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I am submitting herewith a thesis written by Stanley William Telega entitled "Comparison of Soybean Meal and a Liquid Nonprotein Nitrogen Supplement Fed in a Complete Ration with Corn Silage." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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COMPARISON OF SOYBEAN MEAL AND A LIQUID NONPROTEIN NITROGEN
SUPPLEMENT FED IN A COMPLETE RATION WITH CORN SILAGE

A Thesis
Presented for the
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Degree
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ABSTRACT

Six Holstein steers were used in two 3 x 3 latin squares balanced for estimating residual effects to determine nutrient utilization and physiological response of animals consuming unsupplemented, soybean meal (SBM) supplemented, and liquid molasses-urea (LPS) supplemented corn silage complete rations. Supplements were mixed at time of feeding to raise the ration to 12% crude protein on a dry matter basis. Total animal excreta was collected for a five-day period, after which, blood and rumen fluid samples were drawn.

Differences in dry matter intake and digestibility of dry matter, acid-detergent fiber, and acid-detergent cellulose were not significant. Organic matter and crude protein digestibility of the unsupplemented, SBM supplemented, and LPS supplemented rations were 66.7 and 57.5%; 69.0 and 62.4%; and 66.4 and 67.6%, respectively. LPS supplementation decreased ether extract digestibility. Fecal and urinary nitrogen (N) excretion was not affected by supplement. N-retention (g./day) of animals consuming the unsupplemented, SMB supplemented, and LPS supplemented rations was 19.1, 11.0, and 17.7, respectively. Calculated values of true N digestibility, biological value, and net protein utilization for the supplemented rations were not significantly different. Mineral balance and plasma mineral concentrations were not affected by supplement. Concentration and molar percent of isovalerate in the rumen fluid was significantly elevated by consumption of the LPS supplemented ration.

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CHAPTER I

INTRODUCTION

Within the past decade, several major feed manufacturers have introduced liquid supplements into the Southeast. These supplements utilize various types of molasses as carriers for nonprotein nitrogen (NPN) compounds along with an array of minerals, vitamins, and antibiotics used in livestock feeds. Specific formulation of a liquid supplement varies widely among commercial brands. Generally, however, liquid supplements and animal performance in each case is related to the ability of the product to adequately supplement the other feeds in the total ration.

Feeding of liquid supplements have been more widely accepted by the beef industry than the dairy industry. The self-feeding of liquid supplements from a "lick-wheel" feeder offers a labor-saving method of supplying supplemental nitrogen and energy to range cattle. However, variation in consumption of the supplement in a self-feeding system is large. This system of feeding liquid supplements offers dairymen no assurance that high producing cows will consume enough supplement to meet nitrogen and mineral requirements for milk production. Furthermore, excessive consumption of a self-fed liquid supplement by low producing or late-lactation cows will tend to decrease the profit margin of the herd.

The blending of liquid supplements with forage into a complete ration eliminates variable intake of the supplement by animals. Because

of the high energy to nitrogen ratio in corn silage, proper nitrogen and mineral supplementation will produce a high-quality complete feed. The purpose of this study was to determine the effects on nutrient utilization and physiological response of animals consuming a commercial liquid supplement mixed with corn silage in a complete ration.

CHAPTER II

REVIEW OF LITERATURE

I. HISTORY OF NPN FEEDING TO RUMINANTS

In 1879, Weiske first demonstrated that ruminants can utilize nonprotein nitrogen (NPN) compounds by feeding asparagine to cattle (31). However, the role of the rumen microflora in the digestion of cellulose and utilization of NPN was first realized in 1891 by Zuntz and Bohlmann of Germany (83). Subsequent research gave conflicting results and theories concerning the ability of the ruminant to utilize NPN were vigorously contested among European researchers. In 1911, Armsby (3) published a comprehensive review of work on the role of the rumen microorganisms in converting NPN to proteins, their digestion in the lower alimentary tract, and ultimate conversion to proteins for production.

The bulk of early research concerning NPN utilization by ruminants was carried on by German workers. Development of the Haber-Bosh ammonia synthesizing process during World War I led to the development of a commercial process of synthesizing urea from ammonia and carbon dioxide. By the mid 1930's, Europe was reported to be using 10,000 tons of urea annually in livestock feed (83).

Studies on NPN utilization by ruminants in the United States began in the late 1930's at Wisconsin. Hart et al. (29) reported that NPN was utilized by growing dairy heifers and that soluble carbohydrates in the diet increased NPN utilization. This initial work was followed

by a series of experiments at Illinois (27, 28), Massachusetts (2), and Cornell (53). These early studies showed that a significant amount of urea nitrogen could be substituted for protein nitrogen resulting in no adverse effects on production.

Commercial production of urea began in the United States in 1935, making urea available at prices allowing its use by the feed industry (83). During World War II, stress on the supply of natural protein caused an increased use of NPN by feed manufacturers and farmers. Urea became the most commonly used NPN product, being first accepted and most widely used by those fattening beef cattle. The dairy industry was more reluctant to use these compounds. However, of the one million tons of NPN annually fed livestock today, approximately 245,000 tons are consumed by dairy cattle (64).

II. METABOLISM OF NPN COMPOUNDS IN THE RUMEN

Rumen Degradation and Ammonia Formation

NPN compounds are a normal constituent in rumen fluid, even when these compounds are absent from the diet. Pearson and Smith (66) demonstrated that rumen contents have a high urease activity, concluding that the rumen microbes possess the ability to degrade urea and other NPN compounds. Using in vitro techniques, they found that 100 g. of rumen contents could convert 100 mg. of urea to NH_3 and CO_2 in one hour at 39°C .

Ammonia is the common intermediate between NPN hydrolysis and microbial protein synthesis. If an NPN compound cannot yield free NH_3 in the rumen, it is useless as a nitrogen source to the microflora (64). Studies

with ^{15}N -urea have shown the addition of ^{15}N -ammonia to the rumen ammonia pool (46). The potent urease enzyme production is attributed to several species of bacteria present in the rumen. Ruminal ammonia can also arise from the degradation of dietary proteins and peptides, microbial proteins, and recycled urea in the saliva.

Ammonia Metabolism by Rumen Microbes

It has been well established that a wide variety of NPN compounds will support in vitro growth of ruminal microbes (12, 31, 64). Loosli et. al. (54) demonstrated the microbial synthesis of the ten amino acids essential to rats in lambs fed purified diets with urea as the sole source of dietary nitrogen. Chalupa (12) suggests that general amination and transamination reactions of keto acids are the primary mechanism of ammonia utilization by rumen microbes in the synthesis of amino acids. The presence of NAD- and NADP-linked glutamic acid dehydrogenase in rumen microbes (35) and the rumen mucosa (35, 38) suggests that ammonia incorporation into glutamic acid is the major mechanism of ammonia fixation by rumen bacteria (1). Subsequent transaminase reactions of amino-nitrogen to α -keto acids enable the synthesis of other amino acids for microbial protein synthesis (12).

Quality of microbial protein synthesized in the rumen. Microbial protein synthesis from dietary nitrogen depends upon the amount and nature of the dietary constituents fed to the ruminant. The amount of high-energy compounds, primarily ATP, that can be derived from the rumen contents is the major factor determining microbial growth (13). The main

energy yielding substrate in the ruminant diet is carbohydrate. Because of the anaerobic condition of the rumen, the efficiency of microbial growth is greatly reduced when compared to an aerobic system. Hungate (42) estimated that a microbial cell yield of 10-20% would be expected in anaerobic fermentation. Using the mean of 15%, a calculated yield of 18.3 g. of digestible microbial protein could be produced for each digestible megacalorie ingested (71). Chalupa (18) calculated that a dairy cow depending entirely upon microbial protein synthesized in the rumen would produce only 10 kg. milk per day. Cows averaged 8.6-12.1 kg. milk per day over several lactations when fed a protein-free, urea-containing diet (92).

In vivo estimates of rumen microbial protein synthesis has been hampered by the technical problem of separating microbial protein from partially degraded feed protein. Despite this obstacle, a considerable effect has been initiated in recent years. In a review by Thomas (85) considering 27 different rations, rumen protein synthesis as measured by α - ϵ -diaminopimelic acid, ranged from 15.3-53.1 g. N per kg. digestible organic matter. Using a protein-free diet, Hume (41) measured 27 g. of microbial N per kg. digested organic matter entering the omasum. Kropp et al. (51), feeding low-protein hay with varying percentages of protein and NPN, found a constant rate of microbial N flow into the abomasum corresponding to 8.4 g. microbial protein synthesized per 100 g. digested dry matter. A commonly accepted value of 22 to 23 g. microbial protein synthesized per 100 g. organic matter digested in the rumen has been expressed (60, 85).

Satter and Slyter (81) demonstrated in vitro that under nitrogen-limiting conditions, microbial protein yield increased linearly with increasing supplementary urea. However, accumulation of ammonia in the incubated ingesta above the concentration of 5 mg. $\text{NH}_3\text{-N}/100$ ml. had no effect on microbial protein synthesis. It has been suggested that NPN supplementation to ruminant rations already promoting rumen ammonia concentration greater than 5 mg. $\text{NH}_3\text{-N}/100$ ml. does not further increase microbial protein yield, and, therefore, is useless to the ruminant animal (10, 74, 80, 81).

Quality of microbial protein. Amino acids leaving the rumen for digestion in the lower alimentary tract consists of bacterial, protozoal, and undegraded dietary protein. With increasing levels of dietary NPN, bacterial and protozoal protein quality becomes an important component in the nutrition of the host animal. Microbial protein quality has been studied by amino acid analysis of bacterial and protozoal hydrolysates (72, 93) and by feeding microbial preparations to laboratory animals for assessment of nutritive value (59).

Weller (93) demonstrated the constancy of the amino acid composition of rumen microorganisms by examining preparations from animals fed four different rations. His results were confirmed by Purser and Buechler (72) in their amino acid analysis of 22 strains of rumen bacteria grown in pure culture. Protozoal protein contains greater amounts of the essential amino acids isoleucine, leucine, phenylalanine and lysine compared to rumen bacterial protein (71).

Though amino acid composition of rumen microbes may be constant, indications are that release and availability of specific amino acids may vary (71). In vitro digestion of bacterial protein with pepsin and pancreatic proteases showed that methionine may be limiting in cellulolytic bacteria, while leucine may be limiting in non-cellulolytic bacteria (7). Furthermore, proportions of essential amino acids released during digestion varied greatly with different bacterial strains. Purser (71) concluded that modifications in the microbial population of the rumen may be responsible for differences in utilization of different dietary nitrogen sources.

