RUMINAL CRUDE PROTEIN, TRUE PROTEIN AND VOLATILE FATTY ACIDS ON SILAGE RATIONS SUPPLEMENTED WITH SOYBEAN MEAL OR UREA

by 5414

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B. S., National Taiwan University, 1953

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Husbandry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

Approved by:

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INTRODUCTION

The large capacity of the ruminant digestive tract, together with the presence of its large microbial population, makes the ruminant especially adapted for digestion of fibrous feeds, most of which are of little use to monogastric animals. The microbial breakdown of such feeds provides the host with readily available volatile fatty acids, high quality proteins and many vitamins. Rumen microorganisms can utilize inexpensive non-protein nitrogen (NPN) compounds, such as urea, to synthesize proteins for the construction of their own bodies, which are passed out of the rumen and are digested and absorbed. These facts have made it possible for the ruminant to occupy a predominant position in modern agriculture.

Numerous studies have concentrated on factors which will promote the maximum bacterial synthesis of proteins in the rumen to provide for more effective use of rations poor in protein quality and, particularly, of non-protein sources of nitrogen, e.g., urea. The practical objectives are a cheaper ration and improved animal performance.

Because of its low cost per unit of nitrogen, urea has become the most accepted NPN compound for protein substitution in ruminant rations. It has been estimated that about 100,000 tons of urea is used annually for such purposes in the United States (Stangel, 1963).

In addition to its use as a substitute for protein feeds, urea can also be thought of as an extender to the feed supply. It will permit greater utilization of most roughages, the digestion of which is generally hampered by a shortage of nitrogen. By using urea to aid in digestion and in rate of removal of feed from the rumen, the voluntary intake of feed can be enhanced.

The purpose of this research was to obtain information on the amounts

of readily available energy, supplied as grain, necessary for the utilization of urea as supplemental protein equivalent in a high roughage ration. Results were evaluated by live animal weight gains and crude protein, true protein and volatile fatty acids found in rumen contents.

REVIEW OF LITERATURE

Nitrogen Metabolism in the Rumen

The Degradation of Protein. Nitrogen, which is destined to be formed into microbial protein in the rumen, is consumed in two basic forms, namely, natural proteins and non-protein nitrogen. Protein is degraded by the action of bacterial proteolytic enzymes in the rumen (Sym, 1938). Peptides and amino acids are thus produced which in turn are attacked by deaminases to yield ammonia (McDonald, 1948, 1952). It is estimated that 50 percent of the crude protein in the ruminant diet is broken down to ammonia. Ammonia may also be formed from sources other than amino acids, e.g., amide, amidine, urea from the diet and from saliva, which is hydrolyzed by microorganisms to form ammonia (Pearson and Smith, 1943; Annison, 1956; Warner, 1956; El-Shazley, 1958; Lewis and McDonald, 1958; Stallcup and Looper, 1958; Blackburn and Hobson, 1960). From this ammonia rumen microorganisms synthesize varying quantities of all the amino acids.

The Synthesis of Protein. Pearson and Smith (1943) first reported that both synthesis and breakdown of protein occur simultaneously in the rumen. Ammonia N as well as amino acids and peptides can be used in these synthetic reactions (Phillipson et al., 1959). The extent to which dietary nitrogen is converted to microbial nitrogen depends on: (1) the rate at which dietary nitrogen is broken down to amino acids and ammonia, (2) the rate of

absorption of amino acids and ammonia, (3) the rate of passage of material out of the rumen to the abomasum, and (4) the synthetic powers of the microorganisms. In fact, the great majority of the proteolytic rumen bacteria prefer ammonia to amino acids or peptides for protein synthesis (Akkada and Blackburn, 1963).

The net effect of microbial protein synthesis within the rumen is that a large percent of dietary nitrogen, whether protein, urea, or ammonium salts, is converted to microbial protein of a reasonably high biological value. This is obviously an advantage when the dietary nitrogen is of low biological value and a disadvantage when it is of high biological value.

Nutritive Value of Urea in Ruminant Rations

The Effect of the Proportion of Carbohydrates in the Diet. There is considerable interdependence between carbohydrate source and concentration and protein digestion in the rumen. The amount of ammonia formed is dependent on both the nature of the dietary protein and the proportion of carbohydrate in the diet. Protein is better utilized in the presence of added carbohydrates. When the protein intake is increased, there is a more rapid attack upon the fibrous components of the ration. McDonald (1952) demonstrated a decrease in rumen ammonia concentration when starch was added to the rumen. The rate of production of volatile fatty acids (VFA) from starch also increased when small amounts of casein were added.

Bell et al. (1953) indicated that urea nitrogen was utilized with equal efficiency in rations of several cereal grains and sweet potatoes, and with less efficiency in molasses-containing rations. On the other hand, Morimoto et al. (1957) reported in Japan that when goats were fed a ration consisting

mainly of wheat bran and rice bran supplemented with urea at 3% of the ration, urea was not utilized at all and was excreted in the urine. Insight into the mechanism by which starch increases urea utilization has been provided by Orth and Rohr (1963) who stated that rations rich in starch caused lower rumen pH values and more numerous forms of bacteria. The effect of low pH was a slower diffusion of ammonia from the rumen, thus increasing its chances for utilization by rumen microorganisms. A pH depression below 6.5 may also depress urease activity to some extent and thus slow the release of ammonia from urea. Andrec (1963) indicated that when urea was pelleted with soluble starch, the release of urea from such pellets into ruminal juice was very slow and the danger of ammonia intoxication was reduced.

Feeding Urea with Roughages. It is now well established that digestion in the rumen of poor roughages such as straw is impeded by a shortage of nitrogen. William et al. (1959) reported that sheep fed a poor quality oat straw ration lost significantly less weight when the ration was supplemented with 10 gm. urea and 38 gm. molasses per pound of straw. Colovos et al. (1963) showed that when the level of urea in the concentrate mixture was increased to 40 lb. per ton, the high-fiber ration fed with early-cut hay was comparable to the low fiber, more expensive concentrate mixture. Raleigh and Wallace (1963) observed also that the level of nitrogen significantly affected the digestibility of cellulose.

Effects of Other Compounds on Urea Utilization. Thomas and Loosli (1951) reported that lambs fed a diet containing sulfur gained in weight from the start. Lambs on a sulfur-deficient diet were always in negative balance for both sulfur and nitrogen. Starks et al. (1952) reported that lambs receiving added sulfur (0.062%) retained significantly (P 0.01) more

of the dietary nitrogen than did the lambs receiving the basal ration of starch, wheat straw, etc. Lambs that received sulfur were strong, alert and had a good appetite. Starks et al. (1953) again demonstrated that utilization of feed nitrogen was definitely improved by the addition of elemental sulfur. Through five years of feeding experiments with cattle and sheep, Altona et al. (1960) found that additional sulfur was, however, of no benefit.

Richardson et al. (1956) noted that the addition of ethyl alcohol tended to increase the consumption of molasses-urea mixture but not the rate of gain. Smith et al. (1957) reported that diethylstilbestrol increased nitrogen retention and the apparent digestibility of protein. Hatfield and Smith (1963) demonstrated that nitrate reduced gains in lambs fed soybean meal (SBM), but increased gains in lambs fed urea. The interaction was significant ($P < 0.05$).

