EVALUATION OF TALLOW AND FORMALDEHYDE APPLIED TO PROTEIN FOR WINTER SUPPLEMENTATION OF BEEF COWS CONSUMING LOW QUALITY FORAGES

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1975

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1977

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
December, 1980

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ACKNOWLEDGMENTS

Sincere appreciation and thanks are extended to Dr. Keith Lusby, my major adviser, for contributing his enthusiasm, guidance, and expertise to my graduate program.

I would like to give a special thanks to Dr. Fred Owens for his critical review of this manuscript along with Dr. Joe Whiteman, Dr. Gerald Horn, and Dr. E. C. Nelson while serving on the guidance committee.

Further appreciation is extended to Mike Dvorak, Ken Poling, and Leon Knori for their care of the experimental animals.

Thanks are also extended to my fellow graduate students for their friendship and assistance.

Deep gratitude is also extended to my parents for their support and encouragement.

Finally, a very special thanks goes to my wife, Renate, whose understanding and encouragement made this work possible.

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CHAPTER I

INTRODUCTION

The value of the food ingested by the ruminant is modified by the microbial action of the rumen. The nature and extent of modification are largely dependent on the ratio and origin of the constituent materials in the diet.

Infusing different sources of protein directly into the duodenum or abomasum of sheep has resulted in improved protein utilization, indicating that the microbial degradation of proteins may be wasteful. The extent of deamination was found to be related to the amount of ammonia which was formed in the rumen and the rate of ammonia released by different protein sources. The difference in the ability of various protein sources to improve protein utilization should be related to the extent of degradation in the rumen and the differences in the amino acids absorbed from the intestines.

Since excessive degradation of proteins in the rumen is wasteful, altering the solubility and degradability of various dietary protein sources for ruminants by modification of the protein structure, may have the potential to increase ruminant performance and the profitability of the beef cow enterprise. The purposes of this study were: 1) to evaluate the effects of adding tallow to cottonseed meal and soybean meal with heat and either pressure or vacuum on ruminal ammonia concentrations, digestion, and growth by lambs; 2) to determine the effect of

pelleting (and concurrent heating) on ruminal bypass of protein as indicated by winter weight change of lactating cows; and 3) to determine the effect of formaldehyde treatment of soybean meal on nitrogen balance of lambs and winter weight change of cows grazing winter native range.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Ruminants have a very unique and complex system for protein metabolism. This is primarily due to the symbiotic relationship of the ruminal microorganisms with the host. This relationship allows utilization of less expensive nonprotein nitrogen by the host when adequate energy is present in the rumen for bacterial protein synthesis. However, fermentation of dietary protein in the rumen results in protein quality loss to the host animal. Any method of altering higher quality dietary protein to become more resistant to rumen microbial degradation while maintaining high digestibility in the lower gastrointestinal tract should improve protein utilization provided bacterial protein synthesis is not inhibited. Physical and chemical treatment of dietary protein has been shown to reduce ruminal protein degradation and increase nitrogen retention in ruminants. This review will deal with physical and chemical treatment of dietary protein and the reported responses of ruminants fed these products.

The ruminant absorbs its amino acids from two primary sources: first, from the digestion of microbial protein synthesized in the rumen and, secondly, from the digestion of dietary protein which escapes fermentation in the rumen. Although amino acids from the proteolysis of dietary protein can be incorporated directly into bacterial protein, the

major pathway in the fermentation of protein appears to involve deamination of the amino acids and de novo synthesis of the amino acids by bacteria (Hungate, 1966). In addition, approximately 30% of the bacterial protein produced in the rumen is also degraded therein (Hungate et al., 1959). The factors influencing the extent of rumen degradation are not completely understood, but work with four different feeding levels (Orskov and Fraser, 1973) showed convincingly that for a given protein source the rumen retention time was probably the most important single factor, since with a low level of feeding (below maintenance energy level) protein from soybean meal appeared to be completely degraded, while with a high level of feeding (more than twice the maintenance energy level) the degradation appeared to be only in the region of 40%. Conclusions of several reviewers (Hogan, 1974; Leng, 1973; Lewis et al., 1971; Satter and Roffler, 1974; Smith, 1969, and Smith et al., 1974) indicate that as little as 40% or as much as 80% of the dietary protein might be degraded in the rumen and transferred into microbial protein. Although Purser and Buechler (1966) reported that 22 strains of rumen bacteria were similar in amino acid composition, Bergen et al. (1967) suggested that the availability of the amino acids from different strains varied markedly and suggested that modification of the bacterial population could alter the nitrogen status of the animal. There is some evidence that certain strains of bacteria have a need for amino acids in addition to ammonia (Hatfield, 1970). Pittman and Bryant (1964) and Wright (1967) found that some strains of rumen bacteria preferred peptide nitrogen over other sources of nitrogen.

Proteins with a balanced amino acid compostition have a higher biological value when digested post-ruminally (Little and Mitchell, 1967).

But hemicellulose and cellulose are poorly used past the rumen (Hogan and Weston, 1967). Thus, the optimal digestion site differs with different nutrients. Ideally, the rumen should be used to digest cellulose and hemicellulose and build bacterial protein using non-protein nitrogen and proteins of low biological value. Physically bypassing the rumen for intestinal digestion of the other nutrients would improve efficiency (Owens and Isaacson, 1977).

The change in quantity and quality of protein between the diet and the duodenum is perhaps the most important aspect of nitrogen metabolism. Results from a number of experiments indicate that rumen output of microbial protein is a function of the amount of organic matter fermented in the rumen and thus of the amount of energy available to the microbes for their growth (McGilliard, 1972).

Because the rumen microbial protein production is an energy-dependent mechanism, the amount of dietary protein transformed into microbial protein must be an important aspect of the nitrogen economy of the animal and should be an important factor in whether or not to decrease ruminal degradation by artificial procedures (Chalupa, 1975).

Fat Addition to Protein

Attempts have been made to increase the amount and quality of protein reaching the ruminants intestine. Black (1971) illustrated the advantage of preventing degradation of protein in the rumen. Calculations were made for the theoretical utilization of dietary protein for a 20 kg lamb whose entire diet was fermented in the rumen (ruminant lamb) and for another lamb whose diet was digested by host enzymes in the lower gut (nonruminant lamb). Based upon a daily gross energy intake of 1780 kcal and protein intake of

61 g, the net daily dietary protein value[1] was 24.3 g for the ruminant lamb and 45.4 g for the nonruminant lamb. Thus, fermentation of dietary protein in the rumen reduced the protein available for utilization by about 50%. There seem to be several alternatives for reducing or preventing the degradation of proteins in the rumen so that they would pass to the abomasum and intestines for subsequent digestion. Certain natural proteins such as zein, which are very insoluble in rumen fluid, are not readily degraded in the rumen (Ely et al., 1967) but are also poorly digested in the small intestine (Little and Mitchel, 1967).

Supplementation of ruminant diets with free oil alters the composition, mobility, and metabolic activity of rumen microflora (Czerkawski, 1967; Marwaha et al., 1972) and results in changes in cellulose and protein breakdown (Czerkawski, 1966; Czerkawaski et al., 1966, and Robertson and Hawke, 1964) and in production of methane (Clapperton and Czerkawski, 1969; Czerkawski, 1966; Czerkawski et al., 1966, and Czerkawski and Breckenridge, 1969), volatile fatty acids (Storry, 1970; Sutton, 1965), and ammonia (Chalmers, 1960). Microbial synthesis and hydrogenation of fatty acids may also be affected and appetite may be depressed. Protection of dietary protein may increase the efficiency of nitrogen utilization in the ruminant by reducing ruminal proteolysis and allowing bypass to the lower gastrointestinal tract with a subsequent increase in nitrogen retention.

If fat (oil), which contains more energy than carbohydrates, could be used as a method of decreasing dietary protein degradation in the rumen, efficiency of production could also possibly be increased because

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[1]Net dietary protein value = CP x BV x Protein Digestibility

of the concomitant addition of energy to the diet.

Bohman et al. (1959) evaluated range beef cattle response to animal fat and protein supplements. Winter treatments used were two levels of animal fat, 0 and 227 g per animal daily and three protein supplements, none, suncured alfalfa meal, and cottonseed meal. Protein supplementation increased rate of gain during the winter period. Animal fat had no effect on gains of wintering beef calves. During the summer, the cattle fed fat the preceeding winter gained more than those not fed fat in the winter. The response to fat appeared to be independent of amount or type of protein. In an earlier study (Bohman et al., 1957) fattening cattle fed 5% fat gained 28% more than control animals; 10% fat was not as effective in promoting gains as the lower level. In this study it was suggested that the level of protein might be an important factor influencing the utilization of fat by ruminants. Since protein-calorie ratios are important for other species (Combs, 1956), it is possible that the amount of protein may be limiting for the utilization of fat.

When corn oil or tallow has been fed to ruminants, rates of gain have decreased (Brethour et al., 1958; Ward et al., 1957) and the digestibility of the ration has been reduced (Brethour et al., 1958; Grainger et al., 1957; Pfander and Verma, 1957; Ward et al., 1957). The addition of alfalfa, alfalfa ash or calcium overcame this depression in some studies (Granger et al., 1957; Ward et al., 1957) but not in others (Brethour et al., 1958). The method of administering corn oil to the animal has also been shown to affect digestibility (Pfander and Verma, 1957).

The composition of blood was influenced by both protein supplement and by fat (Bohman et al., 1959). Fat temporarily decreased the level

of plasma phosphorus. Bohman and Wade (1958) earlier observed this effect in feed-lot cattle; thus this effect appears to be consistent under two widely different conditions. Protein decreased the level of plasma fat when fat was fed; without extra dietary fat, protein had no effect on plasma fat.

Nottle and Rood (1963) observed little effect when linseed cake replaced linseed meal containing 270 g oil/726 g dry matter (e.g., 37% fat) in the high roughage diet of dry cows. Fat sources were also added through a fistula. Cod-liver oil caused a pronounced fall in the molar proportion of acetic acid and a complementary increase in propionic acid whereas tallow tended to give the reverse changes. There was a slight decrease in the digestibility of crude fiber and of fat-free dry matter in the diet during the period of addition of tallow.

Lipids have been reported to inhibit some rumen microbes, in particular the cellulolytic bacteria, thus producing a low acetate fermentation (Henderson, 1973). Therefore, an increase in the proportion of propionic acid in the rumen fluid has been noted when ruminant diets were supplemented with fat (Shaw and Ensor, 1959). Also, the digestibility of cellulose and protein has been reduced (Brooks et al., 1954) and the rate of voluntary intake depressed (Kowalczyk et al., 1977) by fat feeding.

Jayasinghe (1961) reported that the addition of 12% groundnut oil to undenatured groundnut meal decreased ruminal ammonia concentration in sheep. He suggested that the protein may not have been broken down as rapidly or that degradation rate was the same and there was greater utilization of the degradation products because of the higher energy provided by the fat.

Peterson et al. (1975) fed 80% concentrate diets containing

expeller-extracted linseed meal treated with corn oil, lard, or coconut oil to lambs to determine the effectiveness of coating the protein in reducing its ruminal degradation. Fat was added to linseed meal at the rate of .3 kg of fat per kg of linseed meal (i.e., 30%) which resulted in 78.4 g of fat being consumed per day (i.e., 5.6% dietary fat). Lambs consuming coconut oil-treated linseed meal had a lower concentration of ruminal ammonia (average of .5 mg/dl across all collection times) than those fed normal linseed meal (10.3 mg/dl). Lambs fed corn oil-treated and lard-treated linseed meal had ruminal ammonia levels intermediate to those fed normal and coconut oil-treated linseed meal. Levels of plasma ammonia and plasma urea nitrogen for normal linseed meal were significantly higher than the same measures with the oil-treated linseed meals.

In a similar study Glen et al. (1977), reported similar findings as did Peterson et al. (1975). Lambs consumed 90 g of fat per day in treatments that contained a 5.6% fat addition. Lambs consuming coconut oil-coated linseed meal had significantly lower ruminal ammonia (2.7 mg/dl) than all other treatments across all collection times. However, energy was not equal across treatments. Treatments had 3.9, 4.2, 4.5, and 4.3 kcal/g for the normal linseed meal, corn oil coated linseed meal, lard coated linseed meal, and coconut coated linseed meal, respectively. Corn oil and lard-coated linseed meal diets were lower ($P < .05$) than the normal linseed meal diets and intermediate between normal and coconut oil-coated linseed meal. Digestibilities of dry matter and cellulose were lowest when coconut oil-coated linseed meal was fed. Nitrogen digestibility was lowest ($P < .05$) for the coconut oil treatment. Nitrogen retention was significantly higher for the corn oil treatment than for normal linseed meal.

Decreased microbial activity may explain the low dry matter, cellulose and nitrogen digestibilities from the coconut oil-coated linseed meal, since ruminal ammonia levels were below the levels that Satter and Slyter (1974) suggested as being optimal (e.g., 5 mg/dl) for maximal microbial growth.

The problems associated with fat supplementation in ruminants are due to problems of rumen fermentation and not to a limited capacity for absorption by the host animal (Kowalczyk et al., 1977). When the liquid suspension of fat was given so that the rumen was by-passed there was no reduction in voluntary food intake and digestibility by sheep. When fat entered the rumen both intake and digestibility of dried grass fell. Bailey (1972) showed that lambs weighing about 20 kg were able to absorb about 100 g lipid daily from the small intestine. This would equal approximately 500 g fat/day for a 454 kg cow. The reduction in rate of digestion which was observed with dried grass (Kowalczyk et al., 1977) can have a profound effect on ruminal retention time and consequently on voluntary intake. These researchers also reported a rapid fall in ammonia concentration as the fat supplement increased suggesting that the overall inhibition of digestibility may be the result of low ruminal ammonia concentration.

Chamlers (1960) used the ammonia curve technique to determine the effect of oil on the utilization of protein in the rumen. Groundnut oil, copra oil, and linseed oil were evaluated and their effect depended upon the nitrogen content of the basal ration. At 3% nitrogen the ammonia curve was depressed and at 4.5% nitrogen there was an increased concentration of ruminal ammonia.

Kowalczyk et al. (1977), posed the question as to whether the low ruminal ammonia levels, associated with feeding a high fat supplement to lambs given dried grass ad libitum were the cause of the accompanying decreases in digestion rate and intake. Henderson et al. (1977) investigated this further and found that addition of 10% tallow to the dried grass hay reduced dry matter digestion rate, cotton thread digestion rate, ruminal ammonia concentration, and numbers of protozoa in the rumen. Addition of 5% tallow reduced cotton thread digestion rate but no other significant changes were observed. When urea was added to increase the ruminal ammonia levels no significant changes in dry matter digestion rate, cotton thread digestion rate, or protozoal numbers were found. Based on this work, Henderson et al. (1977) concluded that the depression of ammonia levels could not account for the depressed fiber digestion in the rumen but is presumably a related effect. He suggested that the coating of the feed by fat was likely to restrict colonization of the fiber by cellulolytic and proteolytic bacteria and the depression of ammonia levels may result from decreased proteolysis. Also, the rate of cellulolysis on dried grass diets probably does not provide energy at a rate sufficient for microorganisms to respond to the elevated ammonia levels present on the urea supplemented diets.

