EFFECT OF NITROGEN SOURCE UPON CRUDE AND TRUE PROTEIN AND VFA'S IN RUMEN CONTENTS OF STEERS FED FINISHING RATIONS

by

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INTRODUCTION

One advantage of the rumen function is that inferior proteins and non-protein nitrogen compounds can be converted into presumably high quality microbial proteins. Urea can be used as a source of supplemental nitrogen in the ruminant ration. The nature of the ration to which urea is added influences the growth and multiplication of ruminal bacteria, and therefore, the extent to which ruminants are able to utilize urea nitrogen. A low level of true protein and high level of starch in the ration favors urea utilization. Since urea is soluble in water and rapidly hydrolyzed to ammonia, the need for available carbohydrates at the time of ingestion is indicated (Reid 1953, Akram 1964, Abe 1965).

Volatile fatty acids are important to the ruminant, they arise through starch and cellulose digestion by rumen microorganisms. These acids are utilized by the host animal for production and body maintenance. Since several methods of fatty acid separation have been perfected to the point of becoming acceptable laboratory technique. The knowledge about the total volatile fatty acid and proportion of the individual volatile fatty acids as affect by the rations, conditions, etc. is highly interesting (Ward 1962).

This study was designed to compare the differences in the crude protein, true protein, non-protein nitrogen, total and individual volatile fatty acid formation that occurred in the rumen of fattening steers fed high levels of milo grain

and two pounds of prairie hay supplemented with soybean meal or urea, with and without added alfalfa hay.

REVIEW OF LITERATURE

The Breakdown and Synthesis of Protein in the Rumen:

Sym (1938) reported that rumen contents possess strong proteolytic properties which degrade the protein. Pearson et al., (1943) carefully incubated casein or gelatin with rumen contents, and found definite evidence that proteolysis occurred in the rumen. This subject was further examined by McDonald (1948, 1952) who established that the breakdown of protein by the proteolytic enzymes of rumen microorganisms was of quantitative importance to the host animal. Ammonia was shown to be the major end product of the degradation of several different proteins and was the main component of the non-protein nitrogen fraction. The amount of ammonia formed was dependent on both the nature of the protein and the proportion of carbohydrates in the diet.

Further information on the proteolytic enzymes has become available by in vitro studies. Annison (1956) found rumen proteolytic enzymes to be resistant to changes in conditions and even retained their activity following toluene treatment or extraction after the preparation of acetone powder. In these respects they differed from the deaminases, the enzymes responsible for the final stages of ammonia production, which were inactivated by organic solvents.

Amino acids are undoubtedly intermediates in the fermentation of protein in the rumen, and under the action of deaminases they give rise to ammonia. el-Shazly (1952) showed that the amino acids of an acid hydrolysate of casein were attacked under anaerobic conditions by washed suspensions of rumen organisms. The reaction products were ammonia, carbon dioxide and volatile fatty acids. el-Shazly also concluded that the Sticklan reaction is important in the breakdown of amino acids in the rumen. This reaction is a coupled deamination of two amino acids, one of which functions as a hydrogen donor, and the other as a hydrogen acceptor. This finding was confirmed by Sirotnak et al., (1953) and Lewis (1955).

Oginsky et al., (1959) pointed out that, for amino acid production, the only pathways available for conversion of ammonia to alpha-amino groups are (1) amination of alpha-keto glutamic acid to glutamic acid, and (2) amination of furmaric acid to aspartic acid, the enzymes involved here are glutamic dehydrogenase and aspartase, respectively. Both are operative in anaerobic condition, and consequently, should be available in rumen microorganisms.

Mortenson (1962) on the other hand, discussed an enzyme alanine dehydrogenase, which catalyzes the formation of alanine from pyruvate via a series of reactions involving iminopropionate. This further pathway indicates that many more mechanisms for non-protein nitrogen incoporation into

amino acids are available than was formerly believed. However, ammonia still appears to be the intermediate in non-protein nitrogen metabolism. Following incorporation of ammonia into alanine, aspartic acid and glutamic acid and several of the amides, various interconversions occur leading to formation of other amino acids necessary for syntheses of complete proteins.

Volatile Fatty Acid Formation and Absorption in the Rumen:

It has long been known that volatile fatty acids are among the chief non-gaseous products of bacterial breakdown of the carbohydrates in the rumen. As long ago as, 1883 Tappeiner demonstrated that the fermentation of cellulose in the rumen of ox resulted in the formation of a large amount of volatile fatty acids, which contained at least 50 percent acetic acid. The total concentration of volatile fatty acids in the rumen and the amount of the individual acids present varied from different rations and conditions by different investigators. Gray et al., (1951) concluded the concentration at any one time is dependent on the rate of (1) production in the rumen, (2) absorption in the rumen, (3) passage from the rumen to the omasum, (4) dilution with saliva, (5) utilization by rumen microorganisms, (6) conversion to other metabolites.

Volatile fatty acids can arise not only from carbohydrate fermentation, but from deamination of amino acids. el-Shazly

(1952) conducted an *in vitro* study showing that the products of amino acid fermentation were ammonia, carbon dioxide and volatile fatty acids in roughly equimolar amounts. Acetic acid was the main volatile fatty acid with small amounts of propionic, n-butyric, n-valeric, and branched chain fatty acids.

The presence of propioic acid in the rumen contents was first conclusively demonstrated by Elsden (1945). Diets rich in starch or sucrose favor propionic acid production. Balch *et al.*, (1957) found under these conditions that rumen liquid pH was lower than normal, which encourged proliferation of propionic acid producing organisms.

Bornstein *et al.*, (1948) showed that addition of acetate, propionate or butyrate during fermentation of pyruvate increased the production of butyrate, valerate and caproate respectively. Gray *et al.*, (1952) incubated carbon-14 labelled acetate or propionate with rumen contents. The results suggested that synthesis of the butyrate and valerate acid occurred by the condensation of acetate and propionate with a C_2 compound existing in equilibrium with acetic acid. Elsden *et al.*, (1953) isolated a large Gram-negative coccus, which fermented certain sugars and pyruvate, with the production of acetic, propionic, butyric, valeric and caproic acid.

el-Shazly (1952) using washed sheep rumen suspension, incubated with casein hydrolysate, found that the branched chain acids arise chiefly as end products of protein degradation,

and that isobutyric, isovaleric and 2-methy butyric acids arose from the deamination of valine, leucine and isoleucine, respectively.