Urea vs. Oil Seed Meals. Johnson et al. (1942) stated that the nitrogen in the products formed in the rumen from urea was as well utilized in metabolism as the nitrogen of SBM and somewhat better utilized than the nitrogen of casein. He concluded that a considerable proportion of the protein ultimately utilized by the ruminant is microbial protein, regardless of the nature of the nitrogen compounds contained in the ration as consumed. In a comparison of urea with soybean meal in barley rations, Bell and Gallup (1953) reported that small differences in nutrient digestibility and nitrogen retention favored the SBM supplement. Davis et al. (1956) observed that when one-third of the nitrogen in the basal ration (SBM) was replaced by urea, no significant differences in milk production were noted. Light et al. (1956) found that SBM was significantly ($P < 0.01$) better in increasing

feed consumption and gain than urea, when urea (42% N) was fed at the rate of 3% of the concentrate mixture with poor quality non-legume hay. (Urea furnished 41% of the nitrogen in the ration.) Merrill et al. (1959) demonstrated significantly greater gains for heifers fed rations of molasses and SBM versus those fed molasses and urea. When urea was compared to soybean grist as a nitrogen supplement to cows, Shmanenkov et al. (1959) reported no significant differences in health, weight gain or nitrogen metabolism were observed. He concluded that urea could be used for milk cows in the ratio of 1 gm. urea to 4 kg. liveweight. Drori (1959) found that organic nitrogen concentration and turbidities (a potential index of protein content) of rumen liquor of sheep showed no evidence of more bacterial protein or higher bacterial counts on a SBM diet vs. a urea diet (basal: chopped timothy hay). Sellers et al. (1960) reported that with either sorghum silage or cottonseed hulls, high urea supplementation produced less gain than SBM when fed at the same crude protein level. Richardson and Tsien (1963), studying the amino acid make-up of bacterial protein, found the rumen liquor of steers supplemented with SBM contained substantially more amino acids than rumen liquor from steers fed urea.

When urea was compared with cottonseed meal in high silage and high hay rations for wintering beef cattle, Reynolds et al. (1956) noted that urea and cottonseed meal were of comparable value for milk production.

Chalupa et al. (1963) observed that the protein of corn gluten meal was insoluble in the rumen and passed undegraded to the lower digestive tract. He attributed the inferiority of urea diets to the combination of (1) urea hydrolysis occurring faster than synthesis of microbial protein, and (2) a portion of plant proteins escaping proteolysis and passing intact to the

abomasum.

Other Non-protein Compounds in Ruminant Nutrition

In addition to urea, non-protein compounds such as diammonium phosphate, biuret and ammoniated products may also be used as protein substitutes in ruminant rations.

Diammonium Phosphate (DAP). Russell et al. (1961) evaluated diammonium phosphate as a source of nitrogen for lambs and found that DAP could be used up to 40 gm. urea equivalent per 100 lb. body weight without toxicity being observed. Urea produced toxicity at 15 gm. per 100 lb. body weight. No significant difference in nitrogen retention was found between lambs receiving the two compounds. Russell et al. (1962) reported again that DAP was less toxic and that considerably more DAP than urea was required to cause death in lambs. Lassitter et al. (1962) observed that while some problem with palatability might exist, the results of digestion and nitrogen balance trials indicated that DAP nitrogen was utilized as well as urea or SBM. In a sheep metabolism trial, however, Oltjen et al. (1963) found DAP to be a satisfactory source of phosphorus but the nitrogen of this compound was not retained as well as that supplied by urea or SBM. Laboratory tests indicated that DAP released ammonia when placed in contact with water or ruminant saliva, thereby reducing feed consumption. If the feed was pelleted, 50% of the nitrogen was lost due to effects of heat and pressure.

Biuret. Biuret, a condensation product of urea, has been compared with urea and SBM as a source of nitrogen. When crude biuret (45.5% urea) supplied 50% of the NPN, Anderson et al. (1959) found that protein digestibility was not significantly changed, but when it supplied 100%, protein

digestibility and nitrogen retention were significantly depressed. Hatfield et al. (1959) stated that biuret fed at levels sufficient to supply a significant part of the nitrogen requirement was not toxic to sheep, and that the utilization of biuret nitrogen appeared to be influenced by level of feed intake. In trials with dairy cows, Campbell et al. (1960) reported that biuret and urea supplements increased gains over those of animals on the basal rations to almost the same degree as SBM. Biological values for SBM, urea and biuret were 90.4, 89.6 and 78.2, respectively. In another experiment, Campbell (1962) stated that biuret proved to be slightly inferior to urea in growth trials with Holstein calves and heifers, but the differences were not significant.

Ammoniated Products. Otagaki et al. (1956) reported that milk production was nonsignificantly lower for cows on urea and ammoniated pineapple bran than those on the SBM ration. Tillman et al. (1957) observed that the nitrogen in the ammoniated cane molasses was not well utilized and that only in wintering trials did it compare favorably with urea. Hershberger et al. (1959) also compared ammoniated products with urea and stated that only the "free" ammonia in the materials could be utilized by rumen bacteria and that the "bound" nitrogen was not utilized but was not toxic. In an evaluation of ammoniated bagasse pith as a source of protein for milk production, Shimabukuro et al. (1959) found that dairy cattle could utilize nitrogen in ammoniated pith for growth and milk production. However, the total feed requirements were higher than for the control ration containing natural protein. TDN values were not enhanced with ammoniation, although others have suggested that ammoniation under pressure and heat may have a beneficial effect on lignin-cellulose complex digestibility. Broster et al. (1960)

compared ammoniated sugar beet pulp with peanut meal and found both supplements increased milk production and solids-not-fat. They concluded that the nitrogen in the ammoniated pulp was capable of correcting the nitrogen deficiency but was utilized much less efficiently than peanut meal. Chomyszyn et al. (1960) found that a ration containing ammoniated straw was more acceptable, more digestible and supported higher gains and feed efficiency than soaked straw. These authors concluded that 30 to 40 percent of the crude protein of lamb rations can be effectively replaced by ammoniated straw. They also found that gain and feed efficiency were greatest in the SBM group but feed intake and digestibility of crude fiber and protein were higher in the group fed ammoniated beet pulp.

Volatile Fatty Acids (VFA) in the Rumen

In ruminant nutrition, the major source of energy is volatile fatty acids resulting from rumen fermentation. Silage, containing VFAs formed during the ensiling process, is probably the most important dietary source of preformed fatty acids. The remainder are formed in the rumen mainly from the degradation of cellulose, starch and other carbohydrates and to a lesser extent from the deamination of amino acids. The latter source helps to explain the presence of various branched-chain fatty acids in the rumen.

Effects of Nitrogen Sources on VFA Production. Using a serial dilution type in vitro rumen fermentation, Belasco (1954) reported that urea as the sole source of nitrogen in cellulolytic fermentation promoted formation of higher levels of propionate and lower levels of butyrate and valerate than did natural protein. Acetic acid and total VFAs were not affected. Satapathy and Leffel (1962) showed that in lambs fed a low quality roughage,

the total rumen VFAs were increased when liquid supplements containing 10 to 20% urea were provided ad libitum. Karr et al. (1963) observed that when lambs were fed SBM, urea, or biuret, there were no apparent differences in the concentration of rumen VFAs (C_2 - C_5) or the ratio of acetate to propionate. Oltjen and Davis (1965) found that cattle receiving SBM rations had greater ($P < 0.05$) molar percent of acetate and a wider ($P < 0.01$) acetate/propionate ratio than steers receiving the urea-containing rations. Huber and Boman (1966) reported that higher protein rations resulted in higher concentrations of total VFAs in rumen fluid. Addanki et al. (1966) stated that the propionic acid in fresh rumen juice of calves was higher in the group fed soy bran flakes than those fed alfalfa or beet pulp. Davis and Stallcup (1967) noted that SBM was associated with the highest, and urea the lowest, quantity of total VFAs (C_2 - C_4).

VFAs are absorbed in large quantities from the rumen (Barcroft, 1944; Pfander, 1953). The influence of the pH of rumen contents on the rate at which each of the acids is absorbed was demonstrated by Gray (1948) and it is now generally agreed that rates of absorption are considerably lowered at pH values above 7.0 - 7.5. Annison (1959) stated that this dependence on hydrogen ion concentration is probably related to the proportion of the acid present in the undissociated form. In the pH range normally associated with rumen contents (5.5 - 6.5) a substantial amount of each VFA exists as the free acid but above pH 7.0 - 7.5 the acids are largely undissociated.

Effects of Roughages on VFA Production. Karr et al. (1963) reported that lambs fed dehydrated alfalfa meal tended to have a higher concentration of rumen VFAs (C_2 - C_5) and a narrower acetate/propionate ratio than those on the corncob diets. Moore (1964) indicated a greater concentration of

volatile fatty acids in rumen liquor when roughage was finely ground or pelleted. Of perhaps more interest is the change in ratio of acetate to propionate. Bath and Rook (1965) reported that hays, silages and a single batch of dried grass gave high values for the molar proportion of acetic acid (about 70%). The silages tended to give a lower proportion of propionic acid and a higher proportion of butyric acid than did the hays or dried grass. A close association between cellulose digestibility and VFA production was found by Oellermann (1965). He concluded that VFA production might serve as criteria in the assessment of the nutritive value of forages. In the case of calves, Stobo et al. (1966) reported that VFA concentration tended to be higher on a concentrate diet than a roughage diet.

