environment for nutrient fermentation, including fiber digestion (Hoover, 1986).

Table 2: Ruminal temperature levels, ruminal pH, ruminal NH^+_4 and ruminal NO^-_3 as affected by time

| Item | Time (h) | | | | | | |
	2	4	6	8	10	12	14
[a]RT	38.450	38.66	41.96	38.55	38.48	38.62	38.53
pH	6.910[b]	7.01[a]	6.93[b]	6.80[de]	6.76[e]	6.79[de]	6.81[cde]
[q]NH^+_4	0.890[b]	2.07[a]	2.49[a]	2.34[a]	2.20[a]	2.27[a]	2.19[a]
[q]NO^-_3	331.22	322.89	275.16	225.42	211.08	197.97	222.90
Item	16	18	20	22	24	S.E.M.	Sig.
[a]RT	38.44	38.48	38.59	38.52	38.40	0.94	NS
pH	6.86[bcd]	6.90[b]	6.79[de]	6.86[bcd]	6.88[bc]	0.03	***
[q]NH^+_4	1.22[b]	1.15[b]	1.01[b]	1.02[b]	0.99[b]	0.17	***
[q]NO^-_3	286.08	328.04	277.17	346.12	274.53	20.94	NS

[a,b,c,d,e]: Means within the same row having different superscripts differ significantly ($p<0.05$); ***: $p<0.001$; NS: Not significant; [q]: ppm; [a]: RT Rumen temperature; S.E.M.: Standard error of mean
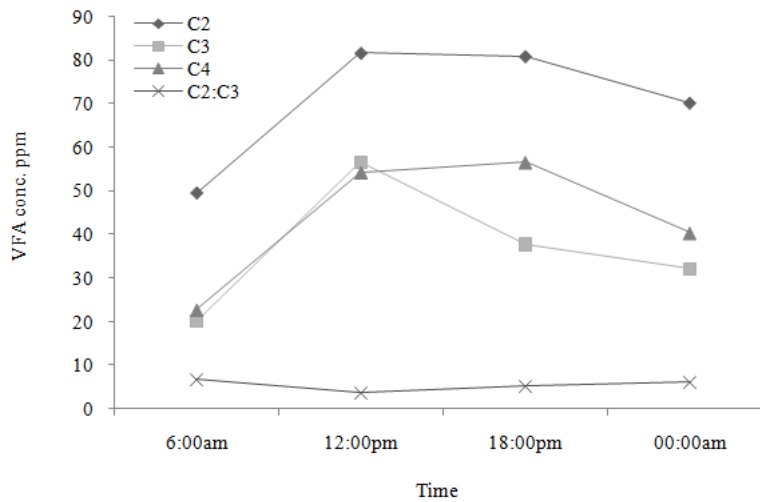
Fig. 1: Change in concentrations of major volatile fatty acids and their ratio in ruminal fluid of sheep over time
C2: Acetic acid; C3: Propionic acid; C4: Butyric acid

Concentrations of the major VFA and their ratio are shown in Fig. 1. C2, C3, C4 and C2/C3 ratio (expressed in ppm) were all influenced at a statistically significant level by sampling period. C2 concentration differed ($p<0.001$) whereas C3, C4 and C2/C3 ratio exhibited moderate defference ($p<0.05$). Concentrations of volatile isocids were reduced during the evening sampling times which corresponds to the lower degradability of their precursors (Val and Leu) respectively. Reduced proteolysis could explain the lower isoacids concentrations and the tendency for high dietary protein escape. Pena *et al*. (1986) suggested decline in concentrations of isoacids is linked to heat treatment, which reduces degradability of their precursors, the branched-chained AA. Other reports (Scholljegerdes *et al*., 2005), showed increased ($p<0.02$) molar proportions of valerate, isovalerate and isobutyrate with rumen undergradable inclusion, which likely reflect ruminal degradation of specific branch-chained AA from the rumen undergradable supplement. The significant decline in C2 and an increase in C4 at a time when lower pH was observed may be due to a shift in metabolism of C2 to C4. This would explain partly the reduced concentration of C2 as pH declines. The high concentration of VFA at the 12 h sampling period is consistent with the low ruminal pH after this period. The trend for increased C2 at higher rumen pH in this study is consistence with other reports Fulton *et al*. (1979) and Andrea *et al*. (2008). Our study is also in agreement with studies in which lower C2 and increased C4 at high pH were observed (Fulton *et al*., 1979). On the contrary, Koenig *et al*. (2003) revealed that lower molar proportion of acetic acid ($p = 0.005$) combined with a higher molar proportion of propionic acid ($p = 0.002$) resulted in a lower C2/C3 ratio for the 5% silage diets ($p<0.001$), reflecting the typical end-products of ruminal starch vs. fiber digestion with

greater cereal grain digestion (Eastridge *et al*., 2009). According to McMurphy *et al*. (2011) this may arise because the ionized state of NH_4^+ is utilised by bacteria that favour the production of more propionic acid as opposed to acetic acid.

