Urea Utilization by Cattle as Influenced by Casein

By

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SUMMARY

THE influence of casein on urea-nitrogen utilization by cattle was investigated in two studies. In a fattening study, the inclusion of casein to a soybean meal (SBM) or urea-supplemented ration depressed both average daily gain (ADG) and average daily feed intake ($P < .05$). Casein addition, on the other hand, significantly ($P < .05$) increased rumen ammonia concentration. The second and metabolism study involved the use of identical rations as in the fattening study. Cattle fed rations with casein did not show any significant decrease in nitrogen retention as compared to cattle fed rations without casein. However, there was a non-significant drop in nitrogen retention, and an increase in urinary nitrogen excretion attributable to casein. Ruminal histamine observed for steers on the fattening study was higher than that noted for steers on the metabolism study. Part of the poor performance of steers on the casein diets could be attributed to the higher urinary nitrogen excreted and the elevated ruminal histamine levels observed.

INTRODUCTION

A NUMBER of factors have been shown to influence urea utilization (Chalupa, 1968). Briggs, (1967) and McDonald, (1948) demonstrated that available energy enhanced microbial protein synthesis. And the report of LEWIS, (1961) adequately illustrated the effect of various carbohydrates on ammonia disappearance from the rumen and established that starch is most useful in enhancing ammonia utilization by ruminal microorganisms.

There seems also to be a need for peptides (Pittman and Bryant, 1964; Wright, 1967), and branched-chain carbon skeletons (Allison and Bryant, 1962; Oltjen, Slyter, Williams and Kern, 1971; Umunna, Klopfenstein and Woods, 1975). Barth, McLaren, Anderson, Welch and Smith (1959) reported improvement in the percentage of absorbed nitrogen retained by lambs when urea-nitrogen was replaced by up to 17 per cent and 11 per cent of methionine and/or tryptophan respectively. McLaren, Anderson, Barth and Welch, (1962) have also shown improvement in nitrogen economy of the animal with partial replacement of urea-nitrogen with casein-nitrogen in the form of enzyme and/or acid hydrolyzed casein but not with intact casein. On the other hand, Hume (1970) showed improved nitrogen retention and ruminal protein production of lambs supplemented with intact casein. The experiments to be discussed were undertaken to further study the influence of casein on urea-nitrogen utilization.

EXPERIMENTAL METHODS

Fattening study

Twenty Hereford, Angus and Hereford X Angus crossbred steer calves of average weight 303.8 kg were used in this study which lasted 119 days. On the basis of breed and initial weights, the steers were assigned at random to the four treatments; SBM; SBM + casein; Urea; Urea + casein (Table 1). These animals were selected from a group of about 300 weaned calves. Prior to the start of the study, they were fed corn silage and a SBM based supplement. The experimental rations were based on maize with SBM and urea as the two sources of supplemental nitrogen,

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TABLE 1

Percentage Composition of Rations fed in Both Fattening and Metabolism Studies

	Treatments			
	1 *SEM*	2 *SEM + Casein*	3 *Urea*	4 *Urea + Casein*
Molasses	3.50	3.50	3.50	3.50
Maize cobs	10.00	10.00	10.00	10.00
Maize	77.17	78.19	83.95	83.22
Soyabean meal	8.05	6.00	—	—
Urea	—	—	1.075	0.82
Casein	—	1.00	—	1.00
Dicalcium phosphate	0.475	0.475	0.675	0.67
Limestone	0.50	0.525	0.443	0.445
Vitamin A[1]	+	+	+	+3
Salt	0.25	0.25	0.25	0.25
Trace minerals premix[2]	0.025	0.025	0.025	0.025
Stilboesterol (Stilbosol — 2)	0.025	0.025	0.025	0.025
TM-50 (Antibiotic)	0.0075	0.0075	0.0075	0.0075
Potassium Chloride	—	—	0.05	0.05
Chemical composition				
Dry matter[3]	87.75	87.75	87.88	87.62
Crude protein[3]	12.70	12.93	12.93	12.94
Calcium[4]	0.40	0.41	0.40	0.41
Phosphorus[4]	0.35	0.35	0.35	0.36

1 Vitamin A added to provide 30,000 IU per steer per day.
2 Premix contained 10% Manganese, 10%, Iron, 1% Copper, 10% Zinc, 0.3% Flourine and 0.1% Cobalt.
3 Analyzed.
4 Calculated.

with or without casein addition (at 1% of the total ration). The chemical composition of the experimental rations are shown in Table 1.