Branched-chain VFAs. The branched-chain acids arise chiefly as end-products of protein degradation. El-Shazly (1952) observed a correlation between the concentration of ammonia and the C_4 and C_5 branched-chain acids in the rumen after the ingestion of protein rich foods. Annison (1954) suggested that the isobutyric, isovaleric and 2-methyl butyric acids arise from the deamination of valine, leucine and isoleucine. It was reported that C_4, C_5 and C_6 VFAs have a stimulatory effect on cellulose digestion (Bentley et al., 1954). Many strains of microorganisms have an absolute requirement for branched-chain VFAs (Bryant, 1954; Annison, 1958) due apparently to an impaired ability to incorporate or synthesize the carbon chain of these acids. Richardson and Tsien (1963) observed lower concentrations of valine, leucine and isoleucine in rumen fluid from twin steers fed urea, compared to those fed SBM. Matrone et al. (1965) noted a lower concentration of isovaleric acid in rumen contents of sheep fed a purified diet containing primarily urea, compared to one of casein. Oltjen and Putnam (1966)

suggested that the lower concentration of valine, isoleucine, leucine and phenylalanine in the blood serum of steers fed a purified diet containing urea, compared to isolated soy protein, was related to an insufficiency of branched-chain VFAs needed for microbial synthesis of protein in the rumen. The addition of these acids significantly increased the apparent nitrogen digestibility and retention. Cellulose and dry matter digestion were also improved by the amino acid addition. In using corn silage as the only forage, Huber et al. (1967) found rumen valerate and isovalerate tended to be lower for urea-containing rations, but acetate and propionate were not affected.

EXPERIMENTAL PROCEDURE

Fifty Hereford steer calves averaging 437 pounds were divided into five lots and fed all the corn silage they would eat each day. The corn silage consisted of 39.2% dry matter of which 27% was grain. Therefore a calf consuming 28 lb. of silage per day would ingest approximately 3 lb. of grain. Treatments of the five lots were as follows: Lot 13 -- 1.25 lb. of urea supplement. Lot 14 -- 1.25 lb. urea supplement and 2 lb. of alfalfa hay. Lot 15 -- 1.25 lb. SBM and 2 lb. alfalfa hay. Lot 16 -- 1.25 lb. urea supplement and 3 lb. milo grain. Lot 17 -- 1.25 lb. urea supplement, 3 lb. milo grain and 2 lb. alfalfa hay.

The urea supplement consisted of 83% milo, 14% urea and 3% dicalcium phosphate, and the SBM consisted of 97% SBM and 3% dicalcium phosphate.

Percent composition and chemical analyses of rations fed during the period rumen samples were taken are shown in Table 1.

Rumen samples were taken after the steers had been maintained on these

Table 1. Chemical Analyses of Rations Fed at the Time Rumen Samples Were Taken

	Dry matter lb.	Crude prot. lb.	Crude fiber lb.	Ether extr. lb.	Ash lb.	N.F.E. lb.
Lot 13						
Silage, 28.3 lb.	11.09	1.10	1.91	.66	1.70	5.72
Urea suppl., 1.25 lb.	1.12	.56	.02	.04	.08	.42
Total	12.21	1.66	1.93	.70	1.78	6.14
% of dry matter	100%	13.7%	15.8%	5.7%	14.6%	50.2%
Lot 14						
Silage, 29.8 lb.	11.68	1.15	2.01	.70	1.79	6.03
Urea suppl., 1.25 lb.	1.12	.56	.02	.04	.08	.42
Alfalfa hay, 2.00 lb.	1.81	.33	.53	.04	.14	.79
Total	14.61	2.04	2.56	.78	2.01	7.24
% of dry matter	100%	13.9%	17.5%	5.3%	13.8%	49.5%
Lot 15						
Silage, 29.9 lb.	11.70	1.16	2.03	.70	1.79	6.03
SBM suppl., 1.25 lb.	1.13	.50	.06	.03	.10	.44
Alf. hay, 2 lb.	1.81	.33	.53	.04	.14	.79
Total	14.64	1.99	2.62	.77	2.03	7.26
% of dry matter	100%	13.6%	17.9%	5.3%	13.9%	49.6%
Lot 16						
Silage, 30.3 lb.	11.90	1.17	2.05	.71	1.82	6.15
Urea suppl., 1.25 lb.	1.12	.56	.02	.04	.08	.42
Milo, 3 lb.	2.63	.30	.05	.08	.04	2.15
Total	15.65	2.03	2.12	.83	1.94	8.72
% of dry matter	100%	13.0%	13.5%	5.3%	12.4%	55.8%
Lot 17						
Silage, 22.3 lb.	8.74	.86	1.51	.52	1.34	4.51
Urea suppl., 1.25 lb.	1.12	.56	.02	.04	.08	.42
Milo, 3 lb.	2.63	.30	.05	.08	.04	2.15
Alf. hay, 2 lb.	1.81	.33	.53	.04	.14	.79
Total	14.30	2.05	2.11	.68	1.60	7.87
% of dry matter	100%	14.6%	14.8%	4.8%	11.2%	55.0%

rations for 112 days. The steers were fed at 8 a.m. and samples were drawn by vacuum through a stomach tube between 1 and 3 p.m. The samples were transferred to plastic bags and were immediately chilled and frozen for analysis at a later date.

Nitrogen Determination for Rumen Samples

Frozen rumen samples were thawed and immediately strained through 4 layers of cheesecloth. Fifty percent sulfuric acid was added at the ratio of 0.5 ml. to about 20 ml. of filtrate to stop the action of the micro-organisms.

Crude Protein. Nitrogen concentration of the rumen liquor, representing the crude protein, was determined by the micro-Kjeldahl method as follows:

(1) Pipette 2.0 ml. of rumen liquor filtrate into a digestion flask and add 3 ml. of conc. H_2SO_4, 1 ml. 10% $CuSO_4$, 1 gm. Na_2SO_4 and a glass bead.

(2) Digest until clear; cool and add distilled water.

(3) Distill ammonia for 5 minutes following addition of 25 ml. 50% NaOH.

(4) Collect ammonia in 4% boric acid solution containing 20 mg./liter brome cresol green sodium salt as an indicator.

(5) Titrate with 0.1 N standardized H_2SO_4 until a green end point is reached. Make appropriate blank corrections.

(6) Calculation:

$$\% \text{ N} = \frac{(\text{ml. acid}) (\text{N acid}) (14)}{\text{sample wt. in mg.}}$$

$$\% \text{ crude protein} = (\% \text{ N}) (6.25)$$

True Protein. It was first necessary to establish a reliable method for the accurate determination of the true protein fraction in the rumen liquor. The two protein precipitants which appeared most suitable were trichloroacetic acid (TCA) and sodium tungstate in H_2SO_4 (Pearson and Smith, 1943). Preliminary tests with rumen liquor showed that tungstic acid precipitated much more protein than did the TCA. Turbidity in the TCA supernatant indicated the failure of TCA to precipitate cellular nitrogen, and appeared largely responsible for the lower nitrogen values observed with TCA. As TCA precipitation tended to underestimate the bacterial protein, tungstic acid was used in this study. The procedure is as follows:

(1) To 2 ml. filtrate of rumen liquor add 1 ml. 10% sodium tungstate solution and 2 ml. 1.0 N H_2SO_4.

(2) Centrifuge at 1,000 g. for five minutes and decant the supernatant.

(3) Determine nitrogen concentration of the precipitated protein by the micro-Kjeldahl method as above. This represents the true protein fraction of the rumen liquor.

Determination of Volatile Fatty Acids in Rumen Liquor

VFAs were separated and quantitated using a Beckman GC-4 gas chromatograph. Separations were carried out on a 15% FFAP (free fatty acid polyester) in a 1/8 in. x 6 ft. Teflon column. Equipment parameters were:

Carrier gas: Nitrogen

Oven temperature: 150°C

Detector: Hydrogen flame ionization

Flow rates:

Carrier gas column:	35 ml./min.
Carrier make-up:	85
Total	120 ml./min.
Hydrogen:	55 ml./min.
Air:	250-300 ml./min.