Navarro-Villa *et al*. (2011) associated decreased CH_4 production with lower C2 and C4 acids production and a higher production of C3 acid. On the contrary, our result indicated a more methanogenic rumen fermentation pathway (higher production of C2 and C2/C3 ratio and lower C3 production). Fortunately, the concentration of NO_3^- in this experiment was high enough to counteract methanogenesis since NO_3^- serves as a hydrogen sink and thus decrease the availability of hydrogen for the formation of CH_4 (Rodriquez *et al*., 2011). This situation is favorable as CH_4 production represents a significant gross energy loss from 0.2 to 0.12 (2-12%) of total feed intake (Rodriquez *et al*., 2011). In this case, our study should have positive impact on energy utilization as well as demonstrate both long-term environmental and short-term economic benefits (Lopez *et al*., 2010; Rodriquez *et al*., 2011). Wanapat (2000) studied the diurnal patterns during 24 h of rumen fermentation characteristics in beef cattle and swamp buffaloes fed on untreated and ureatreated rice straw. In both animals, rumen pH and temperature were maintained at constant values of 6.5-6.7 and 38-39°C, respectively. However, VFA production patterns did either fluctuate (C2) or remained similar (C3 and C4) indicating an active role of rumen microbes and ongoing fibre fermentation of cellulolylic bacteria. They also reported that rumen NH_3-N was very consistent and relatively low (<5 mg/dL) throughout the period. The decrease in ammonia in this study, during the second (0.89 ppm) and twenty fourth (0.99 ppm) h of sampling times may be an indication that treated SBM lowered proteolysis, degradation of peptides and deamination of AA in the rumen (Soltan, 2009).

Table 3: Concentration of AA remaining in the bags after 6 h of incubation as influenced by time and day

Item/%	Day									S.E.M.	Sig.
	1	2	3	4	5	6	7	8	9		
Asp	1.51[ab]	1.14[bc]	0.93[d]	1.22[c]	1.27[bc]	1.69[a]	1.51[ab]	1.28[bc]	1.08[cd]	0.08	***
Glu	1.96[ab]	1.52b[bcd]	1.12[d]	1.80[abc]	1.65[bc]	2.20[a]	1.90[abc]	1.54[bcd]	1.36[cd]	0.03	*
Ser	0.47[ef]	0.41[f]	0.51[def]	0.62[cde]	0.73[bc]	0.91[a]	0.80[ab]	0.65[bcd]	0.58[cde]	0.05	***
Gly	1.03[bc]	0.88[cd]	0.64[e]	0.80[de]	0.98[bcd]	1.31[a]	1.14[ab]	0.84[d]	0.83[d]	0.06	***
Thr	0.62[abcd]	0.52[cd]	0.45[d]	0.70[abc]	0.73[ab]	0.79[a]	0.79[a]	0.66[abc]	0.55[bcd]	0.06	*
Arg	0.83[bc]	0.72[c]	0.79[bc]	0.99[b]	0.67[c]	0.79[bc]	0.99[b]	0.98[b]	1.21[a]	0.07	***
Ala	1.34[cd]	1.28[d]	1.12[d]	1.27[d]	1.53[c]	2.13[a]	1.76[b]	1.21[d]	1.32[d]	0.07	***
Tyr	1.22	0.60	0.49	0.60	0.62	0.86	0.77	0.62	0.62	0.14	NS
Cys	0.31	0.39	0.25	0.30	0.32	0.40	0.36	0.30	0.32	0.04	NS
Val	1.04[a]	0.96[ab]	0.44[e]	0.62[d]	0.75[cd]	0.87[bc]	0.78[cd]	0.66[d]	0.66[c]	0.06	***
Met	0.39	0.35	0.29	0.37	0.46	0.53	0.50	0.43	0.41	0.05	NS
Phe	0.78[a]	0.78[a]	0.45[c]	0.56[bc]	0.64[ab]	0.77[a]	0.74[a]	0.58[bc]	0.53[bc]	0.05	***
Ile	0.96[a]	0.95[a]	0.39[c]	0.46[bc]	0.50[bc]	0.63[b]	0.61[b]	0.50[bc]	0.82[a]	0.06	***
Leu	1.36[ab]	1.23[abc]	0.86[d]	1.00[cd]	1.23[abc]	1.45[a]	1.31[ab]	1.13[bc]	1.02[cd]	0.08	***
Lys	1.07[a]	0.76[bcd]	0.56[d]	0.76[bcd]	0.90[bcd]	1.05[a]	0.94[ab]	0.80[bc]	0.71[cd]	0.06	***
Pro	0.46[cd]	0.38[d]	0.34[d]	0.56[bcd]	0.70[bcd]	0.89[b]	0.80[bc]	0.63[bcd]	1.30[a]	0.11	***