Kowalczyk et al. (1977) observed that successive increments of tallow decreased fiber digestibility and NH_3-N concentrations in the rumen, suggesting that decreased fiber digestibility may be mediated by low NH_3. However, data of Orskov et al. (1978) fail to support this hypothesis, and Palmquist and Conrad (1978) have observed increased NH_3-N on high fat diets.

The addition of fat to ruminant diets has depressed fiber

digestibility in both cattle (Ward et al., 1957) and sheep (Brooks et al., 1954). Devendra and Lewis (1974) summarized four theories to explain this effect: (1) physical coating of the fiber with fat preventing microbial attack; (2) a modification of the rumen microbial population from possible toxic effects of fat on certain microorganisms; (3) inhibition of microbial activity from surface-active effects of fatty acids on cell membranes; (4) reduced cation availability from formation of insoluble complexes with long chain fatty acids. The last effect could be directly on availability of cations for microbial function or indirectly by affecting rumen pH (Palmquist and Jenkins, 1980). Most data support an inhibitory effect on microbial activity, perhaps sufficient to change competitiveness and, therefore, populations (Palmquist and Jenkins, 1980). Fatty acids inhibit rumen bacteria in pure culture (Henderson, 1973; Maczulak, 1979). Fatty acids bind to microbial cells (Henderson, 1973; Maxcy et al., 1967; Nieman, 1954) and this binding can be reduced by adding fiber (Harfoot et al., 1974), reducing inhibition in pure cultures (Maczulak, 1979). Bacterial numbers may increase when fat is fed, usually in conjunction with a decrease in protozoal numbers (Czerkawski, 1973; Maczulak, 1979). Thus, protozoa may be more sensitive to fatty acid, allowing bacteria to move into the voided ecological niche (Palmquist and Jenkins, 1980).

Czerkawski et al. (1975) reported that feeding 90 g linseed oil per day to sheep increased rumen dilution rate and the volume of rumen contents while decreasing the synthesis of diaminopimelic acid in the rumen. The number of protozoa in the rumen of sheep during the period on the high-fat diet decreased considerably but the total number of bacteria increased. The decrease in the number of protozoa could have

been due to toxicity of linseed-oil fatty acids to these organisms, but it could also have been the result of reduced sequestration of these microorganisms in the rumen (Hungate et at., 1971).

A purely physical effect on cellulose digestion would be alleviated by passage of the fat-coated cellulose from the rumen. Since recovery from the decreased cellulose digestibility did not occur until after 17 days, supplemental fat may decrease certain microbial metabolic activity and/or modify the rumen microbial population concerned with cellulose digestion (White et al., 1958). It is postulated that calcium improves fiber digestibility of high fat rations by forming insoluble soaps which remove the fatty acids from solution so they are no longer available to bind to the rumen microbiota (Palmquist and Jenkins, 1980). Although rumen pH may affect fiber digestibility, evidence for added dietary fatty acids changing pH or cation availability is lacking; indeed, Beitz and Davis (1964) found no change in rumen pH of cows fed 225 g cod-liver oil per day. Nevertheless, bacterial uptake of fatty acids in buffered test systems is decreased by increasing pH (Galbraith et al., 1973; 1971). This may occur because increasing pH increases ionization, hydrophilicity, and solubility. Bacterial uptake of fatty acids is increased by increasing hydrophobicity (Bean, 1967).

Formaldehyde Treatment of Protein

Chemical Reactions

Reactions between HCHO and proteins have been surveyed by Van Dooren (1972). In most instances the initial step appears to be the

rapid formation of a methylol compound:

$$R - XH + HCHO \rightarrow R - X - CH_2OH$$

XH can be terminal amino groups ($-NH_2$), the ε-amino group of lysine, the primary amide groups of asparagine and glutamine, the guanidyl group of arginine, the hydroxy groups of threonine and serine, the sulphydryl group of cysteine, the phenol group of tyrosine, the phenyl group from phenylalanine, the indole group of tryptophan, and the imadazole group of histidine. Condensation reactions then take place slowly over time, with the formation of stable methylene cross-linkages between protein chains:

$$R - X - CH_2OH + R' - NH_2 \rightarrow R - X - CH_2 - NH - R' + H_2O;$$

$$R - XH + R' - NH - CH_2OH \rightarrow R - X - CH_2 - NH - R' + H_2O$$

These reactions of HCHO with the amino acid groupings take place under differing conditions of pH and temperature. At neutral pH and room temperature Ferguson (1975) considered the principal reactions to be those involving terminal amino groups, the primary amide groups of asparagine and glutamine and the ε-amino and guanidyl groups of lysine and arginine, respectively. Ferguson et al. (1967) first demonstrated that these chemical reactions could be utilized to protect cosein from rumen degradation, the methylene linkages being hydrolysed under acid-pepsin conditions in the abomosum and the animal's amino acid supply therefore being increased.

In production trials the best possibilities for responses to HCHO treatment must exist where there is a potential for high rates of protein synthesis in the animal tissues (i.e., lactation and wool growth) but

where amino acid supply from the digestive tract is low. Low supply of amino acids will occur in situations where dietary protein contributes little to the amino acids arriving at the duodenum (i.e., diets of low protein concentration and those where most of the nitrogen is either non-protein-nitrogen (NPN) or very soluble protein), and situations where microbial protein production is restricted due to low levels of energy intake.

Casein

The successful application of formaldehyde-treated proteins to practical ruminant feeding is dependent on a thorough examination of the technique and its effect on digestion and utilization of nutrients. Ferguson et al. (1967) soaked casein in dilute aqueous formaldehyde followed by washing and drying the product. Hemsley et al. (1973) compared this method (high-volume treatment) with another procedure in which casein was mixed with small volumes of more concentrated formaldehyde solutions, any unreacted formaldehyde being allowed to remain in the product (low-volume treatment). [^{14}C] Formaldehyde was used in studies by Hemsley et al. (1973) to assess the total amount of HCHO carbon which remained in the products after subjecting them to a standard washing procedure.

The rate and extent of reaction of HCHO with casein depended on the duration of the reaction, the concentration of HCHO solution, and the ratio of solution : casein. The extent of binding increased with HCHO concentration and was greater with a small volume of concentrated solution than with a larger volume of less concentrated solution containing the same amount of formaldehyde. Amounts of bound formaldehyde up to

3.8 g/100 g dry product were obtained.

Untreated casein was readily degraded to ammonia by rumen microorganisms in vitro. Dietary supplements of untreated casein were almost completely digested but produced only slight increases in wool growth rate. Formaldehyde-treated casein which contained 0.5-1.5% bound formaldehyde increased wool growth rate and fiber diameter substantially when included in the diet. The greatest wool growth response was obtained with casein preparations containing about 1% bound formaldehyde, regardless of the treatment procedure. Casein containing amounts of bound formaldehyde outside the range 0.5-1.5% was of little value for wool growth. The preparations that were most effective in stimulating wool growth corresponded to treatments that afforded good protection in vitro without producing an appreciable reduction in digestibility. Ineffective casein preparations were either incompletely protected in the rumen or had a lower digestibility. Casein could be rendered virtually indigestible by excessive formaldehyde treatment (Hemsley et al., 1973).

Partial protection from ruminal degradation has been achieved by treating with vegetable tannins (Le Roy et al., 1965; Driedger et al., 1969), with heat (Tagari et al., 1962; Sherrod and Tillman, 1962), and with aldehyde (Spears et al., 1980; Peter et al., 1971; Hemsley et al., 1970; Faichney, 1971; Ferguson et al., 1967; Reis and Tunks, 1969). A dramatic response with aldehyde treatment has been reported when formaldehyde (HCHO)-treated casein has been fed to sheep at a maintenance level of energy intake (Ferguson et al., 1967; Reis and Tunks, 1969; Langlands, 1971a). Protection of casein, ordinarily 90% degraded in the rumen (McDonald and Hall, 1957), substantially increases total protein reaching the intestine (Faichney and Weston, 1971). Thus, increases of 51 to 70%

in wool production have been reported following supplementation with HCHO-treated casein (Langlands, 1971a; Reis and Tunks, 1969; Ferguson et al., 1967).

Kempton et al. (1979) found that the flow of microbial non-ammonia N (NAN) into the duodenum of lambs fed the HCHO-casein diet (27 g N/day) was more than twice that for the basal diet of oat hulls and sulfa floc (11 g N/day) or basal diet supplemented with either urea, or urea plus casein. The flow of NAN through the ileum and excretion of total N in the feces was also greater with the HCHO-casein diet versus the others. Urea and casein supplements were apparently completely degraded in the rumen. In contrast, the HCHO-casein was almost completely resistant to rumen degradation and only 65% of the HCHO-casein was digested in the small intestine.

It has been demonstrated that, in lambs given a low protein-cellulose-based diet, responses in food intake and growth were obtained to supplementation with N forms that are rapidly degraded to ammonia in the rumen (i.e., urea or casein) and greater responses were obtained when a protein was given in a form which escaped rumen fermentation (as formaldehyde-treated casein, Kempton and Leng, 1979). Growth responses were attributable to an increased food intake. The increases in food intake and growth in lambs were probably attributable to an increased supply of amino acids to the animal. It is not known how much of the increased supply of amino acids was derived from the supplement and how much was from an increase in microbial outflow from the rumen. As well as altering the amount of dietary protein that enters the duodenum, provision of soluble or insoluble proteins also may affect the efficiency of microbial protein synthesis and the flow of microbial protein from the

rumen (Hume, 1970). For example, changes in the microbial associations in the rumen as a result of changes in the flow through the rumen, and also changes in the rumen environment which may be brought about directly by supplementation or indirectly by increased food intake can increase the availability of microbial protein (Kempton et al., 1979). Williams and Moir (1951) found that the bacterial counts from the rumen of lambs were greater when whole egg or casein was the source of supplemental nitrogen than when linseed meal or subterranean clover furnished the nitrogen. They suggested that the microbial population may have a significant effect on the nitrogen status of the ruminant.

Several factors may affect the efficiency of microbial protein synthesis, e.g., dilution rate (Hobson and Summers, 1967; Sutherland, 1976), turnover of microorganisms in the rumen (Thomas, 1973), availability of peptides and amino acids in addition to ammonia (Portugal et at., 1966; Wright et al., 1967; Hume, 1970; Maeng et al., 1976) and availability of branched-chain and higher fatty acids (Hemsley and Moir, 1963; Hume, 1970).

Prevention of the degradation of dietary protein in the rumen might lead to reduced microbial population and the possibility of secondary effects such as reduced cellulolytic activity and volatile fatty acid (VFA) production. Reduced levels of VFA in the rumen were reported by Faichney and Weston (1971) when casein in the diet of lambs was treated, and were associated with a decrease in organic matter digestion in the rumen and a matching increase in the amount of crude protein and starch passing out of the stomach to the intestines.

Infusion of casein into the abomasum of lambs revealed that formaldehyde-treated casein and casein per abomasum were of similar

value (Reis et al., 1970). MacRae et al. (1972) reported increases in the amounts of individual amino acids reaching the duodenum of sheep when a casein supplement to a basal diet of dried grass was treated with formaldehyde.

Treatment of casein with HCHO reduced the apparent digestibility of nitrogen but was associated with increased nitrogen retention at two levels of intake (Faichney, 1974). These findings are consistent with previous results with lambs (Faichney, 1971) and reflect an increase in the digestion of protein in the intestines as a result of the treatment (Faichney and Weston, 1971).

Natural Protein

Treatment of SBM and other proteins with HCHO has been shown to increase the quantity of protein entering the lower digestive tract of ruminants (Faichney and White, 1977; Miller, 1972). Nishimuta et al. (1974) found an increased passage of HCHO-treated sunflower-meal (SFM) protein from the rumen to the lower GIT. The increase in recovery of dietary protein ($P < .01$) in the solid digesta with 1% formaldehyde treated SFM (74.8 g recovered compared to 59.0 g with SFM) indicated that HCHO treatment was effective in preventing the rumen microbial population from degrading this supplementary protein (Amos et al., 1976). The HCHO-SFM treatment effectively increased the quantity of abomasal methionine and cystine, amino acids which are particularly high in SFM; these results are opposite of Nishimuta et al. (1974) who found heating SBM with HCHO increased the quantity of lysine reaching the abomasum daily but did not affect the quantity of methionine, which might be explained by the relatively high lysine and low methionine contents of SBM.

Lambs consuming formalin-treated SBM had lower ($P < .05$) dry matter and crude protein digestibility coefficients, although cellulose digestibility was equal to that observed when normal SBM was fed. The resistance of formalin-treated SBM to postruminal enzymatic degradation was indicated by the lower percentage dietary nitrogen retained (Nishimuta et al., 1973). Decreased digestion and a trend for decreased percent dry matter digestibility was observed by Amos et al. (1974) who fed lambs 1.1% formaldehyde-treated SBM versus untreated SBM fed in a 70% concentrate diet.

Protein supplements used in a 42 day lamb feeding trial with 85% concentrate diets were treated with formaldehyde equivalent to .6% and .5% of the fish and soybean meals, respectively. Daily gain was improved 7% by aldehyde treatment (Nimrick et al., 1972).

Treatment of linseed and meatmeal was accomplished by adding 18-20 liters of 2.5% commercial formalin (1% HCHO) to 10-14 kg batches and mixing to a smooth paste for 1 hr. Batches were then spread out on trays and dried at 80 C (Rattray et al., 1970). In the treated linseed meal 93.8% of the N remained after rumen liquor fermentation compared with 30.6% for untreated. The treated and untreated meatmeals were 81.1% and 60.3%, respectively. After pepsin digestion 14.3% and 46.6% of the N for the untreated and treated linseed meal respectively remained, 26.9% and 62.2% of the N for the untreated and treated meatmeal remained. Treatment of linseed meal with formalin resulted in an increase in N retention. However, the increased N retention was not accompanied by any change in wool growth or live weight gain. The reverse occured with the treatment of meatmeal, untreated meatmeal promoting higher levels of N retention (Rattray et al., 1970).

Nitrogen retention has been increased in digestibility and nitrogen balance trials with sheep even though nitrogen digestibility was reduced by formaldehyde treatment of protein (MacRae et al., 1972; McGilliard, 1972). The more efficient utilization of absorbed nitrogen by cows (Clark et al., 1974) and by sheep in other studies fed formaldehyde-treated protein perhaps is due to the lower amount of nitrogen absorbed, a better balance of amino acids from the intestine, or both.

Wilson (1970) found that formaldehyde-treated casein when offered to dairy cows had no effect on the milk yield, but increased the percentage of protein in the milk. Clark et al. (1971; 1974) reported no effect on milk or protein yield when cows were fed formaldehyde-treated SBM. The SBM may have been overtreated with formaldehyde. It is possible that less formaldehyde applied to the SBM, as suggested by Peter et al. (1971) would improve animal performance. Bypassing SBM to the lower gut, even if it is highly digestible, may not improve lactation performance if its amino acid content does not improve the amino acid pattern available for milk protein synthesis.