The steers were individually tied twice daily, morning and late afternoon, for a 2-hr period each time for feeding. Otherwise, they were free in the pens in groups of four. Some hay was fed at the start of the experiment but was gradually withdrawn as the intake of the experimental ration increased. All the animals were completely withdrawn from hay and were on full feed of the experimental ration by the 16th day. At the termination of the study on day 119, rumen fluid samples were taken from the ventral sac region with a suction strainer (Raun and Burroughs, 1962) at 1, 2 and 3 hr post-feeding for ammonia analysis. One ml of 5 % mercuric chloride was added to each 50 ml sample and stored at -5°C until analyzed for ammonia (Conway, 1955). The initial and final weights were taken after the steers were taken off feed and water for a 16 hr period (shrunk weight).

Metabolism study

Twelve Hereford steer calves of average weight 263.4 kg were used in this study which consisted of two collection periods, a total of eight steers per treatment. The steers were randomly assigned to the four treatments which were identical to those used in the fattening study.

The steers were individually fed the experimental rations for a 14-day preliminary period, immediately followed by a

7-day collection period in metabolism stall similar to those described by Briggs and Gallup (1949). Urine and faecal collection procedures were similar to those described by Woods, Thompson and Grainger, (1956). Faeces from each steer was collected daily and stored in a 95 -1 plastic bucket with a lid at 4°C. At the end of each period, the total collection from each steer was weighed and then thoroughly mixed in a stainless steel food mixer before a 500 g sample was taken for dry matter and nitrogen determinations.

Rumen fluid samples were taken from each steer at the end of each 7-day collection period at 1,2,4 and 6 hr. post-feeding for ammonia determinations. Blood was also taken from each steer at 1, 2 and 4 hr. post-feeding by jugular puncture into heparinized tubes for blood urea analyses. Ammonia and urea determinations were by the method of Convay (1955). All nitrogen and dry matter determinations were according to the A.O.A.C. (1970). Rumen fluid samples from both studies were analyzed for histamine by a modification of the methods of Kremzer and Wilson, (1961), Shelley and Juhlin, (1966), Shore, Burkhalter and Cohn, (1959) and Yusem, Delaney, Lindberg and Fashing, (1969).

Data were analyzed by the analysis of variance and orthogonal comparisons (Steel and Torrie, 1960).

RESULTS

Fattening

ADG was significantly higher ($P<.05$) for cattle fed the SBM and SBM-casein rations than for cattle fed the urea and urea-casein rations (Table 2). ADG and average daily feed intake were significantly higher ($P<.05$) for the cattle on treatments without casein than for cattle on treatments with casein. However, cattle on SBM with or without casein significantly ($P<.05$) consumed more feed than cattle on urea with or without casein. Feed required per unit of gain was significantly less ($P<.05$) for the SEM or SBM-casein group than for the urea or urea-casein group.

