RELATION OF FEEDLOT PERFORMANCE AND CERTAIN PHYSIOLOGICAL RESPONSES TO THE METABOLIZABLE PROTEIN AND UREA CONTENT OF CATTLE DIETS[1,2]

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Summary

An experiment was conducted to test the accuracy of the metabolizable protein system in predicting the amount of urea that would be useful in a corn-based cattle diet. Treatment diets included a basal, low-protein (7.8% CP) negative control (NC) with no supplemental N and a positive control (PC) that contained soybean meal. Urea was added to the NC diet in quantities calculated to be either 25% deficient (LU), equal to (MU) or 25% in excess (HU) of the urea fermentation potential (UFP). In vitro rumen fermentation studies were used to determine sequential ammonia production and digestible dry matter content of the diets. In a growth trial, 12 individually-fed Angus, Hereford and Angus × Hereford steers weighing an average of 213 kg were assigned randomly to each treatment diet. At the conclusion of the 112-d trial, rumen ammonia and jugular blood urea N (BUN) concentrations were determined on two steers from each treatment before feeding and at 1, 2, 3, 4 and 5 h postfeeding. In vitro ammonia concentrations of the NC and PC treatments were lower ($P<.05$) than that of urea containing diets. In vivo rumen ammonia concentrations at 1 h postfeeding and BUN levels at 3 h postfeeding were low for both the NC and PC diets compared with urea-containing diets. Both of these values increased with each successive increase of added urea to the NC diet. During the initial 70 d of the growth trial, daily gains were improved ($P<.05$) by addition of urea up to the MU level, which fulfilled the calculated UFP. Urea in excess (HU) of that level was of no further value. Daily gains for the entire 112-d trial were improved ($P<.05$) over the NC diet by the addition of urea, although no differences ($P>.05$) existed among levels of urea. It was concluded that the metabolizable protein system accurately predicted the amount of urea that would be of value in these diets.
(Key Words: Ruminants, Cattle, Urea, Protein, Metabolizable Protein, Urea Fermentation Potential.)

Introduction

The degree to which diets supplemented with urea are capable of supporting live weight gains of feedlot cattle has been inconsistent. Haskins et al. (1967) and Bolsen et al. (1968) reported that urea-supplemented animals performed similarly to those fed alpha-amino N sources such as soybean meal. Oltjen and Putnam (1966) and Braman et al. (1973) found that urea-fed cattle utilized dietary N less efficiently than did cattle fed N supplements containing largely alpha-amino sources. To more accurately define the amount of urea that would be useful in cattle diets, mathematical systems were developed that consider both the amount of fermentable energy as well as the amount of feed or diet protein degraded in the reticulorumen of cattle (Burroughs et al., 1975; Roffler and Satter, 1975a,b). Although refinement of the basic model is needed, these feeding standards provide the framework for a more precise approach to protein nutrition of ruminants that recognizes the N needs of the rumen microorganisms as well as the amino acid requirements of the host animal. The purpose of this study was to test the accuracy of the metabolizable protein system in predicting

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the amount of urea that can be utilized in high energy diets and to determine the relationship between in vivo rumen ammonia concentrations and the quantity of urea in the diet with respect to the urea fermentation potential (UFP). A third objective included use of in vitro rumen fermentation studies to determine the effect of different levels and sources of supplemental N upon ammonia production and dry matter digestibility.

Experimental Procedure

Selection of Diets. Five treatment diets (table 1), included: a corn grain-cottonseed hull negative control (NC) diet that contained no supplemental N; three diets in which urea was added in amounts calculated to be 25% deficient (LU), equal to (MU) or 25% in excess (HU) of the urea fermentation potential (UFP) of the basal diet, and a positive control (PC) that was supplemented with soybean meal at the expense of corn. A positive UFP indicates sufficient energy in the diet for the utilization of urea N. All diets were isocaloric, having a calculated (NRC, 1976) NE_m and NE_g equal to 1.80 and 1.02 Mcal/kg, respectively.