Standards were prepared from pure VFAs in 0.1 N H_2SO_4. Linearity was checked, and one of the standards closest to the pattern of rumen VFAs was chosen and used. These were as follows:

	Standard used, micromoles/ml.	Other standards, micromoles/ml.				
Acetic	30	90	60	90	60	30
Propionic	7	14	21	60	40	20
Butyric	6	18	12	18	12	6
Isobutyric	1	2	3	-	-	-
Isovaleric	1	2	3	6	4	2
Valeric	1	2	3	6	4	2

As all the rumen samples were settled completely thereby leaving a clear supernatant, no centrifugation was necessary.

Injection size for both samples and standard solution was 2 microliters. Peak heights were determined for the standard solution, and samples were quantitated by simple proportionality. Since sample size was the same with both standard and unknowns, VFAs in the unknowns were reported as micromoles per ml. A typical separation of VFAs of the rumen liquor is shown in Fig. 1.

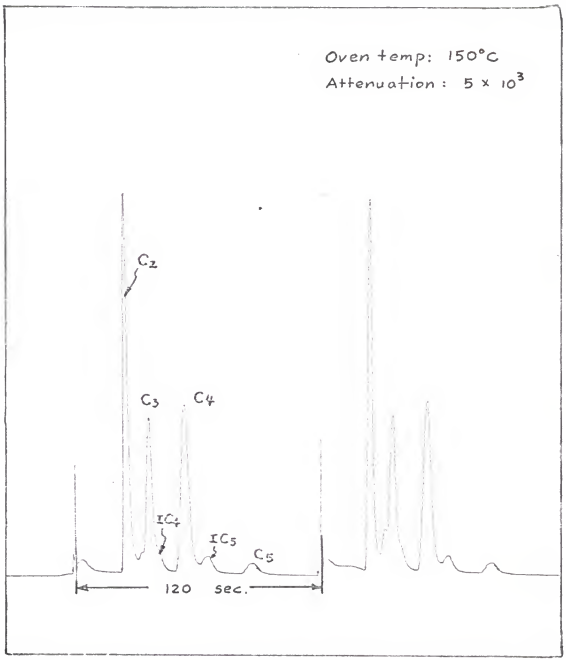

Fig. 1. Separation of VFAs by gas chromatography. C_2: acetic acid. C_3: propionic acid. IC_4: isobutyric acid. C_4: butyric acid. IC_5: isovaleric acid. C_5: valeric acid.

Statistical Analysis

All data were subjected to analysis of variance and multiple comparisons by Fisher's Least Significance Difference (Fryer, 1966). Percentages were treated by Arcsin transformation prior to analysis. All the individual data and the analysis of variance are presented in the Appendix tables.

RESULTS AND DISCUSSION

Feedlot Trials

After 112 days on feed, the steers were weighed. Average gains and feed efficiency are shown in Table 2 and Fig. 2. Individual animal results and analysis of variance are given in Appendix Table 5.

Statistical analysis showed that the average daily gain of lot 13 was significantly lower ($P < 0.05$) than all the other lots. No significant differences were observed among lots 14, 15, 16 and 17. Average daily feed consumption converted to a 90% dry matter basis was highest for lot 16. Feed efficiency of 7.51 in lot 15 was the highest, and 9.91 in lot 13 was the lowest.

Lot 16 had the highest daily gain and the greatest daily feed consumption but feed efficiency was lower than lots 15 and 17.

Theoretically, lot 17 should have outperformed lot 16 because of the addition of 2 lb. of alfalfa hay. Lack of significant difference may have been due to two cases of severe chronic bloat in lot 17.

Lot 13 received only urea as a supplement, and its protein intake of 1.53 lb. was below that of the other lots (Tables 1 and 2). However, silage consumption of lot 13 was comparable to that of the others. This indicates

Table 2. Average Daily Gain and Feed Efficiency of Steers Fed Corn Silage with Different Protein Supplements

Lot no.	Treatment	Initial wt. lb.	Final wt. lb.	Av. da. gain lb.	Av. da. silage cons'd lb.	Av. da. feed cons'd (90% D.M.) lb.	Prot. equiv. intake lb.	Av. feed efficiency (90% D.M.) lb.
13	1.25 lb. urea suppl.	436.5	574.0	1.23[a]	25.05	12.2	1.53	9.91
14	1.25 lb. urea suppl. 2.00 lb. alf. hay	437.5	631.0	1.73[b]	25.44	14.3	1.87	8.26
15	1.25 lb. SBM 2.00 lb. alf. hay	438.5	653.0	1.92[b]	25.53	14.4	1.88	7.51
16	1.25 lb. urea suppl. 3.00 lb. milo	436.5	654.0	1.94[b]	25.07	15.3	1.86	7.89
17	1.25 lb. urea suppl. 3.00 lb. milo 2.00 lb. alf. hay	437.5	637.5	1.78[b]	18.40	14.2	1.90	7.98

[ab] Any two ADG means not bearing a common superscript letter differ significantly ($P < 0.05$).

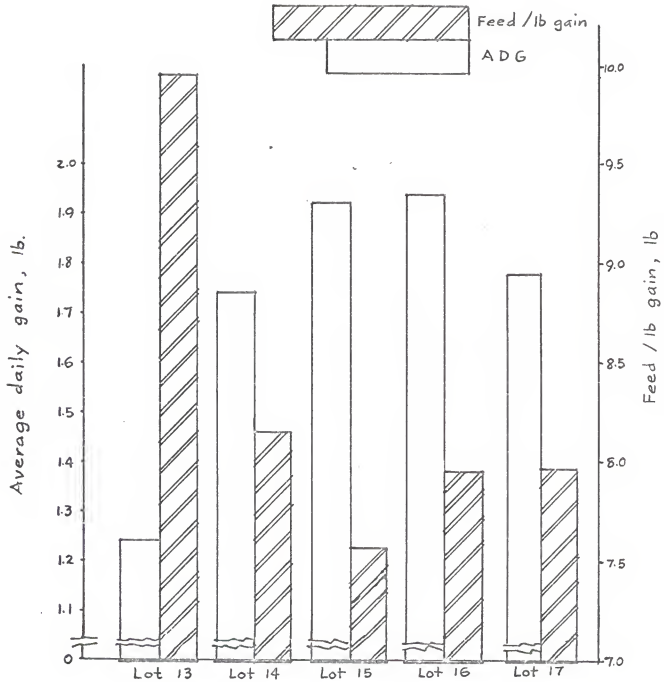

Fig. 2. Average daily gain and feed efficiency.

that there was not enough readily available energy for proper utilization of nitrogen from urea even though the protein equivalent intake was greater than the NRC[1] standard. The addition of alfalfa hay, milo, or both improved growth rate significantly ($P < 0.05$).

Altona et al. (1960) and Karr et al. (1963) reported that alfalfa hay appeared to have a significant effect on the utilization of urea. The present experiment showed that while alfalfa hay improved the utilization of urea (lot 14) its effect was not as marked as that of milo (lot 16). Since the protein intakes of these two lots were about the same, the difference probably could be attributed to the higher readily available carbohydrate content of milo.

McDonald (1952) established that there was considerable interdependence between carbohydrate and protein digestion in the rumen. Protein utilization increased in the presence of added carbohydrate, and the microbial attack upon the fibrous components of the ration was more rapid as the protein intake was increased. Results of the present study confirm this statement.

Merrill et al. (1959) observed a significant ($P < 0.05$) increase in gains for heifers fed rations of molasses and SBM as compared with those fed molasses and urea. Sellers et al. (1960) also reported that with sorghum silage, the high urea supplement produced less gain than SBM when steers were fed at the same crude protein level. Results of the present study, however, showed no significant difference in growth between SBM and urea groups of iso-nitrogenous intake.

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[1]NRC -- National Research Council.