[a,b,c,d,e,f]: Means within the same row having different superscripts differ significantly (p<0.05); *: p<0.05; **: p<0.01; ***: p<0.001; 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; Thr: Threonine; Arg: Arginine; Val: Valine; Met: Methionine; Ile: Isoleucine; Leu: Leucine; Phe: Phenylalanine; Lys: Lysine; Asp: Aspartic acid; Glu: Glutamic acid; Ser: Serine; GIy: Glycine; Ala: Alanine; Pro: Proline; Tyr: Tyrosine; S.E.M.: Standard error of mean

Concentrations of AA remaining in the bags after 6 h of ruminal incubation are shown in Table 3. There were very high significant differences (p<0.001) between the daily AA profiles 6 h post ruminal incubation. Exceptions of note were the lower, yet, non-significant Try, Cys and Met production each day after 6 h of ruminal incubation compared with the other AA measured. Opposite results were obtained by O'Mara *et al.* (1997) possibly due to the slightly different feedstuffs involved. Met was reported by Tamminga (1979) as one of the AA most resistant to ruminal degradation and it increased, or tended to increase, as a proportion of total AA after ruminal incubation with all feedstuffs in that study. Stern *et al.* (1983) ranked Agr, Thr and Lys as the most degradable essential AA in the rumen *in vivo* because microbial proteases are tryptic, tending to preferentially cleave Lys and Agr linkages, leading to greater exposure and consequent destruction of Lys and Agr. However, our study revealed low concentrations of these essential AA possibly because two out of the three ingredients employed were corn based which is considerably low in Lys and has a potential resistance to ruminal degradation (Harstad and Prestlokken, 2000). Another factor to explain this could be the result of processing since Lys is said to be most susceptible to heat damage (Cabrita *et al.*, 2011).

The trend towards a lower degradability of branch-chained AA corresponds to the decline in concentration of isoacids in the rumen. Differences (p<0.001) but lower concentrations of Arg also correspond with those observed for Pro since the former is used as a precursor for the later (Soltan, 2009). Harstad and Prestlokken (2000) cited studies in which the concentration of Arg tended to decreased in residues after rumen incubation of 9-16 h and that the branch-chained AA (Leu, Ile and Val) tended to increase. A similar pattern was observed in the present study except for the branch-chained AA Leu, which showed a slightly lower degradability. Arg is implicated as very sensitive to fermentation, whereas

peptide bonds with branch-chained AA are highly resistant to hydrolysis (Harstad and Prestlokken, 2000).

Daily Concentration of AA remaining in the bags after 12 h of incubation are given in Table 4. The entire AA measured showed differences (p<0.001) except Val (p>0.05). The branch-chained AA, Leu and Ile, in particular, seemed to be more resistant to microbial degradation in the rumen, which agrees with several other reports (Ouellet *et al.*, 2010). Met is said to be one of the AA that is most resistant to rumen degradation thus being the most liming in all the feedstuffs in this study. Along with Lys, Met is often considered one of the limiting AA of corn-based rations, particularly when heated soybeans make up most of the protein source (Bequette *et al.*, 1998). The value for Met though different (p<0.001), were low probably due to destruction of sulfur AA during acid hydrolysis or because of differences in ruminal solubility of the feed ingredients. Met is involved in a multitude of pathways leading to synthesis of specialized compounds, such as phospholipids, carnitine, creatine and the polyamines or may be involved in certain reactions for the synthesis of Cys. Lys, which often has been implicated as a first-limiting AA for dairy cows, showed decreased concentrations in the rumen in this study. It showed a lower extent of degradation for the 12 h compared to the 6 h incubation period. A possible explanation to this could be the involvement of Lys in the Maillard reaction, which is responsible for decrease protein degradation in processed meals. The 12 h ruminal exposure affected (p<0.001) the concentration of 8 out of the 16 AA studied. This was observed for Asp, Ser, Gly, Thr, Ala, Ile and Pro. Only Lys was influenced (p<0.01) by rumen incubation (Table 4). Conversely, Asp, Glu, Gly, Arg, Ala and Leu increased numerically more than average in both sampling periods. The only exception was Val, which showed lower rumen degradability than average for the 6 h incubation (Table 3 and 4). Nonessential AA concentrations increased more after 12 h of ruminal exposure while those of

Table 4: Concentration of AA remaining in the bags after 12 h of incubation as influenced by time and day