Barcroft et al., (1944) examined the concentration of volatile fatty acids in the blood draining the rumen, abomasum, small intestine, and suggested that absorption of these acids took place from the rumen and omasum but not the abomasum and the small intestine. Danielli et al., (1945) concluded that the rate of disappearance of free fatty acid from the rumen increased with the length of the hydrocarbon chain. Pfander et al., (1953) studied cannulated anethetized sheep, and found that acetic, propionic and butyric acids were absorbed from the physiological concentration of these acids in the rumen at pH 5.6 to 6.5 in the order acetic > butyric > propionic. However, when Annison et al., (1954) determined the volatile fatty acid concentration in the portal vein during and after the addition of each of the volatile fatty acids to the sheep rumen, the results suggested that the rates of absorption of the acids were in the order of acetate > propionate > butyrate. The results of Dobson et al., (1956) could be an explanation of the contradiction. They showed that the blood supply to the rumen was influenced by the hydrogen ion concentration of the contents. Addition of volatile fatty acids, which lowered the pH, increased blood flow from the rumen and changed the concentration of volatile fatty acids in the blood.

Urea vs. Soybean Meal as a Source of Nitrogen:

Johnson et al., (1942) investigated the efficiency of urea as a protein substitute in the ration of ruminants. They found that the nitrogen in products formed in the rumen from urea was as well utilized in metabolism as the nitrogen of soybean meal.

Harris et al., (1943) studied the comparative value of urea and soybean meal nitrogen for steers, and observed that the apparent digestion coefficient of nitrogen when fed to 6 to 8 months old steers, was 74 for urea and 78 for soybean meal. The biological value of urea nitrogen was 34 and that of the soybean meal nitrogen was 60 when fed at 12 and 14 percent protein equivalent level.

Ray (1953), while comparing the nutritive value of urea and soybean meal as supplements to corn silage rations for beef steers, observed that steers receiving the natural protein supplement made slightly faster and more efficient gain than those receiving urea, but the differences were not statistically significant.

Bell et al., (1953), using Hereford steers determined the value of urea as a source of nitrogen in rations containing different carbohydrate feeds. They showed that addition of urea improved nitrogen retention to a significant extent ($p < 0.05$) with all rations. Small differences in nutrient digestibility and nitrogen retention favored the soybean meal supplement.

Smith et al., (1957) compared a self-fed urea-molasses mixture to molasses self-fed plus 1 to 3 pounds of soybean meal, in an effort to determine if the urea-molasses mixture self-fed on dry grass would serve as an adequate source of protein and energy. Steers receiving soybean meal and molasses gained 87 pounds per head more over a period of 109 days than a comparable lot self-fed urea and molasses. The difference appeared due to increased energy intake and utilization of protein.

Richardson et al., (1953) used two pair of fistulated identical twin steers to compare, by column chromatography, levels of 19 amino acids in the rumen fluid after feeding urea or soybean meal. Steers fed a ration supplemented with soybean meal synthesized a greater amount of all amino acids than steers fed urea.

Akram (1964) compared the protein nitrogen content of rumen ingesta of fistulated steers fed isnitrogenous rations containing urea or natural proteins, with and without added grain. He found that the percent protein nitrogen in the rumen ingesta of steers fed rations supplemented with urea was significantly ($p < 0.05$) lower than that of steers fed soybean meal, in both experiments.

Relationship between Carbohydrates and Protein Digestion in the Rumen:

In 1891, Zuntz noted that excess soluble carbohydrate

produced a decrease in celluloses digestion. Hart et al., (1939) conducted a feeding experiment with growing calves and showed that most efficient utilization of non-protein nitrogen occurred when some solutle sugar such as corn molasses was fed in the ration. Johnson (1942), using lambs, confirmed the work of both Zuntz and Hart et al., showing that corn molasses depressed the digestibility of the cellulose, but enhanced the utilization of urea nitrogen.

McDonald (1952) demonstrated a fall in rumen ammonia concentration when starch was added to the rumen. Volatile fatty acid production from starch increased when small amounts of casein were added. Lewis et al., (1958) confirmed that active fermentation was encouraged in the presence of both supplements and that growth and synthetic reactions were increased by a more nitrogen to carbohydrate balanced system.

el-Shazly (1952) showed that the depressing effect of large amounts of starch on cellulose digestion may not be due to starch but to a low level of available nitrogen in the ration, producing competition between starch-fermenting and ecllulose-fermenting organisms.

The above results have been confirmed by: Mills et al., (1942), Arias (1951), Hunt et al., (1954), Annison (1956), Belasco (1956), Bloomfield et al., (1958).

From these experiments, two conclusions are drawn: first, there is better utilization of protein in the presence of added carbohydrate, and second, there is a more rapid

attack upon the fibrous components of the ration as the protein intake is increased.

Some Factors Which May Alter Volatile Fatty Acids Production:

Numerous investigations have shown that there are many different factors which may influence the total production as well as proportions of volatile fatty acids in the rumen. Clark et al., (1956), using sheep, studied the effect of changing rations. They found that sudden major changes in the ration resulted in production and absorption changes and in metabolic disorders. Adaptation of rumen flora to even minor changes in diet was reported to take three to four weeks. Shaw et al., (1959) and Davis et al., (1957) studied the effect of protein level on volatile fatty acid production of dairy cows. They showed that high protein produced higher total volatile fatty acid levels. Changing from a high to a low protein concentration increased the production of acetic and decreased the production of propionoc acid. Thus, the acetate and propionate ratio was decreased.

Davis (1957), Beitz (1959), Shaw (1960), Martin Jr. (1966) and many other workers found that high concentrate rations caused a decrease in acetic acid, and an increase in propionic acid. Balch (1957) reported increased acidity of the rumen liquor also reflected an increase in total volatile fatty acids.