Crude Protein, True Protein and Non-protein Nitrogen

Average rumen fluid crude protein and true protein, determined by the micro-Kjeldahl method, and non-protein nitrogen, determined by difference, are presented in Table 3 and Fig. 3. Individual animal results and the analysis of variance are given in Appendix Tables 6 - 9. The average crude protein content in lot 13 (urea) and lot 15 (SBM + alfalfa) were 4.68 mg./ml. and 4.86 mg./ml., respectively. These were significantly lower ($P < 0.10$) than the 6.59 mg./ml. in lot 17 (urea + milo + alfalfa) and the 6.01 mg./ml. in lot 14 (urea + alfalfa), but not the 5.76 mg./ml. in lot 16 (urea + milo).

The average true protein content in lot 13 and lot 15 were 3.15 and 3.46 mg./ml., respectively, which were also significantly lower ($P < 0.10$) than the 4.76 mg./ml. in lot 17, but not the 4.01 mg./ml. in lot 14, or the 3.99 mg./ml. in lot 16.

The average percentage of true protein were 66.1, 68.0, 70.1, 69.9 and 71.6% for lots 13, 14, 15, 16 and 17, respectively. No significant differences were observed among these values.

No significant differences were observed in the NPN fraction among the groups.

Feeding a basal ration of chopped timothy hay to sheep, Drori (1959) reported that rumen liquor organic nitrogen concentrations showed no evidence of more bacterial protein on a SBM diet than on a urea diet. Davis and Stallcup (1967), however, indicated that the concentrations of total nitrogen and protein nitrogen in the rumen fluid were highest when SBM was fed. In the present experiment, the crude protein and true protein on the SBM diet were all lower than the urea diets.

Table 3. Fractions of Crude Protein, True Protein and Non-protein Nitrogen in Rumen Liquor

	Lot no.				
	13	14	15	16	17
Crude protein, mg./ml.	4.68^{b}	6.01^{a}	4.86^{b}	5.76^{ab}	6.59^{a}
True protein, mg./ml.	3.15^{b}	4.01^{ab}	3.46^{b}	3.99^{ab}	4.76^{a}
% true protein	66.1^{a}	68.0^{a}	70.1^{a}	69.9^{a}	71.6^{a}
NPN, mg./ml.	1.53^{a}	2.00^{a}	1.40^{a}	1.77^{a}	1.82^{a}

^{abc}Any two means not bearing a common superscript letter differ significantly ($P < 0.10$).

To explain this situation, it must first be pointed out that rumen samples were obtained from animals on an experiment designed primarily to study the effect of adding alfalfa hay and/or milo to the urea supplement. Therefore, the rations were not isonitrogenous. This situation was aggravated by the fact that the crude protein content of the SBM turned out to be only 39.98%. As a result, the crude protein intake in lot 15 (SBM + alfalfa) was lower than calculated. Table 1 shows the intake of protein in absolute value and in percentage of the dry matter intake. It appears that protein intake was closely related to the protein contents of rumen liquor of various lots.

Since most of the nitrogenous substances present in the rumen are metabolic end-products or intermediates, their concentrations would vary widely. The variations are related to the nature of the diet and the time after feeding. In this case, the calves were fed the supplements at 8 a.m. and samples were taken at 1 - 3 p.m. Therefore, the effect of feeding time

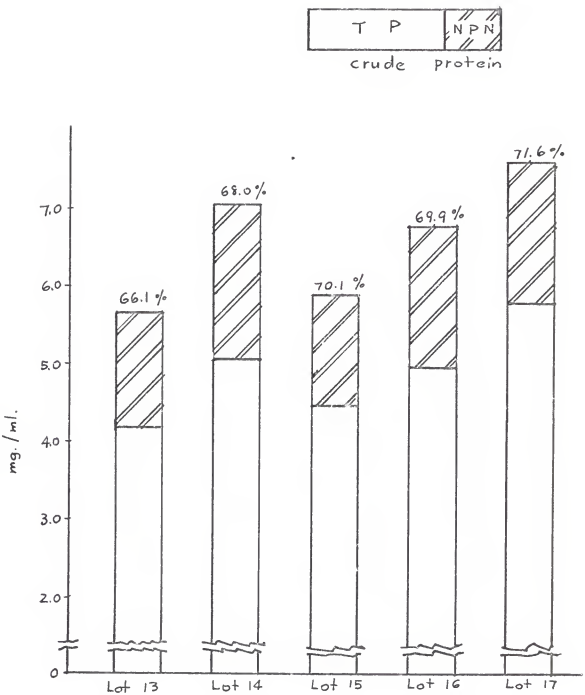

Fig. 3. Fractions of crude protein (CP), True protein (TP), non-protein nitrogen (NPN) in rumen liquor, mg. /ml. Figures on top of columns show percentages of true protein.

on this should be greatly reduced.

Sampling by means of stomach tube could pose a considerable problem. Variation of 20% in crude protein were obtained in sampling different parts of the rumen (Pearson and Smith, 1943; McDonald, 1948). Pearson and Smith (1943) stated that the concentration of crude protein, true protein and NPN were higher in the dry matter of the liquid ingesta than in that of the semisolid material. Rumen samples from the present study showed a wide variation in turbidity (by gross observation) and in nitrogen content as can be seen in Appendix Table 6.

Volatile Fatty Acids

Total micromoles per ml. of volatile fatty acids in rumen liquor, average molar percent of acetic, propionic, butyric, isovaleric and n-valeric acids and the average acetic/propionic ratio are presented in Table 4 and Figs. 4, 5 and 6.

Concentrations of total VFAs as well as the acetic/propionic ratio were similar for all lots. This indicated normal rumen function of the steers under different treatment.

Lots 16 and 17 receiving 3 lb. of milo grain produced higher acetic acid. The average molar percent of acetic acid in lot 13 was significantly lower ($P < 0.05$) than in lot 16 and lot 17. The average molar percent of butyric acid, on the other hand, was significantly higher ($P < 0.10$) in lot 13 than lots 16 and 17. It was suggested by Gray et al. (1952) that the synthesis of butyric and valeric acids occur by the condensation of acetate and propionate, namely, two molecules of acetate to form one of butyrate, and one molecule of acetate and one of propionate to form one of valerate.

Table 4. Concentrations of Total Volatile Fatty Acids, Molar Percent of Individual Acids and Acetic/Propionic Ratio

	Lot no.				
	13	14	15	16	17
Acetic, %	60.6^{a}	63.6^{abc}	61.5^{ab}	64.0^{bc}	65.8^{c}
Propionic, %	19.3^{a}	18.5^{a}	19.2^{a}	19.3^{a}	17.8^{a}
Butyric, %	17.7^{c}	15.9^{abc}	16.6^{bc}	14.4^{a}	14.3^{ab}
Isovaleric, %	1.0^{a}	1.1^{a}	1.3^{a}	1.1^{a}	1.1^{a}
Valeric, %	1.4^{c}	1.1^{ab}	1.5^{c}	1.2^{bc}	0.9^{a}
Total VFAs, micromoles/ml.	67.5^{a}	67.1^{a}	65.9^{a}	62.1^{a}	63.9^{a}
Acetic/propionic ratio	3.20^{a}	3.50^{a}	3.21^{a}	3.41^{a}	3.77^{a}

^{abc}Any two means not bearing a common superscript letter differ significantly ($P < 0.05$) in all values except butyric acid in which $P < 0.10$.

Table 4 shows lot 13 had the highest total VFAs but the lowest acetic acid. Its butyric and valeric acids, however, were significantly higher than lots 16 and 17. It can therefore be postulated that a larger part of acetic acid produced under the condition of lot 13 was eventually condensed to form butyric and valeric acids.

Karr et al. (1963) observed that when lambs receiving 40% roughage were fed SBM or urea, there was no apparent difference in the concentration of VFAs ($C_2 - C_5$) or the ratio of acetate to propionate. In the present study, in which high roughage rations were also used, the same result occurred.

As the supplemental protein or NPN constituted only a small fraction (less than 15%) of the total nitrogen intake (Tables 1 and 2), these results should not be regarded as differing from the work of Oltjen and Davis (1965),

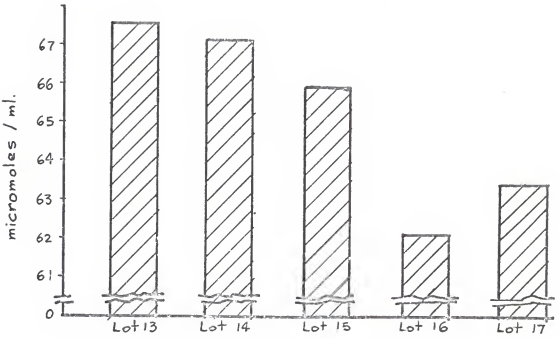

Fig. 4. Total VFA concentration, micromoles/ml.