Chicks fed SBM treated with .3% formaldehyde gained as well as those fed the control diet (Spears et al., 1980). Gain to feed ratios in chicks were slightly decreased even by the low level of formaldehyde treatment, suggesting that, even though growth was not depressed when SBM was treated with .3% formaldehyde, digestibility may have been reduced slightly. This agrees with Schmidt et al. (1973), who found that growth and nitrogen retention by rats was not depressed when SBM was treated with .4% formaldehyde, but that higher levels of formaldehyde treatment reduced both growth and nitrogen retention.

Spears et al. (1980) suggested from <u>in vitro</u> work that treating SBM

with .3 to .6% formaldehyde decreased protein degradation to almost the same extent as treatment with a higher concentration of formaldehyde. Similar results were reported by Peter et al. (1971), who found .6% formaldehyde to be optimal in preventing ruminal degradation of protein from SBM.

The response to dietary protein protection when the level of feed intake is at or near ad libitum, or where proteins other than casein have been used, is much less consistent. The number of studies showing no beneficial results (Wachira et al., 1974; Faichney et al., 1972; Langlands, 1971b; Saville et al., 1971; Clark et al., 1971; Hall et al., 1971; Schmidt et al., 1972) outnumbered those claiming a positive response (Peter et al., 1971; Faichney, 1971; Hemsley et al., 1970).

The desirability of treating proteins would consequently depend on their natural ability to resist ruminal degradation, the extent to which enzymatic digestion is impaired, their amino acid composition, and the nature of the demands of the body for specific growth functions.

CHAPTER III

IN VITRO RUMINAL DEGRADATION OF TEMPERATURE AND PRESSURE TREATED TALLOW-PROTEIN MIXTURES

Summary

Addition of tallow to cottonseed meal (CSM) and soybean meal (SBM) with heat, pressure and/or vacuum was evaluated using an experimental mixer with water jacket, steam and vacuum inlets and temperature control. In vitro ammonia nitrogen (NH_3-N) release showed pressure and heat (using live steam) reduced NH_3-N release in vitro. Vacuum with dry heat was ineffective. In a metabolism study, 15 lambs were fed winter harvested range grass and .07 kg/day of: SBM + 15% tallow (SBM-T), SBM + 15% tallow heated to 121 C, 1.36 atmospheres, for 5 min (HSBM-T), and HSBM-T plus 1% urea (HSBM-T+U). Dry matter digestibility and nitrogen retained tended to be reduced with HSBM-T and HSBM-T+U. Ruminal NH_3-N was not different ($P > .05$) between treatments.

Ruminal NH_3-N was determined from 16 lambs fed a 33% corn, 50% alfalfa diet plus SBM, SBM heated for 5 min at 121 C under 1.36 atmospheres (HSBM), SBM + 15% tallow (SBM-T), and SBM-T heated similar to HSBM (HSBM-T). Heating SBM reduced ($P < .05$) ruminal NH_3-N at all times evaluated when fed with concentrate rations but not with roughage rations. Tallow addition reduced ($P < .05$) ruminal NH_3-N at 8 hr post-feeding vs SBM when fed with concentrate. However, when 16 lambs were fed SBM, HSBM, SBM-T or HSBM-T with low quality hay, ruminal NH_3-N

1 or 8 hr postfeeding was not reduced ($P > .05$) by heat and/or tallow addition to SBM. Heating tended to lower ruminal NH_3-N at 4 hr postfeeding.

SBM, HSBM, SBM-T, and HSBM-T were incubated *in vitro* with rumen fluid from fistulated steers fed a roughage (R) or a 40% concentrate (C) ration. Heating reduced ($P < .05$) NH_s-N in both inocula.

Introduction

Several methods have been developed to reduce ruminal protein degradation. Solubility has been decreased by treatment of soybean meal (SBM) with heat (Little et al., 1963; Glimp et al., 1967; Hudson et al., 1970). Heat plus steam treatment of SBM improved nitrogen retention of growing lambs (Sherrod and Tillman, 1962). Ruminal ammonia concentrations of sheep have been reduced by adding lipids to ground nut or coconut meal (Jayasinghe, 1961) or coating linseed meal with 30% corn oil, lard, or coconut oil (Peterson et al., 1975). The latter treatment also increased nitrogen retention in lambs (Glen et al., 1977). When dietary nitrogen levels were 3% of dry matter the addition of oil depressed ruminal ammonia concentration whereas at a dietary nitrogen level of 4.5% the added oil increased ammonia concentration (Chalmers, 1960). This suggests the relationship between lipids and nitrogen metabolism in the rumen is complex.

This study was designed to evaluate the effects of adding tallow to CSM and SBM with heat and either pressure or vacuum on ruminal ammonia concentrations and digestion by growing lambs.

Experimental Procedure

A roto-cone vacuum dryer[1] was used to apply moist heat and either pressure or vacuum to tallow-protein mixtures. The mixer was water jacketed with steam and vacuum inlets allowing temperature and pressure control. The treatment chamber rotated at 4 RPM and had a capacity of approximately 12 kg of SBM.

Cottonseed meal (CSM) and soybean meal (SBM) were evaluated with two types of tallow (IRN 4-08-127), beef tallow (T) and hydrogenated tallow (HT) with melting points of 43 C and 48 C, respectively. In subsequent abbreviations, H before SBM will designate heat treatment. Preliminary studies indicated that the maximum amount of tallow cows would readily consume in a supplement was about 15%. Therefore, 15% was considered to be the practical maximum level of addition for this evaluation.

Trial 1

Fermentation in vitro was used as a screening method using inoculum from a ruminally fistulated steer (544 kg) fed 5.4 kg/day of the following 50% concentrate ration (diet A): 32.8% corn (IRN 4-02-931); 50% ground alfalfa (IRN 1-00-063); 7.18% cottonseed hulls (IRN 1-01-599); 3.08% dehy (IRN 1-00-023); 5.13% SBM (IRN 5-04-604); 1.54% molasses (IRN 4-04-696); .25% limestone (IRN 6-02-632); .25% dicalcium phosphate (IRN 6-01-808); .05% urea and .2% trace mineralized salt.[2] Inoculum was

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[1]Manufactured by Paul O. Abbe, Inc., Little Falls, New Jersey.

[2]Ingredients in TM salt, %: NaCl, 99; Mn, .25; Fe, .20; S, .10; Cu, .033; Co, .01; I, .007 and Zn, .005.

strained through 4 layers of cheesecloth and mixed 1:1 with buffer (McDougall, 1948). The buffer contained no nitrogen source. Twenty mg nitrogen (N) from CSM, CSM plus 15% beef tallow (CSM-T), and CSM plus 15% hydrogenated tallow (CSM-HT) heated for 5 or 30 min under either 1.36 atmospheres of steam pressure or .6 atmospheres of dry heat vacuum were incubated in vitro for 4 hr. Tallow concentration was 227 mg/dl in tubes containing CSM-T and CSM-HT. At the end of 4 hr fermentation, 2 ml of 20% HCl was added to the 30 ml inoculum in each tube to stop microbial activity. Tubes were then centrifuged at 7,000 x g for 10 min at 0 C. The supernate was removed and refrigerated until ammonia nitrogen (NH_3-N) could be determined by modification of the colormetric procedure of Chaney and Marbach (1962). Duplicate samples of .02 ml undiluted rumen fluid were added to test tubes. One ml of phenol reagent was then added followed by mixing and addition of 1 ml sodium hypochlorite reagent prior to mixing again. Samples were incubated at room temperature for 30 min. Distilled water (8 ml) was then added to the tubes prior to mixing and reading on a spectrophotometer (630 nm).

Trial 2

Heating periods of 5 min or less were evaluated in vitro to determine a minimum effective heating time. Steam pressure of 1.36 atmospheres was used to heat CSM, CSM-T, CSM-HT, SBM, SBM mixed with 15% beef tallow (SBM-T), or SBM mixed with 15% hydrogenated tallow (SBM-HT). CSM was added to in vitro tubes to provide 20 mg N/tube and 227 mg tallow/dl while SBM provided 30 mg N/tube and 230 mg tallow/dl in tubes containing SBM-T or SBM-HT. The in vitro procedure was conducted as described in trial 1.

Trial 3

A nitrogen balance study was conducted using 15 crossbred wether lambs (27 kg) fed ground winter harvested range grass hay (3% CP) ad libitum and 70 g once daily of SBM + 15% tallow (SBM-T), SBM + 15% tallow heated for 5 min at 1.35 atmospheres and 121 C (HSBM-T) or HSBM-T plus 1% urea (HSBM-T+U).[3] A 7 day preliminary period was followed by a 5 day urine and feces collection period. At the end of the digestion study, lambs were ruminally sampled via stomach tube for ruminal ammonia analysis at 1, 4, and 8 hr after supplement feeding. Ruminal fluid was treated as in the in vitro procedure.

Trial 4

To investigate inconsistencies observed between in vitro and in vivo results of trials 2 and 3, ruminal ammonia concentrations were evaluated with 16 crossbred lambs (32 kg). Lambs were randomly assigned to 4 treatments which provided 50 g supplemental crude protein daily from: soybean meal (SBM), SBM heated for 5 min at 1.36 atmospheres and 121 C (HSBM), SBM plus 15% tallow (SBM-T), and SBM-T heated similarly to HSBM (HSBM-T). Supplements were individually fed once daily with low quality ground range grass hay (3% CP) for 5 days. All rations contained 1.5% nitrogen. On day 6, lambs were fed the supplement only, and ruminally sampled for NH_3-N at 1, 4, and 8 hr postfeeding, as previously described. In a similar study, 15 crossbred lambs (23 kg) were fed 50 g supplemental crude protein daily from: SBM, HSBM, SBM-T, HSBM-T plus

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[3]Treatments also included: Dicalcium phosphate, 4.0% and trace mineralized salt[2] .2%.

.6 kg/day of diet A for 7 days. Each ration contained 2.3% nitrogen. On day 8, lambs were fed the supplement only and ruminally sampled for NH_3-N at 1, 4, and 8 hr postfeeding.

Trial 5

In order to compare effects of type of inoculum on *in vitro* NH_3-N concentrations, 4 supplemental treatments (SBM, HSBM, SBM-T and HSBM-T) were evaluated *in vitro*. Inoculum was obtained either from a steer fed 5.4 kg of diet A or a steer fed 8.2 kg of baled winter range grass hay plus 1.75 kg of a supplement which consisted of 78.2% SBM (IRN 5-04-604); 13.8% tallow (IRN 4-08-127); 6.75% dicalcium phosphate (IRN 6-01-080) and 1.23% trace mineralized salt[2]. SBM with or without added tallow was heated for 5 min at 121 C and 1.36 atmospheres prior to addition of other ingredients into the supplement. Fermentation time of 1, 4, and 8 hr were evaluated *in vitro*.

Data were subjected to analysis of variance (Steele and Torrie, 1960) and treatment means were compared statistically by the LSD test.

Results and Discussion

Trial 1

Heating CSM-T under 1.36 atmospheres of steam pressure was more effective in reducing ($P < .05$) 4 hr NH_3-N concentration than was .6 atmospheres of dry vacuum (18.2 vs 22.6 mg/dl). Five min steam pressure treatment of CSM-HT reduced ($P < .05$) *in vitro* NH_3-N (21.0 vs 16.2 mg/dl) when compared to .6 atmospheres of dry vacuum (Table I). Heating CSM under 1.36 atmospheres of steam pressure or .6 atmospheres of dry vacuum

TABLE I

PRESSURE AND TIME EFFECTS ON IN VITRO (FOUR HR) AMMONIA ACCUMULATION FROM COTTONSEED MEAL (TRIAL I)

	Heating time, min				
	5		30		
	Pressure, atm				
Starting[e] Material	.6 (dry heat)	1.36 (steam)	.6 (dry heat)	1.36 (steam)	SE[f]
	mg NH_3-N/dl				
CSM	20.9[abc]	22.0[ac]	18.6[abd]	16.7[bc]	1.5
CSM-T	21.2[abc]	18.0[bcd]	24.0[ac]	18.3[bc]	1.5
CSM-HT	21.0[ac]	16.2[bd]	20.4[abcd]	17.0[abc]	1.5

[ab]Means on the same line with different superscripts are different ($P < .05$).

[cd]Means in same column with different superscripts are different ($P < .05$).

[e]Starting material x heating time interaction ($P < .10$).

[f]Standard error of mean with three tubes/treatment, 20 mg N/tube and 227 mg tallow/dl.

did not significantly reduce NH_3-N accumulation *in vitro*. Heating CSM alone for 30 min with 1.36 atmospheres of steam pressure tended to reduce ($P < .05$) NH_3-N when compared to 5 min heating. However, little NH_3-N reduction ($P < .05$) was observed with 5 or 30 min heating of CSM alone with .6 atmospheres of dry vacuum. Heating either the CSM-T or CSM-HT mixture for 30 min had no further effect ($P < .05$) in reducing 4 hr *in vitro* NH_3-N concentration compared to 5 min heating. The protein mixture by heating time interaction ($P < .10$) was due to increased NH_3-N concentration observed with 5 min heating of CSM while heating the protein-tallow mixtures for 5 min resulted in reduced NH_3-N concentration.

Trial 2

Based on these results, 1.36 atmospheres of steam pressure was evaluated further. Heating of CSM, or CSM-T, or CSM-HT mixtures for 2.5 min or longer reduced ($P < .05$) *in vitro* 4 hr NH_3-N concentration when compared to zero min heating of these materials (Table II). Heating SBM, SBM-T, or SBM-HT mixtures under 1.36 atmospheres for 5 min reduced ($P < .05$) NH_3-N concentration (Table II) with the reduction in NH_3-N levels greater ($P < .05$) with SBM-T and SBM-HT than with SBM. The treatment by heating time interaction ($P < .001$) was attributable to the greater reduction in NH_3-N concentration with SBM-T and SBM-HT than SBM at 5 min. Heating for 5 min at 121 C under 1.36 atmospheres of steam pressure was selected for further study. Given that T and HT had similar effects *in vitro*, the high cost of hydrogenated tallow and the fact that fat digestibility decreases as the melting point exceeds 50 C (Crockett et al., 1974), testing of HT was discontinued.

TABLE II

TIME EFFECTS ON IN VITRO (FOUR HR) AMMONIA ACCUMULATION FROM PROTEIN SOURCES (TRIAL 2)

	Heating time, min				
	0	1.0	2.5	5.0	SE[g]
	mg NH_3-N/dl				
CSM	30.4[ad]	26.8[abd]	25.7[bd]	26.0[bd]	1.3
CSM-T	28.1[ad]	27.1[ad]	20.0[bd]	19.5[be]	1.3
CSM-HT	27.0[ad]	26.8[ad]	20.4[be]	20.4[be]	1.3
SBM[f]	32.6[ad]	33.6[ad]	---	26.6[bd]	1.2
SBM-T	34.8[ad]	30.6[bde]	---	17.9[ce]	1.2
SBM-HT	31.6[ad]	28.9[ae]	---	14.3[be]	1.2

[abc]Means on the same line with different superscripts are different ($P < .05$).

[de]Means in the same column within an individual protein source (CSM or SBM) with different superscripts are different ($P < .05$).