The feeding of rumen microbes to laboratory animals suggests that protozoal protein is slightly higher in nutritive value than bacterial protein. Rumen bacteria average 55% crude protein with a true digestibility of 66%, a biological value of 78%, and a net protein utilization of 52% rumen protozoa average 38% crude protein with a true digestibility of 88%, a biological value of 77%, and a net protein utilization of 67% (59). Superior net utilization of protozoal protein over bacterial protein resulted in better growth in chicks fed a diet supplemented with dried protozoal protein (12). Since cell walls constitute about 15% of the dry weight of bacteria, Chalupa (13) suggests that the proteolytic-resistant cell walls reduce the availability of bacterial protein during digestion.

III. METHODS OF FEEDING NPN

A major problem with direct substitution of urea and other NPN compounds for natural protein is the resulting feed intake depression.

This is apparently due to the undesirable taste (25) of ammonia containing compounds when fed a certain concentrations to ruminants. The limit for feeding urea to dairy cattle without depressing feed intake is about 45 g./100 kg. body weight (38). Sheep consistently refused solutions of 2.5 to 5.0% urea, even when fed a protein-deficient ration (25). Reduced feed intake has been observed when concentrates contain greater than 2% urea (12, 25). However, Plummer (68) fed a concentrate containing 3% urea with no adverse effect on milk production or feed intake. Generally, urea-containing rations are acceptable to cattle when urea nitrogen composes not more than one third of the rations total nitrogen (12).

Several methods of feeding NPN compounds to ruminants have been devised to obtain a high level of NPN intake without depression of total feed intake. Missouri workers (11) showed increasing the frequency of feeding urea-containing rations from two to six times daily increased total NPN and dry matter intakes, resulting in increased rate of gain in dairy heifers. Much interest has been given to mixing NPN with highly palatable feeds. Silages have been the most widely used feed to mix with NPN compounds (38). An expansion processed mixture of urea and starchy grains (Starea) has been shown to increase urea utilization by ruminants (30). Additions of urea and other NPN compounds to molasses has produced variable results when fed in various conditions (8, 14, 36, 50, 57, 65, 73, 75, 78, 87, 98).

IV. FEEDING NPN IN CORN SILAGE BASED RATIONS

Corn silage is a major forage for livestock primarily because of its high energy yield per unit of land. It is also an excellent medium for feeding NPN supplements. Corn silage is high in digestible energy, very palatable to cattle, but relatively low in crude protein. Supplementation with nitrogen is required to balance the crude protein to energy ratio if corn silage is to be a ration's main component. Due to rising costs for natural protein sources, use of urea as a nitrogen supplement for corn silage may be economically profitable for many farmers.

NPN products may be included with corn silage rations either by addition at time of ensiling or mixed at time of feeding. Urea is the common NPN compound added to corn silage because of cost and possibility of acquirement (37, 38), though biuret (16), ammonia (17, 24), and ammonia salts (32) have been used.

NPN Addition at Ensiling

Several advantages are associated with nitrogen supplementation of corn silage at ensiling: (1) masking of undesirable odor and taste of ammonia compounds; (2) even distribution of supplement in the silage, minimizing the possibility of toxicities; (3) even intake of NPN by ruminants over the whole day, increasing nitrogen utilization; and (4) take advantage of the complete ration concept and automated feeding systems (38).

German researchers reported the initial work on addition of urea to corn silage at ensiling (79). Early work in the United States showed slightly lower consumption by dairy cows of corn silage containing 0.5%

urea compared to untreated silage (86). No effect on milk production was detected, however, Polan (69) fed corn silage treated with 0, 0.5, and 0.75% urea as the only forage for lactating cows consuming grain in the milk parlor according to the production. Results showed that level of urea in the silage did not influence silage intake, total feed intake, or milk production. Huber and Thomas (40) and Montgomery et al. (62) concluded that 0.5% urea-treated corn silage could warrant the feeding of a low-protein concentrate to lactating cows without adverse effect on feed intake or milk yield. Work at Michigan showed milk yields of cows fed NPN-treated corn silages have been equal to or better than yields for cows fed isonitrogenous rations from all natural protein, even when over 60% of the supplemental nitrogen of the treated rations came from NPN (37). However, milk production was greatly depressed when corn silage treated with urea was greater than 40% dry matter.

Giardini et al. (24) treated corn silage with several commercial pre-ensiling preparations containing urea and/or ammonia to increase the silage to 11-13% crude protein. Though differences existed among commercial products, NPN-treated corn silage promoted the same rate of gain as corn silage and soybean meal when fed to feedlot steers.

Addition of urea to corn silage at ensiling produces a more stable silage with higher utilization coefficients for nitrogen and little chance for toxicity (33, 37, 38). The added urea is hydrolyzed in the silo to ammonia and CO_2 . Released ammonia is then available to combine with organic fermentation acids, forming organic ammonia salts, or for bacterial protein synthesis. Henderson et al. (33) demonstrated a slower rumen

degradation of organic salts and, therefore, a smaller quantity of ammonia entering the blood of cattle fed urea-treated silages. Johnson et al. (45) reported corn silage treated with 0.5% urea at ensiling contained 30-40% of the total nitrogen as NPN, while untreated silage contained 40-50% of the total nitrogen as NPN. This suggests the incorporation of urea nitrogen into bacterial protein in the silo.

NPN Mixed at Feeding

Addition of NPN to corn silage at time of feeding enables control of the nitrogen content of the silage for individual or group feeding systems. Little or no loss of NPN occurs, though the task may require addition labor to ensure proper mixing.

Feed grade urea or other NPN products may be mixed dry with corn silage or dissolved in a liquid medium and sprinkled through the forage (79). Huber et al. (39) found that a ration where urea was the sole nitrogen supplement of corn silage depressed milk yields in high producing dairy cows, but dry matter intake was not affected. An isonitrogenous supplementation of corn silage with oil seed meals and urea, however, had no adverse affect on milk production when compared to an all oil seed meal supplementation. Van Arsdell et al. (88) found that fattening steers fed soybean meal mixed with corn silage gained 10% more weight than steers fed an isonitrogenous, isocaloric, corn silage ration supplemented with ground corn and urea. Several researchers have concluded that urea is not acceptable as the only nitrogen supplement for corn silage based rations when mixed at time of feeding (28, 37, 38, 89).

Utilization of urea is improved when included in a multi-ingredient supplement with corn silage (13, 18, 38, 79). High-producing cows fed a concentrate containing 2.5% urea blended with corn silage prior to feeding yielded the same amount of milk as cows fed a concentrate containing soybean meal (18). However, substitution of ammonia salts for urea decreased nitrogen utilization and depressed milk yields. Barth et al. (5) found increasing nutrient digestibility coefficients when urea furnished increasing percentages of dietary nitrogen in steers fed a complete ration of corn silage, ground corn, cotton-seed meal, and urea. When urea constituted 48% of the dietary nitrogen, however, increased urinary excretion of nitrogen decreased net nitrogen utilization, resulting in lower nitrogen retention values.

Dissolving NPN compounds in an aqueous solution facilitates mixing with corn silage and reduces the risk of ammonia toxicity (79). Harris and Mitchell (27) improved the growth rate of lambs fed corn silage by adding urea solutions to increase the crude protein of the ration. They noted that lambs consuming the treated silage took a longer time to consume the forage than lambs eating the untreated silage. Molasses has been used as a solvent for NPN to increase the palatability of the mixture and supply needed energy and sulfur for NPN utilization. King et al. (49) found only small differences between the acceptance of corn silage mixed with blackstrap molasses and blackstrap molasses containing 5% urea by dairy heifers.

V. MOLASSES-NPN LIQUID SUPPLEMENTS FOR CATTLE .

The use of liquid supplements to provide additional energy, crude protein, minerals, and other nutrients has markedly increased in recent years. Perhaps the savings on labor with the self-fed supplements have been attractive to many farmers. Other advantages of using liquid supplements are: (1) increased palatability of finely ground and course roughages; (2) decreased dustiness of feeds; (3) minimum amount of product waste; (4) ease of handling and storage; and (5) they offer a means of utilizing many good by-products of industry (47).

Most liquid supplements contain a molasses base with urea added to supply the major portion of the crude protein equivalent. The addition of other ingredients such as phosphorus sources, trace minerals, and antibiotics, vary with the specific brand of supplement. In 1970, 99 known brands of liquid supplements were being distributed throughout the southeast United States (47).

Bently et al. (6) reported that cane molasses is a substance that increases rumen microbial activity in vitro. Mills et al. (58), using fistulated heifers, showed that addition of corn molasses to a hay-urea ration decreased rumen ammonia, while increasing percipitable proteins. Potter et al. (70) investigated the quantity of nitrogenous material entering the abomasum of finishing steers fed a corn-corn cob ration supplemented with: (1) soybean meal, (2) urea, (3) urea and 2.5% molasses, and (4) urea and 10% molasses. The addition of 2.5% molasses to the urea diet increased daily abomasal nitrogen by 23%, but the addition of 10%

molasses to the urea ration was of no greater benefit. It has been suggested that molasses may be a source of essential factors that stimulate rumen microbial growth, thus increasing NPN incorporation into microbial protein (70, 98). Because of the extremely rapid digestion of sugars in the rumen, Johnson (44) suggests that starches, rather than molasses, would be a more adequate source of carbohydrate for maximal NPN utilization by the rumen microbes.

Comparison of Liquid vs. Dry Supplements

Much data is available on the comparison of liquid and dry supplements of beef cows on low quality forage. Wintering range cows in Oklahoma performed equally well on self-fed liquid and dry supplements containing molasses and either urea or biuret (87). However, calf weaning weights and cow condition scores were higher for cows receiving natural protein supplements. Similar results were obtained in Virginia with beef cows fed corn silage and a liquid dry urea-containing supplement (56). Cows on low quality pasture consuming a dry molasses urea supplement containing starchy grains performed better than cows consuming a liquid molasses-urea supplement (78). Apparently starch in the dry supplement supported greater NPN utilization than sugars in the molasses of the liquid supplement (40, 78).

McClure et al. (57) compared dry supplements containing urea and biuret spread over corn silage to a liquid supplement fed separately with corn silage to finishing steers fed grain. Steers consumed only 0.64 kg. daily of the liquid supplement, about one-fourth of the desired amount.

Consequently, feeding of the liquid supplement resulted in reduced carcass weight.

Michigan work suggests that a liquid molasses-urea supplement added to corn silage either at ensiling or at feeding results in performance equal to that of a dry urea-mineral supplement (36). In high-producing dairy cows, lower milk yields and feed intake were observed on rations where NPN furnished all the supplemental nitrogen compared to a control ration of natural protein supplements.