TABLE 2

Results of the Fattening Study

	Treatments				
	1	2	3	4	
	SEM	*SEM + Casein*	*Urea*	*Urea + Casein*	*SE*
No. of steers	5	5	5	5	
Initial weight, kg.	303.0	300.0	304.0	307.5	
Final weight, kg	436.5	421.3	412.7	400.4	
Total gain, kg	133.5	120.4	108.7	92.8	
Average daily gain, kg[a, b]	1.12	1.01	0.91	0.78	0.055
Average daily feed intake, kg[a,b]	8.09	7.59	7.23	6.56	0.266
Feed required per kg gain, kg[b]	7.21	7.50	7.91	8.41	0.321

S.E. = Standard Error
a: 1, 3 vs. 2, 4 significant ($P<0.05$)
b: 1, 2 vs. 3, 4 significant ($P<0.05$)
c: 1, 4 vs. 2, 3 significant ($P<0.05$)

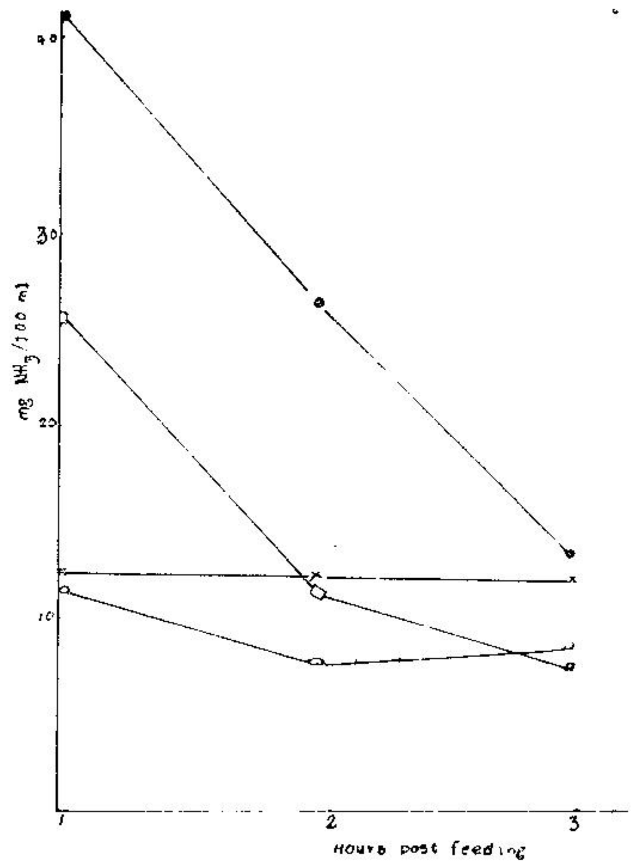

Figure 1: Fattening of Study : Rumen ammonia levels in Steers.

Rumen ammonia concentration was significantly higher ($P<.01$) for the urea and urea-casein treatments than for the SBM and SBM-casein treatments. However, the addition of casein to either urea or SBM supplemented ration resulted in significantly higher ($P<.01$) rumen ammonia levels than was observed for treatments which had urea or SBM alone. The interaction of treatment and time with regard to rumen ammonia levels was significant ($P<.01$).

Metabolism study

Cattle fed the SBM or SBM-casein ration had significantly higher ($P<.01$) nitrogen retention than cattle on urea with or without casein. Nitrogen retention as percent of absorbed nitrogen was also in favour of cattle fed the SBM ration with or without casein. (Table 3). Urinary nitrogen loss was significantly lower ($P<.05$) for the SBM or SBM-casein group than for the urea or urea-casein group.

Nitrogen digestibility was significantly higher ($P<.01$) for the urea or urea-casein treatment than for the SBM or SBM-casein treatment. A highly significant ($P<.01$) increase in rumen ammonia and blood urea levels was observed for the urea and urea-casein fed steers than for the SBM or SBM-casein fed steers. Inclusion of casein to the SBM supplemented ration was associated with higher levels of rumen ammonia and urinary nitrogen. Ammonia levels decreased significantly ($P<.01$) with time across treatments, and the interaction of treatment and time with regard to the rumen ammonia levels was significant ($P<.01$)