In Vitro Rumen Fermentation Studies. Sequential ammonia-N accumulation was determined on two replications with triplicate samples for each treatment diet in a modified version of the Tilley and Terry (1963) in vitro rumen fermentation technique. The incubation mixture consisted of .5 g diet ground through a 1-mm screen, 40 ml of McDougall's phosphate-bicarbonate buffer at pH 6.8 (McDougall, 1948) and 10 ml of rumen fluid previously strained through eight layers of cheesecloth. The rumen fluid was collected at 1000 h from a fistulated steer fed (at 0800 h) a daily diet consisting of Coastal Bermuda grass hay (IFN 1-00-716), corn (IFN 4-02-931) and a mineral mixture. Control incubations consisted of the McDougall's buffer-rumen fluid mixture in the absence of dietary substrate. One-milliliter samples of the incubation mixture supernatant were withdrawn via a disposable pipette (Thomas et al., 1979) at 4 h of incubation time. An equal volume of McDougall's buffer was returned to each tube following sampling. Samples were stored in acidified auto-analyzer cups until analyzed for ammonia-N by an automated alkaline phenol-hypochlorite procedure (Technicon Corporation, 1960). Control ammonia-N values were subtracted from the ammonia-N values obtained with substrate to provide an estimate of net ammonia accumulation. In a separate study, digestible dry matter was determined after a 20-h fermentation period.

Cattle Studies. Angus, Hereford and Angus x Hereford steer calves, averaging 213 kg initially, were used in this study. Upon arrival, an adaptation diet based on corn and cottonseed hulls was fed for a period of 21 d during which time the steers were trained to eat from the

TABLE 1. FEED COMPOSITION OF DIETS USED DURING IN VITRO RUMEN FERMENTATIONS AND CATTLE GROWTH TRIALS[a]

Ingredient	Negative control	Low urea	Medium urea	High urea	Positive control
			%		
Cottonseed hulls (IFN 1-01-599)	30.0	30.0	30.0	30.0	30.0
Sugarcane molasses (IFN 4-04-696)	8.0	8.0	8.0	8.0	8.0
Corn grain, cracked (IFN 4-02-931)	59.4	58.8	58.5	58.3	51.6
Soybean meal (IFN 5-04-604)					8.1
Urea		.70	.95	1.2	
Vitamins and minerals	2.6	2.6	2.6	2.6	2.3
Estimated chemical composition					
Crude protein, %	7.8	9.5	10.0	10.8	10.8
Urea fermentation potential[b], g/100 kg	+976	+269	0	−234	+11.3
Metabolizable protein, %[b]	5.15	6.67	7.20	7.20	7.92

[a]Feed and estimated chemical composition of diets are expressed on a dry matter basis.

[b]Based on calculations outlined by Burroughs et al. (1975).

TABLE 2. EFFECT OF NITROGEN SOURCE AND LEVEL UPON IN VITRO AMMONIA PRODUCTION AND DRY MATTER DIGESTIBILITY

Treatment	Length of incubation, h[a]				Digestible DM[b]
	1	2	3	4	
	mg NH_3-N/dl				%
Blank	1.92	1.80	1.76	1.75	
Negative control	.14[c]	0[c]	−.15[c]	−.69[c]	59.5
Low urea	1.76[d]	1.71[d]	1.06[cd]	.57[cd]	59.5
Medium urea	2.98[d]	2.45[d]	1.95[d]	1.24[cd]	62.7
High urea	2.64[d]	2.55[d]	2.17[d]	1.67[d]	59.8
Positive control	.07[c]	.02[c]	−.40[c]	−.57[c]	61.6
SE	.15	.20	.10	.05	1.3

[a]Values represent the mean of two replications with triplicate samples. The blank values, shown on the first row and consisting of rumen fluid and McDougall's buffer without dietary substrate, have been subtracted.

[b]Values represent the mean of four determinations.

[c,d]Values within the same column with different superscripts differ ($P<.05$).

individual feeding doors[5]. The cattle were weighed on 2 consecutive days following a 12-h period without feed and water. The average of these weighings served as the initial test weight. Steers were divided into six outcome weight groups (10 animals/group) then assigned randomly to the dietary treatments (table 1). Pens were 8.5 x 7.3 m with a large dirt lot outside and contained 10 steers including two steers/dietary treatment. Steers were subsequently weighed at 14-d intervals throughout the trial.

At the conclusion of the 112-d growth trial, rumen fluid and jugular blood samples were taken from two randomly selected steers within each treatment group. On the day before sample collection, feed consumption by the steers was restricted to approximately one-half normal amounts to assure rapid intake of normal amounts of feed on the day of sampling. Rumen samples were obtained by stomach tube and jugular blood samples by veinipuncture 30 min before feeding and at 1, 2, 3, 4 and 5 h after feeding. Upon collection, fermentation in the rumen samples was terminated with saturated mercuric chloride after which they were placed on ice, transported to the laboratory and analyzed for ammonia content (Technicon Corporation, 1960). Jugular blood samples were processed according to the procedure of Schmidt et al. (1973) in which 9 ml of blood were deposited into heparinized tubes containing 3 ml of 40% trichloroacetic acid solution. The tubes were agitated and stored on ice until they could be transported to the laboratory and centrifuged at 1,000 x g for 10 min. Urea content of the plasma was determined by an automated procedure (Technicon Corporation, 1974).