Self-Feeding of Liquid Supplements

Basically, there are two methods of feeding liquid supplements: (1) self-feeding tanks where intake is limited by phosphate compounds or salts; and (2) mixed with a forage, usually a silage. The self-feeding method has been used extensively for supplementing range cattle (26, 77, 78, 87). Rush et al. (77) found that heifers consuming a liquid molasses-urea supplement loss more weight than those supplemented with a self-fed natural protein supplement on winter range grass. These findings are contrary to those of Grelen and Pearson (26) when comparing a liquid supplement with cottonseed cake. Replacement dairy heifers on a good quality ryegrass pasture showed no extra gains when supplemented with a free choice liquid supplement (75). Using chromium oxide as a marker, Whitlow et al. (94) demonstrated that nearly all the liquid supplement consumed from a lick-wheel feeder passes into the reticulorumen and does not bypass it via the esophageal groove as suggested by some.

In sugarcane-growing countries where cereals and oil seed meals

are scarce, liquid molasses-urea mixtures may compose the majority of the diet (14, 19, 65, 73). Cuban workers have reported satisfactory gains in beef steers (14) and A.I. bulls (19) when liquid composed greater than 70% of the diet. Reports of cross-bred, dual-purpose cows efficiently produced milk on a ration of 75% molasses-urea liquid have come from India (65).

Mixing Liquid Supplements with Forage

Most of the work with feeding liquid supplements mixed with forage has been done with dairy cattle. This method of feeding lends itself to a more exact control of supplement intake than the self-fed method. Silages seem to be the forage of choice because they mask the undesirable taste associated with urea because their organic acids will bind released ammonia (36). The taste of ammonia may render feeds unacceptable to cattle (25).

Addition of molasses-urea liquid supplements to corn silage makes it essentially a complete feed in protein and energy for growing heifers. South Carolina workers reported daily gains of 0.73 kg. in dairy heifers fed a 5% urea-molasses solution (16.7% crude protein equivalent) mixed with corn silage and cottonseed meal (48). However, when a 10% urea-molasses solution (32.4% crude protein equivalent) was the sole supplement for corn silage, heifers gained only 0.46 kg. per day. Nitrogen metabolism studies showed no difference in nitrogen utilization of corn silage rations supplemented with either 5% urea-molasses and cottonseed meal (50). However, decreased nutrient digestibility of forage rations supplemented with liquid urea-molasses mixtures has been reported (8, 22, 55). Montgomery (unpublished data) observed decreased growth by dairy heifers fed a corn

silage ration supplemented with a 35% crude protein commercial liquid supplement when compared to heifers consuming an unsupplemented corn silage ration.

Van Horn and Mudd (90) reported that cows receiving a complete feed of corn silage and a liquid NPN supplement produced 1.5 kg. less milk per day than cows fed an isonitrogenous mix containing soybean meal. Cows fed the diet containing soybean meal consumed more total feed than cows consuming the liquid NPN supplemented diet.

Ammoniated-Molasses Supplements

Early work with feeding ammoniated molasses resulted in reduced gains in growing dairy heifers (48, 49) and decreased digestibility of dietary crude protein (50). In vitro studies have shown rather poor utilization of ammoniated molasses nitrogen by rumen microbes (34). Most liquid supplements on the market today do not contain ammoniated molasses (47).

CHAPTER III

EXPERIMENTAL PROCEDURE

I. OBJECTIVES OF EXPERIMENT

The objectives of this study were: (1) to determine nutrient digestibility and nitrogen utilization of corn silage alone or supplemented with soybean meal (SBM) or a liquid molasses-urea supplement (LPS); and (2) to ascertain if there are any major alterations in urine, blood, or rumen fluid following the consumption of the LPS. This study was a follow-up to a growth trial at the Dairy Experiment Station at Lewisburg which resulted in decreased weight gains in heifers when the LPS, Golden Flo, a product of Tennessee Farmer's Cooperative, was used with either corn or wheat silages.

II. EXPERIMENTAL DESIGN

The experimental design consisted of two 3×3 latin squares balanced for estimating residual effects of the treatments. Six Holstein steers were the experimental animals. The steers were randomly allotted to one of three experimental rations consisting of corn silage alone or supplemented with either SBM or LPS. Ration assignments for the three trials are shown in Table 1.

TABLE 1. Ration Assignments in Latin Squares Balanced for Estimating Residual Effects

<u>Square I</u>				<u>Square II</u>			
<u>Animal</u>	<u>204</u>	<u>197</u>	<u>200</u>	<u>Animal</u>	<u>201</u>	<u>198</u>	<u>205</u>
Trial 1	A	B	C	Trial 1	A	B	C
Trial 2	B	C	A	Trial 2	C	A	B
Trial 3	C	A	B	Trial 3	B	C	A

A = Corn silage alone ration

B = Corn silage + SBM ration

C = Corn silage + LPS ration

III. MATERIALS AND METHODS

Conduct of Trials

Six Holstein steers, averaging 329 kg. in body weight, were confined in individual metabolism crates designed to separate urine and feces. All animals had been used in a previous metabolism study.

Experimental rations consisted of corn silage unsupplemented or supplemented with either SBM or LPS to raise the crude protein content of the silage to 12% on a dry matter basis. The supplements were manually mixed with the corn silage at time of feeding. All rations were offered to ensure ad libitum consumption with at least 10% refusals. Animals were fed and watered twice daily (8 am and 4 pm); refusals were collected, weighed, and recorded daily before the afternoon feeding. An attempt was made to keep the silage fresh. Silage removed from the silo and not used for immediate feeding was stored in plastic garbage cans under refrigeration. Refrigerated silage was never stored longer than one day. A trace mineralized salt block was placed in the feed box of each steer.

All animals were weighed at the beginning of the experiment and between each trial. The three trials consisted of three phases: the first seven days served as a ration adjustment phase, the second seven days constituted an intake measurement phase, and the last five days were for total excreta collection. During the collection phase, fecal output was collected and weighed daily. A 5% aliquot sample from each animal was composited and refrigerated in plastic bags. Total daily urinary excretion from each steer was collected in polyethylene jars containing

50 ml. of 6N HCl to prevent ammonia vaporization. A 1% aliquot sample, drawn to the nearest 25 ml., was composited for each steer and refrigerated in glass jars until analysis. Rations were sampled daily before the afternoon feeding, composited, and refrigerated in plastic bags. Refusals were weighed and 10% aliquot samples were composited and also refrigerated in plastic bags.

After the fifth collection day, the steers were removed from the crates and samples of blood and rumen fluid were drawn. The blood was immediately centrifuged at 1000xg and plasma frozen until analysis. The rumen fluid was strained through several layers of cheese cloth and frozen in plastic containers. A 2-day rest period, in which the steers were group fed corn silage ad libitum, was allowed between trials.

Triplicate samples of ration, refusal, and feces collected from each animal were weighed, dried for 72 hours in a forced-air oven at 60° C, and allowed to air-equilibrate for 48 hours before being reweighed to determine air-equilibrated dry matter. The samples were then ground through a 2 mm. mesh screen of a Wiley Mill and placed in air-tight jars before the beginning of the next collection period of the following trial.

Laboratory Analysis

All laboratory analyses were conducted in triplicate. Ration, refusal, and fecal samples were analyzed for dry matter by drying in a 100° C oven overnight. Crude protein, ether extract, and ash were determined by methods according to A.O.A.C., (4). Acid-detergent fiber and acid-insoluble lignin were determined by the method of Van Soest (91). Acid-

detergent cellulose was determined by difference of acid-detergent fiber and acid-insoluble lignin.

Nitrogen content of urine samples were determined by the Kjeldahl method (4). All samples collected, except rumen fluid, were analyzed for calcium, magnesium, and potassium by atomic absorption and phosphorus by the method of Fiske and SubbaRow (21).

Ration samples were composited across trials for the determination of sulfur and soluble nitrogen, NPN, and precipitable protein. Sulfur was analyzed gravimetrically by Galbraith Laboratories, Inc., Knoxville, Tennessee. Nitrogen solubility was determined in a mineral buffer solution (ionic strength = 0.14, pH = 5.5) similar to the method suggested by Wohlt et al. (97). Soluble proteins were precipitated overnight with sodium tungstate and centrifuged at 12,000xg. The supernate was then analyzed for nitrogen (soluble NPN). Soluble protein values were obtained by the subtraction of soluble NPN from total soluble nitrogen.

Volatile fatty acid (VFA) concentrations in the rumen fluid were determined by gas-liquid chromatography as described by Erwin et al. (20). A 1.8 meter glass column packed with 10% SP1200 washed with 1% H_3PO_4 on Chromasorb W¹ was used for VFA separation. Nitrogen was the carrier gas. Injection portal, flame ionizing detector, and oven temperatures were 210°, 250°, and 120° C., respectively.

¹Supelco Inc., Bellefonte, Pennsylvania.

Statistical Analysis

The data was analyzed by methods developed by Williams (95) for the estimation of residual effects of treatments. The mathematical model for the analysis was:

$$Y_{ijklm} = \mu + t_i + r_j + a_k + tr_1(sq_m) + e_{ijklm}$$

where:

Y_{ijklm} = dependent variable

μ = estimate of population mean

t_i = direct effect of treatments

r_j = residual effect of treatments

a_k = effect of individual animals

$tr_1(sq_m)$ = effect of trials within each square

e_{ijklm} = randomized error effect.

Treatment differences were determined by Duncan's Multiple Range Test (43) on treatment means adjusted for residual treatment effects.

CHAPTER IV

RESULTS AND DISCUSSION

I. RATION ANALYSIS

Because of limited quantities of the LPS left over from the feeding trials at Lewisburg, another batch of LPS from the same supplier was also used in this experiment. The first batch was fed during the first trial and for the first five days of the second trial. The new batch was fed for the remainder of the experiment.

Both batches of LPS analyzed similar for dry matter and nitrogen, approximately 50% and 10.9% (dry matter basis), respectively. The first batch, however, analyzed 1.3% higher in ash content than the second batch (3.4% vs. 2.1%). Differences in sulfur content accounted for most of the difference in ash content. Percentages of sulfur on a dry matter basis for the first and second batch were 3.25 and 1.78, respectively. Calcium, phosphorus, and magnesium were higher in the first batch, while potassium was higher in the second batch. Percentages on a dry matter basis for calcium, phosphorus, magnesium, and potassium were: 0.19, 0.57, 0.07, and 0.49, respectively for the first batch; and 0.05, 0.22, 0.05, and 0.71, respectively for the second batch.