Item/%	Day 1	2	3	4	5	6	7	8	9	S.E.M.	Sig.
Asp	1.59[b]	1.06[c]	1.31[bc]	2.21[a]	1.05[c]	1.12[c]	1.21[c]	1.29[bc]	1.06[c]	0.11	***
Glu	2.10[b]	1.26[c]	1.60[c]	2.81[a]	1.32[c]	1.38[c]	1.67[c]	1.59[c]	1.31[c]	0.15	***
Ser	0.44[c]	0.54[bc]	0.68[b]	1.20[a]	0.54[bc]	0.57[bc]	0.65[b]	0.72[b]	0.69[b]	0.06	***
Gly	2.23[b]	1.49[e]	1.76[cde]	3.17[a]	1.59[de]	1.73[cde]	2.02[bc]	1.92[bcd]	1.85[bcde]	0.13	***
Thr	0.63[b]	0.61[b]	0.71[b]	1.16[a]	0.54[c]	0.57[bc]	0.63[b]	0.67[b]	0.58[b]	0.05	***
Arg	0.89[b]	0.59[c]	0.61[c]	1.29[a]	0.86[b]	0.87[b]	0.84[b]	0.99[b]	0.63[c]	0.07	***
Ala	1.43[b]	1.15[b]	1.34[b]	2.56[a]	1.18[b]	1.37[b]	1.52[b]	1.33[b]	1.23[b]	0.12	***
Tyr	0.60[bc]	0.57[c]	0.78[b]	1.01[a]	0.51[c]	0.58[c]	0.63[bc]	0.65[bc]	0.60[bc]	0.06	***
Cys	0.23[cd]	0.24[d]	0.27[cd]	0.45[a]	0.26[cd]	0.28[cd]	0.33[b]	0.28[cd]	0.30[bc]	0.01	***
Val	1.15	0.62	5.08	1.09	0.47	0.62	0.65	0.69	0.63	1.52	NS
Met	0.35[c]	0.33[c]	0.37[c]	0.66[a]	0.34[c]	0.40[bc]	0.47[b]	0.37[c]	0.38[c]	0.03	***
Phe	0.86[b]	0.56[c]	0.59[c]	1.03[a]	0.48[c]	0.54[c]	0.58[c]	0.58[c]	0.52[c]	0.05	***
Ile	1.00[a]	0.48[d]	0.50[d]	0.82[ab]	0.52[d]	0.91[a]	0.72[bc]	0.56[cd]	0.49[d]	0.06	***
Leu	1.46[b]	0.97[c]	1.02[c]	1.95[a]	0.88[c]	1.02[c]	1.08[c]	1.05[c]	1.09[c]	0.09	***
Lys	1.20[a]	0.57[bc]	0.65[bc]	1.17[a]	0.50[c]	0.59[bc]	1.03[ab]	0.68[bc]	0.85[abc]	0.15	**
Pro	0.30[e]	0.58[cd]	0.68[bcd]	1.12[a]	0.75[bc]	0.88[b]	0.74[bc]	0.77[bc]	0.51[d]	0.07	***

[a,b,c,d,e]: Means within the same row having different superscripts differ significantly (p<0.05); ***: 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; 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; S.E.M.: Standard error of mean

essential AA were in higher concentration after 6 h ruminal exposure. Concentrations of Ala, Asp, Gly and Glu increased (p<0.001) after exposure in the rumen for 12 h. All other nonessential AA decreased after 12 h of exposure in the rumen. Cys makes disulfide bonds that are hard to break permanently, which may be why less Cys was found in the rumen 12 h post incubation. A similar trend was observed for concentration of nonessential AA 6 h post ruminal incubation. According to Bequette *et al.* (1998), net catabolism of the branch-chained AA results in the contribution of NH_3 to nonessential AA synthesis (i.e., Glu and Asp). The daily concentration of Gly was higher than any other AA measured. This may explain the higher rate of ruminal C2 because C2 is known to be partly derived from Gly (Pena *et al.*, 1986). Glu was second in term of relative degradation during the 12 h sampling times and O'Mara *et al.* (1997) support this. There was an ingredient x day interaction (p<0.01) for ruminal AA for both the 6 and 12 h sampling period. Conversely, the 12 h post ruminal AA concentrations contained numerically greater total AA compared to 6 h post ruminal incubation throughout the trial (data not shown).

CONCLUSION

From the results obtained in this study, we conclude that sampling period significantly influenced rumen fermentation patterns, producing higher acetate and lower propionate and butyrate levels, which appeared to be higher during the afternoon than evening sampling times. Greater essential AA were produced during 6 h rumen incubation whereas more nonessential AA were produced after 12 h incubation. There were considerable variations by time and day for ruminal AA production and this fact should be considered when evaluating feed quality. Therefore, understanding the influence of hourly ruminal degradation pattern and daily AA production in the rumen may help in discovery of how to decrease ruminal feed degradation and/or dependably predict assimilation.

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