1989

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EVALUATION OF UREA, CASEIN, SOY PROTEIN ISOLATES OR ZEIN IN A SEMIPURIFIED DIET FED TO LAMBS

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SHEEP 89-3

Summary

Four ruminally cannulated crossbred lambs were utilized with a 4 x 4 Latin square design with repeated measures over time to determine the effect nitrogen source has on nitrogen balance and fermentation. Urea, casein, soy protein isolates or zein were included in semipurified diets and fed at 2.2% body weight (48 kg). After a 3-week adaptation to the urea supplemented semipurified diet, lambs were fed their respective nitrogen source. Orts, feces, urine and feed samples were collected, subsampled and analyzed for nitrogen content. During each period, rumen samples were collected before feeding and 2, 4, 6, 9 and 12 hours postfeeding and analyzed for pH, ammonia and volatile fatty acids. Blood was collected by jugular venipuncture before feeding and postfeeding at 4 and 8 hours and analyzed for plasma urea nitrogen. Apparent biological value was lower ($P<.05$) for urea (16.4) than casein (50.9), soy protein isolates (50.7) or zein (46.1). Rumen volatile fatty acids responded quadratically over time. Nitrogen source did not affect acetate, propionate, acetate:propionate ratio or total volatile fatty acids. Ruminal concentrations of isobutyrate were lower ($P<.05$) with the urea diet, higher ($P<.05$) for butyrate with casein diet and higher ($P<.05$) for valerate with casein and zein diets. Plasma urea nitrogen was higher ($P<.05$) for urea (16.2 mg/dl) than casein (8.63), soy protein isolates (10.9) and zein (8.75). Rumen pH responded quadratically over time. Rumen ammonia was affected by nitrogen source and time ($P<.05$) where the urea diet responded differently over time than pure proteins. Nitrogen source affected nitrogen balance and ruminal fermentation with pure protein differing from urea.

(Key Words: Semipurified diet, nitrogen, lambs, proteins.)

Introduction

The nutritional requirements for nitrogen are usually expressed on a crude protein basis for ruminant animals. However, crude protein is based solely on nitrogen content and nutritional differences between pure proteins are not considered. The nutritional differences which exist between pure proteins relate to their amino acid composition and, for ruminants, the degree of ruminal degradation of the protein. Unfortunately, the ruminal microbes that allow ruminants to use nonprotein nitrogen also prevents ruminants from directly utilizing feed proteins. Proteins which are resistant to ruminal degradation can escape the rumen intact and supply amino acids to the small intestine. The resulting combination of microbial and feed protein presented to the small intestine provide the amino acid nitrogen needed by the animal. Differences in amino acid composition between these proteins may affect the nutritional status of the animal. The objectives of this study were to determine how protein source

Prepared for Sheep Day, June 9, 1989.

would influence nitrogen balance and ruminal fermentation when fed in a semipurified diet to lambs.

Experimental Procedure

Four crossbred wether lambs (48 kg) fitted with permanent ruminal cannulas were fed a semipurified diet[1] (Fritz, 1987) containing nitrogen sources of either urea, casein, soy protein isolates or zein twice daily at 12-hour intervals (Table 1). Lambs were gradually adapted to the semipurified diet containing urea during a 3-week period and then fed for 10 days until intake stabilized at 2.2% body weight. After adaptation, lambs were started in the experimental term. The experimental term consisted of four periods with each period containing 7 days of adaptation to the nitrogen source, 1 day of ruminal and blood collections and two consecutive 2-day fecal and urine collections.

TABLE 1. COMPOSITION OF SEMIPURIFIED DIET SUPPLEMENTED WITH UREA, CASEIN, SOY PROTEIN ISOLATE OR ZEIN AS NITROGEN SOURCES

	Nitrogen source			
Ingredient, % DM	Urea	Casein	Soy protein isolates	Zein
Corn cobs	29.932	27.355	27.355	27.355
Corn starch	30.951	28.374	28.374	28.374
Solka floc	27.931	25.354	25.354	25.354
Urea	4.269	0	0	0
Casein[a]	0	12.000	0	0
Soy protein isolate[b]	0	0	12.000	0
Zein[c]	0	0	0	12.000
Animal fat	2.499	2.499	2.499	2.499
Masonex	2.000	2.000	2.000	2.000
Dical[d]	1.490	1.490	1.490	1.490
K_2SO_4	.584	.584	.584	.584
Trace mineral salt	.300	.300	.300	.300
MgO	.017	.017	.017	.017
KI, ppm	1.000	1.000	1.000	1.000
ZnO, ppm	64.000	64.000	64.000	64.000
$Na_2SeO_3.5H_2O$, ppm	.219	.219	.219	.219
Vitamin ADE premix[e]	.020	.020	.020	.020