[f]Treatment by heat time interaction ($P < .001$).

[g]Standard error of mean with three tubes/treatment: CSM 20 mg N/tube and 227 mg tallow/dl; SBM 30 mg N/tube and 230 mg tallow/dl.

Trial 3

Previous work with cows and lambs showed no significant improvement in performance with addition of tallow to protein (Stanton et al., 1980). A lamb trial was conducted to determine if *in vitro* results with pressure treatment of protein would be apparent *in vivo* (Table III). A trend for decreased nitrogen retention and dry matter digestibility was apparent when SBM-T was heated 5 min at 1.36 atmospheres and 121 C. Ruminal NH_3-N levels tended to be higher ($P > .05$) for HSBM-T and HSBM-T+U than for unheated SBM with tallow. Heating SBM-T in this trial tended to increase ruminal NH_3-N. In all other trials, heating significantly decreased or did not effect NH_3-N concentrations *in vitro* and *in vivo* when compared with an unheated protein-tallow mixture.

Trial 4

Incubating HSBM-T reduced NH_3-N accumulation *in vitro* using a concentrate inoculum, however ruminal NH_3-N levels tended to be higher when feeding HSBM-T than when feeding unheated SBM-T to lambs fed winter range grass hay. Supplements of SBM, HSBM, SBM-T, and HSBM-T were fed to 16 lambs along with winter harvested range grass hay. Heat treatment of SBM with or without tallow moderately reduced ($P > .50$) ruminal NH_3-N 4 hr postfeeding (10.0 vs 16.6 mg/dl), however, heat treatment and/or tallow addition did not reduce ($P > .05$) ruminal NH_3-N at 1 or 8 hr postfeeding, (Figure 1). Ruminal NH_3-N increased ($P < .01$) over 1, 4 and 8 hr when range grass hay was fed except for the reduction in NH_3-N observed at the 4 hr sampling with heated SBM or HSBM-T which was the cause of the treatment by hr interaction ($P < .001$).

TABLE III

NITROGEN BALANCE DATA FROM LAMBS FED SBM-15% TALLOW (SMB-T), SBM-T HEATED FOR FIVE MIN AT 121 C AND 1.36 ATMOSPHERES (HSBM-T) AND HSBM-T PLUS 1% UREA (HSBM-T+U) AND RANGE GRASS HAY (TRIAL 3)

Item	Treatments			SE[c]
	SBM-T	HSBM-T	HSBM-T+U	
	g/head/day			
Hay intake	418.00	394.00	386.00	
Supplement intake	67.00	67.00	68.00	
Nitrogen intake	5.66	5.37	5.43	.07
Urine nitrogen	3.24	3.46	3.50	.19
Fecal nitrogen	2.90	2.58	2.66	.13
Nitrogen retained	-.47	-.68	-.74	.24
% DMD	50.38	47.89	47.80	2.15
Ruminal NH_3-N (mg/dl):				
1 hr postfeeding	8.58[a]	10.65[a]	11.57[b]	1.39
4 hr postfeeding	7.84[a]	8.95[a]	8.38[a]	1.39
8 hr postfeeding	11.52[b]	15.05[b]	12.48[b]	1.39

[ab]Means in a column with similar superscripts are not significantly different ($P < .05$).

[c]Standard error of mean with five lambs/treatment.

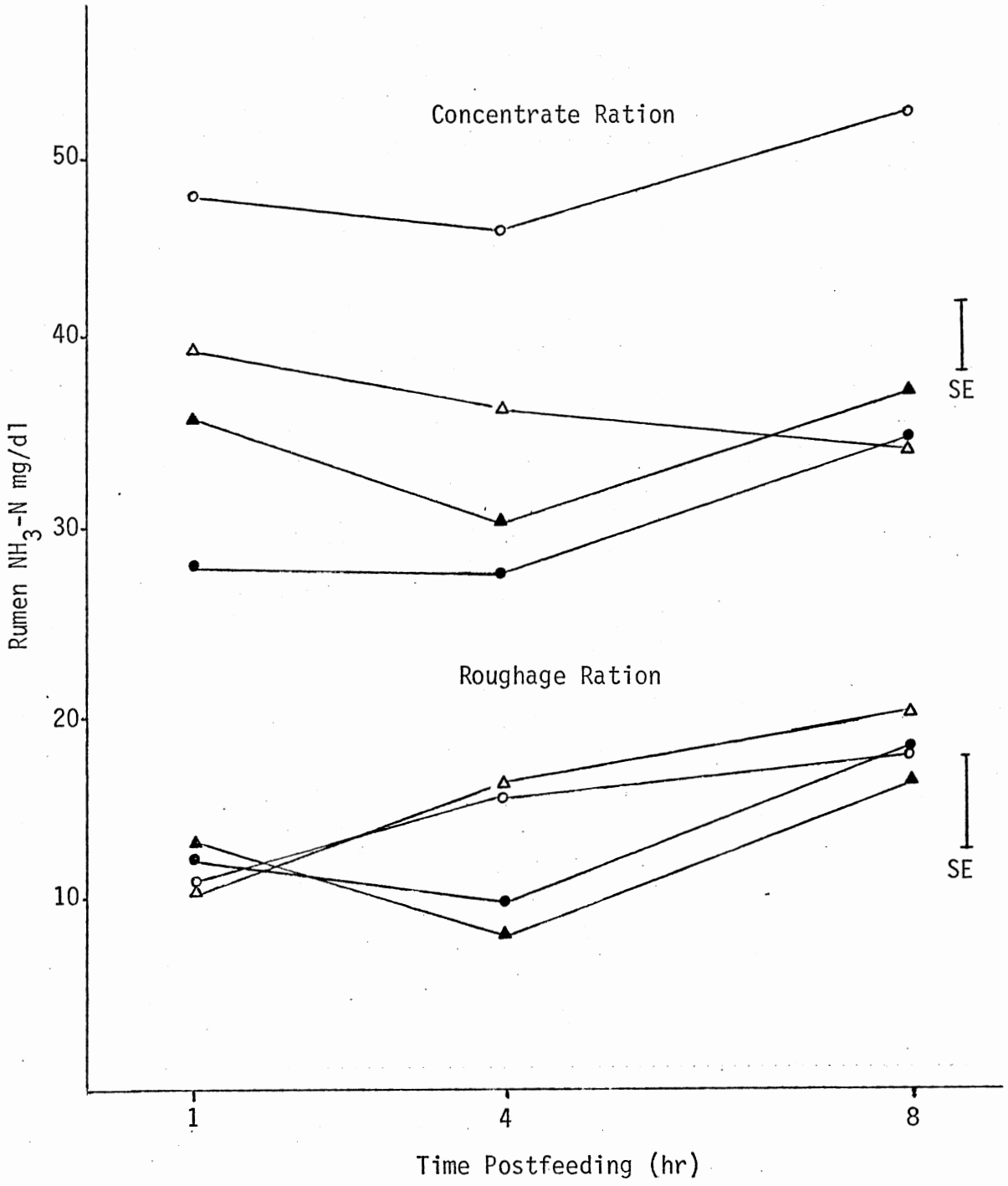

Figure 1. Ruminal Ammonia Concentrations of Lambs Fed the Following Supplements: SBM (-O-), HSBM (-●-), SBM-T (-△-), and HSBM-T (-▲-)

When lambs were fed the same supplements as above, but were fed a 50% concentrate diet (diet A) instead of range grass hay, heat treatment of SBM or SBM-T reduced ($P < .05$) ruminal NH_3-N concentration (30.1, 34.3 vs 47.7 mg/dl) compared to unheated SBM (Figure 1). The addition of tallow to SBM [SBM-T (34.3) vs SBM (51.1 mg/dl)] reduced ($P < .05$) 8 hr NH_3-N concentration to about the same extent as heat treatment. Glen et al. (1977) and Peterson et al. (1975) also observed a reduction in rumen NH_3-N levels at all sampling times postfeeding when linseed meal coated with 30% corn oil, lard, or coconut oil was fed with an 80% concentrate diet. Ruminal NH_3-N increased ($P < .05$) from 1 to 8 hr when lambs were fed range grass hay, however, ruminal NH_3-N concentration was not different ($P > .25$) between 1, 4, or 8 hr sampling times when lambs were fed a 50% concentrate diet (diet A). This suggests that the amount of roughage in the ration may have an effect on ruminal protein degradation when protein is coated with oil or fat.

Trial 5

Complexities of *in vitro* fermentations were further investigated using 2 types of inoculum (i.e. roughage and concentrate) along with 20 mg N/tube of SBM, SBM-T, HSBM, HSBM-T (Figure 2). Protein with tallow provided 160 mg tallow/dl. Heating with either SBM or SBM-T reduced ($P < .05$) 4 and 8 hr NH_3-N concentration when compared to either unheated SBM or SBM-T in either inoculum. Heating SBM-T reduced ($P < .05$) NH_3-N at 8 hr when compared to heating SBM. Heat treatment by fermentation time interaction ($P < .001$) was apparent. This was a result of a more rapid ammonia accumulation from the unheated proteins than the heated proteins. The inoculum source by fermentation time interaction ($P < .001$)

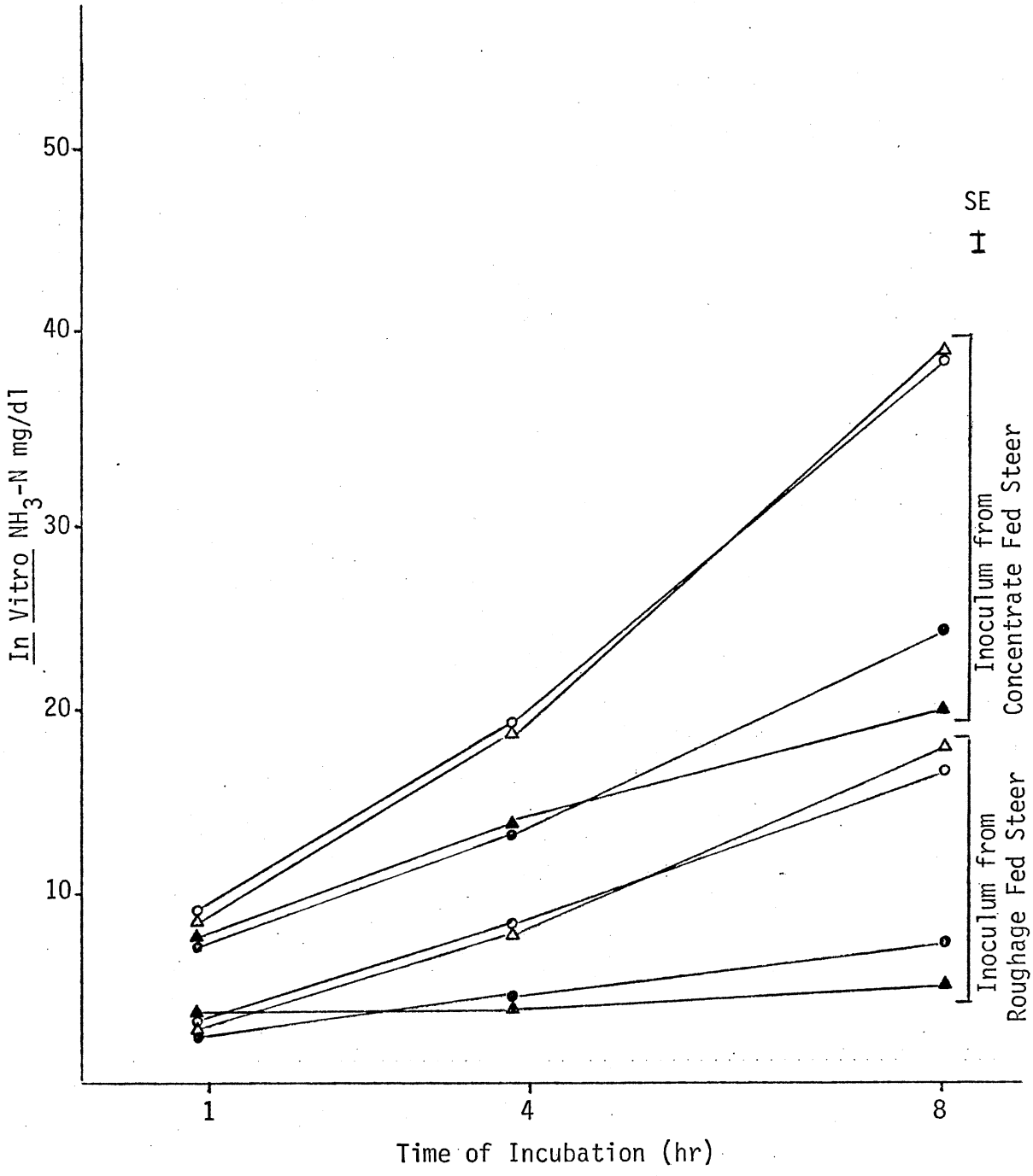

Figure 2. In Vitro Ammonia Concentrations with Various Inocula and the Following Protein Sources: SBM (-O-), HSBM (-●-), SBM-T (-Δ-), and HSBM-T (-▲-)

and the inoculum source by heat treatment interaction ($P < .001$) were apparent with higher ($P < .05$) NH_3-N concentrations associated with the concentrate inoculum than the roughage inoculum.

Czerkawski (1967) observed that incubating 150 mg of free fatty acids from linseed meal/dl significantly reduced protozoal numbers in the rumen of sheep during a period of high fat feeding (90 g of linseed oil/day). This might suggest that when fat is fed at levels that reduce protozoa numbers, NH_3-N could be reduced because of the resultant increase in bacterial numbers and not because of protein protection from ruminal degradation. Tallow concentrations in vitro were more than 150 mg/dl which may have contributed to the inconsistencies observed between in vitro and animal trials. However, free fatty acid concentrations were probably very low relative to tallow concentrations.

Heating SBM consistently reduced NH_3-N concentration when compared with unheated SBM. This is similar to results of Sherrod and Tillman (1962). Addition of tallow to SBM either increased or had little effect on NH_3-N concentration when fermented with inoculum from a roughage-fed animal. However, tallow addition to SBM reduced NH_3-N concentration in inoculum from concentrate-fed steers similar to heating of SBM. Peterson et al., (1975) and Glen et al., (1977) observed an NH_3-N reduction when lambs were fed an 80% concentrate diet with a 30% fat addition to linseed meal.

The synergistic actions of heat and tallow on ammonia production in vitro would support the statement of Chalmers (1960) that the relationship between lipid and nitrogen metabolism in the rumen is complex. Lipids may alter microbial activity while heat and lipid will influence exposure of protein for degradation. The heated CSM and SBM tallow mixtures had

desirable handling and feeding characteristics. Heat treatment may increase the amount of fat which may be handled in a ration and accepted by animals.