Average analyses of the experimental rations are in Tables 2 and 3. The SBM supplemented ration was lower in crude protein (Table 2) than had been expected. The crude protein content of the LPS supplemented

TABLE 2. Chemical Composition of Supplemented Corn Silage Complete Rations

	Supplement		
	Alone	SBM	LPS
Dry matter (%)	30.1 $\pm$ 0.5 ^a	31.7 $\pm$ 0.4	31.1 $\pm$ 0.4
-----(% of Dry Matter)-----			
Organic matter (%)	94.3 $\pm$ 0.1	94.2 $\pm$ 0.1	93.2 $\pm$ 0.3
Crude protein (%)	9.2 $\pm$ 0.3	11.3 $\pm$ 0.9	13.7 $\pm$ 1.0
Ether extract (%)	2.4 $\pm$ 0.1	2.3 $\pm$ 0.1	2.2 $\pm$ 0.1
Acid detergent			
Fiber (%)	35.1 $\pm$ 1.0	33.4 $\pm$ 0.6	31.6 $\pm$ 1.2
Lignin (%)	4.5 $\pm$ 0.2	4.4 $\pm$ 0.3	4.2 $\pm$ 0.4
Cellulose (%)	30.6 $\pm$ 0.8	29.0 $\pm$ 0.8	27.4 $\pm$ 1.0

^aMean $\pm$ SD.

TABLE 3. Mineral Composition of Supplemented Corn Silage Complete Rations^a

	Supplement		
	Alone	SBM	LPS
Nitrogen (%)	1.46±0.05	1.80±0.01	2.19±0.16
Soluble Nitrogen (% of N) ^c	45.9	38.9	68.0
Soluble NPN (% of N) ^c	32.9	32.8	61.2
Soluble Protein Nitrogen (% of N) ^c	13.0	6.1	6.8
Calcium (mg/g dry matter)	3.5±0.3	3.6±0.4	3.9±0.3
Phosphorus (mg/g dry matter)	2.5±0.2	2.7±0.1	4.2±1.2
Magnesium (mg/g dry matter)	2.3±0.1	2.4±0.2	2.4±0.1
Potassium (mg/g dry matter)	14.5±0.8	14.9±0.6	17.3±1.3
Sulfur (%) ^{a,c}	0.21	0.30	0.42
Nitrogen:Sulfur Ratio	7.0	6.0	5.3

^aExpressed on dry basis.

^bMean±SD.

^cAnalysis on composited sample across trials.

ration averaged higher than expected and varied between trials. The variation probably resulted from uneven mixing of the LPS in the ration which hindered the collection of representative samples.

Soluble nitrogen (Table 3) composed 45.9, 38.9, and 68.0% of the total nitrogen in the unsupplemented, SBM supplemented, and LPS supplemented rations, respectively. All the nitrogen supplied by the LPS was soluble, presumably in the form of urea.

Since the phosphorus content of the LPS was generally high, the LPS supplemented ration averaged less than a 1:1 calcium-to-phosphorus (Ca:P) ratio. The Ca:P ratio of the ration was 0.7:1 when the first batch of LPS was used and averaged 1.1:1 when the second batch was used. The Ca:P ratio of the other rations was in accordance with NRC recommendations (63).

II. RATION INTAKE AND AVERAGE WEIGHT CHANGE

Adjusted means for dry matter intake and average weight change are in Table 4. Animals consumed more of the SBM supplemented ration than either the unsupplemented or the LPS supplemented rations. Mean differences, however, were not statistically significant. Generally, consumption of all three experimental rations was high enough to meet growth requirements of a 350 kg. steer gaining 0.25 kg./day (63).

Animals consuming the SBM supplemented ration averaged a positive weight change during the trials, while animals consuming the other experimental rations averaged a negative weight change. This parameter should be considered lightly because of the shortness and nature of such a metabolism study and stress placed upon the animals.

TABLE 4. Effect of Supplement on Voluntary Intake, Weight Change and Nutrient Digestibility Coefficients of Complete Corn Silage Rations^a

	Supplement			SE ^b
	Alone	SBM	LPS	
Dry matter intake				
kg/day	6.43	7.04	6.60	0.21
kg/100kg body weight	1.96	2.13	1.94	0.07
Average weight change (kg/day) *	- 0.08 ^c	0.32 ^d	- 0.14 ^c	0.08
Apparent digestion coefficients (%)				
Dry matter	65.8	68.0	65.4	0.52
Organic matter	66.7 ^c	69.0 ^d	66.4 ^c	0.47
Crude protein	57.5 ^c	62.4 ^d	67.6 ^e	0.60
Acid detergent fiber	56.2	57.5	52.5	1.34
Acid detergent cellulose	62.7	64.1	59.1	1.33
Ether extract	80.4 ^d	79.2 ^d	77.4 ^c	0.39

^aMeans adjusted for residual treatment effects.

^bStandard error of treatment means.

^{c,d,e}Means in same line having different superscripts are significantly different ($P < .05$).

III. APPARENT NUTRIENT DIGESTIBILITY

Adjusted means for apparent digestion coefficients of ration nutrients are in Table 4. Apparent digestibility of dry matter, acid-detergent fiber, and acid-detergent cellulose was not significantly different among rations, though all three nutrients were more digestible in the SBM supplemented ration. This trend is further evident by a significantly higher digestibility of organic matter in this ration compared to the unsupplemented and the LPS supplemented rations. The addition of LPS to corn silage depressed the digestibility of the ether extract fraction of the ration.

Several studies have demonstrated decreased nutrient digestibility in rations containing molasses (8, 22, 55). Martin and Wing (55) showed that a high energy complete ration containing 6% molasses depressed the digestibility of dry matter, cellulose, and energy when fed to growing dairy steers. Other workers found crude fiber and cellulose digestion markedly reduced at relatively high levels of molasses in the diet (8, 22). It has been suggested that highly soluble carbohydrates may depress the activity of cellulolytic bacteria in the rumen (6, 8). There is general agreement among reported data that molasses containing rations decreases ether extract digestibility in forage based rations (8, 22, 50). However, King et al. (50) found no other depressing effect on nutrient digestibility when supplementing corn silage with a molasses-urea solution for dairy heifers.

Apparent digestion coefficients for crude protein of the unsupplemented, SBM supplemented and LPS supplemented rations were 57.5, 62.4,

and 67.6%, respectively. Since apparent digestibility of crude protein is positively correlated with crude protein content of the ration (16), the lower crude protein digestion coefficient for the unsupplemented ration was expected. The LPS supplemented ration had a significantly higher crude protein digestibility than the SBM supplemented ration. King et al. (49) found no differences in apparent crude protein digestibility when comparing corn silage rations supplemented with either molasses-urea or cottonseed meal. Chalupa (13) suggests that rations containing high amounts of soluble nitrogen in their crude protein fraction may exhibit high apparent digestibility of the ration's crude protein. Ammonia released in the rumen from soluble nitrogen sources may be absorbed across the rumen wall, giving the effect of crude protein being digested and absorbed in the gastrointestinal tract.

IV. NITROGEN UTILIZATION

A major consideration of this study was to assess the utilization of supplemental nitrogen in a corn silage complete ration. The lack of response in growth of dairy heifers consuming LPS-silage complete rations (Montgomery, unpublished data) indicated poor utilization of the nitrogen supplied by the LPS.

Mean values of nitrogen balance data and nitrogen utilization calculations adjusted for residual effects of treatment are in Table 5. Differences in nitrogen intake reflect differences in nitrogen content of the rations. Animals consuming the LPS supplemented ration had an average nitrogen intake of 147.6 g./day, while animals fed the SBM

TABLE 5. Effect of Supplement on Dietary Nitrogen Utilization of Complete Corn Silage Rations^a

	Supplement			SE ^b
	Alone	SBM	LPS	
Nitrogen intake (g/day)	93.2 ^c	125.6 ^d	147.6 ^e	2.26
Fecal Nitrogen				
g/day	39.7 ^c	47.1 ^d	48.0 ^d	1.42
% of N intake	42.5 ^e	37.6 ^d	32.4 ^c	0.61
Urinary Nitrogen				
g/day	34.4 ^c	64.3 ^d	72.4 ^d	3.99
% of N intake	38.2	51.4	49.9	3.26
Retained Nitrogen				
g/day	19.1 ^{c,d}	14.2 ^c	27.1 ^d	2.77
% of N intake	19.3	11.0	17.7	3.08
Metabolic fecal N (MFN) ^f	23.9	24.5	24.8	--
Endogenous urinary N (EUN) ^g	8.0	8.0	8.1	--
True N digestibility (%) ^h	83.2	81.9	84.4	0.75
Biological value of N (%) ⁱ	64.2	45.2	47.4	3.76
Net protein utilization (NPU) ^j	53.5	36.9	40.1	3.04

^aMeans adjusted for residual treatment effects.

^bStandard error of treatment means.

^{c,d,e}Means in same line having different superscripts are significantly different ($P < .05$).

^fMFN = 10.9 g/kg fecal dry matter.

^gEUN = 0.44 g/kg body wt.^{.5}

^hTrue N digestibility = $\frac{\text{N intake} - (\text{Fecal N} - \text{MFN})}{\text{N intake}} \times 100$.

ⁱThomas-Mitchell biological value of protein = $\frac{\text{N intake} - (\text{Fecal N} - \text{MFN}) - (\text{urinary N} - \text{EUN})}{\text{N intake} - (\text{Fecal N} - \text{MFN})} \times 100$.

^jNPU = Biological value x true digestibility/100.

supplemented ration averaged only 125.6 g./day. Mean intake of nitrogen of animals consuming the two rations was significantly different.

Fecal nitrogen from animals consuming the supplemented rations averaged higher than from animals consuming the unsupplemented ration. This agrees with observations of Stallcup et al. (82) and Coleman (16) that fecal nitrogen output is highly correlated with nitrogen intake. Differences in the quantity of fecal nitrogen excreted by animals consuming the supplemented rations were small and not significant. Fecal nitrogen output expressed as a percent of total nitrogen intake reflects the apparent digestion of the crude protein of the rations.

Urinary nitrogen from animals consuming the LPS supplemented ration was slightly, but not significantly, higher than from animals consuming the SBM supplemented ration. Animals fed the unsupplemented ration excreted considerably less nitrogen in the urine than animals consuming the supplemented rations. However, when considered as a percent of nitrogen intake, ration did not affect urinary nitrogen excretion. These data are contrary to those of King et al. (5) and Bohman et al. (8) who observed increased urinary nitrogen by animals consuming corn silage rations containing a molasses-urea supplement.

Retained dietary nitrogen was 19.1, 14.2, and 27.1 g./day for animals consuming the unsupplemented, SBM supplemented, and LPS supplemented rations, respectively. The significantly higher quantity of nitrogen retained by animals consuming the LPS supplemented ration compared to those fed the SBM supplemented ration is contrary to several reports (8, 17, 22, 39, 49, 50, 89). In these studies, animals consuming corn silage rations

supplemented with natural sources of protein retained more nitrogen than animals consuming rations supplemented with NPN. Generally, soluble nitrogen sources result in increased urinary nitrogen which reduces the retention of dietary nitrogen by the animal. In this study, elevated urinary nitrogen excretion was not associated with the LPS supplemented ration when compared to the SBM supplemented ration. Furthermore, a higher level of nitrogen intake by animals fed the LPS supplemented ration resulted in significantly higher amounts of dietary nitrogen being retained. Retained nitrogen expressed as a percent of nitrogen intake was not significantly different among rations, though this parameter was higher for the LPS supplemented ration than the SBM supplemented ration (17.7 vs. 11.0).