Evidence presented in reviews by Balch (1960) and Moore (1966) indicated a greater concentration of volatile fatty acids in rumen ingesta liquor when roughage was finely ground and pelleted. They also found a change in ratio of acetate to propionate. Bensadoun (1962) further showed that the increased concentration of propionic acid relative to acetate in rumen ingesta was reflected in the portal blood.

Bloser (1959) working with dairy cows, found no change in total volatile fatty acids when pelleted concentrates were compared with ground concentrates.

Essig et al., (1959) found that salts of volatile fatty acids could be added to certain lamb rations as the major energy source without affecting daily gains. Rations containing free volatile fatty acids were not consumed as readily as those containing their salts.

EXPERIMENTAL PROCEDURE

Animals:

Fifty Hereford steer calves averaging 630 pounds were divided into five lots of 10 steers each, and were group-fed rations as described in Table 2 for 172 days from April 8, 1967 to September 26, 1967. The steers were fed ad libitum, and water was available at all times. They were weighed individually on two consecutive days intially and at termination of the experiment. At the end of the trial, steers

were slaughtered and detailed carcass data were obtained. Rumen samples were taken on August 19, 1967, which was 134th day on fattening ration.

Diets:

All animals received 2 pounds of prairie hay daily, and milo grain was fed ad libitum. Lot 14, 15 and 17 received an additional 2 pounds of alfalfa hay. "Supplement 370" was used for all lots except Lot 15, which was fed "Supplement S 370." The proximate analyses of the feedstuffs and the average daily intake of the ration during the week before rumen samples were taken, and the daily intake of proximate nutrients are given in Tables 1 and 2.

Sampling Procedure:

Since the ingestion of feed just previous to sampling would have affected considerably the chemical composition of the rumen contents, all animals were deprived of feed, but not of water for 4 hours before sampling. Samples were withdrawn by vacum through a suitable length of stomach tube passed down through the esophagus into the rumen. Rumen samples were transfered quickly to plastic bags, which were placed in the freezer until analyzed. The rumen contents were strained through 4 layers of surgical gauze for all the determinations.

TABLE 1

CHEMICAL ANALYSES OF FEEDS USED IN EXPERIMENT (%)

Description	Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Ash	Nitrogen Free Extract
Silage	39.20	3.87	6.76	2.33	6.02	20.22
Grain	87.61	10.16	1.58	2.71	1.35	71.81
Prairie Hay	92.93	4.59	30.40	2.98	7.14	47.82
Alfalfa Hay	90.70	16.60	26.40	2.22	6.81	39.67
Supplement 370 80% milo 4% $CaCO_3$ 3% dical. 13% urea 3000 I.U. vit. A 75 mg aureomycin 10 mg. Stibestrol	89.25	44.25	1.95	3.21	6.00	33.84
Supplement S 370 94% SBOM 3% dical. 3% $CaCO_3$ 30000 I.U. vit. A 75 mg aureomycin 10 mg stibestrol	90.57	39.98	5.16	2.46	8.16	34.81

TABLE 2

DAILY CONSUMPTION OF RATIONS AND PROXIMATE NUTRIENTS DURING SAMPLING (lb.)

Lot Number	13	14	15	16	17
Grain	21.82	19.64	19.43	21.11	19.11
Supplement 370	1.00	1.00	-	1.00	1.00
Supplement S 370	-	-	1.00	-	-
Prairie Hay	2.00	1.71	1.71	2.00	2.00
Alfalfa Hay	-	2.00	2.00	-	2.00
Dry Matter	21.89	21.52	21.31	21.27	21.32
Crude Protein	2.75	2.85	2.78	2.68	2.80
Crude Fiber	1.00	1.43	1.40	0.99	1.49
Ether Extract	0.67	0.64	0.65	0.62	0.61
Ash	0.51	0.61	0.58	0.50	0.60
Nitrogen Free Extract	16.94	16.03	15.95	16.43	15.79

Analysis of the Rumen Contents:

(1). Rumen liquor pH: A Beckman direct reading pH meter with glass electrodes was used to determine pH values.

(2). Crude protein nitrogen: Micro-Kjeldahl method on 2 ml. of the rumen liquid. (Chung, 1968).

(3). True protein nitrogen: Micro-Kjeldahl method, tungstate precipitation from 2 ml. of rumen liquid. (Chung, 1968).

(4). Non-protein nitrogen: The difference between the values of crude protein nitrogen and true protein nitrogen was used as a measure of the non-protein nitrogen.

(5). Total and individual volatile fatty acids: A Beckman GC-4 gas chromatograph machine was used to determine the individual volatile fatty acids. The total volatile fatty acids were determined indirectly by adding all the values of individual volatile fatty acid. (Chung, 1968).

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

Crude Protein: The average crude protein contents of

rumen liquor for five lots are shown in Table 3. The individual animal results and the analysis of variance are shown in Appendix Table 2.

The differences between the crude protein content of five lots were not significant. These findings supported the results obtained by Akram (1964), and Abe (1965), who showed that when grain was added to the prairie hay rations, the crude protein and true protein in rumen contents were quite similar for steers fed rations supplemented with soybean oil meal or urea.

True Protein: The average true protein contents of rumen liquor for different lots are shown in Table 3. The individual animal results and the analysis for variance are shown in Appendix Table 3.

The difference between the rumen liquor true protein concentration of the steers of the five lots were non-significant.

Non-protein Nitrogen: The average non-protein nitrogen contents of rumen liquor for five lots are shown in Table 3. The individual animal results and the analysis of variance are shown in Appendix Table 4.

Lot 15 yielded the highest average non-protein nitrogen values, but produced statistically similiar values to Lots 14 and 17, which were statistically higher than Lots 13 and 16 ($p < 0.05$). These results showed that when steers received an additional 2 pounds of alfalfa hay, they probably formed more rumen ammonia.