TABLE 3

Results of Metabolism Study

Characteristics	Treatments 1 SEM	2 SEM + Casein	3 Urea	4 Urea + Casein	SE
DM digestibility, %	77.9	77.3	78.8	77.7	0.602
Protein digestibility, %	72.0	72.6	75.3	73.7	0.722
N intake, g	90.35	89.74	96.64	89.39	
Faecal N, g	25.29	24.56	23.91	23.48	
Urinary N, g [a]	40.43	42.78	51.44	47.08	1.19
N retained, g[a]	24.63	22.40	21.29	18.83	0.118
As % absorbed N retained	37.83	34.44	29.26	28.59	1.724
Blood urea concentrations (mg Urea/100ml)[a]					
Hours post-feeding					
1	33.56	36.38	42.84	41.05	
2	32.46	35.58	44.08	42.77	
4	31.07	35.65	44.98	45.42	

S.E. = Standard error
a: 1, 2 vs 3, 4 significant (P <.05)
b: 1, 3 vs 2, 4 significant (P <.05)
c: 1, 4 vs 2, 3 significant (P <.05)

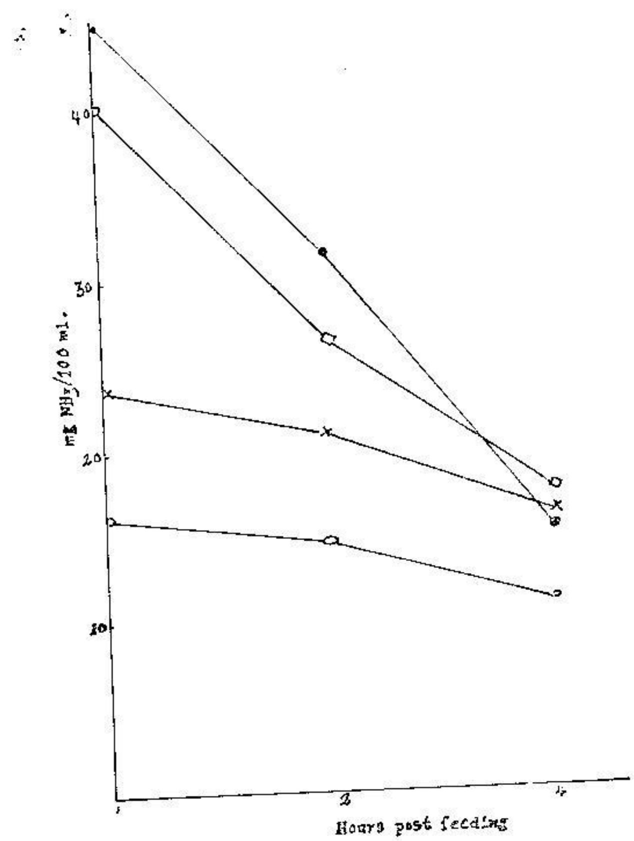

Figure 2: Metabolism Study. Rumen ammonia levels of steers.

DISCUSSION

In both the fattening and metabolism studies, the addition of casein to either a SBM or urea-supplemented ration was detrimental to ADG, average daily feed and nitrogen retention. This observation is not consistent with the results of Hume, (1970) and Umunna *at al.* (1975). Working with sheep fed a 50 % oat hull diet, Hume, (1970) demonstrated increased nitrogen retention and ruminal protein production when approximately 39 % casein-nitrogen replaced urea nitrogen. Umunna *at al.* (1975) also showed improved nitrogen retention of lambs when casein-nitrogen replaced 9 % of urea-nitrogen in a high roughage ration. The reasons for the disparity in the results quoted above and those under discussion are not apparent. Better performance would be expected from the steers in the present study since they were fed a high energy maize ration which would enhance ruminal protein synthesis. But this was not the case. However, the results of the present study are partially consistent with those of McLaren *at al.* (1962) who fed lambs a 48 % maize cob ration and demonstrated failure of intact casein supplementation to improve urea utilization but did not report any depressing effect of casein on nitrogen retention.

In the fattening study, the addition of casein to SBM or urea supplemented ration adversely and significantly affected ADG and average daily feed intake. However, in the metabolism study, even though casein addition depressed nitrogen retention, its effect was non-significant. This could be explained on the basis of intake.