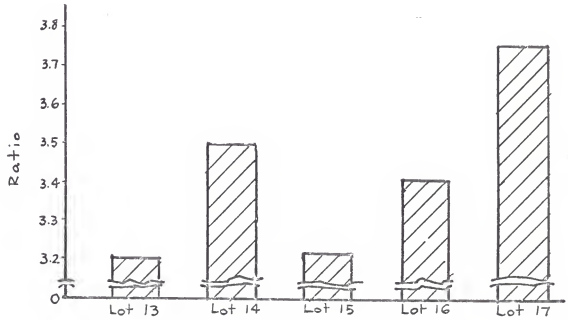

Fig. 5. Acetic/Propionic ratio

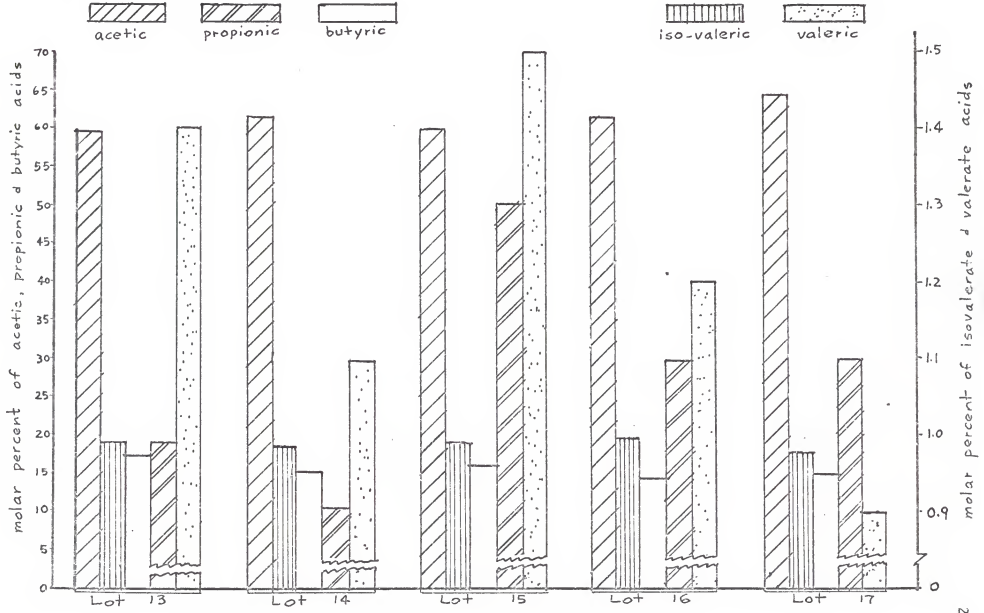

Fig. 6. Molar percent of individual acids in rumen liquor.

Huber and Boman (1966) or Davis and Stallcup (1967), who reported that SBM was associated with higher quantity of total VFAs and wider acetic/propionic ratio. Lot 15, receiving SBM had a higher propionic acid level, agreeing with the reports of Belasco (1954) and Addanki et al. (1966), but this difference was not statistically significant.

El-Shazly (1952) reported a positive correlation between level of protein in the diet and level of branched-chain fatty acids in rumen fluid and concluded that proteins were the primary source of branched-chain fatty acids. Similarly, Huber et al. (1967) found rumen valerate and isovalerate tended to be lower for urea-containing rations. In the study at hand, SBM (Lot 15) produced higher concentrations of isovaleric and valeric acids. However, these, again, were not statistically different, perhaps, because most of the protein intake came from the corn silage and SBM protein constituted only a small fraction of the total protein intake.

The problem of measuring the amounts of VFAs produced in the rumen is indeed a complex one since the concentration of a particular acid at any time is dependent on the rates of production and absorption, passage to the omasum, dilution with saliva, utilization by the microorganisms and conversion to other rumen metabolites. Furthermore, as mentioned before, this was complicated by the sampling with stomach tube. The high mean square for error in the Analysis of Variance in Appendix tables attests to the high degree of variability. The author, therefore, does not attempt to draw any conclusion on the rates of VFA production based on ruminal VFA concentration, but simply presents what he has found.

SUMMARY

Rumen samples drawn from steers were analyzed for fractions of crude protein, true protein and volatile fatty acids in an attempt to obtain information on the amounts of readily available energy, supplied as grain, necessary for utilization of urea as supplemental protein equivalent in a high roughage ration.

Fifty steer calves averaging 437 pounds were divided into five lots and fed all the corn silage they would eat each day. The treatment of the five lots were as follows: Lot 13 -- 1.25 lb. of urea supplement (44% crude protein). Lot 14 -- 1.25 lb. of urea supplement plus 2 lb. of alfalfa hay. Lot 15 -- 1.25 lb. SBM plus 2 lb. alfalfa hay. Lot 16 -- 1.25 lb. urea supplement plus 3 lb. milo. Lot 17 -- 1.25 lb. urea supplement plus 3 lb. milo plus 2 lb. alfalfa hay. The rations were not intended to be isonitrogenous.

The average daily gain of lot 13 was significantly lower ($P < 0.05$) than that of the other lots. No significant differences, however, were observed among the other lots. Feed efficiency in lot 15 was the highest and that in lot 13 the lowest.

The average rumen filtrate crude protein content in lot 13 and lot 15 were significantly ($P < 0.10$) lower than that in lots 17 and 14, but not lot 16. The average true protein content in lot 13 and lot 15 were also significantly ($P < 0.10$) lower than that in lot 17, but not in lot 14 or 16. The average percentages of true protein were essentially the same. No significant differences were observed in NPN fraction among the groups. Protein intakes were closely related to the protein contents of rumen liquor among the lots. The lowest crude protein content in lot 13 also was in agreement with the lowest body gain among the groups.

Concentrations of total volatile fatty acids as well as the acetic/propionic ratios were similar for all lots, indicating normal rumen function of the steers under different treatments. Lots 16 and 17, receiving 3 lb. milo, produced higher acetic acid than the other lots. The average molar percent of acetic acid in lot 13 was significantly lower ($P < 0.05$) and that of butyric and valeric acids were significantly ($P < 0.05$) higher. This suggests more condensation of acetic acid to form the higher acids.

Results of the present study indicated that there was not enough readily available energy from corn silage for proper utilization of nitrogen from urea. Supplementation with milo, alfalfa hay, or both improved the growth rate significantly ($P < 0.05$).

ACKNOWLEDGMENTS

The author wishes to express his sincere thanks and appreciation to his major professor, Dr. Draytford Richardson for his assistance and advice in setting up the experiment and preparing the manuscript.

Appreciation is also extended to Dr. B. E. Brent for his aid in nitrogen determination and gas chromatography.

The author is grateful to his wife, Tuan-chin, for her patience and sacrifice when he was away from home.

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APPENDIX

Table 5. Body Weight Gains of the Steers, lb.

Lot no.	13	14	15	16	17
	210	220	275	245	200
	90	220	200	170	180
	55	120	195	225	200
	95	240	220	215	185
	95	240	165	175	235
	130	215	230	185	160
	130	185	250	225	210
	150	150	175	255	210
	185	175	215	205	205
	235	170	230	235	215
Mean	138	194	216	218	200
S.E.	18.2	12.6	11.5	10.9	6.6

Table 5a. Analysis of Variance for the Data of Table 5

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	42364	10591	6.89	$P < 0.01$
Within	45	69194	1537.6		
Total	49	111558			

Table 6. Contents of Crude Protein in Rumen Liquor, mg./ml.