TABLE 3

MEAN VALUES OF pH, CRUDE PROTEIN, TRUE PROTEIN AND NPN (mg/ml)

Lot Number	13	14	15	16	17
pH	6.7^{a*}	6.5^{a}	6.5^{a}	6.6^{a}	6.8^{a}
Crude Protein	12.68^{a}	14.11^{a}	14.19^{a}	13.86^{a}	12.08^{a}
True Protein	11.43^{a}	11.87^{a}	11.97^{a}	12.30^{a}	10.07^{a}
Non-protein Nitrogen	1.26^{c}	$2.04^{a,b}$	2.22^{a}	$1.56^{b,c}$	$2.02^{a,b,c}$

*Values within each item with the same superscript are not significantly different ($p < 0.05$).

The higher level of non-protein nitrogen in the animals fed alalfa hay might be explained by the work of Lenkeit et al., (1938) and Somers (1961). They showed that the ammonia concentration in the rumen was markedly increased in 30 minutes for animals fed urea, and 4 hours for those fed plant protein.

Total Volatile Fatty Acids: The average total volatile fatty acid concentrations of rumen liquor for all lots are shown in Table 4. The individual animal results and the analysis of variance are shown in Appendix Table 5.

Lot 17 yielded the lowest mean level of total volatile fatty acids ($p < 0.05$). All other lots produced statistically similiar values. It was very interesting, that Lot 14, which had the same treatment during the fattening phase as Lot 17, had the highest concentration among the lots. Since the area where rumen samples were taken was not known, the works of Amith et al., (1956) and Lampila (1955) might be an explanation for this. They showed the concentrations of volatile fatty acid, crude protein, true protein and non-protein nitrogen were higher at the top of the rumen, in some cases the concentration of volatile fatty acids were more than 50 percent higher than that of the lower part. But, in this experiment, samples were taken under similar conditions, thus, this observation should be due only to chance.

pH: The average pH values of rumen liquor from steers in different lots are shown in Table 3. The individual

TABLE 4

MEAN VALUES OF TOTAL AND INDIVIDUAL VFA CONCENTRATIONS (mMole/L)

Lot Number	13	14	15	16	17
Total VFA	97.9^{a*}	100.9^{a}	100.7^{a}	92.5^{a}	78.3^{b}
Acetic Acid	48.6^{a}	49.1^{a}	49.5^{a}	41.5^{a}	45.3^{a}
Propionic Acid	36.3^{a}	35.9^{a}	33.1^{a}	38.0^{a}	19.7^{b}
Butyric Acid	9.5^{b}	$11.2^{a,b}$	13.6^{a}	9.3^{b}	9.8^{b}
Iso-valeric Acid	1.7^{a}	2.3^{a}	2.5^{a}	1.9^{a}	2.3^{a}
Valeric Acid	1.9^{a}	2.2^{a}	2.1^{a}	2.3^{a}	1.3^{b}
Acetate/ propionate	1.55^{b}	1.52^{b}	1.7^{b}	1.22^{b}	2.4^{a}

*Values within each item with the same superscript are not significantly different ($p < 0.05$).

TABLE 5

MEAN VALUES OF MOLAR PERCENTAGE OF INDIVIDUAL VFA (%)

Lot Number	13	14	15	16	17
Acetic Acid	50.3^{b*}	49.2^{b}	49.7^{b}	45.0^{b}	58.0^{a}
Propionic Acid	36.6^{ab}	35.0^{ab}	32.2^{b}	40.3^{a}	25.1^{ac}
Butyric Acid	9.7^{c}	11.3^{abc}	13.5^{a}	10.0^{bc}	12.2^{ab}
Iso-valeric Acid	1.9^{b}	2.3^{ab}	2.5^{ab}	2.1^{b}	2.9^{a}
Valeric Acid	1.9^{a}	2.2^{a}	2.1^{a}	2.4^{a}	1.7^{a}

*Values within each item with the same superscript are not significantly different ($p < 0.05$).

animal results and the analysis of variance are shown in Appendix Table 1.

The difference between the pH value in the rumen of steers of five lots were not significant. Both Lots 14 and 15 had the lower pH values, which correlated with the higher values of total volatile fatty acids and crude protein. Lot 17 had the highest pH value and the lowest total volatile fatty acid and crude protein levels. These findings agreed with Phillipson (1942) and Orth (1958), who reported that rumen pH values decreased with the increasing total volatile fatty acid concentration and crude protein level in the rumen liquor.

Acetic Acid: The average acetic acid concentrations and molar percentages are shown in Tables 4 and 5. The individual animal results and the analysis of variance are shown in Appendix Tables 6 and 7.

The differences between the acetic acid concentration of five lots were not significant. Lot 17 yielded the highest mean molar percentage of any lot ($p < 0.05$). The rest of them produced statistically similar values.

Propionic Acid: The average concentrations and molar percentages of propionic acid are shown in Tables 4 and 5. The individual animal results and the analyses of variance are shown in Appendix Tables 8 and 9.

Lot 17 yielded the lowest mean level of concentration ($p < 0.05$). Animals receiving an additional 2 pounds alfalfa hay produced more propionic acid, although there was no

statistical difference between lots 13 and 16. Lots 16 and 13 (animals without alfalfa hay) yielded a higher mean molar percentages than did Lots 15 and 14 ($p < 0.05$). Lot 17 showed the statistically lowest mean molar percentage ($p < 0.05$). The results agreeded with Belasco (1954), who showed animals fed urea had a higher level of propionate and lower levels of butyrate and valerate.

Butyric Acid: The average concentration and molar percentage of butyric acid are shown in Tables 4 and 5. The individual animal results and analysis of variance are shown in Appendix Table 10 and 11.

Lot 15 (soybean meal as the supplement), yielded a higher mean butyrate concentration ($P < 0.05$), than Lots 13, 16 and 17, but a statistically similar concentration to Lot 14. Animals without the additional 2 pounds alfalfa had a lower mean butyrate concentration, but the differences were not statistically significant. On the molar percentage basis, Lot 15, 17 and 14 yielded a higher mean levels than did Lots 16 and 13 ($p < 0.05$). These findings had a tendency to support the results obtained in in vitro studies by Brent (1966), who showed that acetate and propionate were lower and butyrate was higher on soy protein substrates.