Intake was limited to 3.95 kg dry matter per head for steers used in the metabolism study while steers used in the finishing study were allowed all they could eat when tied twice daily. Under this regime, average intake of dry matter was 7.4 kg. This was approximately double the intake of steers in the metabolism study. The restriction of intake in the metabolism study could have led to a more efficient utilization of available nutrients (Simpson 1965). This is especially true for the treatments which contained the readily soluble nitrogen sources, urea and/or casein. On the other hand when the time allocated for feeding is restricted, (as in the finishing study) there is a tendency for cattle to consume a lot of feed in a very short time. When consumed rapidly and in large quantities, urea or other soluble sources of nitrogen, will be rapidly hydrolyzed to ammonia, much of which could be lost via the urine (Annison, Hill and Lewis, 1957). This was observed. In the finishing study, rumen ammonia was significantly ($P < .01$) higher for the treatments that contained casein when compared to the treatments without casein. In the metabolism study, rumen ammonia level was higher with casein addition than with SBM or urea alone although the difference did not attain significance.

The observations of Simpson, (1965) and Annison *at al.* (1957) would suggest that increased levels of rumen ammonia are accompanied by increased urinary nitrogen loss. The urinary nitrogen loss of the SBM and SBM-casein groups confirmed this observation. The steers fed the SBM-casein ration had higher urinary nitrogen losses than steers fed SBM alone. Unfortunately, the same comparisons could not be made for the urea and urea-casein groups since through error in ration mixing, the nitrogen content of the urea treatment was higher than for the urea-casein treatment. The increased nitrogen intake of steers on the urea treatment, therefore, masked any differences in urinary nitrogen loss that could have been apparent.

Analysis of the fattening data revealed that casein significantly ($P<.05$) and detrimentally affected both the ADG and average daily feed intake. Since some authors (Dain, Neal and Dougherty, 1955) have suggested the detrimental effect of histamine on both feed intake and animal performance, the ruminal histamine levels were determined. Ruminal histamine observed for the fattening study was higher than for the metabolism study (3.5 vs 1.25mg/100 ml). This paralleled the intake of casein in the two studies (33.5 vs 17.6 g per steer per day, respectively). Though there is no agreement on the effect of histamine (Dain *at al.*, 1955; Koers, 1973) on animal performance, the depressions on both performance and feed intake which has been noted by some workers (Dain et al. 1955; Wren, Bitman, Cecil and Mench, 1964) were observed. These studies suggest that part of the poor performance on casein could be attributed to increased urinary nitrogen loss and the effect(s) of elevated ruminal histamine levels.

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REFERENCES

ALLISON, M.J. and BRYANT, M.P. 1962. On the biosynthesis of branched-chain amino acids by Ruminococcus flavifaciens. *VIIIth Intern. Congr. Microbiol; Montreal,* 42 (*Abstr.*).

A.O.A.C. 1970. Official Methods of Analysis of the Association of Official Analytical Chemists (8th Edition) Association of Official Analytical Chemists, Washington, D.C.

ANNISON, E.F. HILL, K.J. and LEWIS, D. 1951 Studies on the portal blood of sheep. I. Absorption of ammonia from the rumen of the sheep. *Biochem. J.* **66**: 587-592.

BARTH, K.M.; MCLAREN, G.A.; ANDERSON, G.C.; WELCH, J.A. and SMITH G.S. 1959. Urea nitrogen utilization in lambs as influenced by methionine and tryptophan supplementation. *J. Anim. Sci.* **18**: 1521 (*Abstr.*).

BLOOMFIELD, R.A.; GARNER, G.B. and MUHRER, M.E. 1960. kinetics of urea metabolism in sheep. *J. Anim. Sci.* **19**, 1248 (*Abstr*).

BRIGGS, H.M. 1967. Urea As A Protein Supplement. Pergamon. New York.