Within the experimental design, dietary nitrogen retained by animals was significantly affected by the ration consumed in the previous trial (Appendix, Table 9). The unsupplemented ration exerted a significant positive effect, while the LPS supplemented ration exerted a significant negative effect on nitrogen retention of animals in the following trial. The SBM supplemented ration had virtually no residual effect associated with the nitrogen retention of animals.

Using estimates of metabolic fecal nitrogen and endogenous urinary nitrogen (84), individual values of true nitrogen digestibility, biological value and net protein utilization were calculated for each observation. Differences among rations for these three parameters of nitrogen utilization were not significant. Unsupplemented corn silage nitrogen, however, had a higher biological value and net protein utilization than the nitrogen

contained in the supplemented rations. According to these calculated values, utilization of the nitrogen supplied by SBM and LPS when consumed in a complete ration with corn silage was similar.

V. MINERAL BALANCE AND PLASMA MINERAL STATUS

Adjusted means for mineral balances and plasma mineral concentrations are in Table 6. Plasma mineral concentrations were within ranges previously reported (15). Balances and plasma levels of calcium, phosphorus, and potassium were not affected by the experimental rations. King et al. (50) concluded that the supplementation of corn silage with a molasses-urea liquid did not effect calcium and phosphorus metabolism, though in one trial, consumption of the ration by dairy heifers resulted in large negative calcium balances.

Magnesium balance was negative (-0.8 g./day) and significantly lower in animals consuming the unsupplemented ration compared to the supplemented rations. This observation, however, did not manifest itself in an altered level of plasma magnesium in animals consuming the unsupplemented ration.

VI. NITROGEN-TO-SULFUR RATIO

All experimental rations had a relatively high nitrogen-to-sulfur (N:S) ratio (Table 3, p. 27). The level of sulfur in the unsupplemented and SBM supplemented rations, however, were within the recommended range of 0.16 to 0.30% (67). Composite samples of the LPS supplemented ration contained 0.42% sulfur with a 5.2:1 N:S ratio. However, the first batch

TABLE 6. Effect of Supplement on Mineral Balance and Plasma Minerals of Steers Consuming a Complete Corn Silage Ration^a

	Supplement			SE ^b
	Alone	SBM	LPS	
Mineral balance (g/day)				
Calcium	5.4	6.0	5.9	1.37
Phosphorus	0.5	1.4	4.4	1.52
Magnesium	-0.8 ^c	4.4 ^d	3.1 ^d	0.67
Potassium	4.1	3.4	6.2	1.44
Plasma minerals (mg/100 ml)				
Calcium	9.7	9.6	9.3	0.14
Phosphorus	7.1	7.8	7.9	0.17
Magnesium	2.12	2.09	2.02	0.05
Potassium	17.9	17.8	17.8	0.52

^aMeans adjusted for residual treatment effect.

^bStandard error of treatment means.

^{c,d}Means in same line having different superscripts are significantly different ($P < .05$).

of LPS contained a higher amount of sulfur than the second batch. The calculated level of sulfur in the LPS supplemented ration was 0.48%, when using the first batch, and 0.37% when using the second batch. Calculated N:S ratios of the LPS supplemented ration were 4.6:1 and 5.7:1 when using the first and second batches of LPS, respectively.

Dry matter intake of the LPS supplemented ration was considerably lower when the ration was mixed with the first batch of LPS (Appendix, Table 13, Trial 1). Throughout the entire experiment, however, there was no adverse affect on dry matter intake due to the high sulfur content of the LPS supplemented ration. Thompson et al. (86) reported reduced feedlot gains in steers consuming rations containing NPN when supplementary sulfur produced a 5:1 N:S ratio. Pendlum et al. (67), however, observed no effect on feed intake or daily gain when sulfur was supplemented to rations containing SBM or urea to produce a N:S ratio of 4:1. Bouchard and Conrad (9) reported that the use of molasses as a sulfur supplement for a ration containing 60% of the dietary nitrogen as NPN increased dietary nitrogen utilization in lactating cows. However, the highest level of sulfur studies was 0.24%.

VII. RUMEN VOLATILE FATTY ACIDS

A summary of the adjusted means of rumen volatile fatty acid (VFA) concentrations and molar percents is shown in Table 7. Differences among the concentration and molar proportions of acetate, proprionate, isobutyrate, butyrate, and valerate were not significant among experimental rations. The majority of variation in rumen VFA concentrations was due

TABLE 7. Effect of Supplement on Rumen Volatile Fatty Acid Composition of Steers Consuming Complete Corn Silage Rations^a

	Supplement			SE ^b
	Alone	SBM	LPS	
----- (μ moles/ml) -----				
Acetate	30.1	31.4	33.1	0.61
Propionate	8.9	8.5	9.9	0.71
Isobutyrate	0.40	0.40	0.40	0.05
Butyrate	4.6	4.2	5.1	0.34
Isovalerate	0.68 ^c	0.60 ^c	1.06 ^d	0.08
Valerate	0.57	0.60	0.63	0.02
Total	45.3	4.57	50.3	3.67
----- (molar %) -----				
Acetate	66.0	67.8	65.4	0.77
Propionate	19.3	18.8	19.1	0.52
Isobutyrate	0.9	0.9	1.0	0.08
Butyrate	10.1	9.0	10.2	0.40
Isovalerate	1.6 ^c	1.3 ^c	2.1 ^d	0.10
Valerate	1.3	1.3	1.2	0.09

^aMeans adjusted for residual treatment effect.

^bStandard error of direct treatment means for complete rations.

^{c,d}Means in same line having different superscripts are significantly different ($P < .05$).

to individual animal effects (Appendix, Table 17).

The concentration and molar percent of isovalerate were higher in animals receiving the LPS supplemented ration. This observation is contrary to previous reports (55, 76). Martin and Wing (55) reported a significant reduction in isovalerate when molasses was added to a high energy ration. Molar percents of all other acids were unaffected by level of molasses in the ration. Rumsey et al. (76) noticed a trend toward proportions of iso-acids in pasturing steers consuming a self-fed liquid molasses-urea supplement.

CHAPTER V

SUMMARY AND CONCLUSIONS

Six Holstein steers, averaging 329 kg. in body weight, were used in a metabolism study to determine the effects of ration on feed intake, nutrient digestibility, nitrogen utilization, mineral balance, plasma mineral status, and rumen volatile fatty acid concentrations. Two 3 x 3 latin squares balanced for residual effects of treatment were employed with three experimental corn silage rations: (1) unsupplemented; (2) soybean meal (SBM) supplemented; and (3) liquid molasses-urea (LPS) supplemented. Supplements were mixed with silage at time of feeding to raise the ration to 12% crude protein on a dry matter basis. All rations were offered to ensure ad libitum consumption with at least 10% refusals. Daily fecal and urinary excretions were collected for five days following a seven-day adjustment and a seven-day intake measurement period. Blood and rumen fluid samples were collected at the end of each trial.

Feed, fecal, and refusal samples were analyzed for dry matter, crude protein, ether extract, ash, acid-detergent fiber, acid-insoluble lignin, and minerals. Urine samples were analyzed for nitrogen and minerals. Samples of rumen fluid were analyzed for volatile fatty acid concentrations. Minerals examined in this study included calcium, phosphorus, magnesium, and potassium. Composite feed samples were subjected to sulfur and soluble nitrogen analysis.

The data indicated that feed intake and digestibility of dry matter, acid-detergent fiber, and acid-detergent cellulose were not significantly

different among rations. Supplementation of corn silage with LPS tended to decrease digestibility of these nutrients and significantly reduced organic matter digestibility when compared to the SBM supplemented ration. Crude protein digestibility was highest in the LPS supplemented ration, but ether extract digestibility was significantly reduced.

A comparison of nitrogen utilization data indicated that nitrogen metabolism was not significantly affected by supplements. Differences in amounts of fecal and urinary nitrogen excreted by animals consuming either supplemented rations were not significant. A higher nitrogen intake by animals consuming the LPS supplemented ration thus resulted in a larger amount of nitrogen retained by these animals. Calculated values of true nitrogen digestibility, biological value, and net protein utilization were not significantly different between supplemented rations.

Mineral balance and plasma mineral concentrations were generally unaffected by experimental ration. Though a significantly lower magnesium balance was noted in animals consuming unsupplemented corn silage, plasma magnesium levels were not significantly altered.

Rumen concentration and molar percent of isovalerate was higher when animals consumed the LPS supplemented ration compared to the other two rations. Concentration and molar percent of the other volatile fatty acids, however, was not affected by ration.

LITERATURE CITED

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1. Allison, M.J. 1969. Biosynthesis of amino acids by ruminal microorganisms. *J. Anim. Sci.*, 29:797.
2. Archibald, J.G. 1941. A study of urea as a partial substitution for protein in the ration of dairy cows. *Mass. Exp. Stat. Bull.*, 378:21.
3. Armsby, H.P. 1911. The nutritive value of the nonprotein nitrogen of feeding stuffs. *USDA Bur. of Anim. Indus., Bull.*, 139:49.
4. A.O.A.C. Official Methods of Analysis. 1975. (12th ed.) Association of Official Agricultural Chemists, Washington, DC.
5. Barth, K.M., J.A. Corrick, P.E. Shumway and S.W. Coleman. 1974. Effects of level of urea plus limestone on N metabolism of corn silage based rations for cattle. *J. Anim. Sci.*, 38:687.
6. Bentley, O.G., R.R. Johnson, S. Vanecko and C.H. Hunt. 1954. Studies on factors needed by rumen microorganisms for cellulose digestion in vitro. *J. Anim. Sci.*, 13:581.
7. Bergen, W.G., D.B. Purser and J.H. Cline. 1967. Enzymatic determination of protein quality of individual rumen bacteria. *J. Nutr.*, 92:357.
8. Bohman, V.R., G.W. Trimberger, J.K. Loosli and K.L. Turk. 1954. The utilization of molasses and urea in the ration of growing cattle. *J. Dairy Sci.*, 37:284.
9. Bouchard, R. and H.R. Conrad. 1973. Sulfur requirements for lactating cows. II. Utilization of sulfates, molasses and lignin-sulfate. *J. Dairy Sci.*, 56:1429.
10. Burroughs, W., D.K. Nelson and D.R. Mertens. 1975. Protein physiology and its application in the lactating cow: The metabolizable protein feeding standard. *J. Anim. Sci.*, 41:933.
11. Campbell, J.R., W.M. Howe, F.A. Martz and C.P. Merilan, 1963. Effects of frequency of feeding on urea utilization and growth characteristics in dairy heifers. *J. Dairy Sci.*, 46:131.
12. Chalupa, W. 1968. Problems in feeding urea in ruminants. *J. Anim. Sci.*, 27:207.