Iso-valeric Acid: The average concentrations and molar percentages are shown in Tables 4 and 5. The individual animal results and analyses of variance are shown in Appendix Tables 12 and 13.

The differences between the concentrations of five lots were not significant. On the molar percentage basis, the animals which had alfalfa hay yielded higher mean values than those which did not have alfalfa hay ($p < 0.05$).

Valeric Acid: The average concentrations and molar percentages are shown in Tables 4 and 5. The individual animal results and analysis of variance are shown in Appendix Tables 14 and 15.

The differences between both concentrations and molar percentages of five lots were not significant.

Acetate/Propionate Ratio: The average acetate/propionate ratios for five lots are shown in Table 4. The individual animal results and analysis of variance are shown in Appendix Table 16.

Lot 17, yielded the highest mean ratio of any lot ($p < 0.05$). The rest yielded statistically similar values. Comparing Lots 13, 14, 15 and 16 the soybean meal lot (15) had a wider acetate/propionate ratio. This agreed with works done by Davis (1963), Oltjin (1965) and Huber (1966). They showed that soybean meal produced a wider acetate/propionate ratio than urea.

Carcass Evaluation and Feed Efficiency: Carcass evaluation for five lots are shown in Table 6. The individual animal results and the analyses of variance of body weight gain (during fattening phase) and loin eye areas are shown in Appendix Tables 17 and 18.

TABLE 6

CARCASS DATA

Lot Number	13	14	15	16	17
Av. Body Weight Gain During (lb) Fattening Phase	410^{a}*	406^{a}	417^{a}	413^{a}	424^{a}
Av. Fat Thickness (inch)	0.51	0.54	0.60	0.71	0.61
Av. Size Loin-eye (inch)	$11.82^{b,c}$	12.16^{b}	$12.60^{a,b}$	11.28^{c}	13.05^{a}
Carcass Grade					
Choice No.	6	9	9	10	6
Good No.	4	1	0	0	4
Dressing %	61.2	62.3	63.3	62.3	62.2

*Values within each item with the same superscript are not significantly different ($p < 0.05$).

TABLE 7

AVERAGE DAILY GAIN AND FEED CONSUMPTION

Lot Number	13	14	15	16	17
Av. Daily Gain, lb					
fattening	2.38	2.36	2.43	2.40	2.52
over-all	1.93	2.11	2.23	2.22	2.23
Av. Daily Ration, lb					
Silage					
fattening	0.90	0.99	1.01	1.06	0.87
over-all	4.90	5.01	5.04	5.00	3.72
Grain					
fattening	14.82	14.36	13.79	14.94	13.73
over-all	8.98	8.70	8.57	10.12	9.39
Supplement					
fattening	0.97	0.97	0.97	0.97	0.97
over-all	1.04	1.04	1.04	1.04	1.04
Prairie Hay					
fattening	1.67	1.53	1.51	1.69	1.70
over-all	1.01	0.93	0.92	1.03	1.03
Alfalfa Hay					
fattening	-	1.80	1.80	-	1.80
over-all	-	1.80	1.80	-	1.80
Total					
fattening	18.36	19.85	19.08	18.66	19.07
over-all	15.93	17.40	17.37	17.19	16.08
Av. lb. Feed Per Unit Gain					
Silage					
fattening	0.27	0.40	0.39	0.42	0.31
over-all	2.54	2.59	2.30	2.30	1.70
Grain					
fattening	6.23	6.06	5.83	6.23	5.45
over-all	4.66	4.12	3.84	4.55	4.20
Supplement					
fattening	0.41	0.41	0.41	0.41	0.39
over-all	0.54	0.48	0.46	0.47	0.46
Prairie Hay					
fattening	0.70	0.65	0.62	0.71	0.68
over-all	0.52	0.44	0.41	0.46	0.46
Alfalfa Hay					
fattening	-	0.77	0.74	-	0.71
over-all	-	0.86	0.81	-	0.81
Total					
fattening	8.31	8.29	7.99	7.78	7.54
over-all	8.26	8.59	7.82	8.18	7.63

The differences between the body weight gain (during fattening phase) were not statistically significant.

Lot 17 yeilded the highest mean loin eye area, which was statistically similar to Lot 15 ($p < 0.05$). Lot 13 and 16, without an additional 2 pounds alfalfa hay, yielded a lower mean level ($p < 0.05$) of loin eye area.

The data concerning feed consumption, average daily gain and feed efficiency for both the fattening phase and over all experiment including the wintering phase are shown in Table 7.

SUMMARY

Fifty Hereford steer calves averaging 630 pounds were divided into five lots, of 10 steers per lot, and used in a feeding experiment to compare the differences in crude protein, true protein, non-protein nitrogen and total and individual volatile fatty acid formation in the rumen liquor of fattening steers. They were self-fed milo grain and two pounds of prairie hay supplemented with the following: Lot 13, urea; Lot 14, urea and 2 pounds of alfalfa hay; Lot 15, soybean meal and 2 pounds of alfalfa hay; Lot 16, urea; Lot 17, urea and 2 pounds of alfalfa hay. Rumen samples were taken by stomach tube four to five hours after feeding.

There were no significant differences in pH value, crude protein and true protein of rumen liquor among lots. Animals receiving 2 pounds alfalfa hay yielded significantly higher

values of non-protein nitrogen than those not receiving alfalfa ($p < 0.05$). Lot 17, which had the same treatment as Lot 14 yielded the lowest total volatile fatty acid concentration ($p < 0.05$), lowest molar percentage of propionic acid ($p < 0.05$) and the highest molar percentage of acetic acid ($p < 0.05$). These findings are believed due to individual animal difference and/or sampling techniques. Animals receiving a higher proportion of concentrates (those without alfalfa hay) yielded a higher molar percentage of propionic acid ($p < 0.05$). Animals receiving additional alfalfa hay yielded higher molar percentages of butyric and isovaleric acids ($p < 0.05$). Lot 17 yielded the highest value of acetate/propionate ratios. Carcass evaluation data and feed consumption data were also detailed.