[a] Alaren 771 Rennet Casein, Dried Whey, Inc., Monticello. IA.
[b] Ardex F Soy Protein Isolate, Archer Daniels Midland Co., Decatur, IL.
[c] Zein, Regular Grade, Freeman Industries, Inc., Tuckahoe, NY.
[d] A mixture of mono and dicalcium phosphate to equal 26.30% Ca and 18.70% P.
[e] Premix contained 1,000,000 USP units vitamin A, 500,000 USP units vitamin D and 1000 international units vitamin E per .45 kg.

[1] Feedstuffs generously donated were casein from International Distributing Co., soy protein isolates from Archer Daniels Midland Co. and masonex from Masonite Corporation.

Total feed consumption was recorded and feed refusals were weighed and subsampled. Following each collection period, daily feces, urine and orts were combined and frozen. Collections were subsampled for dry matter (DM) and nitrogen (N) determination. Feed and orts were dried in a forced air oven at 100 °C for 48 hours and feces were dried at 60 °C for 72 hours. All samples were then ground through a 1-mm screen and stored in air-tight containers.

Blood samples were collected before feeding (0 hour) and postfeeding at 4 and 8 hours and cooled with ice. Plasma was separated by centrifugation at 7800 x g for 30 minutes at 5 °, decanted and analyzed for plasma urea nitrogen (Chaney and Marbach, 1962).

Urine was collected into containers holding 100 ml of 30% HCl. Urine output (<1000 ml) was diluted to 1 liter with distilled water to avoid salt precipitation. Pooled subsamples of the urine (10% of the collected volume) were stored at 2 °C during the collection periods and stored at -18 °C until N analysis.

Nitrogen content of the feed, feces, orts and urine was determined by the Kjeldahl method (AOAC, 1982).

Ruminal samples were collected prior to feeding (0 hour) and at 2, 4, 6, 9 and 12 hours postfeeding. Samples were collected and strained through three layers of cheesecloth before pH was determined. Samples for volatile fatty acid determination were mixed with 25% metaphosphoric acid (5:1, v:v), centrifuged at 22,000 x g at 5 °C for 20 minutes. The supernate was drawn off and stored at -18 °C until analysis. Volatile fatty acids were analyzed by gas-liquid chromatography (HP 5890) with a 10m x .53 mm capillary column coated with acid derivatized polyethylene glycol (Alltech-Superox FA). Temperature was ramped at 18 °C per minute from 80 °C to 130 °C. Ruminal samples were prepared for ammonia determination (Chaney and Marbach, 1962) by $HgCl_2$ additio followed by centrifugation (20,000 x g, 5 °C, 20 minutes). The supernate was decanted and frozen at 18 °C until ammonia analysis.

The experiment was statistically analyzed as a 4 x 4 Latin square split plot design for dependent variables measured over time. The main effects of the Latin square, nitrogen source, lamb and period, were tested by nitrogen source x lamb x period. The split plot factor of time was tested by the lamb x time interaction (Damon and Harvey, 1987). Orthogonal polynomial coefficients for unequal spaces were utilized to determine linear and quadratic effects over time. Nitrogen balance data were analyzed as a 4 x 4 Latin square design with subsampling. Main effects of nitrogen source, lamb and period were tested with collection x period error term. Means were separated by t-tests (SAS, 1982).

Results

Acceptance to the semipurified diet by lambs was reflected by the length of the adaptation period. Lambs were reluctant to consume their daily feeding of the semipurified diet supplemented with urea during the adapation period. Supplementing the diets with pure proteins appeared to stimulate appetite, although intakes were limited to 2.2% body weight.

The corn cob, corn starch and solka floc based diet (Table 1) was adapted from a previous study (Fritz, 1987) which evaluated the relationship between intake and nitrogen retention. The pure proteins, casein, soy protein isolates and zein, used in this experiment were obtained from commercial sources and supplemented in the diet at 12% dry matter basis.