CHAPTER IV

TALLOW TREATED COTTONSEED MEAL FOR GROWING LAMBS AND WINTERING COWS

Summary

The feasibility of decreasing ruminal protein breakdown by treating cottonseed meal with tallow was studied in a growth trial with lambs and a wintering trial with cows. In the lamb trial crossbred lambs (36 kg) were individually fed ad libitum for 56 days a 72% rolled corn ration with 4 supplemental protein treatments: cottonseed meal in the meal form, pelleted cottonseed meal, cottonseed meal in meal form mixed with 15% tallow, and cottonseed meal pelleted with 15% tallow. Differences in daily gain (ADG) and for feed/gain were small ($P > .05$). In the cow wintering trial, 61 lactating Hereford cows (446 kg) grazing dormant, winter tallgrass range were assigned to 3 supplemental protein treatments: cottonseed meal, cottonseed meal mixed with 15% tallow and pelleted into 1.6 cm pellets, and cottonseed meal plus the 15% tallow fed unpelleted with cottonseed hulls. Energy was equalized across treatments with cottonseed hulls and cane molasses so that intake averaged .54 kg crude protein and 1.94 kg TDN/head/day. Treatment differences were small ($P > .50$) in cow or calf weight change or ruminal ammonia concentration. In these trials 15% tallow addition to cottonseed meal did not improve protein utilization by ewe lambs fed a concentrate diet or lactating Hereford cows grazing dormant, winter tallgrass range.

Introduction

Supplementation of ruminant diets with free oil alters the composition, mobility, and metabolic activity of rumen microflora (Czerkawski, 1967; Marwaha et al., 1972) causing changes in cellulose and protein breakdown (Czerkawski, 1966; Czerkawski et al., 1966; and Robertson et al., 1964) and production of methane (Clapperton et al., 1969), volatile fatty acids, and ammonia (Chalmers, 1960).

Addition of 30% oil to protein supplements fed with a 53% corn diet (Glen et al., 1977) reduced ruminal ammonia and increased nitrogen retention of lambs. In a similar study, Peterson et al. (1975), found that mixing oil with protein reduced ruminal ammonia and plasma urea of lambs. In contrast, Bohman et al. (1959), found that a 5% addition of animal fat did not effect rate of gain of steers fed native grass hay, cottonseed meal, or alfalfa.

Heat treatment has been shown to protect dietary protein for ruminants if appropriate temperature and heating times are employed (Danke et al., 1966). Sherrod and Tillman (1964) found that autoclaving of cottonseed meal increased gains and efficiency of feed use.

The objectives of this research were: (1) to evaluate the effect of tallow on animal performance and (2) to determine the effect of pelleting (and concurrent heating) on ruminal bypass of protein as measured by ruminant weight change.

Materials and Methods

Trial 1

Thirty crossbred ewe lambs (36 kg) were randomly assigned to 4

treatment rations, maintained in individual pens and fed ad libitum rations containing 1.86% tallow for 56 days (Table IV). The tallow had a titer of 43 C. The tallow was added to the CSM (15% tallow in the supplement) or to the grain mixture (0% tallow in supplement). The supplement was fed unpelleted or following pelleting through a 2.0 cm die. The 15% level was chosen since preliminary studies with cows indicated that this was the highest level of tallow that cows would readily consume in a supplement. Pellet crumbling became troublesome above this level. Feed intake was measured daily and weights were taken every 28 days after 12 hr withdrawal of feed and water.

Trial 2

Sixty-one lactating Hereford cows (446 kg) were used in a 125 day wintering trial (December 14 - April 18), in Central Oklahoma. Cattle grazed native tallgrass range with predominant grass species of little bluestem (Andropogan scorparius), big bluestem (Andropogan gerardi), Indian grass (Sorghastrum nutans), and switch grass (Panicum virgatum). Cows were randomly assigned to 3 treatments (Table V) and were group fed 6 days each week. Cows were rotated between 3 pastures every 2 weeks to reduce pasture effects. Initial, intermediate (28 day), and final weights were obtained after a 12 hr withdrawal from feed and water. Postpartum interval was calculated by subtracting 283 days from the subsequent calving dates. Ruminal ammonia nitrogen samples were obtained from cows via stomach tube at 1 and 4 hr postfeeding of the supplements on day 105 of the trial. Sixty ml samples were obtained and microbial activity was stopped with 1 ml of 20% HCl and refrigeration. Ammonia nitrogen (NH_3-N) was analyzed by a modification of the colorimetric

TABLE IV

COMPOSITION OF DIETS FOR LAMB GROWTH TRIAL

Supplement form		Meal		Pelleted	
Tallow % in supp.	IRN	0	15	0	15
Ingredients[a]					
Rolled corn	4-02-931	71.63	71.52	71.63	71.52
Cottonseed hulls	1-01-599	14.60	14.58	14.60	14.58
Cottonseed meal	5-01-621	9.36	9.29	9.36	9.29
Tallow	4-00-409	1.86	1.86	1.86	1.86
Wheat midds	4-05-205	1.04	1.24	1.04	1.24
Limestone	6-02-632	.97	.97	.97	.97
Trace mineralized salt[b]		.54	.54	.54	.54
Vitamin A[c]		+	+	+	+

[a]Dry matter basis; 10.5% crude protein in each diet by analysis.

[b]Ingredients in TM salt, %: Mn, .25; Fe, .20; S, .10; Cu, .033; I, .007; Zn, .005; NaCl, 99.

[c]Vitamin A palmitate, to add 1,100 IU/kg of diet.

TABLE V

COMPOSITION OF LACTATING COW SUPPLEMENTS

Ingredients[a]	IRN	Control	15P[b]	15H[c]
			kg/hd/day	
Cottonseed meal	4-00-621	1.24	1.24	1.24
Tallow	4-00-409	0	.22	.22
Cottonseed hulls	1-01-599	1.73	1.02	1.04
Molasses	4-04-696	.75	.44	.45
KCl		.01	.01	.01

[a]Group fed cows received .54 kg crude protein and 1.94 kg TDN/hd/day.

[b]Tallow added to cottonseed meal and pelleted prior to feeding.

[c]Tallow mixed with cottonseed hulls, molasses, and KCl prior to cottonseed meal addition and feeding.

procedure by Chaney and Marbach (1962). Duplicate samples of .02 ml undiluted rumen fluid were added to test tubes. One ml of phenol reagent was then added followed by mixing and addition of 1 ml sodium hypochlorite reagent prior to mixing again. Samples were incubated at room temperature for 30 min. Distilled water (8 ml) was then added to the tubes prior to mixing and reading on a spectrophotometer (630 nm). All data were evaluated by analysis of variance (Steele and Torrie, 1960).

Results and Discussion

Trial 1

Protein intake was under 74% of the NRC requirement for lambs so that improved animal performance due to improved protein utilization should have been easily detected. Addition of 15% tallow to cottonseed meal failed to significantly improve gain or feed efficiency of lambs (Table VI). Previously, increased nitrogen retention has been observed in lambs when 30% corn oil, lard, or coconut oil was added to linseed meal in a 53% corn diet (Glen et al., 1977). Source of fat, level of fat and level of concentrate differed between these two studies. In this trial, intake of tallow was 13 g and nitrogen was 19.2 g compared with 90 g of corn oil, lard or coconut oil with 14.4 nitrogen in the trial of Glen et al. (1977).

Trial 2

In the wintering cow trial, supplemental protein was at 63% of the requirement listed by NRC (1976). This ensured that cows would not gain weight during the trial and would demonstrate readily a protein response if fat addition or the heat from pelleting improved the value

TABLE VI

INTAKE AND PERFORMANCE OF GROWING LAMBS

Supplement form	Meal		Pelleted		
Tallow % in supp.	0	15	0	15	SED[b]
Lambs, no.	8	8	7	7	
Intake (kg/day):					
Dry matter	1.08	1.12	1.18	1.27	.07
Crude protein (%NRC)[a]	.11 (62)	.12 (68)	.12 (68)	.13 (74)	
Weight gain (kg/day)	.19	.20	.20	.22	.02
Feed/gain	5.68	5.60	5.90	5.77	.51

[a]Percentage of protein requirements listed by NRC (1975).

[b]Approximate standard error of difference.

of added protein. Standing dormant, winter range grass (3% CP) contributes little to the protein needs of lactating beef cows (Forero, 1979; Rush, 1974). Addition of tallow to cottonseed meal or cottonseed hulls had no apparent effect on weight loss of cows or on calf gain (Table VII). These results are similar to those observed with weanling steer calves grazing winter range and fed either alfalfa or cottonseed meal with or without 227 g of animal fat (Bohman et al., 1959).

Postpartum interval was not affected ($P > .25$) by the addition of tallow to cottonseed meal or cottonseed hulls to the postcalving ration (Table VII). The short postpartum interval with low conception rates suggests that those cows cycling early in the 60 day breeding season were bred, while those not bred during the 60 day period probably were delayed beyond the short breeding season because of cold temperatures and 47 days of snow cover encountered during the trial.

Adding tallow to cottonseed meal and/or cottonseed hulls fed to cows reduced ruminal NH_3-N only slightly ($P > .50$) 1 hr postfeeding (Table VII). Robertson and Hawke (1964) found ruminal NH_3-N increased when 500 g and 700 g of linseed oil were infused with water into cows fed freshly cut pasture. These authors suggested that the increase in NH_3-N was due to an increased deamination of amino acids or decreased utilization of NH_3-N by rumen micro-organisms with added oil. Chalmers (1960) found that when dietary nitrogen levels were 3% of dry matter, the addition of oil depressed ruminal NH_3-N concentrations whereas at a nitrogen level of 4.5%, added oil increased NH_3-N concentration. In this trial, cows consumed approximately 0.9% dietary nitrogen and 2.4% dietary fat (220 g of tallow daily). No effect on rumen protein metabolism was apparent from ruminal NH_3-N concentrations. Previous

TABLE VII

WEIGHT CHANGE, REPRODUCTIVE PERFORMANCE, AND RUMINAL NH_3-N OF LACTATING COWS FED TALLOW-COTTONSEED MEAL

Item	Control	15P	15H	SED[b]
Cow-calf pairs, no.	20	22	19	
Initial wt, kg:				
Cow	447	446	447	
Calf	55	56	56	
Wt change, kg(125 days):				
Cow	-28.5	-30.3	-31.5	2.8
Calf	47.7	48.1	51.7	1.8
Post-partum interval (days)	55.9	60.3	62.9	4.0
Conception rate, %	65	55	58	
NH_3-N (mg/dl):[a]				
1 hr postfeeding	4.16	3.55	3.56	.58
4 hr postfeeding	2.81	2.82	2.86	.58

[a]Sixteen cows sampled from each treatment.

[b]Approximate standard error of difference.

work suggests that some minimal concentration of NH_3-N (2.2 mg/dl) in the rumen is required for growth of bacteria and a slightly elevated concentration (4.5 mg/dl) will maximize nitrogen retention in steers without altering dry matter digestibility (Slyter et al., 1979). Ruminal NH_3-N concentration in the cow trial was within this range. Since dormant, native range grass is digested slowly in the the rumen and energy available for urea utilization is minimal, addition of urea probably would not have been beneficial. Adding tallow to protein at levels higher than 15%, such as Glen et al. (1977) did, becomes problematic due to cow supplement refusal, storage, and handling. Addition of 15% tallow to cottonseed meal and/or pelleting this mixture were not effective in protecting cottonseed meal from rumen degradation (based on animal performance), increasing lamb growth, or decreasing weight loss in cows maintained on winter native range.

CHAPTER V

FORMALDEHYDE-TREATED SOYBEAN MEAL FOR RUMINANTS GRAZING WINTER RANGE GRASS

Summary

Soybean meal (SBM) treated with formaldehyde (HCHO) was evaluated as bypass protein in 4 trials. Nineteen lactating Hereford cows/treatment were individually fed .59 kg crude protein daily of either SBM, ½ SBM plus ½ SBM treated with .6% by weight of formaldehyde, or .6% HCHO-SBM while grazing a single winter pasture. Substitution of SBM treated with .6% HCHO for SBM increased cow weight loss and decreased calf gain ($P < .05$) with the mixture intermediate between the untreated and .6% treated SBM.

Five lambs/treatment in a metabolism study were fed 160 g daily of SBM, .3% HCHO-SBM, or .3% HCHO-SBM plus 1% urea (.3% HCHO-SBMU) with winter harvested range grass available ad libitum. Treatment of SBM with .3% HCHO decreased nitrogen (N) digestibility ($P < .01$), ruminal ammonia ($P < .05$) and tended to decrease dry matter digestibility and N retention. In a third trial, 17 lactating Hereford cows/treatment were individually fed .75 kg CP daily of high SBM, .51 kg daily from SBM, or .51 kg daily of .3% HCHO-SBM. Cows fed .3% HCHO-SBM had similar ($P < .05$) weight than either low protein group.

In a final trial, 14 or 15 lactating Hereford cows/treatment were individually fed .90 kg CP daily of high SBM, .68 kg CP from SBM, .68 kg

CP from ½ SBM plus ½ .2% HCHO-SBM, or .68 kg CP from .2% HCHO-SBM. Cows fed .2% HCHO-SBM supplement maintained similar weight loss over 70 days as cows fed high SBM, and maintained more weight ($P < .05$) than those fed SBM. Results indicate that treatment levels above .2% formaldehyde are excessive for cows consuming range forage.

Introduction

Physical or chemical treatments of protein to reduce protein degradability in the rumen can increase production. When rumen degradation or proteins was avoided by an abomasal infusion technique, large increases in wool growth were obtained by Reis and Schinkel (1964). Improved nitrogen utilization and performance in growing lambs (Barry 1972; Faichney, 1971; Ferguson et al., 1967; Wright, 1971) and improved feed efficiency in steers (Spears et al., 1980) has been reported with formaldehyde treatment of SBM. However, some scientists (Schmidt et al., 1973b, 1974; Wachira et al., 1974) have reported little or no animal response to treating SBM with formaldehyde. The method and level of application of formaldehyde for treatment of SBM has varied between studies which contributes to conflicting results. Nitrogen must be adequate for microbial growth in the rumen to maintain digestion and microbial protein flow to the small intestine. Under ideal conditions, high quality protein must bypass the rumen and be digested postruminally.

The purpose of this research was to determine the effect of formaldehyde treatment of soybean meal on nitrogen balance of lambs and winter weight change of cows grazing winter native range.

Materials and Methods

Soybean meal (SBM) was commercially treated with 0, .2%, .3%, and .6% by weight of formaldehyde in a closed system that allowed thorough mixing and prevented volatilization of formaldehyde. One batch of SBM was used for all treatments in a trial.

Trial 1

Fifty-nine fall-calving Hereford cows were allotted by weight to 3 treatments and were blocked on calf age. The trial was conducted during a 56 day period from November to January. Animals grazed a single pasture of native tallgrass range in central Oklahoma, where predominant forage species were little bluestem (Andropogan scoparius), switchgrass (Panicum virgatum), big bluestem (Andropogan gerardi), and Indian grass (Sorghastrum nutans).

Six days each week cows were gathered from the pasture and individually offered one of the three supplements in covered stalls. Supplement treatments are shown in Table VIII. Cows were visually scored for body condition (degree of fatness; 1 = very thin, 10 = very fat) at the beginning and end of the trial. Cow weights were taken after overnight withdrawal of feed and water.