The data were analyzed as a randomized complete block design with blocks being pens in the cattle study and replication days in the laboratory studies. Duncan's new multiple range test (Steel and Torrie, 1960) was used to test treatment differences when a significant F-test existed.

Results and Discussion

In Vitro Rumen Fermentation Studies. The effect of level and source of supplemental nitrogen upon ammonia accumulation and dry matter digestibilitiy under in vitro conditions is shown in table 2. Although ammonia concentrations were maximal for all treatments at 1 h of incubation time, peak concentrations may have occurred before this time. At 1 h, ammonia concentrations of the NC and PC treatments were lower ($P<.05$) than the urea-containing diets. The addition of urea at all levels increased ammonia concentrations above that of the NC diet ($P<.05$) for 2 h after which time the difference was only significant for the MU and HU treatments. At 4 h incubations, the concentrations of ammonia for the NC, LU, MU and

[5]Distributed by American Calan, Inc., Route 4, Northwood, NH 03261.

PC treatments were similar (P>.05) in contrast to the HU treatment, which remained higher (P<.05) than those of both control treatments. It was assumed that ammonia levels of the NC and PC treatments became negative as the fermentation progressed due to slow rates of ammonia released in conjunction with accelerated rates of microbial utilization. These findings are in agreement with in vivo urea utilization studies reported by Carver and Pfander (1974). Digestibilitiy of the dry matter (DM) did not differ among treatments (P>.05). Calculations based upon the metabolizable protein system predict that a deficiency of ammonia will exist in the NC, LU and PC diets and relatively lower levels of ammonia were found in these fermentations.

Cattle Studies — Feedlot Performance. Daily feed intake during the initial 70-d period (table 3) did not differ (P>.05) among the five treatment groups. However, the PC group consumed 5.4% more feed than the NC group. Steers fed the NC diet gained 1.24 kg daily, with a feed to gain ratio of 7.1. As shown in table 1, the NC, LU, MU, HU and PC diets had calculated UFP values of +976, +269, 0, −234 and +11.3 g/100 kg DM, respectively (Burroughs et al., 1975). These values are based upon the amount of fermentable energy in the diet and reflects the calculated quantitiy of urea that would be useful to rumen microorganisms for purposes of growth including protein synthesis. The calculated quantities of metabolizable protein (%) contained in each diet were: NC, 5.15; LU, 6.67; MU, 7.20; HU, 7.20 and PC, 7.92. According to the metabolizable protein concept, the PC cattle were expected to perform superior to all other treatments. The HU and MU groups were expected to perform equally and be superior to the LU and NC cattle. The crude protein content of the NC, LU, MU, HU and PC diets was 7.8, 9.5, 10.0, 10.8 and 10.8%. Differing from the metabolizable protein system, the crude protein system (N × 6.25) would predict that feedlot performance of steers fed the NC diet would be poorest, with performance of cattle fed the HU and PC diets being equal to each other and superior to the other treatments. In this study, addition of urea at the lowest level (LU) resulted in a 10.5% nonsignificant increase in daily gain and a 9.5% improvement in feed efficiency compared with the NC group. Steers fed the MU and HU diets had greater (P<.05) daily gains and feed conversion ratios, averaging 15.7% and 8.4%, respectively, compared with the NC. As predicted by the metabolizable protein system, daily gains of steers fed the MU and HU diets were nearly identical, showing no increase from the addition of urea in excess of the positive UFP of the diet. Performance of the PC group was superior to all other treatments (P<.05). The metabolizable protein system also accurately predicted that the protein adequacy of the PC diet would be much better than all other treatments as demonstrated by the 12.6% improvement (P<.05) in daily gains and 9.1% improvement

TABLE 3. PERFORMANCE OF STEER CALVES FED ISOCALORIC DIETS VARYING IN METABOLIZABLE PROTEIN CONTENT

Item	Negative control	Low urea	Medium urea	High urea	Positive control	SE[a]
Initial 70 d						
Avg initial wt, kg	215	213	217	210	213	
Avg final wt, kg	302	309	317	310	326	
Feed intake, kg/d	9.2[b]	9.2[b]	9.4[b]	9.3[b]	9.7[b]	.49
Daily gain, kg	1.24[b]	1.37[bc]	1.43[c]	1.44[c]	1.61[d]	.11
Feed/gain	7.1[b]	6.7[cd]	6.6[d]	6.5[cd]	6.0[c]	.19
Entire 112 d						
Avg final wt, kg	343	358	361	354	377	
Feed intake, kg/d	9.6[b]	9.6[b]	9.8[bc]	9.5[b]	10.1[c]	.34
Daily gain, kg	1.14[b]	1.29[c]	1.28[c]	1.29[c]	1.47[d]	.08
Feed/gain	8.4[b]	7.5[cd]	7.6[bd]	7.4[cd]	6.9[c]	.20

[a]Standard error of the mean (n=12).