The nitrogen sources selected for the study represent four different types. Urea is a nonprotein nitrogen which requires microbial conversion to ammonia. The ammonia is used by the microbes to mak amino acids and protein which are digested

by the ruminant for its amino acid needs. Casein is a natural milk protein estimated to be 90% degraded to amino acids and ammonia in the rumen by microbes. Ruminal microbes may utilize the ammonia to synthesize amino acids or use amino acids directly from casein for producing microbial protein. Soy protein isolate is estimated to be 30 to 70% degraded in the rumen, depending upon the length of time and temperature of heat exposure. The protein which is not degraded in the rumen passes to the small intestine where the protein is digested. The protein present in corn, zein, is relatively undegradable in the rumen by microbes and passes directly to the small intestine for digestion. The degree of protein nondegradability can affect the digestibility of the diet, alter the nitrogen balance of the animal and change the amino acid profile of the digesta.

Dry matter intake was similar between nitrogen sources with an overall mean of 1027.0 g per head per day. The digestibility of the semipurified diet (mean = 77.2%) was not affected (P>.05) by nitrogen source. This indicates adequate nitrogen was present in the diet to sustain microbial fermentation in the rumen regardless of the type of nitrogen source. Proteins which have low degradability may use urea recycling mechanisms to supply degradable nitrogen to the rumen, thus providing ammonia for fermentation.

Nitrogen intake, fecal nitrogen, digested nitrogen and apparent nitrogen digestibility were not affected (P>.05) by nitrogen source (Table 2). The amount of nitrogen excreted in the urine was affected (P<.05) by nitrogen source in the diet. Consequently, the amount of nitrogen retained daily was affected (P<.05), with pure protein diets supporting greater nitrogen retention than the urea diet. Apparent biological value was less (P<.01) for the urea diet (16.4) than the casein (50.9), soy protein isolate (50.7) or zein diets (46.1). The value of urea for supplying protein and amino acids via microbial production for the lamb is inadequate compared to pure proteins. The

TABLE 2. NITROGEN BALANCE OF LAMBS FED SEMIPURIFIED DIET SUPPLEMENTED WITH UREA, CASEIN, SOY PROTEIN ISOLATES OR ZEIN

	Nitrogen source				
Item	Urea	Casein	Soy protein isolates	Zein	SE[a]
Dry matter intake, g/day	920.2	1034.2	1090.2	1063.5	78.44
Dry matter digestibility, %	79.2	73.9	76.5	79.2	2.36
Nitrogen intake, g/day	17.1	19.8	20.5	21.2	1.21
Fecal nitrogen, g/day	4.1	4.8	5.5	5.0	.59
Digested nitrogen, g/day	13.0	15.0	14.9	16.2	.73
Apparent nitrogen digestibility, %	77.9	75.4	72.6	76.2	2.69
Urinary nitrogen, g/day	10.9[b]	7.2[c]	7.2[c]	8.5[bc]	.73
Retained nitrogen, g/day	2.0[b]	7.7[c]	7.6[c]	7.6[c]	.58
Apparent nitrogen retained, %[d]	13.2[b]	38.6[c]	37.1[c]	35.5[c]	2.97
Apparent biological value[e]	16.4[b]	50.9[c]	50.7[c]	46.1[c]	3.08

[a] Standard error.
[b,c] Means with unlike superscripts within a row differ (P<.05).
[d] Percent apparent biological value = (retained nitrogen/intake nitrogen) x 100.
[e] Apparent biological value = (retained nitrogen/digested nitrogen) x 100.

inadequacy may be either inefficient conversion of NPN or an imbalanced amino acid supply to bacterial protein.

Rumen variables were evaluated for fermentation effects from feeding the semipurified diet supplemented with urea, casein, soy protein isolate or zein (Table 3). Although nitrogen source and time did not affect (P<.05) ruminal pH or ammonia, interaction of nitrogen source and time was significant for ruminal pH and ammonia N (P<.05 and P<.01), respectively). The concentrations of acetate, propionate, isovalerate, total volatile fatty acids, total acetate and propionate and acetate:propionate ratio were not affected (P>.05) by nitrogen source. The concentrations of isobutyrate, butyrate and valerate were affected by nitrogen source. The concentrations of isobutyrate for the urea diet (.24 umoles/dl) was less (P<.05) than the pure protein diets (.59, .46, .50 umoles/dl for casein, soy protein