Cows were artificially inseminated over a 41 day period (December 10 to January 20) and subsequently exposed to a bull for 20 days. Estrus was detected by sterile teaser bulls with chin-ball markers during the AI period and breeding bulls during the natural service period. Pregnancy was determined by rectal palpation approximately 54 days after the end of the breeding season. Estrus and pregnancy rates were analyzed by the

TABLE VIII

COMPOSITION OF PROTEIN SUPPLEMENTS INDIVIDUALLY FED TO LACTATING COWS

Item	IRN	Trials 1 and 2			Trial 4		
		SBM	½ .6% HCHO-SBM ½ SBM	.6% HCHO-SBM	High SBM	Low SBM	.3% HCHO Low SBM
				%DM			
Soybean meal	5-04-604	92.34	46.17		49.57	24.63	
.3% HCHO-SBM							24.63
.6% HCHO-SBM			46.17	92.34			
Corn gluten meal	5-02-900				25.34	12.60	12.60
Corn	4-02-915				8.38	43.63	43.63
Alfalfa	1-00-023	3.18	3.18	3.18	5.01	4.98	4.98
Molasses	4-04-696	2.59	2.59	2.59	3.85	3.83	3.83
Dicalcium phosphate	6-01-080				4.28	5.30	5.30
KCl		1.77	1.77	1.77		1.10	1.10
Salt					3.34	3.70	3.70
Trace mineralized salt[a]					.17	.17	.17
Se premix[b]					.06	.06	.06
Vitamin A[c]		.12	.12	.12	+	+	+
Determined crude protein, %		36.80	36.80	36.80	41.50	30.00	28.30

[a]Ingredients of TM salt, %: Fe, 8; Co, 23; Zn, 11; I, .45; Cu, 3.2; and Mn, 6.

[b]Selenium premix contained one mg Se/gram of premix.

[c]Vitamin A supplement provided: Trial 1, 38,000 IU/head/day; Trial 3, 46,000 IU/head/day.

Chi-square procedure (Steele and Torrie, 1960). Cow weight change, cow condition scoring, and calf weight gain were statistically analyzed by procedures outlined by Steele and Torrie (1960) for a randomized block design.

Trial 2

Fifty-seven pregnant Hereford heifers were allotted by weight to 3 treatments and were blocked on heifer origination. The trial was conducted during the 91 day period from November 16 to February 15. Animals grazed a single pasture, were individually fed identical supplements (Table VIII), and were visually scored for body condition as in Trial 1.

Rumen liquor samples were obtained on day 56 of the trial at 1 and 4 hr postfeeding via stomach tube by a slight modification of the technique of Raun and Burroughs (1962). Microbial activity was stopped by adding 1 ml of 20% HCl per 50 ml of rumen fluid. Samples were frozen for later analysis for ruminal ammonia nitrogen (NH_3-N) by a modification of the colorimetric procedure by Chaney and Marbach (1962). Duplicate samples of .02 ml undiluted rumen fluid were added to test tubes. One ml of phenol reagent was then added followed by mixing and addition of 1 ml sodium hypochlorite reagent prior to mixing again. Samples were incubated at room temperature for 30 min. Distilled water (8 ml) was then added to the tubes prior to mixing and reading on a spectrophotometer (630 nm).

Heifer weight change and condition score change were statistically analyzed by procedures outlined by Steele and Torrie (1960) for a completely randomized block design.

Trial 3

Fifteen crossbred wether lambs (27 kg) were randomly assigned, 5 per treatment, in a nitrogen balance trial to 58 g crude protein (CP) from the following supplements[1] plus ad libitum ground winter harvested range grass hay (3% CP): soybean meal (SBM), .3% formaldehyde-treated SBM (.3% HCHO-SBM), and 1% urea-supplemented .3% formaldehyde-treated SBM (.3% HCHO-SBMU). After lambs learned to consume their supplement within 15 to 20 min after offering, hay was provided ad libitum. A 7 day preliminary period was followed by 5 days of collection of urine and feces. At the end of the metabolism study, lambs were fed their respective supplements and were sampled for NH_3-N at 1, 4, and 8 hr postfeeding. Microbial activity was stopped in the 50 ml sample by adding 1 ml of 20% HCl and refrigeration. Ammonia nitrogen was determined by a modification of the colorimetric procedure by Chaney and Marbach (1962).

Trial 4

This trial was conducted during the 100-day period from November 27 to March 6. Fifty-three fall-calving Hereford cows were stratified by weight and assigned to 3 treatments. Animals grazed a single pasture and were individually fed 6 days each week. Supplement compositions are shown in Table I. Animal weights and condition score were obtained as described in Trial 1.

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[1]Ingredients consumed per day, grams: Dicalcium phosphate, 15; NaCl, 10; Iron sulfate, 10; zinc sulfate, .09; calcium carbonate, .09; manganese sulfate, .04; copper sulfate, .01; cobalt sulfate, .8 mg; calcium iodate, .3 mg; vitamin A, 1700 IU; and vitamin D, 300 IU.

Cows were exposed to a bull for 59 days (December 3 to January 31). Estrus was detected by breeding bulls with chin-ball markers. Pregnancy was determined by rectal palpation approximately 55 days after the end of the breeding season.

Rumen liquor samples were obtained on day 45 of the trial via stomach tube by the technique of Raun and Burroughs (1962). Microbial activity was stopped by adding 1 ml of 20% HCl per 50 ml of rumen fluid. Samples were frozen for later analysis for ruminal NH_3-N by a modification of a colorimetric procedure (Chaney and Marbach, 1962). Results of ruminal NH_3-N were analyzed as a split plot on sampling time (Steele and Torrie, 1960). Cow weight change, cow condition score, and calf weight gain (blocked on sire) were statistically analyzed by procedures for a completely randomized block design (Steele and Torrie, 1960). Estrus and pregnancy rates were analyzed by the Chi-square procedure (Steele and Torrie, 1960).

Trial 5

Sixteen crossbred ram lambs (24.7 kg) were randomly assigned in a nitrogen balance trial to ad libitum winter harvested range grass hay (3% CP) plus the following treatments: high SBM (100 g CP), SBM (76 g CP), ½ .2% HCHO-SBM plus ½ SBM (76 g CP), and .2% HCHO-SBM (76 g CP). Composition of supplements was identical to protein supplements fed to lactating cows grazing winter native range (Table IX). Supplements were offered once daily in the absence of hay until consumed. After lambs learned to consume their supplement within 15 to 20 min after offering, hay was provided ad libitum. A 7 day preliminary period was followed by 5 days of collection of urine and feces. At the end of the

TABLE IX

COMPOSITION OF PROTEIN SUPPLEMENTS INDIVIDUALLY FED TO LACTATING COWS

Item	IRN	High SBM	SBM	½ .2% HCHO-SBM ½ SBM	.2% HCHO-SBM
			%DM		
Soybean meal	5-04-604	89.76	59.50	29.75	
.2% HCHO-SBM				29.75	59.50
Corn	4-02-931		29.64	29.64	29.64
Alfalfa	1-00-023	2.07	2.07	2.07	2.07
Molasses	4-04-696	2.01	2.01	2.01	2.01
Monocalcium phosphate		4.92	5.55	5.55	5.55
KCl		1.12	1.12	1.12	1.12
Vitamin A[a]		.11	.11	.11	.11
Determined crude protein, %		41.28	32.78	29.81	33.62

[a]Vitamin A supplement provided 67,000 IU/head/day.

metabolism study, lambs were fed their respective supplements and were sampled for ruminal NH_3-N at 1 and 4 hr postfeeding. Microbial activity was stopped in the 50 ml sample by adding 1 ml of 20% HCl and refrigeration. Ammonia nitrogen was determined by a modification of a colorimetric procedure (Chaney and Marbach, 1962).

Trial 6

Fifty-eight of the 59 fall-calving cows used in Trial 1 were randomly allotted by weight to 4 treatments. Cows were blocked on previous treatment and grazed a single pasture described in Trial 1 for the 70 day period (January 18 to March 29). Six days each week, animals were gathered from the pasture and individually offered 1 of the 4 supplements in covered stalls. Supplement treatments are shown in Table IX. On day 33 of the trial, all cows were fed their supplements and ruminally sampled for NH_3-N at 1 and 4 hr postfeeding. Cows were visually scored for body condition as described in Trial 1 at the beginning and end of the trial. Cow and calf weights were taken after overnight withdrawal from feed and water.

The treatment and block sum of squares for cow weight change and calf gain were adjusted for the covariable cow weight. Cow condition score was statistically analyzed as a completely randomized block design and ruminal NH_3-N results were analyzed as a split plot on sampling time (Steele and Torrie, 1960).

Duplicate 2 g samples of protein supplements were digested in 1 g pepsin per 250 ml of 20% HCl for 24 hr at 39C. Pepsin insoluble nitrogen was calculated from nitrogen contents of the original sample and residue remaining. Remaining residue nitrogen divided by the amount in

the original sample was considered to represent pepsin insoluble nitrogen. Solubilities of protein supplements were determined by extracting approximately .5 g supplement samples in 50 ml .15 N NaCl at 39 C for 6 hr (Waldo and Goering, 1979). Rate of ruminal digestion was estimated using the rumen in situ technique with dacron bags as described by Mehrez (1976), except duplicate bags were incubated for 4, 12, and 24 hr (Zinn et al., 1980). Dry matter remaining in bags at 4 hr incubation was considered the zero time value for estimating rates of protein disappearance.

Results

Trial 1

Substitution of SBM treated with .6% formaldehyde for untreated SBM for lactating cows resulted in greater ($P < .05$) weight loss in cows (-23.0 vs -13.6 kg) and reduced weight gain in calves (20.4 vs 27.3 kg) over 56 days (Table X). Clark et al. (1974) fed .9% formaldehyde treated SBM to lactating cows and found no beneficial effect on milk production or milk protein content. In our trial as well as that of Clark et al. (1974), the SBM may have been overprotected with formaldehyde. It is possible that a lower level of formaldehyde treatment as suggested by Peter et al. (1971) would improve animal performance. Cow condition score change did not differ ($P > .05$) between treatments (-.7 vs -1.0).

Estrus was detected in 71%, 58%, and 53% of the cows fed the SBM, ½ .6% HCHO-SBM plus ½ SBM, and .6% HCHO-SBM supplements, while cows pregnant at palpation 54 days after breeding were 76%, 42%, and 58%,

TABLE X

PERFORMANCE OF LACTATING COWS INDIVIDUALLY FED .6% FORMALDEHYDE TREATED SOYBEAN MEAL

Item	SBM	½ .6% HCHO-SBM ½ SBM	.6% HCHO-SBM	SED[d]
No. cows	21	19	19	
Supp. intake, kg/day;[c]				
Dry matter	1.60	1.60	1.60	
Crude protein	.59	.59	.59	
Cow weight, kg:				
Initial	466.2	466.1	468.0	
56 day change	-13.6[a]	-17.9[ab]	-23.0[b]	3.0
Cow condition score:				
Initial	6.3	6.4	6.6	
56 day change	-.7[a]	-1.0[a]	-1.0[a]	.2
Calf weight gain, kg	27.3[a]	23.9[ab]	20.4[b]	1.9
Cows showing estrus	15[a]	11[a]	10[a]	
Cows pregnant	16[a]	8[a]	11[a]	

[ab]Means in a row with the same superscript letter do not differ significantly ($P < .05$).

[c]Intake based on seven days/week.

[d]Approximate standard error of difference.

respectively. Although reproductive data did not differ ($P > .05$) between treatments, reproductive performance tended to decline as the proportion of formaldehyde treated SBM increased in the supplement.

Trial 2

Feeding .6% HCHO-SBM to pregnant heifers resulted in a trend for greater ($P > .05$) weight loss (-16.9 vs -11.8 kg) over 91 days (Table XI). This trend agrees with the lactating cow performance in Trial 1. Because pregnant heifers have greater energy needs relative to their intake capabilities compared to mature cows, lack of energy may have prevented a weight response to protein supplementation as was observed with lactating cows fed .6% HCHO-SBM in Trial 1. Heifer condition score change was not significantly altered by treatment (-.4 vs -.2).

Ruminal NH_3-N was decreased ($P < .05$) at 1 and 4 hr postfeeding when heifers were fed any .6% HCHO-SBM compared to untreated SBM (Table XI).

Trial 3

Treatment of soybean meal with .3% formaldehyde decreased ($P < .05$) urinary nitrogen, nitrogen digestibility, and ruminal NH_3-N in lambs while increasing ($P < .05$) fecal nitrogen (Table XII). There was also a trend for formaldehyde treatment to decrease ($P > .05$) nitrogen retention, dry matter digestibility, and nitrogen retained as percent of nitrogen absorbed. Faichney (1974) observed reduced nitrogen digestibility and ruminal ammonia with increased nitrogen retention when lambs were fed casein treated with 1.9% formaldehyde and fed a high quality hay. This reflects an increase in intestinal digestion of protein as a

TABLE XI

PERFORMANCE OF HEIFERS INDIVIDUALLY FED .6% FORMALDEHYDE TREATED SOYBEAN MEAL

Item	SBM	½ .6% HCHO-SBM ½ SBM	.6% HCHO-SBM	SED[d]
No. heifers	19	18	20	
Supp. intake, kg/day:[c]				
Dry matter	1.17	1.17	1.17	
Crude protein	.43	.43	.43	
Heifer weight, kg:				
Initial	339.9	334.0	336.6	
91 day change	-11.8[a]	-12.7[a]	-16.9[a]	2.1
Heifer condition score:				
Initial	5.8	5.8	5.8	
91 day change	-.2[a]	-.3[a]	-.4[a]	.1
Ruminal NH_3-N, mg/dl:				
1 hr postfeeding	28.6[a]	16.5[b]	14.9[b]	3.8
4 hr postfeeding	31.8[a]	13.9[b]	11.2[b]	3.8

[ab]Means in a row with the same superscript letter do not differ significantly ($P < .05$).

[c]Intake based on seven days/weeks.

[d]Approximate standard error of difference.

TABLE XII

DIETARY NITROGEN PARTITIONING AND RUMINAL AMMONIA PRODUCTION BY LAMBS FED .3% FORMALDEHYDE-TREATED SOYBEAN MEAL AND HAY

Item	SBM	.3% HCHO-SBM	.3% HCHO-SBM + 1% urea	SE[d]
		g/hd/day		
Supplement intake	150	150	150	
Hay intake	611	591	584	
N intake	13.08[a]	12.84[a]	13.05[a]	.12
Urine N	6.11[a]	5.18[b]	5.40[b]	.19
Fecal N	4.78[b]	6.04[a]	6.22[a]	.24
N retained	2.20[a]	1.60[a]	1.50[a]	.26
% N digestibility	63.44[a]	52.91[b]	49.77[b]	1.73
% dry matter digestibility	49.07[a]	44.97[a]	46.22[a]	1.44
N retained as % of N absorbed	33.90[a]	27.50[a]	23.60[a]	35.20
NH_3-N (mg/dl):				
1 hr postfeeding	17.64[a]	9.04[b]	10.77[b]	2.14
4 hr postfeeding	16.85[a]	7.70[b]	7.27[b]	2.14
8 hr postfeeding	19.94[a]	12.31[b]	8.96[c]	2.14

[abc]Means in a row with same superscript letter do not differ significantly ($P < .05$).