BRIGGS, H.M.; GALLUP, W.D. 1949. Metabolism stalls for wethers and steers. *J. Anim. Sci.* **8**: 479-482.

CHALUPA, W. 1968. Problems in feeding urea to ruminants. *J. Anim. Sci.* **27**: 207-249.

CONWAY, E.J. 195_. Microbiffusion Analysis and Volumetric Error. New York. Macmillan Co.

DAIN, J.A., NEAL, A.L. and DOUGHTERY, R.W. 1955. The occurrence of histamine and tryamine in rumen ingesta of experimentally over-fed sheep. *J. Anim. Sci.* **78**: 15-19.

HUME, I.D. 1970. Synthesis of microbial protein in the rumen. III. The effect of dietary protein. *Aust. J. Agr. Res.* **21**: 305-314.

KOERS, W. 1973. Digestive disturbances in ruminants as related to ruminal histamine and lactate. *Ph.D. Thesis. University of Nebraska, Lincoln, Nebraska.*

KREMZER, L.T. and WILSON, I.B. 1961. A procedure for the determination of histamine. *Biochem. Biophys. Acta* **50**, 364-367.

LEWIS, D. 1961. The fate of nitrogenous compounds in the rumen. In Digestive Physiology and Nutrition of the Ruminant. Butterworths, Lond.

MCDONALD, I.W. 1948. The absorption of ammonia from the rumen of the sheep. *Biochem. J.* **42**, 584.

MCLAREN, G.A.; ANDERSON, G.C. BARTH, K.M. and WELCH, J.A. 1962. Casein and its degradation products in the utilization of urea nitrogen by lambs. *J. Anim. Sci.* **21**: 258-261.

OLTJEN, R.R., SLYTER, L.L., WILLIAMS, JR. E.E. and KERN, D.L. 1971. Influence of branched-chain volatile fatty acids and phenylacetate on ruminal microorganisms and nitrogen utilization by steer fed urea or isolated soy protein. *J. Nutr.* **101**: 112-118.

PITMAN, K.A. and BRYANT, M.P. 1964. Peptides and other nitrogen sources for growth of Bacteroides ruminocola. *J. Bacteriol.* **88:** 401-410.

SHELLEY, W.B. and JUHLIN, L. 1966. Histamine chromatography and electrophoresis-the-o-Phthaldehyde fluorogram. *J. Chromatog.* **22,** 130-138.

SHORE, P.A., BURKHALTER, A. and COHN JR., V.H. 1959. A method for the flourometric assay of histamine in tissues. *J. Pharmacol. and Exptl. Therap.* **127,** 182-186.

SIMPSON, O.C. 1965. Influence of rate of ammonia and energy release on protein synthesis by rumen bacteria. *Ph.D. Thesis. University of Nebraska, Lincoln, Nebraska.*

STEEL, A.R.D. and TORRIE, J.H. 1960. Principles and Procedures of Statistics. New York, McGraw-Hill Book Co., Inc.

UMUNNA, N.N ; KLOPFENSTEIN, T.J. and WOODS, W.R 1975. Influence of branched-chain volatile fatty acids on nitrogen utilization by lambs fed urea containing high roughage rations *J. Anim. Sci.* **40:** 523-529.

WOODS, W.R., THOMPSON, C.M. and GRAINGER, R.B. 1956. The effect of varying levels of protein and cerelose on the utilization of mature timothy hay by sheep. *J. Anim. Sci.* **15:** 1141-1146.

WREN, T.R., BITMAN, J., CECIL, H.C. and MENCH, M.L. 1964. Histamine excretion of cattle fed various diets. *J. Dairy Sci.* **47:** 882-884.

YUSEM, M., DELANEY, W.E., LINDBERG, M.A. and FASHING, E.M. 1969. An improved fluorometric determination of histamine by stabilizing the o-Phthaldehyde reagent. *Anal. Chem. Acta* **44,** 403-409.