TABLE 3. MEAN CONCENTRATION OF RUMEN METABOLITES, pH AND PLASMA UREA NITROGEN BY NITROGEN SOURCE

	Nitrogen source				
Item	Urea	Casein	Soy protein isolates	Zein	SE[a]
pH	6.39	5.96	6.19	6.39	.129
Ammonia N, mg/dl	21.6	17.0	14.1	12.6	4.43
Volatile fatty acids, umoles/ml					
Acetate	38.15	38.83	34.96	35.96	1.730
%[b]	72.9	66.0	70.3	71.9	
Propionate	8.67	11.79	8.35	6.59	1.440
%	16.2	20.3	16.6	13.2	
Isobutyrate	.24[c]	.59[d]	.46[d]	.50[d]	.053
%	.6	1.0	.9	1.0	
Butyrate	3.54[c]	5.07[d]	3.84[c]	3.68[c]	.332
%	6.8	8.3	7.8	7.5	
Isovalerate	1.67	1.77	1.68	2.33	.324
%	3.1	3.0	3.4	4.8	
Valerate	.25[c]	.66[d]	.38[c]	.59[d]	.068
%	.5	1.1	.8	1.2	
Total VFA	52.55	58.74	49.71	49.68	2.952
Acetate:propionate ratio	4.87	3.31	4.50	5.67	.720
Plasma urea nitrogen, mg/dl	16.21[c]	8.63[d]	10.09[d]	8.75[d]	2.007

[a] Standard error.
[b] Percentage of total VFA within column.
[c,d] Means with unlike superscripts within row differ (P<.05).

isolates and zein, respectively). Butyrate concentration was higher (P<.06) when lambs were fed casein than either urea, soy protein isolates or zein. Higher concentration of valerate occurred (P<.02) with casein and zein diets than urea or soyprotein isolate diets. Nitrogen source did affect concentration of longer chain volatile fatty acids, although concentration or ratio of the major volatile fatty acids, acetate and propionate, was not affected.

Plasma urea nitrogen for lambs was affected by nitrogen source, with urea diet having higher (P<.11) plasma levels of urea than casein, soy protein isolates or zein (Table 3). A significant interaction occurred (P<.05) between time and nitrogen source for plasma urea nitrogen (Table 4).

TABLE 4. PLASMA UREA NITROGEN MEAN CONCENTRATIONS OVER TIME FOR LAMBS FED SEMIPURIFIED DIET SUPPLEMENTED WITH UREA, CASEIN, SOY PROTEIN ISOLATE OR ZEIN

Nitrogen source	Time, hours		
	0	4	8
	Plasma urea nitrogen, mg/dl		
Urea	14.4[a]	18.6[a]	15.5[a]
Casein	10.7[b]	6.9[b]	8.2[b]
Soy protein isolate	10.5[b]	10.8[c]	8.8[b]
Zein	9.4[b]	9.2[c]	7.5[b]
SE[d]	.69	.69	.69
Mean[e]	11.29	11.42	10.04

[a,b,c] Means with unlike superscripts within column differ (P<.05).
[d] Standard error.
[e] Linear effect over time (P<.05).

When urea was fed, plasma urea nitrogen concentration was higher (P<.05) before feeding (0 hour) compared to 4 and 8 hours postfeeding. Casein, soy protein isolates and zein had similar (P>.05) concentrations of plasma urea nitrogen before feeding and 8 hours postfeeding. At 4 hours postfeeding, plasma urea nitrogen levels were lower for the casein diet than soy protein isolate and zein diets. Decreased nitrogen retention and higher circulating plasma urea nitrogen values for the urea diet indicate a decrease in utilization of the urea compared to pure protein diets. Possibly the decrease in utilization is coupled with the decrease in mean concentrations of isobutyrate and valerate. Also possible is the production of amino acids by microbial fermentation which could not be utilized by the ruminant due to an insufficiency of limiting amino acids. The result would be amino acid catabolism which would produce elevated levels of plasma urea.

Presented in Table 5 are the mean concentrations of ruminal ammonia nitrogen and volatile fatty acids and mean pH values over time. Volatile fatty acid concentration and pH responded quadratically (P<.01) over time. Ruminal ammonia nitrogen responded quartically (P<.10), although a significant interaction between nitrogen source and time occurred. The urea diet caused high rumen ammonia nitrogen levels following feeding with levels decreasing after the 2-hour peak (Figure 1). The pure proteins responded differently with ammonia nitrogen levels decreasing after feeding followed by subsequent higher levels (Table 6). Possibly the decreasing levels after feeding may have resulted from a dilution effect caused by feed consumption, saliva production and water consumption. The subsequent higher levels could have resulted from microbial proteolytic activity in converting the protein to ammonia for microbial protein synthesis or reduced microbial ammonia nitrogen uptake 9 hours after feeding. The observed divergence in trend by soy protein isolate from casein and zein may indicate the soy protein isolates used in this experiment were more readily degradable through the first 4 hours and then moderately degradable providing for the subsequent sustained release of ammonia. Conversely, the trend by zein suggests resistance to degradation in the rumen. The trend by