[d]Standard error of mean with five lambs per treatment.

result of treatment (Faichney and Weston, 1971). Method of HCHO application and level of formaldehyde differed between their studies and the present study. The addition of 1% urea to .3% HCHO treated SBM reduced ($P < .05$) 8 hr ruminal NH_3-N compared to untreated SBM or .3% HCHO-SBM without urea but failed to increase nitrogen retention. Other digestibility and nitrogen balance trials with sheep have shown increased nitrogen retention even though nitrogen digestibility was reduced by formaldehyde treatment of protein (MacRae et al., 1972; McGillard, 1972). Since nitrogen retention was not increased ($P > .05$) in lambs fed .3% HCHO treated SBM, this level of formaldehyde treatment may have over-protected SBM for this type of roughage diet for lambs.

Trial 4

Treating a low level of SBM with .3% formaldehyde did not reduce ($P < .05$) 100-day weight losses of lactating cows (Table XIII). Cows consuming a higher level of untreated SBM lost less weight ($P < .05$) than cows fed low SBM (-42.8 vs -61.4 kg), indicating that cows fed the low protein levels were deficient in dietary protein and therefore would have responded favorably if .3% formaldehyde treatment of SBM had improved protein utilization.

Cow condition score changes were not different ($P > .05$) between treatments. Calf gain, as expected, followed the same pattern as cow weight change. Calves nursing cows in the higher protein group had greater gains ($P < .05$) than calves nursing cows fed less protein (48.8 vs 37.9 kg).

Past research in Oklahoma (Pope et al., 1963) showed a high relationship ($r = .65$ to $.80$) between the amount of milk produced and spring

TABLE XIII

PERFORMANCE OF LACTATING COWS INDIVIDUALLY FED .3% FORMALDEHYDE TREATED SOYBEAN MEAL

Item	High SBM	Low SBM	.3% HCHO Low SBM	SED[f]
No. cows	18	18	17	
Supp intake, kg/day:[d]				
Dry matter	1.80	1.70	1.80	
Crude protein	.75	.51	.51	
Cow weight kg:				
Initial	453.30	451.10	451.30	5.3
100 day change	-42.8[a]	-61.4[b]	-58.6[b]	
Cow condition score:				
Initial	6.3	6.2	6.2	
100 day change	-1.0[a]	-1.2[a]	-.8[a]	.2
Cows showing estrus	10[a]	12[a]	14[a]	
Cows pregnant	17[a]	18[a]	16[a]	
Calf weight gain, kg	48.8[a]	37.9[b]	39.6[b]	3.5
Ruminal NH_3-N, mg/dl:[e]				
1 hr postfeeding	8.8[a]	4.7[b]	3.0[b]	.9
4 hr postfeeding	11.0[a]	3.3[b]	1.3[c]	.9

[abc]Means in a row with the same superscript letter do not differ significantly ($P < .05$).

[d]Intake based on seven days/week.

[e]Treatment by hr interaction ($P < .05$); 18 cows sampled per treatment.

[f]Approximate standard error of difference.

weight of fall born calves suggesting that greater milk production by cows fed the high SBM supplement may be responsible for greater calf gains.

Estrus was detected in 55%, 67%, and 82% of the cows fed the high protein, low protein, and .3% formaldehyde low protein supplements. Cows pregnant at palpation 55 days after breeding were 94%, 100%, and 94% of cows exposed respectively. The good reproductive performance was probably due to the mild snowless winter combined with the excellent condition of the cows at the beginning of the trial. Since pregnancy rates on all treatments were high, the low number of cows detected in estrus reflects poor estrus detection.

Treatment of SBM with .3% formaldehyde tended to reduce ruminal NH_3-N at 1 hr and reduced ($P < .05$) ruminal NH_3-N at 4 hr postfeeding. (Table XIII). Ruminal NH_3-N concentration was higher ($P < .05$) at 1 and 4 hr postfeeding with the higher protein than the low protein supplements.

The reduction in ruminal ammonia concentration with formaldehyde indicates that protein degradation was substantially reduced by treatment. Such reductions have been found consistently with treatment of protein with forage based diets (Ferguson et al., 1967; Faichney and Weston, 1971), although with concentrate diets the response is more variable (Faichney, 1972) and appears related to the pattern of fermentation (Faichney, 1974).

Trial 5

One ram lamb fed the SBM treatment stopped eating and was taken off test. Because lambs in this trial did not consume as much low quality hay as desired, the amount of supplement consumed made up over 40% of

the diet. This probably accounted for the confusing nitrogen retention results. Feeding lambs .2% HCHO-SBM tended to reduce ($P > .05$) urinary N and increased ($P > .05$) fecal N and percent dry matter digestibility compared to the SBM supplement (Table XIV).

Ruminal NH_3-N concentration was very high overall due to low hay intake. However, .2% HCHO-SBM reduced ($P < .05$) ruminal NH_3-N at 1 and 4 hr postfeeding compared to the SBM treatment. The results from this study should be discounted due to low hay intakes and ruminal NH_3-N concentrations approaching toxicity levels.

Trial 6

Treatment of SBM with .2% formaldehyde decreased ($P < .05$) 70 day weight loss (-38.3, -38.5 vs -47 kg) of lactating cows (Table XV). Weight loss with formaldehyde treated SBM was numerically similar to that of cows fed a higher protein supplement (-38.3, -38.5 vs -38.8 kg). Since the lactating cows that lost the most weight in Trial 1 (those fed .6% HCHO-SBM) lost the least weight in this trial, there was a significant block effect. Feeding cows a low SBM treatment increased ($P < .10$) cow condition score change compared to feeding formaldehyde treated SBM to cows (-.7 vs -.3). Calf weight gain was not different ($P > .05$) between treatments. Ruminal NH_3-N levels were progressively reduced ($P < .05$) at 1 and 4 hr postfeeding with formaldehyde treatment. In contrast, Spears et al., (1980) observed only a tendency for NH_3-N concentrations to be lower in steers fed .3, .6, or .9% formaldehyde treated SBM with a high concentrate diet.

Cows fed the high SBM produced higher ($P < .05$) ruminal NH_3-N levels than any of the other treatments. A treatment by hr interaction

TABLE XIV

DIETARY NITROGEN PARTITIONING AND RUMINAL AMMONIA PRODUCTION BY LAMBS FED .2% FORMALDEHYDE-TREATED SOYBEAN MEAL AND HAY

	High SBM	SBM	½ .2% HCHO-SBM ½ SBM	.2% HCHO-SBM	SE[f]
			g/hd/day		
Supplement intake	210	214	188	207	
Hay intake	309.75[a]	333.25[a]	328.75[a]	298.00[a]	42.32
N intake	17.76[a]	14.02[b]	14.18[b]	14.19[b]	.23
Urine N	12.73[a]	9.77[b]	10.03[b]	9.07[b]	.68
Fecal N	4.73[a]	4.45[a]	5.20[a]	4.53[a]	.30
N retained	.30[a]	.53[a]	-1.60[a]	.59[a]	.76
% N digestibility	73.00	68.00	63.00	68.00	
% dry matter digestibility	53.69[a]	55.48[a]	53.70[a]	56.69[a]	1.70
Ruminal NH_3-N (mg/dl):					
1 hr postfeeding	70.60[ae]	50.17[be]	31.38[cd]	28.62[cd]	7.82
4 hr postfeeding	98.41[ad]	57.15[bd]	30.05[cd]	30.68[cd]	7.82

[abc]Means in a row with the same superscript letter do not differ significantly ($P < .05$).

[de]Means in a column with the same superscript letter do not differ significantly ($P < .05$); treatment by hr interaction ($P < .05$).

[f]Standard error of mean with four lambs/treatment.

TABLE XV

PERFORMANCE OF LACTATING COWS INDIVIDUALLY FED .2% FORMALDEHYDE TREATED SOYBEAN MEAL

Item	High SBM	SBM	½ .2% HCHO-SBM ½SBM	.2% HCHO-SBM	SED[j]
No. cows	15	14	15	14	
Supp. intake, kg/day:[h]					
Dry matter	2.18	2.07	2.28	2.02	
Crude protein	.90	.68	.68	.68	
Cow weight, kg:					
Initial	488.10	445.80	440.90	448.20	
70 day change	-38.8[a]	-47.2[b]	-38.3[a]	-38.5[a]	2.7
Cow condition score:					
Initial	5.60	5.50	5.30	5.60	
70 day change	-.5[de]	-.7[d]	-.3[e]	-.3[e]	.2
Calf weight gain, kg	23.7[a]	24.4[a]	24.0[a]	22.5[a]	1.4
Ruminal NH_3-N, mg/dl:[i]					
1 hr postfeeding	15.5[af]	9.9[bf]	8.2[bcf]	4.5[cg]	2.2
4 hr postfeeding	20.6[ag]	11.7[bg]	9.7[bf]	2.7[cf]	2.2

[abc]Means in a row with the same superscript letter do not differ significantly ($P < .05$).

[de]Means in a row with the same superscript letter do not differ significantly ($P < .10$).

[fg]Means in a column with the same superscript letter do not differ significantly ($P < .05$).

[h]Intake based on seven days/week.

[i]Treatment by hr interaction ($P < .05$).

[j]Approximate standard error of difference.

($P < .05$) was due to a very low 4 hr NH_3-N concentration of cows fed .2% HCHO-SBM. The low ruminal NH_3-N value observed at 4 hr postfeeding with .2% HCHO-SBM could have limited microbial growth (Slyter et al., 1979). However, based on equivalent cow weight losses observed with the high SBM and ½ .2% HCHO-SBM plus ½ SBM treatments which also had higher ruminal NH_3-N concentrations than the .2% HCHO-SBM, NH_3-N may have been adequate under these feeding conditions.

Schmidt et al. (1973b, 1974) and Wachira et al. (1974) found with lambs, steers, and lactating dairy cows that .3%, .6%, and .8% formaldehyde treatment of SBM fed to ruminants resulted in no beneficial effect. This agrees with our studies with .3% and .6% formaldehyde treated SBM fed to lambs and cows. Working with similar levels and method of application of formaldehyde, Spears et al. (1980) suggested from in vitro work that treating SBM with .3% to .6% formaldehyde decreased protein degradation to almost the same extent as treatment with a higher concentration of formaldehyde (i.e. .9%) compared to untreated SBM.

Treating SBM with .6% HCHO substantially increased percent pepsin insoluble nitrogen compared to the SBM supplement (21.1 vs 8.5%, respectively) in Trial 1 (Table XVI). This decrease in nitrogen digestibility was reflected in greater cow weight loss when fed .6% HCHO-SBM. Treatment of SBM with .2% and .3% HCHO increased percent pepsin insoluble nitrogen to similar extents. However, feeding .2% HCHO-SBM reduced ($P < .05$) cow weight loss (-38 vs -47 kg) while feeding .3% HCHO to cows did not (-59 vs -61 kg). Since corn gluten meal was included in supplements in Trial 4, the failure to obtain a significant cow weight response may have been due to meeting the bypass protein requirements of the cow with corn gluten meal and therefore, being unable to detect a

TABLE XVI

RUMINAL DEGRADATION *IN VIVO*, *IN VITRO* SOLUBILITY AND PEPSIN INSOLUBLE NITROGEN OF PROTEIN SUPPLEMENTS

Protein Supplements	Pepsin Insoluble Nitrogen (%)	.15 N NaCl Soluble N (%)	*In situ* digestion rate % per hour		Intestinal supply of digestible protein, %[a]
			0-8 hr	8-20 hr	
SBM	8.5	8.9	1.20	7.06	15.9
½ .6% HCHO-SBM	10.2	.6	.28	1.78	73.1
.6% HCHO-SBM	21.1	2.7	.26	1.15	63.2
High SBM	9.4	8.5	3.12	1.84	46.6
SBM	9.6	12.2	0	5.33	31.4
½ .2% HCHO-SBM	11.1	7.3	1.61	1.11	60.7
.2% HCHO-SBM	12.2	5.2	1.10	.26	72.0
High SBM	8.3	11.1	4.15	6.10	14.9
Low SBM	9.0	4.6	0	2.63	61.3
.3% HCHO Low SBM	11.1	3.6	0	3.06	55.8

[a]Calculated assuming pepsin insoluble nitrogen is not digestible while soluble N is totally digested in the rumen and *in situ* ruminal digestion continues for 20 hr.

response to feeding .3% HCHO-SBM because of protein excess to the intestines.

Percent soluble nitrogen in .15 N NaCl was reduced with formaldehyde treatment. The amount of reduction reflected the level of formaldehyde treatment (Table XVI).

The in situ digestion rates appeared to generally be reduced with formaldehyde treatment especially during the 8 - 20 hr period. The zero digestion rate values encountered during the 0 - 8 hr period suggest either very little digestion when incubating samples in the rumen from 4 to 12 hr or some procedural problems.

The estimated intestinal supply of digestible protein appeared to increase with formaldehyde treatment compared to untreated SBM. However, in the .3% HCHO-SBM trial values were similar (55.8 vs 61.3%) and may have been due to corn gluten meal addition to the supplements.

Discussion

Ideally, one would like to protect dietary protein from ruminal degradation with formaldehyde treatment, yet maintain high digestibility of treated protein in the lower gastrointestinal tract. Treatment of SBM with greater than .3% formaldehyde for lactating range cows may be reducing both ruminal degradation and intestinal digestibility of treated protein as indicated by pepsin digestibility in this trial. Chicks fed SBM treated with .3% formaldehyde gained as well as those fed the control diet (Spears et al., 1980). Gain to feed ratios in chicks were slightly decreased even by the low level of formaldehyde suggesting that, even though growth was not depressed when SBM was treated with .3% formaldehyde, digestibility may have been reduced slightly. It is unclear

from their report whether protein was or was not limiting in their basal ration. Similar findings with rats fed .4% formaldehyde treated SBM were reported by Schmidt et al. (1973b).

Treatment of SBM and other proteins with formaldehyde has been shown to increase the quantity of protein entering the lower digestive tract of ruminants (Faichney and White, 1977; MacRae et al., 1972; Miller, 1972; Nishimuta et al., 1974). Spears et al. (1980) reported HCHO treatment resulted in a linear increase in average daily gain in steers and improvement in feed efficiency.

Lactating cow performance and pepsin insoluble nitrogen suggests that a lower level of formaldehyde treatment may be more desirable when ruminants are fed low quality roughage diets. These studies indicate that lactating beef cows are highly sensitive to over protection of protein and that weight gain of calves is very responsive to change in protein level. Since the level of fermentable energy in poor quality roughage diets is low, ruminal ammonia concentration is probably not as critical to microbial fermentation as it would be in a grain diet. It is unlikely that a single level of protein protection with formaldehyde will be suitable for a wide range of diets.