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APPENDIX

APPENDIX TABLE 1

pH VALUES

Lot No.	13	14	15	16	17
	7.0	7.0	6.9	6.7	6.7
	6.8	6.3	6.7	6.7	7.0
	6.2	6.6	6.5	6.2	6.6
	6.5	7.1	6.1	6.2	7.0
	7.2	6.6	6.6	5.9	7.1
	6.5	5.0	6.8	7.0	7.0
	7.4	6.4	6.6	6.8	6.9
	6.4	6.5	6.8	6.2	6.5
	6.7	7.1	6.0	7.2	6.3
	7.0	6.5	6.8	6.9	7.0
Mean	6.7	6.5	6.5	6.6	6.8

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	0.7	0.18	1.00	N.S.
Within	45	7.9	0.18		
Total	49	8.6			

N.S.: Non significance.

APPENDIX TABLE 2

CONTENTS OF CRUDE PROTEIN IN RUMEN LIQUOR (mg/ml)

Lot No.	13	14	15	16	17
	11.51	15.55	14.08	15.31	14.45
	13.61	10.62	17.33	13.78	13.38
	13.67	13.49	14.22	16.08	14.43
	12.03	15.29	9.04	14.31	11.73
	10.92	13.00	11.93	11.25	12.09
	16.63	16.27	16.82	13.35	7.70
	11.47	17.06	17.17	13.93	12.10
	10.94	15.91	13.38	16.25	8.45
	14.83	10.44	16.09	9.86	12.06
	11.21	13.46	11.79	14.47	14.41
Mean	12.68	14.11	14.19	13.86	12.08

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	35.86	8.97	1.71	N.S.
Within	45	235.85	5.24		
Total	49	271.71			

N.S.: Non significance

APPENDIX TABLE 3

CONTENTS OF TRUE PROTEIN (mg/ml)

Lot No.	13	14	15	16	17
	9.91	11.49	11.69	13.43	12.60
	12.36	9.83	14.83	12.37	9.83
	12.02	11.23	11.86	15.37	12.03
	11.02	13.09	6.98	11.49	9.95
	9.47	11.75	9.63	9.39	10.48
	15.49	12.79	14.83	11.72	6.22
	10.28	14.92	14.37	12.68	11.10
	10.21	13.24	11.37	14.00	6.28
	13.37	9.00	14.07	9.12	10.36
	10.13	11.35	10.04	13.46	11.86
Mean	11.43	11.87	11.97	12.30	10.07

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	30.43	7.61	1.72	N.S.
Within	45	198.80	4.42		
Total	49	229.23			

N.S.: Non-significance

APPENDIX TABLE 4

CONTENTS OF NON-PROTEIN NITROGEN (mg/ml)

Lot No.	13	14	15	16	17
	1.60	2.06	2.39	1.88	1.85
	1.25	0.79	2.50	1.41	3.55
	1.65	2.26	2.36	0.71	2.78
	1.01	2.20	2.06	2.82	1.78
	1.45	1.25	2.30	1.86	1.61
	1.14	3.48	1.99	1.63	1.48
	1.19	2.14	2.80	1.25	1.00
	0.73	2.67	2.01	2.25	2.17
	1.46	1.44	2.02	0.74	1.70
	1.08	2.11	1.75	1.01	2.32
Mean	1.26	2.04	2.12	1.56	2.02

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	6.36	1.59	5.13	$P < 0.01$
Within	45	14.05	0.31		
Total	49	20.41			

APPENDIX TABLE 5

CONCENTRATIONS OF TOTAL VFA (mMole/l)

Lot No.	13	14	15	16	17
	84.7	72.6	88.8	97.1	86.8
	74.8	51.7	111.2	63.2	66.0
	112.5	120.6	129.0	87.2	92.4
	111.1	88.3	87.9	69.8	86.8
	101.7	116.5	109.8	97.1	47.3
	111.5	88.3	87.9	69.8	86.8
	85.4	128.2	87.7	103.4	82.1
	103.4	103.6	109.9	122.1	71.3
	111.0	88.9	90.2	95.6	52.9
	82.5	113.4	95.1	104.4	129.8
Mean	97.9	100.9	100.7	92.5	78.3

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	11869.9	2967.4	15.6	P< 0.01
Within	45	8545.0	189.9		
Total	49	20414.9			

APPENDIX TABLE 6

CONCENTRATION OF ACETIC ACID (mMole/l)

Lot No.	13	14	15	16	17
	55.4	40.0	46.8	40.0	49.5
	41.1	23.2	53.2	10.5	37.3
	49.5	52.6	54.7	34.0	62.1
	53.7	49.5	56.8	34.7	48.9
	47.9	50.0	44.2	52.1	26.8
	41.6	64.2	44.2	42.1	45.8
	42.1	57.4	39.5	58.9	42.1
	47.9	53.2	52.6	48.4	44.2
	60.0	54.2	49.5	37.9	27.9
	46.8	46.3	53.2	47.4	67.9
Mean	48.6	49.1	49.5	41.5	45.3

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	1104.3	276.1	2.83	$P < 0.05$
Within	45	4393.8	97.6		
Total	49	5498.1			

APPENDIX TABLE 7

MOLAR PERCENTAGES OF ACETIC ACID (%)

Lot No.	13	14	15	16	17
	65.4	55.1	52.7	41.2	57.0
	54.9	44.9	47.8	30.9	56.5
	44.0	43.6	42.4	44.6	67.2
	48.3	56.1	64.6	48.7	56.3
	47.1	42.9	40.3	53.7	56.7
	37.3	51.3	45.2	49.6	68.2
	49.3	44.8	45.0	57.0	51.3
	46.3	51.4	47.9	39.6	62.0
	54.1	61.0	54.9	39.6	52.7
	56.7	40.8	55.9	45.4	52.3
Mean	50.3	49.2	49.7	45.0	58.0
transform to Arcsin data	45.20	44.54	44.88	42.11	49.65

ANALYSIS OF VARIANCE (ARCSIN DATA)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	298.66	74.67	4.36	$p < 0.01$
Within	45	770.81	17.13		
Total	49	1069.47			

APPENDIX TABLE 8

CONCENTRATIONS OF PROPIONIC ACID (mMole/l)