TABLE 5. MEAN CONCENTRATIONS OF RUMINAL AMMONIA AND VOLATILE FATTY ACIDS AND MEAN VALUES FOR RUMEN pH OVER TIME

Item	Time, hours 0	2	4	6	9	12	SE[a]
Ruminal ammonia N, mg/dl[b]	14.8	22.6	15.5	14.2	16.4	14.3	1.77
Volatile fatty acids, umoles/ml							
Acetate[c]	36.2	33.6	41.8	41.8	38.9	29.2	.87
Propionate[c]	8.8	8.6	9.8	9.6	9.0	7.2	.27
Isobutyrate[d]	.5	.3	.3	.4	.5	.4	.03
Butyrate[c]	3.6	3.5	4.4	43.9	4.6	3.0	.33
Isovalerate[c]	1.7	1.5	1.9	2.2	2.1	1.5	.10
Valerate[c]	.4	.4	.4	.5	.6	.3	.02
Total VFAs[c]	51.4	48.1	58.9	59.7	55.8	41.9	1.21
Acetate:propionate ratio[c]	4.4	4.3	4.8	4.9	4.7	4.3	.14
pH[c]	6.55	6.19	5.86	5.96	6.33	6.51	.039

[a] Standard error.
[b] Quartic response over time (P<.10).
[c] Quadratic response over time (P<.01).
[d] Cubic response over time (P<.01).

TABLE 6. MEAN CONCENTRATIONS FOR RUMINAL AMMONIA AND TOTAL FATTY ACIDS AND MEAN VALUES FOR RUMEN pH OVER TIME

Nitrogen source	Time, hours 0	2	4	6	9	12
Rumen ammonia N, mg/dl[a]						
Urea	9.3	48.1[b]	32.0[b]	19.6[b]	10.7[b]	9.6
Casein	17.1	9.7[c]	10.7[cd]	20.3[c]	26.4[c]	17.4
Soy protein isolates	14.0	21.1[bc]	14.3[c]	11.2[bc]	12.2[b]	11.9
Zein	18.8	11.6[c]	4.8[d]	5.6[c]	16.2[b]	18.3
Total VFAs, umoles/ml[e]						
Urea	56.4	52.6	64.3	66.0	58.3	48.8
Casein	55.0	49.7	63.9	59.8	63.1	46.7
Soy protein isolates	40.7	36.8	46.6	50.9	47.3	31.7
Zein	53.6	53.4	60.7	62.0	54.4	40.2
Ruminal pH[f]						
Urea	6.62[b]	6.80[b]	5.98[b]	6.01[b]	6.37[bc]	6.57[bc]
Casein	6.26[c]	5.79[c]	5.61[c]	5.69[c]	6.12[b]	6.31[b]
Soy protein isolates	6.52[bc]	6.02[d]	5.85[bc]	6.00[b]	6.30[c]	6.46[c]
Zein	6.81[b]	6.15[d]	6.01[b]	6.13[b]	6.54[c]	6.71[c]

[a] Standard error = 3.22.
[b,c,d] Means with unlike superscripts within column and dependent variable differ (P<.05).
[e] Standard error = 3.69.
[f] Standard error = .105.