13. Chalupa, W. 1972. Metabolic aspects on nonprotein nitrogen utilization in ruminant animals. *Fed. Proc.*, 31:1152.
14. Chicco, C.F., T.A. Shulta, E. Shultz, A.A. Carnevali and C.B. Ammerman. 1972. Molasses-urea for restricted forage fed steers in tropics. *J. Anim. Sci.*, 35:859.
15. Claypool, D.W. 1976. Factors affecting calcium, phosphorus, and magnesium status of dairy cattle on the Oregon coast. *J. Dairy Sci.*, 59:2005.
16. Coleman, S.W. 1972. N-utilization by cattle fed rations consisting mainly of biuret and low quality hay and factors affecting N-utilization by cattle fed urea-corn silage rations. Ph.D. Dissert. Univ. of Tenn., Knoxville.
17. Cook, R.J. and D.G. Fox. 1976. Anhydrous ammonia addition to corn silage. *J. Anim. Sci.*, 43:317.
18. Dutrow, N.A., J.T. Huber and H.E. Henderson. 1974. Comparison of ammonia, salts and urea in rations for lactating dairy cows. *J. Anim. Sci.*, 38:304.
19. Elias, A. and A. Delgado. 1976. Intensive beef production from sugarcane. 12. Effects of maize-wheat supplementation to the molasses/urea diet of bulls. *Cuban J. Agr. Sci.*, 10:81.
20. Erwin, E.S., G.J. Marce and E.M. Emerly. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.*, 44:1768.
21. Fiske, C.H. and Y. SubbaRow. 1925. The colormetric determination of phosphorus. *J. Biol. Chem.*, 66:375.
22. Foreman, C.F. and H.A. Herman. 1953. Effects of carbohydrate feeding levels on roughage digestion in dairy cattle. *Mo. Agr. Exp. Stat., Bull.* 535.
23. Garrigus, V.S. 1960. Pinch hitting for protein. *Feedstuffs*. Sept.
24. Giardini, A., F. Lambertini, F. Gaspari and A. LoBruno. 1976. Levels, sources, and methods of nitrogen supplementation of maize silage for beef production. *Anim. Feed Sci. and Tech.*, 1:465.
25. Goatcher, R.S. and D.C. Church. 1970. Taste response in ruminants. 11. Reactions of sheep to acids, quinine, urea and sodium hydroxide. *J. Anim. Sci.*, 30:784.

26. Grelen, H.E. and H.A. Pearson. 1977. Liquid supplements for cattle on southern forest range. *J. Range Manag.*, 30:94.
27. Harris, L.E. and H.H. Mitchell. 1941. The value of urea in the synthesis of protein in the paunch of the ruminant. I. In maintenance. *J. Nutr.*, 22:167.
28. Harris, L.E. and H.H. Mitchell. 1941. The value of urea in the synthesis of protein in the paunch of the ruminant. II. In growth. *J. Nutr.*, 22:183.
29. Hart, E.B., G. Bohstedt, H.J. Deobald and M.L. Wagner. 1939. The utilization of simple nitrogenous compounds such as urea and ammonia bicarbonate by growing calves. *J. Dairy Sci.*, 22:785.
30. Helmer, L.G., E.E. Bartley, C.W. DeYoe, R.M. Meyer and H.B. Pfof. 1970. Feed processing. V. Effect of an expansion-processed mixture of grain and urea (Starea) on nitrogen utilization in vitro. *J. Dairy Sci.*, 53:330.
31. Hendrickx, H.K. 1967. The effectiveness of urea and other non-protein nitrogen compounds in ruminant feeding. In: *Urea as a protein supplement.*, M.H. Griggs, ed. Pergamon Press, New York.
32. Henderson, H.E., W.G. Bergen and C.M. Hausen. 1972. Corn silage additives compared. *J. Anim. Sci.*, 35:229. (Abstr.).
33. Henderson, H.E. and M.R. Geasler. 1970. Anhydrous ammonia, urea, and mineral additives to corn silage. *Mich. Agri. Exp. Stat. Res. Rep.*, 108:3.
34. Hershberger, T.V., P. Engle, O.G. Bentley and A.L. Moxan. 1955. Utilization of the nitrogen in some ammoniated feeds by rumen microbes. *J. Anim. Sci.*, 14:1907.
35. Hoshino, S., S. Katsuharu and K. Morimoto. 1966. Ammonia anabolism in ruminants. *J. Dairy Sci.*, 49:1523.
36. Huber, J.T. 1972. Research on liquid nitrogen supplements for dairy cattle. *J. Anim. Sci.*, 34:166.
37. Huber, J.T. 1975. Protein and non-protein nitrogen utilization in practical dairy rations. *J. Anim. Sci.*, 41:954.
38. Huber, J.T., C.E. Polan and D. Hillman. 1968. Urea in high corn silage rations for dairy cattle. *J. Anim. Sci.*, 27:220.

39. Huber, J.T., R.A. Sandy, C.E. Polan, H.T. Bryant and R.E. Blaser. 1967. Varying levels of urea for dairy cows fed corn silage as the only forage. *J. Dairy Sci.*, 50:1241.
40. Huber, J.T. and J.W. Thomas. 1971. Urea-treated corn silage in low protein rations for dairy cattle. *J. Dairy Sci.*, 54:224.
41. Hume, I.D. 1970. Synthesis of microbial protein in the rumen. III. The effect of dietary protein. *Austr. J. Agric. Res.*, 21:305.
42. Hungate, R.E. 1966. The rumen and its microbes. Academic Press, New York.
43. Huntsberger, P.V. and P. Billingsley. 1976. Elements of Statistical Inference (3rd ed.) Allyn and Bacon, Inc., Boston.
44. Johnson, R.R. 1976. Influence of carbohydrate solubility on non-protein nitrogen utilization in the ruminant. *J. Anim. Sci.*, 43:184.
45. Johnson, R.R., K.E. McClure, E.W. Klosterman and L.J. Johnson. 1967. Corn plant maturity. III. Distribution of nitrogen in corn silage treated with limestone, urea or diammonium phosphate. *J. Anim. Sci.*, 26:394.
46. Jones, G.A. 1967. Ureolytic rumen bacteria. In: Urea as a protein supplement. M.H. Briggs, ed. Pergamon Press, New York.
47. Kennedy, B. 1970. Liquid supplements for beef? *Tenn. Farmer*. Sept.
48. King, W.A., J.P. LaMaster, G.D. O'Dell and D.B. Roderick. 1956. Feeding blackstrap molasses to dairy cattle. *S.C. Agr. Exp. Stat. Bull.*, 433.
49. King, W.A., G.D. O'Dell, J.P. LaMaster and D.B. Roderick. 1955. Comparison of cottonseed meal, molasses containing urea and ammoniated molasses as protein supplements for dairy heifers. *J. Dairy Sci.*, 38:624. (Abstr.).
50. King, W.A., G.D. O'Dell and D.B. Roderick. 1957. Utilization of blackstrap molasses, urea in molasses, and ammoniated molasses by dairy heifers. *J. Dairy Sci.*, 40:810.
51. Kropp, J.R., R.R. Johnson, J.R. Miles and F.N. Owens. 1977. Microbial protein synthesis with low quality roughage rations: Isonitrogenous substitution of urea for soybean meal. *J. Anim. Sci.*, 45:837.

52. Little, C.O., G.E. Mitchell Jr. and G.D. Potter. 1965. Dietary influences of ruminal fluid proteins. *J. Anim. Sci.*, 22:893. (Abstr.).
53. Loosli, J.K., C.M. McCay and L.A. Maynard. 1942. Utilization of urea by calves less than four months of age. *J. Dairy Sci.*, 25:680.
54. Loosli, J.K., H.H. Williams, W.E. Thomas, F.H. Ferris and L.A. Maynard. 1949. Synthesis of amino acids in the rumen. *Science*, 110:144.
55. Martin, R.J. and J.M. Wing. 1966. Effects of molasses level on digestibility of a high concentrate ration and on molar proportions of volatile fatty acids produced in the rumen of dairy steers. *J. Dairy Sci.*, 49:846.
56. McClaugherty, F.S. and J.P. Fontenot. 1974. Wintering beef cattle on corn silage supplemented with biuret or liquid supplement. *Va. Polytec. Inst. and St. Univ. Res. Div., Rep.* 158:34.
57. McClure, W.H., H.J. Genken Jr., M.B. Wise, J.P. Fontenot and R.C. Carter. 1973. Liquid and dry non-protein nitrogen supplements for finishing steers fed corn silage and grain. *Va. Polytec. Inst. and St. Univ. Res. Div., Rep.* 153:151.
58. McLaren, G.A., G.C. Anderson, W.G. Martin and W.K. Cooper. 1961. Fixation of ammonia nitrogen by rumen mucosa. *J. Anim. Sci.*, 20:942.
59. McNaught, M.L., E.C. Owen, K.M. Henry and S.K. Kon. 1954. The utilization of non-protein nitrogen in the bovine rumen. 8. The nutritive value of the proteins of preparations of dried rumen bacteria, rumen protozoa, and brewer's yeast for rats. *Biochem. J.*, 56:151.
60. Miller, E.L. 1973. Evaluation of foods as sources of nitrogen and amino acids. *Proc. Nutr. Soc.*, 32:79.
61. Mills, R.C., C.C. Lardinois, I.W. Rupel and E.B. Hart. 1944. Utilization of urea and growth of heifer calves with corn molasses and cane molasses as the only readily available carbohydrate in the ration. *J. Dairy Sci.*, 27:571.
62. Montgomery, M.J., H.D. Baxter and B.J. Bearden. 1976. Corn silage supplementation for maximum intake and milk production. *J. Dairy Sci.*, 59:1915.
63. N.R.C. 1975. Nutrient requirements of domestic animals. Nutrient requirements of beef cattle. *Nat. Acad. Sci., Washington, DC.*
64. N.R.C. 1976. Urea and other nonprotein nitrogen compounds in animal

nutrition. Nat. Acad. Sci., Washington, DC.