Lot No.	13	14	15	16	17
	14.7	20.0	24.0	39.7	23.6
	25.0	20.0	42.6	32.0	13.3
	48.7	49.7	52.0	34.0	16.3
	45.0	22.0	16.7	26.0	24.7
	40.7	52.7	42.3	34.7	12.3
	50.3	38.3	37.3	30.0	11.7
	33.7	55.3	31.7	22.7	29.3
	40.7	37.3	38.0	56.0	14.0
	37.0	20.7	26.3	48.0	17.3
	26.7	45.3	20.0	47.7	34.0
Mean	36.3	35.9	33.1	38.0	19.7

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	2202.7	550.7	4.98	$p < 0.01$
Within	45	4978.7	110.6		
Total	49	7181.4			

APPENDIX TABLE 9

MOLAR PERCENTAGES OF PROPIONIC ACID (%)

Lot No.	13	14	15	16	17
	17.4	27.5	27.0	40.9	27.2
	33.4	38.7	38.4	50.6	20.2
	43.4	41.2	40.3	39.0	17.6
	40.5	24.9	19.0	37.2	28.5
	40.0	45.2	38.5	35.7	26.0
	45.1	30.6	38.2	35.3	17.4
	39.5	43.1	36.1	22.0	35.7
	39.4	36.0	34.6	45.9	19.6
	33.3	23.3	29.2	50.2	32.8
	32.4	39.9	21.0	45.7	26.2
Mean	36.6	35.0	32.2	40.3	25.1
transform to Arcsin data	36.99	36.17	34.43	39.26	29.92

ANALYSIS OF VARIANCE (ARCSIN DATA)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	489.95	122.48	5.27	$p < 0.01$
Within	45	1047.19	23.27		
Total	49	1537.14			

APPENDIX TABLE 10

CONCENTRATIONS OF BUTYRIC ACID (mMole/l)

Lot No.	13	14	15	16	17
	10.6	10.0	14.2	12.3	9.5
	6.3	5.9	11.6	8.7	11.2
	9.8	12.8	17.3	8.9	10.3
	8.8	12.1	10.6	6.4	9.5
	9.5	8.9	17.3	7.5	5.6
	14.5	17.3	11.7	9.9	7.3
	8.1	10.6	12.3	15.6	7.3
	10.9	8.7	14.0	11.7	10.0
	10.0	11.2	10.4	7.0	5.0
	6.4	14.8	16.5	4.6	22.3
Mean	9.5	11.2	13.6	9.3	9.8

ANALYSIS OF VARIANCE

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	129.5	32.38	2.82	$p < 0.05$
Within	45	517.1	11.49		
Total	49	646.6			

APPENDIX TABLE 11

MOLAR PERCENTAGES OF BUTYRIC ACID (%)

Lot No.	13	14	15	16	17
	12.5	13.8	16.0	10.6	10.9
	8.4	11.4	10.4	13.8	17.0
	8.7	10.6	13.4	10.2	11.1
	7.9	13.7	12.1	9.2	10.9
	9.3	7.6	15.8	7.7	11.8
	13.0	13.8	12.0	11.7	10.9
	9.5	8.3	12.0	15.1	8.9
	10.5	8.4	12.7	9.6	14.0
	9.0	12.6	11.5	7.3	9.5
	7.8	13.1	17.4	4.4	17.2
Mean	9.7	11.3	13.5	10.0	12.2
transform to Arcsin data	18.05	19.57	21.52	18.19	20.35

ANALYSIS OF VARIANCE (ARCSIN DATA)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	86.42	21.60	3.93	$p < 0.01$
Within	45	246.91	5.49		
Total	49	333.33			

APPENDIX TABLE 12

CONCENTRATIONS OF ISO-VALERIC ACID (mMole/l)

Lot No.	13	14	15	16	17
	2.4	1.9	2.5	2.6	2.6
	1.5	0.7	2.1	1.4	3.1
	1.7	2.7	2.2	1.3	2.4
	2.1	3.4	3.1	1.6	2.1
	0.8	1.3	2.4	1.9	1.9
	1.5	3.5	1.9	1.6	1.4
	1.6	2.4	1.4	4.7	1.9
	1.4	2.5	2.6	2.1	2.0
	2.7	1.7	2.7	0.5	1.8
	1.7	2.9	3.8	1.3	3.4
Mean	1.7	2.3	2.5	1.9	2.3

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	3.7	0.93	1.45	N.S.
Within	45	28.8	0.64		
Total	49	38.9			

N.S.: Non-significance

APPENDIX TABLE 13

MOLAR PERCENTAGES OF ISO-VALERIC ACID (%)

Lot No.	13	14	15	16	17
	2.8	2.6	2.8	2.7	3.0
	2.0	1.4	1.9	2.2	4.7
	1.5	2.2	1.7	1.5	2.6
	1.9	3.9	3.5	2.3	2.4
	0.8	1.1	2.2	2.0	4.0
	1.3	2.8	1.9	1.9	2.1
	1.9	1.9	1.6	4.5	2.3
	1.8	2.4	2.4	1.7	2.8
	2.4	1.9	3.0	0.5	3.4
	2.1	2.6	4.0	1.2	2.6
Mean	1.9	2.3	2.5	2.1	2.6
transform to Arcsin data	7.73	8.57	9.00	7.99	9.87

ANALYSIS OF VARIANCE (ARCSIN DATA)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	29.04	7.26	2.96	$p < 0.05$
Within	45	110.04	2.45		
Total	49	139.08			

APPENDIX TABLE 14

CONCENTRATIONS OF VALERIC ACID (mMole/l)

Lot No.	13	14	15	16	17
	1.6	0.7	1.3	2.5	1.5
	0.9	1.9	1.6	1.6	1.1
	2.8	2.8	2.8	4.1	1.3
	1.5	1.3	0.7	1.1	1.6
	2.8	3.6	3.6	0.9	0.7
	3.6	1.9	2.6	1.3	1.0
	0.9	2.5	2.8	1.5	1.5
	2.5	1.9	2.7	3.9	1.1
	1.3	1.1	1.3	2.2	0.9
	0.9	4.1	1.6	3.4	2.2