[b,c,d]Values in the same row with different superscripts differ (P<.05).

(P>.05) in feed efficiency of the PC cattle compared with the MU cattle.

For the entire 112-d feeding period (table 3), the quantitiy of feed consumed was similar for the NC, LU, MU and HU treatment groups, while the PC cattle consumed more feed (P<.05) than the NC, LU and HU cattle. Over the entire period, urea-supplemented steers grew approximately 12.8% faster than the NC group. Daily gains of the urea-supplemented cattle were nearly identical regardless of the level of urea. The PC diet supported gains 28.9% greater (P<.05) than the NC treatment (1.47 vs 1.14 kg/d) and 14.4% faster (P<.05) than the urea-containing diets (1.47 vs 1.28 kg/d). The addition of urea improved (P<.05) the efficiency of feed conversion by an average of 10.7% over the NC, with no differences existing among the three urea-containing diets. The PC diet improved feed efficiency (P<.05) by 17.8% over the NC treatment and tended to improve feed efficiency over the urea-containing diets by an average of 8%. Most of the improvement in gains and feed efficiency by the PC group, when summarized over the entire trial, was achieved during the first 70 d of the trial. During the first 70 d, the composition of live weight gain by the lightweight steers probably consisted of a greater percentage of protein compared with that in the latter phases in which a greater percentage of fat was being deposited (Eversole et al., 1981). Thus, performance achieved during the initial 70 d is a better indicator of the protein adequacy of these treatment diets.

Similar performance of steers fed the urea-containing diets during the entire trial and increased performance of the HU and MU steers over the LU steers during the initial 70 d of the trial were predicted by the metabolizable protein system and supports the validity of this system in predicting the degree to which urea was useful in supporting ruminal fermentation.

Rumen Observations. Rumen ammonia concentration as a function of time after feeding (table 4) showed large differences in the amount of ammonia produced by each treatment diet, with all ammonia concentrations peaking at or before the first hour of collection. The relative order of magnitude of ruminal ammonia concentration was in close agreement to that found in the in vitro fermentation studies of this experiment, with the HU group having a maximal value of 19.5 mg NH_3-N/dl at 1 h, followed in order by the MU, LU, PC and NC groups. Satter and Slyter (1974) stated that a minimum of 5 mg NH_3-N/dl was needed to support maximal amounts of microbial growth in the rumen. It appears from these data that the HU and MU diets both provided this minimal amount of ammonia during the first 3 to 4 h postfeeding. In contrast, the LU diet appeared to provide sufficient ammonia for only 1 to 2 h. According to Satter and Slyter (1974), the PC diet, which supported the best feedlot performance, appeared to provide insufficient amounts of ammonia needed to maximize microbial growth throughout the 5-h postprandial period. This is in close agreement with the results from the in vitro fermentation studies and implies that addition of a small quantity of urea to the PC diet might have been utilized by the rumen microorganisms, thus, resulting in even greater amounts of absorbable amino acids and further improvement in feedlot performance.

Blood Data. Changes in blood urea nitrogen (BUN) following feeding are summarized in table 5 and are a reflection of the conversion of absorbed ammonia into urea by the liver during a 5-h period after eating. Changes in postprandial levels of BUN ranked in the same order as rumen ammonia levels, with HU being the highest, followed by MU, LU, PC and NC dietary treatments, respectively. Feeding the LU and MU diets resulted in rapid increases in BUN within 1 to 2 h, after which they leveled off and slowly declined. Blood urea N levels continued to increase for 3 h postfeeding in cattle fed the HU diet. The PC diet resulted in a small increase in BUN during the first hour, then gradually declined. There was a continuous decline for the basal diet, the greatest concentration being at the initial reading. These findings support those of Lewis (1957), in which infusion of increased levels of urea into the rumen resulted in increased rumen ammonia levels and a subsequent increase in BUN content.