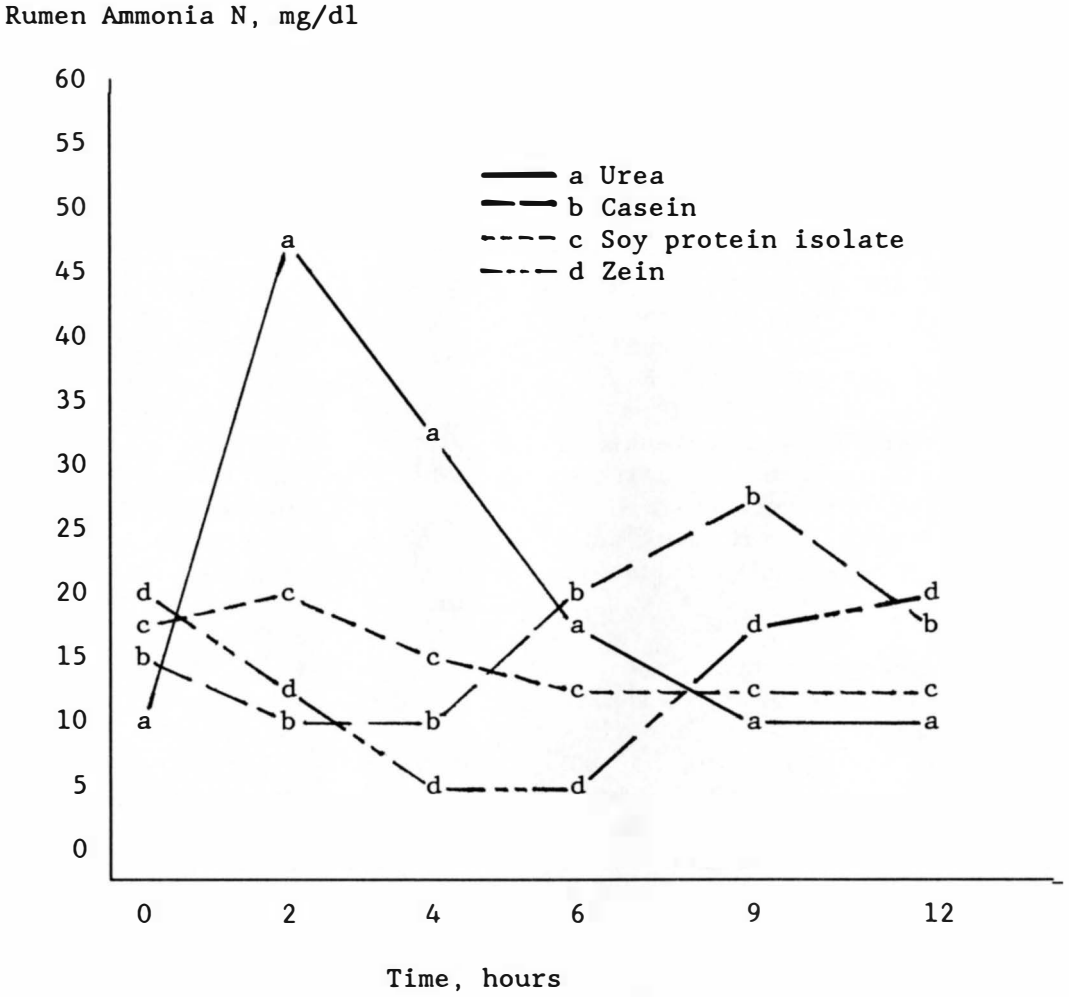

Figure 1. Mean concentration of rumen ammonia by time for lambs fed a semipurified diet supplemented with urea, casein, soy protein isolate or zein.

zein for rumen ammonia nitrogen production coupled with the plasma urea nitrogen response suggests the rise in rumen ammonia may be due to urea clearing from the blood to the rumen to aid in fermentation or more likely partial proteolysis of zein occurs after time in the rumen.

Volatile fatty acid concentrations in the rumen follow a quadratic response over time for acids (Table 5) except isobutyrate. The quadratic response could be explained by the increase in volatile fatty acid production in response to a meal, with the subsequent decrease due to decreasing substrate. The effect of nitrogen source on total volatile fatty acid production over time is shown in Table 6. The response over time by ruminal pH follows a quadratic curve which may be due to the increase in metabolites in response to the meal causing a decrease in pH (Table 5). The differences between nitrogen sources over time for rumen pH is shown in Table 6. Overall, urea and zein diets caused higher ruminal pH than casein and soy protein isolates.

Nitrogen sources have been shown to affect the nitrogen status of the animal. The nitrogen sources used in this experiment indicate pure proteins have a higher biological value than urea regardless of the degree of degradation by the protein in the rumen. Although the ruminant can retain nitrogen with a nonprotein nitrogen diet, productivity is diminished. The effects of the pure protein on nitrogen balance may occur either ruminally or duodenally or both. Possible rumen effects are supplying needed substrate to the rumen microbes in the form of a sustained release nitrogen source, a carbon skeleton source or amino acids, dipeptides or tripeptides. Alternately, the duodenal effect could result from either an increase in total protein as microbial and feed protein presented to the small intestine or differences in amino acid profiles between feed and microbial proteins. This experiment shows pure proteins and urea have different responses in the rumen and nitrogen retention is greater with pure proteins compared to the semipurified diet containing only urea.

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