ANALYSIS OF VARIANCE

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	5.9	1.48	1.63	N.S.
Within	45	41.1	0.91		
Total	49	47.0			

N.S.: Non-significance

APPENDIX TABLE 15

MOLAR PERCENTAGES OF VALERIC ACID (%)

Lot No.	13	14	15	16	17
	1.9	1.0	1.5	2.6	1.8
	1.2	3.7	1.4	2.5	1.7
	2.5	2.3	2.2	4.7	1.4
	1.4	1.5	0.8	1.6	1.8
	2.8	3.1	3.3	0.9	1.5
	3.2	1.5	2.7	1.5	1.5
	1.1	2.0	3.2	1.5	1.8
	2.4	1.8	2.5	3.2	1.6
	1.2	1.2	1.4	2.3	1.7
	1.1	3.6	1.7	3.3	1.7
Mean	1.9	2.2	2.1	2.4	1.7
transform to Arcsin data	7.73	8.31	8.14	8.72	7.38

ANALYSIS OF VARIANCE (ARCSIN DATA)

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	11.75	2.94	1.07	N.S.
Within	45	123.92	2.75		
Total	49	135.67			

N.S.: Non-significance

APPENDIX TABLE 16

ACETATE TO PROPIONATE RATIOS

Lot No.	13	14	15	16	17
	1.86	2.00	1.95	1.01	2.10
	1.66	1.15	1.24	0.61	2.80
	1.02	1.06	1.05	1.14	3.82
	1.14	2.25	3.40	1.31	1.98
	1.18	0.95	1.05	1.50	2.18
	0.53	1.68	1.18	1.41	3.92
	1.25	1.04	1.25	2.59	1.44
	1.18	1.43	1.38	0.86	3.16
	1.62	2.62	1.88	0.79	1.61
	1.75	1.02	2.66	0.99	2.00
Mean	1.55	1.52	1.70	1.22	2.40

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	7.74	1.94	2.73	$p < 0.05$
Within	45	23.20	0.52		
Total	49	30.90			

APPENDIX TABLE 17

BODY WEIGHT GAINS DURING FATTENING PHASE (lb)

Lot No.	13	14	15	16	17
	370	385	-	400	405
	400	425	480	385	460
	465	415	445	420	370
	430	380	390	445	480
	420	370	405	430	355
	405	465	405	430	355
	395	400	400	380	415
	385	400	405	435	480
	450	400	425	375	405
	375	415	400	470	370
Mean	410	406	417	413	424

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	2037	509.3	0.40	N.S.
Within	44	56102	1275.0		
Total	48	58139			

N.S.: Non-significance

APPENDIX TABLE 18

LOIN EYE AREAS ($inch^2$)

Lot No.	13	14	15	16	17
	12.27	11.18	-	12.26	14.75
	12.23	11.71	11.93	11.79	12.94
	10.94	12.01	12.95	11.03	13.65
	13.32	14.45	13.86	11.92	12.95
	13.58	-	12.94	11.05	13.70
	11.47	13.93	11.02	9.14	10.94
	10.95	12.63	12.94	11.31	11.87
	10.40	11.86	13.03	11.44	13.18
	11.92	10.62	-	11.61	13.65
	11.08	11.06	12.17	11.27	12.88
Mean	11.82	12.16	12.60	11.28	13.05

ANALYSIS OF VARIANCE

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F	Level of Significance
Between	4	28.36	7.09	8.65	$p < 0.01$
Within	43	35.12	0.82		
Total	47	63.48			

EFFECT OF NITROGEN SOURCE UPON CRUDE AND TRUE PROTEIN AND VFA'S IN RUMEN CONTENTS OF STEERS FED FINISHING RATIONS

by

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Considerable work has been conducted to compare protein and non-protein nitrogen feeds for ruminants. As evidenced by its widespread use, urea by far has been the non-protein nitrogen feed of most interest. The nature of the ration to which urea is added affects the growth and multiplication of ruminal bacteria. It is believed that an adequate amount of urea with sufficient grain in the ration is comparable to plant protein sources as a nitrogen supplement for ruminants.

A cattle feeding experiment was conducted to compare differences in rumen liquor crude protein, true protein, non-protein nitrogen, and total and individual volatile fatty acid formation in the rumen that occurred during the fattening of steers fed high level of milo grain and two pounds of prairie hay supplemented with soybean meal or urea with and without added alfalfa hay. Fifty Hereford steer calves averaging 630 pounds were divided into five lots, of 10 steers per lot. They were self-fed milo grain and two pounds of prairie hay supplemented with the following: Lot 13, urea; Lot 14, urea and 2 pounds alfalfa hay; Lot 15, soybean meal and 2 pounds alfalfa hay; Lot 16, urea; Lot 17, urea and 2 pounds alfalfa hay. Rumen samples were taken by stomach tube four to five hours after feeding on the 134^{th} day of the fattening diet.

There were no significant differences in pH, crude protein and true protein of rumen liquor among lots. Animals receiving 2 pounds alfalfa hay yielded significantly higher

values of non-protein nitrogen than those not receiving alfalfa ($p < 0.05$). Lot 17, which had the same treatment as Lot 14 during the experiment yeilded the lowest total volatile acid concentration ($p < 0.05$), lowest molar percentage of propionic acid ($p < 0.05$) and the highest molar percentage of acetic acid ($p < 0.05$). These findings are believed due to individual animal difference and/or sampling techniques. Animals receiving a higher proportion of concentrates (those without alfalfa hay) yielded a higher molar percentage of propionic acid ($p < 0.05$). Animals receiving additional alfalfa hay yielded higher molar percentages of butyric and iso-valeric acids ($p < 0.05$). Lot 17 yielded the highest value of acetate/propionate ratio. Animals without 2 pounds of alfalfa hay yielded a lower mean level of loin eye area ($p < 0.05$).

There were minor, but non-significant differences between animals fed urea and soybean meal in criteria analyzed. The addition of 2 pounds alfalfa hay produced statistically significant differences in several metabolites as shown above.