These relative changes in BUN concentrations also rank in accordance with predictions based upon the observed in vivo rumen ammonia concentrations and with the calculated UFP values of the treatment diets. The HU diet had a high negative UFP (−234), followed by the MU and LU diets, which had positive UFP values of 0 and 269, respectively. The PC and NC diets had positive UFP values of 11.3 and 976, respectively. The large negative UFP of the HU diet would serve as a predictor of a large

TABLE 4. EFFECT OF SOURCE AND LEVEL OF DIETARY NITROGEN UPON RUMEN AMMONIA CONCENTRATION MEASURED AT HOURLY INTERVALS AFTER FEEDING STEERS[a]

Treatment	Time, h					
	0	1	2	3	4	5
	mg NH_3-N/dl					
Negative control	.04[b]	.29[b]	.23[b]	.11[b]	.02[b]	.04[b]
Low urea	3.34[c]	6.17[bc]	4.30[bc]	1.86[bc]	.07[b]	.15[b]
Medium urea	3.93[cd]	14.01[cd]	11.62[cd]	7.36[c]	5.46[b]	2.55[b]
High urea	3.58[c]	19.50[d]	14.62[d]	6.97[c]	.89[b]	.75[b]
Positive control	4.47[d]	3.81[b]	2.76[b]	1.76[b]	1.20[b]	1.49[b]
SE	.21	4.49	3.94	2.91	2.11	1.78

[a]Values represent the mean of two animals sampled at hourly intervals postfeeding after adjustment for time of sampling by multiple regression analysis.

[b,c,d]Values with different superscripts in the same column differ ($P<.05$).

excess of ammonia existing in relation to the ability of the rumen microbes to utilize it for growth. Thus, a large quantitiy of this ammonia would be expected to leave the rumen and be converted into urea, contributing to the high BUN values. The UFP values of the MU and PC diets suggest that BUN levels would be lower than that from the HU diet because there is no large excess of ammonia N compared with the amount of fermentable energy in these diets. The large positive UFP of the LU and NC diets suggests an even greater depression of BUN compared with the other treatments due to the large excess of fermentable energy in relation to quantities of ammonia in the rumen ammonia pool. Of interest is the difference in BUN values of the MU and PC groups, considering the similar magnitude of their UFP values. Initially, it would appear that similar UFP values should result in similar BUN values. However, a review of the in vivo and in vitro rumen ammonia data showed that the rate of release of ammonia from the two diets was vastly different. In the case of the MU diet, excess rumen ammonia levels (exceeding 5 mg/100 ml) existed for approximately 3.5 to 4 h postfeeding. In the case of the PC diet, ruminal ammonia concentrations were consistently very low and never exceeded 5 mg/100 ml during the 5-h period. Although the total N content of the MU and PC diets was identical, large differences in BUN resulted following feeding. These data support the findings of Lewis (1957) in which fluctuations in BUN are

TABLE 5. EFFECT OF SOURCE AND LEVEL OF NITROGEN UPON CHANGES IN BLOOD UREA NITROGEN AFTER FEEDING[a]

Treatment	Time, h					
	0	1	2	3	4	5
	mg BUN/dl					
Negative control	4.07[b]	−.90[d]	−1.23[b]	−1.47[b]	−1.60[b]	−1.59[b]
Low urea	3.99[b]	1.98[cd]	1.99[cd]	1.63[cd]	1.09[bc]	.60[bc]
Medium urea	6.94[b]	2.51[c]	2.69[c]	2.47[c]	2.09[c]	1.80[c]
High urea	5.20[b]	2.90[c]	3.47[c]	3.57[c]	3.24[c]	2.58[c]
Positive control	7.10[b]	.34[bd]	−.18[bd]	−.83[bd]	−1.37[b]	−1.54[b]
SE	1.06	.96	1.24	1.44	1.45	1.24

[a]Prefeeding concentrations (zero time values) have been subtracted from the hourly concentrations.

[b,c,d]Values with different superscripts in the same column differ ($P<.05$).

a reflection of rumen ammonia concentrations rather than overall N intake.

Conclusion

Rumen fermentation studies effectively ranked the different diets in terms of ruminal ammonia concentrations when fed in vivo. There was a close relationship between levels of urea in the diet and the change in ruminal ammonia concentration after feeding. However, BUN concentrations were not closely related to the UFP of the treatment diets probably because of differences in both the rate and extent to which the alternate N sources were converted to ammonia in the rumen. Differences in growth rate of lightweight cattle during the early part of the trial adds further verification of the accuracy by which the metabolizable protein system predicts the quantity of urea that would be useful in high energy diets. Also, the superiority of this system in predicting the protein adequacy of these experimental diets compared with the crude protein system was observed.

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