65. Pathak, N.N., D.V.G. Krishna Mohan, S.K. Ranjhan, R.C. Katiyar and P.N. Bhat. 1976. Utilization of urea-molasses liquid diets along with limited amounts of intact protein and cereal forage for milk production in cross-bred (B. indicus x B. taurus) cows, 2. Effects on digestibility coefficients and efficiency of energy and nitrogen utilization for milk production. Indian J. Anim. Sci., 46:396.
66. Pearson, R.M. and J.A.B. Smith. 1943. The utilization of urea in the bovine rumen. 2. The conversion of urea to ammonia. Biochem J., 37:148.
67. Pendlum, L.C., J.A. Boling and N.W. Bradley. 1976. Plasma and ruminal constituents and performance of steers fed different nitrogen sources and levels of sulfur. J. Anim. Sci., 43:1307.
68. Plummer, J.R., J.T. Miles and M.J. Montgomery. 1971. Effects of urea in the concentrate mix on intake and production of cows fed corn silage as the only forage. J. Dairy Sci., 54:1861.
69. Polan, C.E., J.T. Huber, R.A. Sandy, J.W. Hall Jr. and C.N. Miller. 1968. Urea-treated corn silage as the only forage for lactating cows. J. Dairy Sci., 51:1445.
70. Potter, G.D., C.O. Little, N.W. Bradley and G.E. Mitchell Jr. 1971. Abomasal nitrogen in steers fed soybean meal, urea and urea plus two levels of molasses. J. Anim. Sci., 32:531.
71. Purser, D.B. 1970. Nitrogen metabolism in the rumen: Micro-organisms as a source of protein for the ruminant animal. J. Anim. Sci., 30:988.
72. Purser, D.B. and S.M. Buechler. 1966. Amino acid composition of rumen organisms. J. Dairy Sci., 49:81.
73. Ranjhan, S.K., N.N. Pathak, V.B. Singh, D.N. Verma and A. Verma. 1974. Utilization of urea/molasses liquid feed as the main source of nitrogen and energy for cattle and buffalo. Proc. of Tracer Studies on Non-Protein Nitrogen for Rumin. II. IAEA, Vienna.
74. Roffler, R.E. and L.D. Satter. 1975. Relationship between ruminal ammonia and nonprotein nitrogen utilization by ruminants, 1. Development of a model for predicting nonprotein nitrogen utilization by cattle. J. Dairy Sci., 58:1880.
75. Roussel, J.P., L.F. Gomula, L. McNabb and J.N. Garnett Jr. 1973. Liquid feed supplement and ryegrass for replacement dairy heifers.

- J. Dairy Sci., 56:314. (Abstr.).
76. Rumsey, T.S., J. Blood, R.H. Hart and G.E. Carlson. 1971. Ruminant NH_3 , pH, and VFA of beef cattle on orchardgrass pasture and self-fed liquid molasses-urea or ground corn-fat supplements. J. Anim. Sci., 33:507.
 77. Rush, I.G., R. Helderman, W.E. Sharp and R. Totuske. 1973. Liquid protein supplement vs. dry natural protein supplement for wintering heifer calves. Okla. Agr. Exp. Stat., Misc. Pub., 90:51.
 78. Rush, I.G. and R. Totuske. 1976. Supplemental value of feed grade biuret and urea-molasses for cows on dry winter grass. J. Anim. Sci., 42:497.
 79. Ryley, J.W. 1967. Silage with urea. In: Urea as a protein supplement. M.H. Briggs, ed. Pergamon Press, New York.
 80. Satter, L.D. and R.E. Roffler. 1975. Nitrogen requirement and utilization by dairy cattle. J. Dairy Sci., 58:1219.
 81. Satter, L.D. and L.L. Slyter. 1974. Effect of ammonia concentration on rumen microbial production in vitro. Brit. J. Nutr., 32:199.
 82. Stallcup, O.T., G.V. Davis and L. Shields. 1975. Influence of dry matter and nitrogen intakes on fecal nitrogen losses in cattle. J. Dairy Sci., 58:1301.
 83. Stangel, H.J. 1967. History of the use of urea in ruminant feeds. In: Urea as a protein supplement. M.H. Briggs, ed. Pergamon Press, New York.
 84. Swanson, E.W. 1977. Factors for computing requirements of protein for maintenance of cattle. J. Dairy Sci., 60:1583.
 85. Thomas, P.C. 1973. Microbial protein synthesis. Proc. Nutr. Soc., 32:82.
 86. Thompson, L.B., M.B. Wise, R.W. Harvey and E.R. Barrick. 1972. Starea, urea, and sulfur in beef cattle rations. J. Anim. Sci., 35:474.
 87. Totuske, R., J.W. Hollaway and W.E. Sharp. 1971. Self-fed liquid and dry supplements for wintering range cows. Okla. Agr. Exp. Stat., Misc. Pub., 85:36.
 88. Van Arsdell, W.J., J.A. Hoefer, G.A. Branaman and R.W. Lueche. 1953. Supplementing corn silage for fattening steers. J. Anim. Sci., 12:1934. (Abstr.).

89. Van Horn, H.H., D.R. Jacobson and A.P. Graden. 1969. Influence of level and source of nitrogen on milk production and blood components. *J. Dairy Sci.*, 52:1395.
90. Van Horn, H.H. and J.S. Mudd. 1971. Comparison of a liquid supplement of non-protein nitrogen with urea and soybean meal for lactating cows. *J. Dairy Sci.*, 54:58.
91. Van Soest, P.J. 1963. The use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *J. Assn. Offic. Agr. Chemists*, 46:829.
92. Virtanen, A.I. 1967. The production of milk on protein free diets. In: Urea as a protein supplement. M.H. Briggs, ed. Pergamon Press, New York.
93. Weller, R.A. 1957. The amino acid composition of hydrolysates of microbial preparations from the rumen of sheep. *Austr. J. Biol. Sci.*, 10:384.
94. Whitlow, L.H., S.P. Marshall, H.H. Van Horn and J.R. Florus. 1976. Liquid feed passage route into stomach compartments, influence of abomasal infusions on plasma glucose, and supplementation on dry rations with liquid feeds from lick wheel feeders. *J. Dairy Sci.*, 59:675.
95. Williams, E.J. 1949. Experimental designs balanced for the estimation of residual effects of treatments. *Austr. J. Sci. Res.*, 2:149.
96. Wise, G.H., J.H. Mitchell, J.P. LaMaster and D.B. Roderick. 1944. Urea-treated corn silage vs. untreated corn silage as a feed for lactating cows. *J. Dairy Sci.*, 27:649. (Abstr.).
97. Wohlt, J.E., C.J. Sniffen and W.H. Hoover. 1973. Measurement of protein solubility in common feedstuffs. *J. Dairy Sci.*, 56:1052.
98. Young, A.W., N.W. Bradley, C.O. Little and J.A. Boling. 1969. Level of feed intake and molasses addition to urea rations for feedlot steers. *Ky. Agr. Exp. Stat., Prog. Rep.*, 181:32.

APPENDIX

TABLE 8. Analysis of Variance of Digestibility Coefficients, Dry Matter Intake, and Average Weight Change Data

Source of Variation	Degrees of Freedom	Mean Squares						
		Apparent Digestibility (%)			Daily DM Intake (kg)		Weight Change (kg)	
		DM ^a	OM ^b	CPC	ADFD	ADCE	EEF	
Total	17							
Direct TMT								
Unadjusted	2	9.8	10.3*	152.1**	33.4	34.2	14.1*	0.18
Adjusted	2	9.9	9.9*	123.2**	33.4	32.3	11.0*	0.30*
Residual TMT								
Adjusted	2	1.3	1.7	1.6	2.6	2.7	0.2	0.15
Unadjusted	2	1.2	2.1	30.1	2.7	4.6	3.2	0.03
Animal	5	3.6	3.3	24.6*	2.5	1.0	9.1*	0.27
Trial (square)	4	1.2	1.2	8.5	2.7	3.7	33.4**	1.52*
Error	5	1.6	1.6	2.2	10.8	10.6	0.9	0.04

*P < .05.

**P < .01.

^aDry matter.

^bOrganic matter.

^cCrude protein.

^dAcid-detergent fiber.

^eAcid-detergent cellulose.

^fEther extract.

TABLE 9. Analysis of Variance of Nitrogen Balance and Utilization Data

Source of Variation	Degrees of Freedom	Mean Squares					NPU ^a
		Intake	Nitrogen (g/day)		Retained	True Digest. (%)	Biological Value (%)
Total	17						
Direct TMT							
Unadjusted	2	4583.3**	123.8*	1578.0*	507.3*	5.2	220.3
Adjusted	2	3593.4**	98.5*	1930.5**	205.6*	7.4	374.9
Residual TMT							
Adjusted	2	7.0	3.2	360.7	389.6*	2.7	266.7
Unadjusted	2	996.9**	28.5	8.1	691.5*	0.5	112.0
Animal	5	186.5	65.4	69.4	179.3	11.8	109.8
Trial (square)	4	183.0	58.5	42.6	12.2	7.3	20.1
Error	4	30.8	12.1	95.5	46.2	3.4	55.6

*R < .05.

**P < .01.

^aNet Protein Utilization.

TABLE 10. Analysis of Variance of Mineral Retention and Plasma Mineral Concentration Data

Source of Variation	Degrees of Freedom	Mean Squares					
		Mineral Retained (g/day)			Plasma Minerals (mg/100 ml)		
		Ca	P	Mg	Ca	P	K
Total	17						
Direct TMT							
Unadjusted	2	0.7	9.4	28.7**	0.17	0.84	0.02
Adjusted	2	0.4	21.7	27.0**	0.18	1.09	0.01
Residual							
Adjusted	2	1.3	21.5	1.8	0.11	0.26	0.01
Unadjusted	2	1.6	9.2	3.5	0.11	0.00	0.02
Animal	5	5.8	9.9	5.2*	0.51	1.41*	0.04
Trial (square)	4	12.2	37.8	5.9*	0.13	2.62*	0.01
Error	4	11.3	13.8	0.5	0.11	0.18	0.02

*P < .05.

**P < .01.

TABLE 11. Analysis of Variance of Rumen Fluid Volatile Fatty Acid Concentration Data

Source of Variation	Degrees of Freedom	Mean Squares						
		VFA Concentrations (μ moles/ml)					Total	
		Acet.	Prop.	Isobut.	Buty.	Isoval.		
Total	17							
Direct TMT								
Unadjusted	2	9.0	4.4	0.05	1.8	0.36*	0.01	51.0
Adjusted	2	11.2	2.3	0.02	1.0	0.29*	0.01	41.1
Residual TMT								
Adjusted	2	105.0	6.8	0.07	3.0	0.02	0.02*	223.5
Unadjusted	2	102.7	8.9	0.10*	3.8	0.08	0.03*	233.4
Animal	5	260.7	24.0*	0.04	5.3*	0.11	0.09*	592.9*
Trial (square)	4	11.6	0.9	0.01	0.3	0.04	0.01	6.4
Error	4	40.9	3.0	0.01	0.7	0.04	0.01	80.8

***P < .05.**

TABLE 12. Analysis of Variance of Rumen Fluid Volatile Fatty Acids Expressed as Molar Percent of Total VFA Concentrations

Source of Variation	Degrees of Freedom	Mean Squares			
		Acet.	Prop.	VFA Concentration (molar %)	Isoval. Val.
Total	17				
Direct TMT					
Unadjusted	2	13.2	0.9	0.14	2.4
Adjusted	2	7.5	0.3	0.03	2.2
Residual TMT					
Adjusted	2	2.3	1.2	0.15	0.6
Unadjusted	2	8.1	1.6	0.26	0.9
Animal	5	1.9	2.8	0.05	0.6
Trial (square)	4	17.9	6.7	0.29*	1.3
Error	4	3.6	1.6	0.04	1.0

*P < .05.