Lot no.	13	14	15	16	17
	4.49	5.88	3.70	5.09	6.35
	4.58	8.09	3.69	5.19	7.06
	4.16	5.44	6.80	6.83	5.84
	5.04	9.29	4.38	5.33	9.19
	3.18	3.88	7.30	3.31	5.31
	5.08	2.86	6.20	9.61	6.40
	5.78	7.45	2.54	7.65	5.20
	3.36	4.23	3.95	3.41	7.71
	6.48	7.00	5.13	5.45	4.96
	-	-	-	-	7.84
Mean	4.68	6.01	4.85	5.76	6.59
S.E.	0.35	0.71	0.54	0.67	0.43

Table 6a. Analysis of Variance for the Data of Table 6

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	24.04	6.01	2.17	$P < 0.10$
Within	41	113.72	2.77		
Total	45	138.76			

Table 7. Contents of True Protein in Rumen Liquor, mg./ml.

Lot no.	13	14	15	16	17
	3.14	4.13	2.64	3.15	4.96
	2.28	4.15	2.54	3.10	5.05
	2.91	3.03	5.35	5.39	4.36
	3.75	5.45	3.89	3.84	6.84
	1.93	2.21	5.43	2.21	4.03
	4.00	2.41	3.98	6.20	4.99
	3.08	6.46	1.36	4.75	3.08
	1.84	3.33	2.90	2.88	6.84
	5.43	4.91	3.01	4.35	2.95
	-	-	-	-	4.53
Mean	3.15	4.01	3.46	3.99	4.76
S.E.	0.37	0.47	0.44	0.43	0.46

Table 7a. Analysis of Variance for the Data of Table 7

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	14.56	3.64	2.14	$P < 0.10$
Within	41	69.90	1.70		
Total	45	84.46			

Table 8. Percentages of True Protein

Lot no.	13	14	15	16	17
	69.9	70.2	71.3	61.8	78.1
	49.7	51.2	68.8	59.7	71.5
	69.9	55.6	78.6	78.7	74.6
	74.4	58.6	88.8	72.0	74.4
	60.6	56.9	74.3	66.7	75.8
	78.7	84.2	64.1	64.5	77.9
	53.2	86.7	53.5	62.0	59.2
	54.7	78.7	73.4	84.4	88.7
	83.7	70.1	58.6	79.8	59.4
	-	-	-	-	57.7
Mean	66.1	68.0	70.1	69.9	71.7
S.E.	4.0	4.4	3.5	3.2	3.2

Table 8a. Analysis of Variance for the Data of Table 8

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	176	44.0	0.4	NS[1]
Within	41	5010	108.9		
Total	45	5186			

[1] Nonsignificant

Table 9. Non-protein Nitrogen (crude protein - true protein) in the Rumen Liquor, mg./ml.

Lot no.	13	14	15	16	17
	1.35	1.75	1.06	1.94	1.39
	2.30	3.94	1.15	2.09	2.01
	1.25	2.41	1.45	1.44	1.48
	1.29	3.84	.49	1.49	2.35
	1.25	1.67	1.87	1.10	1.28
	1.08	.45	2.22	3.41	1.41
	2.70	.99	1.18	2.90	2.12
	1.52	.90	1.05	.53	.87
	1.05	2.09	2.12	1.10	2.01
	-	-	-	-	3.31
Mean	1.53	2.00	1.40	1.77	1.82
S.E.	0.19	0.41	0.18	0.30	0.69

Table 9a. Analysis of Variance for the Data of Table 9

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	2.10	0.53	0.79	NS[1]
Within	41	27.48	0.67		
Total	45	29.58			

[1] Nonsignificant

Table 10. Total VFA's in Rumen Liquor, micromoles/ml.

Lot no.	13	14	15	16	17
	96.0	79.3	30.4	81.4	79.1
	55.6	89.4	56.8	21.3	56.7
	33.7	65.0	65.9	72.4	52.1
	68.9	79.0	87.4	65.6	52.6
	62.4	61.4	67.1	48.2	52.9
	82.4	37.7	75.1	64.9	76.3
	75.1	30.0	68.9	62.3	51.6
	66.6	79.3	64.7	79.5	39.1
	79.0	74.0	65.8	72.1	107.5
	55.4	75.4	76.7	53.0	68.5
Mean	67.5	67.1	65.9	62.1	63.6
S.E.	5.6	3.7	4.7	5.6	6.3

Table 10a. Analysis of Variance for the Data of Table 10

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	214.4	53.6	0.17	NS[1]
Within	45	14387.2	319.7		
Total	49	14601.7			

[1] Nonsignificant

Table 11. Molar Percentages of Acetic Acid in Rumen Liquor

Lot no. 13		14		15		16		17	
%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]
58.8	49.60	62.3	52.48	59.6	50.53	62.6	52.06	62.6	52.18
66.6	54.70	57.6	49.20	59.9	50.36	72.3	57.54	69.9	56.79
64.4	53.37	68.5	55.86	63.8	53.01	69.2	56.23	66.3	54.51
59.0	50.36	64.3	53.25	56.3	48.56	61.4	51.41	67.0	54.94
58.3	50.01	64.1	53.25	62.9	52.42	69.5	60.47	66.6	54.45
60.6	50.94	63.2	52.65	63.1	52.53	62.2	52.06	63.8	53.07
59.8	50.48	63.3	52.89	58.7	49.95	61.6	51.71	66.8	54.82
59.0	50.07	62.4	52.18	64.7	53.31	62.3	51.88	72.0	58.05
55.7	48.16	68.0	55.55	67.0	54.88	60.0	50.71	58.0	49.43
64.0	53.01	62.6	52.42	58.7	49.95	59.6	50.48	64.9	53.07
Mean:									
60.6	51.1	63.6	53.0	61.5	51.6	64.0	53.5	65.79	54.2
S.E.									
1.2		1.2		1.4		1.7		1.2	

[1]Tr. % — percentage transformed to Arcsin data.

Table 11a. Analysis of Variance for the Transformed Data of Table 11

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	69.0	17.25	2.87	$P < 0.05$
Within	45	270.9	6.02		
Total	49	339.9			

Table 12. Molar Percentages of Propionic Acid in Rumen Liquor

Lot no. 13		14		15		16		17	
%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]
23.2	28.79	19.7	26.35	20.4	26.85	18.7	25.62	19.4	26.13
18.2	25.25	16.1	23.50	19.7	26.35	15.5	23.42	17.2	24.50
14.8	22.63	14.3	22.22	17.6	24.80	21.9	27.90	17.1	24.43
19.8	26.42	17.6	24.80	21.0	27.28	23.5	29.00	17.1	24.50
18.6	25.55	20.2	26.71	17.2	24.50	17.4	19.55	16.4	23.89
17.2	24.50	19.4	26.13	20.5	26.92	15.9	23.50	18.2	25.25
22.0	27.97	18.0	25.10	17.8	24.95	18.0	25.10	17.4	24.65
19.8	26.42	21.2	27.42	19.2	25.99	17.5	24.73	14.7	22.71
20.1	26.64	17.7	24.88	16.8	24.20	21.1	27.35	23.9	29.27
18.9	25.77	20.4	26.85	22.1	28.04	23.2	28.79	16.7	24.12
Mean:									
19.3	26.0	18.5	25.4	19.2	26.0	19.3	25.5	17.8	25.0
S.E.:									
0.9		0.8		0.7		1.1		0.8	

[1]Tr. % -- percentage transformed to Arcsin data.

Table 12a. Analysis of Variance for the Transformed Data of Table 12

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	7.7	1.93	0.53	NS[1]
Within	45	164.6	3.66		
Total	49	172.3			

[1]Nonsignificant

Table 13. Molar Percentages of Butyric Acid in Rumen Liquor

Lot no. 13		14		15		16		17	
%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]
15.9	23.50	15.5	23.19	16.8	24.20	16.9	24.23	16.0	23.58
13.2	21.30	24.7	29.60	18.3	25.33	8.5	17.05	10.8	19.19
18.4	25.40	15.5	23.19	15.8	23.42	7.2	15.56	14.6	22.46
18.5	25.48	16.3	23.81	19.9	26.49	12.8	20.96	13.7	21.72
20.1	26.71	13.8	21.81	17.2	24.50	11.0	18.91	15.0	22.79
19.6	26.28	15.2	22.95	13.7	21.72	19.6	26.28	15.7	23.34
16.2	23.73	16.7	24.12	21.1	27.35	18.2	25.25	12.8	20.96
18.7	25.62	14.6	22.46	13.4	21.47	18.0	25.10	11.3	19.64
21.7	27.76	11.5	19.82	13.3	21.39	16.1	23.66	16.5	23.97
14.9	22.71	14.9	22.71	16.2	23.73	15.6	23.26	16.5	23.97
Mean:									
17.7	24.9	15.9	23.4	16.6	24.0	14.4	22.0	14.3	22.2
S.E.:									
1.0		1.5		0.7		1.4		0.6	

[1] Tr. % -- percentage transformed to Arcsin data.