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APPENDIX

TABLES

TABLE XVII

ANALYSIS OF VARIANCE (*IN VITRO* AMMONIA NITROGEN TRIALS, CHAPTER III)

	AOV Table for *In Vitro* Trial I		
Source of Variation	df	Mean Squares	P Value
Total	35	- - -	- - -
Treatments	11	17.460	P < .05
Mixture	2	8.621	P > .10
Time	1	4.333	P > .10
Pressure	1	81.270	P < .005
Mixture x Time	2	22.893	P < .10
Mixture x Pressure	2	15.315	P > .10
Pressure x Time	1	3.966	P > .10
Mixture x Pressure x Time	2	4.414	P > .10
Days	2	419.376	P < .001
Error	22	6.686	- - -

TABLE XVII (Continued)

AOV Table for *In Vitro* Trial II (CSM)			
Source of Variation	df	Mean Squares	P Value
Total	35	- - -	- - -
Treatments	11	42.074	P < .001
Mixture	2	52.143	P < .001
Time	3	101.783	P < .001
Mixture x Time	6	8.863	P > .10
Error	24	5.361	- - -
AOV Table For *In Vitro* Trial II (SBM)			
Source of Variation	df	Mean Squares	P Value
Total	26	- - -	- - -
Treatments	8	154.553	P < .001
Mixture	2	80.664	P < .001
Time	2	472.345	P < .001
Mixture x Time	4	32.599	P < .001
Error	18	4.324	- - -

TABLE XVIII

ANALYSIS OF VARIANCE (LAMB NITROGEN BALANCE TRIAL, CHAPTER III)

AOV Table for Lamb Nitrogen Intake (Grams/Day)			
Source of Variation	df	Mean Square	P Value
Total	14	- - -	- - -
Treatments	2	.1181	P > .10
Error	12	.0223	- - -
AOV Table for Lamb Urine Nitrogen Excreted			
Source of Variation	df	Mean Square	P Value
Total	14	- - -	- - -
Treatments	2	2.748	P > .10
Error	12	4.488	- - -
AOV Table for Lamb Fecal Nitrogen Excreted			
Source of Variation	df	Mean Square	P Value
Total	14	- - -	- - -
Treatments	2	3.245	P > .10
Error	12	1.980	- - -
AOV Table for Lamb Nitrogen Retained			
Source of Variation	df	Mean Square	P Value
Total	14	- - -	- - -
Treatment	2	.1031	P > .10
Error	12	.2932	- - -

TABLE XVIII (Continued)

AOV Table for Lamb Percent Dry Matter Digestibility			
Source of Variation	df	Mean Square	P Value
Total	14	- - -	- - -
Treatments	2	10.580	P > .10
Error	12	23.138	- - -
AOV Table for Lamb Ruminal Ammonia Nitrogen			
Source of Variation	df	Mean Square	P Value
Total	44	- - -	- - -
Whole plot	14	11.083	P > .10
Treatments	2	19.473	P > .10
Error	12	9.684	- - -
Subplot	30	9.565	P < .005
Time	2	81.294	P < .001
Treatment x Time	4	5.232	P > .10
Error	34	3.042	- - -

TABLE XIX

ANALYSIS OF VARIANCE (LAMB RUMINAL AMMONIA NITROGEN TRIAL, CHAPTER III)

AOV Table for Lambs Fed SBM-Tallow Plus Roughage			
Source of Variation	df	Mean Squares	P Value
Total	47	- - -	- - -
Whole Plot	15	77.758	P > .10
Treatments	3	18.281	P > .10
Error	12	92.627	- - -
Subplot	32	16.223	P < .001
Time	2	175.173	P < .001
Treatments x Time	6	26.223	P < .001
Error	24	.478	- - -
AOV Table for Lambs Fed SBM-Tallow Plus Concentrate			
Source of Variation	df	Mean Squares	P Value
Total	47	- - -	- - -
Whole Plot	15	178.463	P < .05
Treatments	3	678.282	P < .001
Error	12	53.508	- - -
Subplot	32	70.747	P > .10
Time	2	80.783	P > .10
Treatments x Time	6	28.817	P > .10
Error	24	80.393	- - -

TABLE XX

ANALYSIS OF VARIANCE (IN VITRO AMMONIA NITROGEN STUDY, TRIAL FIVE, CHAPTER III)

	AOV Table for In Vitro Ammonia Nitrogen Using Roughage or Concentrate Inoculum		
Source of Variation	df	Mean Squares	P Value
Total	47	- - -	- - -
Mixture	3	141.969	P < .001
Inoculum	1	1322.265	P < .001
Time	2	842.141	P < .001
Mixture x Inoculum	3	6.921	P < .001
Mixture x Time	6	59.273	P < .001
Inoculum x Time	2	164.215	P < .001
Mixture x Time x Inoculum	6	.834	P > .10
Error	24	.834	- - -

TABLE XXI

ANALYSIS OF VARIANCE (LAMB TRIAL, CHAPTER IV)

AOV Table for Lamb Feed Intake (Grams)			
Source of Variation	df	Mean Squares	P Value
Total	29	- - -	- - -
Treatments	3	186045003.3	P > .10
Error	26	123687042.0	- - -
AOV Table for Lamb Gain (KG)			
Source of Variation	df	Mean Squares	P Value
Total	29	- - -	- - -
Treatments	3	4.3219	P > .10
Error	26	5.7506	- - -
AOV Table for Lamb Feed Per Gain			
Source of Variation	df	Mean Squares	P Value
Total	29	- - -	- - -
Treatments	3	1.8795	P > .10
Error	26	1.9140	- - -

TABLE XXII

ANALYSIS OF VARIANCE (SBM-TALLOW COW TRIAL, CHAPTER IV)

AOV Table for Cow Weight Change (125 Days)			
Source of Variation	df	Mean Squares	P Value
Total	60	- - -	- - -
Treatments	2	449.322	P > .10
Error	58	780.961	- - -
AOV Table for Calf Weight Gain (lbs)			
Source of Variation	df	Mean Squares	P Value
Total	60	- - -	- - -
Treatments	2	447.487	P > .10
Error	58	670.677	- - -
AOV Table for Post-Partum Interval			
Source of Variation	df	Mean Squares	P Value
Total	35	- - -	- - -
Treatments	2	151.237	P > .10
Error	33	188.803	- - -

TABLE XXII (Continued)

AOV Table for Ruminal Ammonia Concentration			
Source of Variation	df	Mean Squares	P Value
Total	95	- - -	- - -
Whole plot	47	2.660	P > .10
Treatments	2	.904	P > .10
Error	45	2.738	- - -
Subplot	48	3.179	P > .10
Time	1	20.740	P < .05
Treatments x Time	2	1.076	P > .10
Error	45	2.880	- - -

TABLE XXIII

ANALYSIS OF VARIANCE (.6% FORMALDEHYDE COW TRIAL, CHAPTER V)

	AOV* Table for Cow Weight Change (56 Days)		
Source of Variation	df	Mean Squares	P Value
Total	58	- - -	- - -
Treatments	2	2432.894	P < .01
Calf Birth Date	20	532.851	P > .10
Error	36	426.413	- - -
	AOV* Table for Calf Weight Gain (56 Days)		
Source of Variation	df	Mean Squares	P Value
Total	58	- - -	- - -
Treatments	2	1061.702	P < .01
Calf Birth Date	20	179.681	P > .10
Error	36	185.016	- - -
	AOV Table for Cow Condition Score Change		
Source of Variation	df	Mean Squares	P Value
Total	57	- - -	- - -
Treatments	2	.8237	P > .10
Blocks	2	.5282	P > .10
Treatments x Blocks	4	.1880	P > .10
Error	49	.3798	- - -

*Adjusted for covariable calf birth date.

TABLE XXIV

CHI-SQUARE ANALYSIS FOR COW REPRODUCTIVE DATA
(.6% HCHO-SBM COW TRIAL, CHAPTER V)

	Analysis of Post-Partum Estrus Data			
	SBM	½ .6% HCHO-SBM ½ SBM	.6% HCHO-SBM	Total
Cows Detected in Estrus	16	8	11	35
Cows not Detected in Estrus	5	11	8	24
Total	21	19	19	59

$$\chi^2 = \frac{\Sigma\ (\text{Expected-Observed})^2}{\text{Expected}} = 1.596 \text{ with 2 d.f. ns}$$

	Analysis of Pregnancy Data			
	SBM	½ .6% HCHO-SBM ½ SBM	.6% HCHO-SBM	Total
Cows Showing Estrus	15	11	10	36
Cows not Showing Estrus	6	8	9	23
Total	21	19	19	59

$$\chi^2 = \frac{\Sigma\ (\text{Expected-Observed})^2}{\text{Expected}} = 4.826 \text{ with 2 d.f. ns}$$

TABLE XXV

ANALYSIS OF VARIANCE (.2% HCHO-SBM LAMB METABOLISM TRIAL, CHAPTER V)

AOV Table for Lamb Nitrogen Intake (g/day)			
Source of Variation	df	Mean Squares	P Value
Total	15	- - -	- - -
Treatment	3	13.213	P < .001
Error	12	.208	- - -
AOV Table for Lamb Urinary Nitrogen (g/day)			
Source of Variation	df	Mean Squares	P Value
Total	15	- - -	- - -
Treatment	3	10.287	P < .025
Error	12	1.860	- - -
AOV Table for Lamb Fecal Nitrogen (g/day)			
Source of Variation	df	Mean Squares	P Value
Total	15	- - -	- - -
Treatment	3	.4568	P > .10
Error	12	.3509	- - -
AOV Table for Lamb Nitrogen Retention (g/day)			
Source of Variation	df	Mean Squares	P Value
Total	15	- - -	- - -
Treatment	3	2.080	P > .10
Error	12	2.328	- - -

TABLE XXV (Continued)

AOV Table for Lamb Percent Dry Matter Digestibility			
Source of Variation	df	Mean Squares	P Value
Total	15	- - -	- - -
Treatment	3	8.600	P > .10
Error	12	11.598	- - -
AOV Table for Lamb Hay Intake (g/day)			
Source of Variation	df	Mean Squares	P Value
Total	15	- - -	- - -
Treatment	3	1086.560	P > .10
Error	12	7164.188	- - -
AOV Table for Lamb Ruminal Ammonia			
Source of Variation	df	Mean Squares	P Value
Total	31	- - -	- - -
Whole plot	15	1256.853	P < .005
Treatment	3	5306.982	P < .001
Error	12	244.321	- - -
Subplot	16	137.273	P < .05
Hour	1	630.303	P < .005
Treatment x Hour	3	341.850	P < .005
Error	12	45.040	- - -

TABLE XXVI

ANALYSIS OF VARIANCE (.2% FORMALDEHYDE
COW TRIAL, CHAPTER V)

	AOV* Table for Cow Weight Change (70 Days)		
Source of Variation	df	Mean Squares	P Value
Total	57	- - -	- - -
Treatments	3	1122.232	P < .05
Blocks	2	1465.596	P < .05
Treatments x Blocks	6	269.834	- - -
*Adjusted for covariable initial cow weight.			
	AOV* Table for Total Calf Gain (70 Days)		
Source of Variation	df	Mean Squares	P Value
Total	57	- - -	- - -
Treatments	3	32.476	P > .10
Blocks	2	165.537	P > .10
Treatments x Blocks	6	61.059	- - -
*Adjusted for covariable initial calf weight.			
	AOV Table for Cow Condition Score Change		
Source of Variation	df	Mean Squares	P Value
Total	57	- - -	- - -
Treatments	2	.6492	P = .10
Error	55	.2730	- - -

TABLE XXVI (Continued)

AOV Table for Cow Ruminal Ammonia Nitrogen Concentration			
Source of Variation	df	Mean Squares	P Value
Total	79	- - -	- - -
Whole plot	39	78.254	
Treatments	3	718.512	P < .001
Error	36	24.898	- - -
Subplot	40	16.417	P > .10
Time	1	53.600	P < .05
Treatments x Time	3	40.120	P < .05
Error	36	13.409	- - -

TABLE XXVII

ANALYSIS OF VARIANCE (HEIFER TRIAL, CHAPTER V)

AOV Table for Heifer Total Weight Change (91 Days)			
Source of Variation	df	Mean Squares	P Value
Total	56	- - -	- - -
Treatments	2	692.390	P > .05
Blocks	2	1587.685	P < .10
Blocks x Treatments	4	913.499	P < .10
Error	48	410.190	- - -
AOV Table for Heifer Condition Score Change			
Source of Variation	df	Mean Squares	P Value
Total	56	- - -	- - -
Treatments	2	.2196	P > .10
Blocks	2	.0828	P > .10
Blocks x Treatments	4	.2376	P > .10
Error	48	.1497	- - -

TABLE XXVII (Continued)

AOV Table for Heifer Ruminal Ammonia Nitrogen Concentration			
Source of Variation	df	Mean Squares	P Value
Total	59	- - -	- - -
Whole plot	29	182.965	P < .01
Treatment	2	1660.000	P < .001
Error	27	73.555	- - -
Subplot	30	37.252	P > .10
Time	1	24.950	P > .10
Treatment x Time	2	66.810	P > .10
Error	27	35.518	- - -

TABLE XXVIII

ANALYSIS OF VARIANCE (.3% FORMALDEHYDE COW TRIAL, CHAPTER V)

AOV Table for Cow Total Weight Change (100 Days)			
Source of Variation	df	Mean Squares	P Value
Total	52	- - -	- - -
Treatments	2	8725.517	P < .005
Blocks	3	5040.740	P < .025
Treatments x Blocks	6	932.342	P > .10
Error	41	1209.656	- - -
AOV Table for Cow Condition Score Change			
Source of Variation	df	Mean Squares	P Value
Total	52	- - -	- - -
Treatments	2	.4315	P > .10
Blocks	3	1.1309	P < .01
Treatments x Blocks	6	.4482	P > .10
Error	41	.2756	- - -

TABLE XXIX

CHI-SQUARE ANALYSIS FOR COW REPRODUCTIVE DATA
(.3% HCHO-SBM COW TRIAL, CHAPTER V)

	Analysis of Post-Partum Estrus Data			
	High SBM	Low SBM	.3% HCHO-Low SBM	Total
Cows detected in Estrus	10	12	14	36
Cows not detected in Estrus	8	6	3	17
Total	18	18	17	53

χ^2 = 2.90 with 2 d.f. ns

	Analysis of Cow Pregnancy Data			
	High SBM	Low SBM	.3% HCHO-SBM	Total
Cows Pregnant	17	18	16	51
Cows not Pregnant	1	0	1	2
Total	18	18	17	53

χ^2 = 1.055 with 2 d.f. ns

TABLE XXIX (Continued)

AOV Table for Cow Ruminal Ammonia Nitrogen Concentration			
Source of Variation	df	Mean Squares	P Value
Total	107	- - -	- - -
Whole plot	54	29.173	P < .005
Treatments	2	588.238	P < .001
Error	52	7.671	- - -
Subplot	53	6.595	P > .10
Time	1	2.510	P > .10
Treatments x Time	2	41.996	P < .001
Error	50	5.261	- - -
AOV Table for Calf Weight Gain (100 Days)			
Source of Variation	df	Mean Squares	P Value
Total	52	- - -	- - -
Treatments	2	2956.519	P < .01
Blocks	3	1336.517	P < .10
Treatment x Blocks	6	738.264	P > .10
Error	41	530.964	- - -

VITA[2]

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