TABLE 13. Body Weights, Dry Matter Intake, and Nutrient Digestibility for Individual Animals

Supplement and Animal Number	Body Weight (kg)		Dry Matter Intake (kg)	Digestibility Coefficients (%)					
	Initial	Final		DMA ^a	OMB ^b	CPC ^c	ADF ^d	ADCE ^e	EE ^f
TRIAL I									
Alone									
201	305	306	5.95	67.4	68.1	62.1	58.5	64.0	78.7
204	345	345	6.14	64.7	65.2	59.3	52.8	59.4	75.4
SBM									
197	332	337	6.88	67.1	68.2	61.5	59.4	66.1	74.6
198	337	345	6.42	67.0	67.5	61.9	57.3	63.8	75.4
LPS									
200	313	300	4.97	66.3	66.8	69.4	49.6	57.0	76.8
205	341	329	5.27	62.4	63.6	69.2	51.3	57.7	73.4
TRIAL II									
Alone									
198	345	365	6.66	63.7	64.7	49.2	54.2	60.4	80.3
200	300	311	5.82	66.8	67.4	59.5	58.4	64.9	86.7
SBM									
204	345	365	7.47	68.3	69.2	62.4	58.2	65.6	82.1
205	329	335	7.55	66.7	67.9	60.2	55.1	62.0	81.2
LPS									
197	337	353	7.14	65.7	66.9	64.7	51.2	57.4	81.7
201	306	324	7.22	67.5	68.8	70.6	55.4	61.0	81.4
TRIAL III									
Alone									
197	353	335	6.68	65.1	66.1	57.6	53.9	60.6	81.3
205	335	319	7.15	66.7	67.7	56.8	57.7	64.8	80.0
SBM									
200	311	298	6.53	69.5	70.7	64.8	57.0	63.4	81.8
201	324	323	7.16	68.7	67.7	65.4	57.7	63.9	80.4
LPS									
198	365	350	7.58	65.3	66.6	62.5	54.6	62.1	75.6
204	365	361	7.74	66.5	67.5	68.4	54.8	61.1	75.4

^aDry matter.^dAcid-detergent fiber.^bOrganic matter.^eAcid-detergent cellulose.^cCrude protein.^fEther extract.

TABLE 14. Nitrogen Balance and Utilization of Individual Animals

Supplement and Animal Number	Nitrogen (g/day)			True N Digesti- bility (%)	Biological Value (%)	Net Protein Utilization
	Intake	Fecal	Urinary			
TRIAL I						
Alone						
201	90.0	34.5	36.3	85.4	63.1	53.9
204	94.5	38.5	33.3	84.4	68.5	57.8
SBM						
197	125.7	48.4	62.0	81.2	47.1	38.2
198	115.0	43.9	64.3	82.0	40.4	33.2
LPS						
200	124.7	38.2	64.4	84.0	45.9	38.6
205	132.8	40.8	62.6	85.5	51.9	44.4
TRIAL II						
Alone						
198	99.2	50.5	42.3	75.7	54.7	41.5
200	85.1	34.5	55.1	84.2	33.8	28.4
SBM						
204	135.4	51.0	41.3	81.4	70.0	57.0
205	135.0	53.8	76.2	80.5	37.2	30.0
LPS						
197	157.5	55.7	65.7	81.6	55.3	45.1
201	160.6	47.2	76.0	86.5	50.9	44.1
TRIAL III						
Alone						
197	92.4	39.2	30.4	85.1	71.7	61.0
205	96.4	41.7	34.1	83.7	67.6	56.6
SBM						
200	112.1	39.5	56.0	84.1	48.8	41.1
201	127.4	44.0	80.8	84.6	32.3	27.3
LPS						
198	153.0	57.4	68.0	81.2	51.9	42.2
204	160.3	50.6	77.7	86.1	49.7	42.8

TABLE 15. Calcium and Phosphorus Balance for Individual Animals

Supplement and Animal Number	Calcium (g/day)			Phosphorus (g/day)		
	Intake	Fecal	Urinary	Intake	Fecal	Urinary
TRIAL I						
<u>Alone</u>						
201	20.0	13.9	0.6	16.3	14.4	0.07
204	20.1	14.5	0.2	16.6	13.5	0.06
<u>SBM</u>						
197	22.1	17.8	0.2	19.1	18.4	0.03
198	20.9	14.6	0.2	18.0	14.2	0.04
<u>LPS</u>						
200	22.7	12.7	0.1	31.8	18.1	0.04
205	23.7	16.0	3.4	33.0	23.9	0.60
TRIAL II						
<u>Alone</u>						
198	21.7	22.0	0.4	15.0	19.4	0.03
200	18.4	13.3	0.1	12.9	11.3	0.13
<u>SBM</u>						
204	30.1	20.5	0.2	18.5	18.0	0.06
205	29.8	25.7	0.4	18.3	19.9	0.06
<u>LPS</u>						
197	24.5	20.6	0.1	23.5	24.6	0.03
201	23.9	20.8	0.2	22.8	26.1	0.05
TRIAL III						
<u>Alone</u>						
197	26.0	16.7	0.3	15.9	12.5	0.07
205	27.5	18.0	0.3	17.1	13.7	0.05
<u>SBM</u>						
200	21.8	16.1	0.3	14.7	15.4	0.05
201	24.3	15.3	0.5	18.0	12.0	0.05
<u>LPS</u>						
198	30.1	24.4	0.9	26.3	24.8	1.64
204	30.7	22.9	0.1	27.0	23.1	0.06

TABLE 16. Magnesium and Potassium Balance for Individual Animals

Supplement and Animal Number	Magnesium (g/day)			Potassium (g/day)		
	Intake	Fecal	Urinary	Intake	Fecal	Urinary
TRIAL I						
Alone						
201	12.8	6.5	5.3	90.3	30.2	60.6
204	13.3	7.1	5.5	93.1	9.9	80.5
SBM						
197	15.3	8.9	0.9	106.7	23.7	76.5
198	14.4	7.8	1.2	99.9	8.3	79.8
LPS						
200	12.8	7.6	0.5	84.6	18.1	67.4
205	13.5	8.4	1.8	89.9	20.2	60.3
TRIAL II						
Alone						
198	16.3	10.9	6.9	92.4	11.1	81.3
200	13.9	7.8	4.2	80.8	9.7	94.8
SBM						
204	19.0	9.2	7.6	106.3	11.9	88.0
205	19.2	13.9	2.1	107.4	19.3	95.1
LPS						
197	16.6	13.0	1.4	124.1	21.4	96.4
201	16.7	10.9	5.5	125.6	29.0	94.6
TRIAL III						
Alone						
197	14.7	7.4	5.9	97.0	13.4	71.7
205	15.7	12.6	6.3	103.8	21.2	68.5
SBM						
200	14.9	6.2	1.8	97.2	12.8	78.0
201	16.3	8.5	4.9	106.7	22.4	76.9
LPS						
198	18.9	12.8	4.9	129.6	26.6	98.6
204	19.1	10.9	3.5	133.4	30.9	89.7

TABLE 17. Calcium, Phosphorus, Magnesium, and Potassium Concentration of Plasma From Individual Animals

Supplement and Animal Number	Concentration (mg/ml)			
	Calcium	Phosphorus	Magnesium	Potassium
TRIAL I				
Alone				
201	9.5	6.30	2.20	16.9
204	9.0	6.36	2.12	14.2
SBM				
197	10.3	6.61	2.34	18.0
198	8.8	7.50	2.12	16.0
LPS				
200	9.5	5.78	1.70	14.9
205	9.1	6.66	2.00	14.8
TRIAL II				
Alone				
198	9.6	8.80	2.13	19.1
200	9.9	6.90	2.04	18.0
SBM				
204	9.8	7.90	2.03	17.0
205	9.7	8.50	2.09	17.8
LPS				
197	10.2	8.50	2.05	18.2
201	8.8	8.40	2.28	18.8
TRIAL III				
Alone				
197	10.3	7.30	2.22	21.8
205	9.8	7.60	1.91	19.0
SBM				
200	9.5	6.90	1.97	17.0
201	9.1	9.20	2.12	20.4
LPS				
198	8.6	9.60	2.05	18.5
204	9.6	8.60	2.01	20.6

TABLE 18. Volatile Fatty Acid Concentration of Rumen Fluid From Individual Animals

Supplement and Animal Number	VFA Concentration (μ moles/ml)						Total
	Acet.	Prop.	Isobut.	Buty.	Isoval.	Val.	
TRIAL I							
Alone							
201	14.5	3.9	0.26	2.5	0.59	0.34	22.1
204	35.8	11.1	0.35	4.8	0.69	0.74	53.6
SBM							
197	23.7	12.3	0.32	3.2	0.57	0.64	40.7
198	44.0	8.1	0.37	5.8	0.82	0.90	60.0
LPS							
200	26.8	8.0	0.56	4.1	0.90	0.60	41.0
205	34.0	8.6	0.47	5.9	1.59	0.71	51.3
TRIAL II							
Alone							
198	50.2	13.6	0.86	7.8	1.08	0.91	74.5
200	23.5	6.1	0.27	3.5	0.38	0.41	34.2
SBM							
204	31.6	7.0	0.31	3.6	0.49	0.49	43.5
205	37.2	8.6	0.35	5.1	0.66	0.57	52.5
LPS							
197	43.9	12.1	0.60	5.5	0.93	0.73	63.8
201	14.3	3.6	0.23	2.0	0.61	0.29	21.0
TRIAL III							
Alone							
197	35.8	11.1	0.33	6.0	0.77	0.55	54.6
205	35.2	11.2	0.52	5.3	0.74	0.67	53.6
SBM							
200	17.8	5.7	0.37	2.6	0.47	0.41	27.4
201	26.6	6.9	0.29	3.7	0.44	0.29	38.2
LPS							
198	37.8	11.3	0.59	5.6	1.08	0.71	57.1
204	35.0	11.1	0.69	6.0	1.17	0.73	54.7

VITA

Stanley William Telega was born in Clairton, Pennsylvania on October 25, 1954. He attended St. Clare Parish elementary school in Clairton and Serra Catholic High School in McKeesport, where he graduated in 1972. In September of that year, he enrolled in The Pennsylvania State University, University Park.

He completed the Bachelor of Science degree in June, 1976, majoring in Animal Science. He accepted a research assistantship at the Animal Science Department of the University of Tennessee and completed the Master of Science degree in August, 1978, with emphasis in ruminant nutrition.