Table 13a. Analysis of Variance for the Transformed Data of Table 13

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	58.1	14.53	2.11	$P < 0.10$
Within	45	309.9	6.89		
Total	49	368.0			

Table 14. Molar Percentages of Isovaleric Acid in Rumen Liquor

Lot no.									
13		14		15		16		17	
%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]
1.0	5.74	1.0	5.74	1.6	7.27	.6	4.44	.9	5.44
.9	5.44	.8	5.13	.9	5.44	2.8	9.63	1.6	7.27
.9	5.44	.8	5.13	1.4	6.80	1.0	5.74	1.0	5.74
1.2	6.29	.9	5.44	1.0	5.74	1.1	6.02	1.1	6.02
1.3	6.55	.8	5.13	1.2	6.29	.6	5.44	1.1	6.02
1.1	6.02	1.1	6.02	1.1	6.02	1.2	6.29	1.1	6.02
.7	4.80	1.0	5.74	1.2	6.29	1.1	6.02	1.0	5.74
1.0	5.74	.8	5.13	1.2	6.29	.9	5.44	1.0	5.74
.9	5.44	1.4	6.80	1.5	7.04	1.1	6.02	.7	4.80
1.1	6.02	.8	5.13	1.6	7.27	.9	5.44	1.2	6.29
Mean:									
1.0	5.8	.9	5.5	1.3	6.5	1.1	6.1	1.1	5.9
S.E.:									
.05		.05		.05		.18		.05	

[1]Tr. % -- percentage transformed to Arcsin data.

Table 14a. Analysis of Variance for the Transformed Data of Table 14

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	4.6	1.15	1.85	NS[1]
Within	45	28.1	0.62		
Total	49	32.7			

[1]Nonsignificant

Table 15. Molar Percentages of Valeric Acid in Rumen Liquor

Lot no. 13		14		15		16		17	
%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]	%	Tr. %[1]
1.1	6.02	1.5	7.04	1.6	7.27	1.2	6.29	1.1	6.02
1.1	6.02	.8	5.13	1.2	6.29	.9	5.44	.5	4.05
1.5	7.04	.9	5.44	1.4	6.80	.7	4.80	1.0	5.74
1.5	7.04	.9	5.44	1.8	7.71	1.2	6.29	1.1	6.02
1.6	7.27	1.1	6.02	1.5	7.04	1.5	7.50	.9	5.44
1.5	7.04	1.1	6.02	1.6	7.27	1.1	6.02	1.2	6.29
1.3	6.55	1.0	5.74	1.2	6.29	1.1	6.02	1.0	5.74
1.5	7.04	1.0	5.74	1.5	7.04	1.3	6.55	1.0	5.74
1.6	7.27	1.4	6.80	1.4	6.80	1.7	7.50	.9	5.44
1.1	6.02	1.3	6.55	1.4	6.80	1.7	7.50	.7	4.80
Mean:									
1.4	6.8	1.1	6.0	1.5	6.9	1.2	6.4	.9	5.5
S.E.:									
.03		.07		.03		.09		.07	

[1]Tr. % -- percentage transformed to Arcsin data.

Table 15a. Analysis of Variance for the Transformed Data of Table 15

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	12.9	3.23	7.69	$P < 0.01$
Within	45	18.8	0.42		
Total	49	31.7			

Table 16. Acetic/Propionic Ratio for the Rumen Liquor

Lot no.	13	14	15	16	17
	2.51	3.20	2.92	3.33	3.21
	3.66	3.58	3.00	4.66	4.09
	4.34	4.78	3.63	3.15	3.89
	2.98	3.65	2.67	2.60	3.91
	3.14	3.20	3.62	3.99	4.04
	3.52	3.27	3.06	3.92	3.54
	2.70	3.50	3.28	3.46	3.89
	2.96	2.94	3.34	3.54	4.84
	2.77	3.84	3.96	2.84	2.41
	3.38	3.06	2.64	2.56	3.86
Mean	3.20	3.50	3.21	3.41	3.77
S.E.	.17	.17	.07	.25	.19

Table 16a. Analysis of Variance for the Data of Table 16

Sources of variation	Degree of freedom	Sum of square	Mean square	F value	Level of significance
Between	4	2.21	0.55	1.72	NS[1]
Within	45	14.61	0.32		
Total	49	16.82			

[1] Nonsignificant

RUMINAL CRUDE PROTEIN, TRUE PROTEIN AND VOLATILE FATTY ACIDS ON SILAGE RATION SUPPLEMENTED WITH SOYBEAN MEAL OR UREA

by

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B. S., National Taiwan University, 1953

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Husbandry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

Rumen samples drawn from steers were strained and analyzed for fractions of crude protein, true protein and volatile fatty acids in an attempt to relate metabolic aspects of different rations with the performance of the animal. Protein nitrogen was determined by the micro-Kjeldahl method and the volatile fatty acids by gas chromatography.

Fifty Hereford steer calves averaging 437 lb. were divided into five lots and fed all the corn silage they would eat each day. The treatments of the five lots were as follows: Lot 13 -- 1.25 lb. of urea supplement (44% crude protein). Lot 14 -- 1.25 lb. urea supplement plus 2 lb. of alfalfa hay. Lot 15 -- 1.25 lb. SBM plus 2 lb. alfalfa hay. Lot 16 -- 1.25 lb. urea supplement plus 3 lb. milo. Lot 17 -- 1.25 lb. urea supplement plus 3 lb. milo plus 2 lb. alfalfa hay. Rations were not intended to be isonitrogenous.

The average daily gain of lot 13 was 1.23 lb., which was significantly lower ($P < 0.05$) than the 1.73, 1.92, 1.94 or 1.78 lb. of lots 14, 15, 16 or 17, respectively. No significant differences were observed among the other lots. Feed efficiency of 7.51 (on 90% dry matter basis) in lot 15 was the highest, and the 9.91 in lot 13 the lowest.

The average crude protein content in rumen filtrate from lot 13 and lot 15 were 4.68 and 4.86 mg./ml., respectively. These were significantly ($P < 0.10$) lower than 6.59 mg./ml. in lot 17 and 6.01 mg./ml. in lot 14, but not 5.76 mg./ml. in lot 16. The average true protein levels in lot 13 and lot 15 were 3.15 and 3.46 mg./ml., respectively, which were also significantly ($P < 0.10$) lower than 4.76 mg./ml. in lot 17, but not 4.01 mg./ml. in lot 14, or 3.99 mg./ml. in lot 16. The average percentages of true protein were 66.1, 68.0, 70.1, 69.9 and 71.6% for lots 13, 14, 15, 16 and 17,

respectively. No significant differences were observed in percent of true protein and NPN. Protein intake related closely to the protein contents of rumen liquor among the lots. The lowest crude protein content in lot 13 was associated with the lowest body weight gain among the groups.

Concentrations of total volatile fatty acids were 67.5, 67.1, 65.9, 62.1 and 63.9 micromoles per ml. for lots 13, 14, 15, 16 and 17, respectively. The acetic/propionic ratios were 3.20, 3.50, 3.21, 3.41 and 3.77 for lots 13, 14, 15, 16 and 17, respectively. Neither total VFAs or acetic/propionic ratios showed any significant difference between any lots, indicating normal rumen function of the steers under different treatments. Lots 16 and 17, receiving 3 lb. milo, produced higher acetic acid than the other lots. The average molar percent of acetic acid in lot 13 was significantly lower ($P < 0.05$) and that of butyric and valeric acids were significantly ($P < 0.05$) higher. This suggests more condensation of acetic acid to form the higher acids.

Results of the present study indicated that there was not enough readily available energy from corn silage for proper utilization of nitrogen from urea. Supplementation with milo, alfalfa hay, or both improved the growth rate significantly ($P < 0.05$).