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Modifying soybean meal protein to reduce rumen microbial destruction and increase urea utilization in cattle diets

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INTRODUCTION

Most previous protein feeding standards for cattle and other ruminant species have been based upon nitrogen times 6.25 (crude protein) with little or no consideration given to specific tissue amino acid needs and their fulfillment from dietary sources. The widespread usage of nonprotein nitrogen as urea in cattle diets during the past 30 years and the ease and simplicity of applying a nitrogen standard probably was responsible for the usage of this type of standard.

The first attempt to develop an amino acid feeding standard for cattle and sheep was the metabolizable protein (MP) feeding standard as published by Burroughs et al. (1974). This standard established net tissue needs for specific amino acids and assigned amino acid values for a wide variety of feedstuffs. The assigning of these values recognized that each feedstuff incorporated and fed in a ruminant's diet supplied net absorbable amino acids from two sources. The first source was directly from the feedstuff protein that escaped microbial destruction within the rumen and was digested post-ruminally by body enzymes. The second source of net amino acids from a feedstuff was the digested microbial protoplasmic protein synthesized from the feedstuff protein destroyed during rumen fermentation.

It was observed in applying this 1974 feeding standard that the quantity of net absorbable amino acids from microbial protein synthesis was a constancy for any given feedstuff but dependent upon two considerations. The first consideration was the quantity of fermentable energy possessed as measured in total digestible nutrient (TDN) units. The second consideration in assuring this constancy was the supplying of supplementary nonprotein nitrogen for microbial protein synthesis whenever inadequate nitrogen was supplied from the microbial destruction of the feedstuff protein.

A second observation in applying this 1974 feeding standard was that the quantity of net absorbable amino acids originating from feedstuff protein escaping rumen destruction was not a constancy based upon its energy content but was dependent upon the vulnerability of the protein to being attacked by rumen microorganisms. This vulnerability is believed to be due to solubility characteristics of a given protein when subjected to fairly high hydrogen ion concentrations within the rumen above a minimum pH of about 5.5.

A final observation in attempting to apply this 1974 feeding standard was the possibility that major improvements in crude protein (N x 6.25) usefulness in cattle diets might be achieved by purposely decreasing rumen solubility of certain feedstuff proteins. This lowered solubility would be expected to permit more of the protein to escape rumen

destruction and thus make available more absorbable amino acids post-ruminally, provided the altered feedstuff protein was fed in diets with adequate supplementary urea or feedstuff ammonia. This adequacy was deemed essential such that rumen microbial protein synthesis could be maintained at a constant rate without reducing absorbable amino acids from this source.

The purpose of the present research was an attempt to favorably modify soybean meal (SBM) proteins for usage in cattle diets containing urea, keeping in mind the above observations.

LITERATURE REVIEW

Destruction and Synthesis of Alpha Amino Nitrogen During Rumen Fermentation

Wegner et al. (1940) established that the saliva of ruminants contains no proteolytic enzymes and that the mucosa of the rumen and reticulum possess no secretory glands. Therefore, it is evident that digestion of protein in the rumen can be due only to proteolytic enzymes contained in the food or produced by microbial fermentation (McDonald, 1952).

Blackburn and Hobson (1960) isolated several different kinds of rumen bacteria and concluded that only a small proportion were actively proteolytic.

Abou Akkada and Blackburn (1963) surveyed bacteria isolated from the rumen of sheep and found Bacteroides amylophilus, Bacteroides ruminicola, species of Bacteroides, Selenomonas, Butyrivibrio, Bacillus, Eubacterium, Clostridium, and Gram-positive cocci to be proteolytic. B. amylophilus strains had mostly high proteolytic activity and low exopeptidase activity while the B. ruminicola has consistently high exopeptidase activity. The end products of casein degradation by these bacteria were mainly amino acids and polypeptides. No dipeptides were found.

Research concerning the location and characteristics of

proteolytic enzymes of rumen bacteria is limited. Blackburn (1968a), working with Bacteroides amylophilus strain H18, concluded that 20% of the protease was located extracellularly. McDonald (1962) conducted studies with Micrococcus and concluded that approximately 50% of the proteinase activity was loosely bound to the cell membrane and that most of the peptidase and deaminase enzymes were contained inside the cell.

Lesk and Blackburn (1970) attempted to purify the protease from Bacteroides amylophilus and established that proteolytic activity existed over a wide pH range (pH 3.0 to 11.5) with optima at pH 6.0 and 11.0. They interpreted this wide range on the basis that more than one protease was involved. However, failure of gel electrophoresis to resolve the protease into bands active at different pH values did not support their interpretation. The bands contained proteolytic active fractions having molecular weights of approximately 30,000 and 60,000. Since the pH range of both proteolytic fractions was the same, the authors concluded that the 60,000-molecular weight species might be a dimer of the 30,000-molecular weight species and that the two molecular-weight species might exist in equilibrium.

Blackburn (1968b) attempted to characterize the protease

and indicated a similarity to trypsin although soybean trypsin inhibitor did not inhibit the protease.

Mycek et al. (1952) examined the specificity of a streptococcal proteinase and found that the site of action was at peptide bonds involving a variety of L-amino acid residues.

Hullah and Blackburn (1971) used [^{14}C]-amino acids and unlabeled casein hydrolysate in the substrate for Bacteroides amylophilus and concluded that the degree of concentration of the [^{14}C]-amino acids within the cell was indicative of active transport. Pardee (1968) suggested that transport proteins are involved because they are the only molecules which have the observed degree of specificity to discriminate between possible substrates. It was suggested that these transport proteins are located on or near the cell membrane.

One of the first studies concerning the degradation and disappearance of purified proteins in the rumen was conducted by McDonald (1948) who found that casein and gelatin disappeared rapidly from the rumen.

Lewis et al. (1957) fed semipurified diets containing protein supplements of either high casein, low casein or no casein to lambs fitted with rumen fistulas and catherized loops of the portal vein and found that changes in rumen ammonia concentration were paralleled by changes in portal-ammonia concentration.

Looper et al. (1959) individually fed five amino acids (alanine, aspartic acid, glutamic acid, glycine and lysine) to a fistulated steer and found that significant deamination occurred with alanine, aspartic acid and glutamic acid as indicated by ammonia levels in the rumen.

Hogan (1964) used lambs fitted with ruminal or duodenal fistulas and fed either a dry roughage consisting of a mixture of lucerne and wheaten hay or a freshly cut mixture of lucerne and ryegrass. The green feed contained more soluble protein and higher rumen ammonia levels were achieved, indicating that a more extensive degradation of alpha amino protein to ammonia had occurred in the rumen.

Van Horn et al. (1969) used lactating cows in a 4 x 3 factorial design with four levels of urea and 3 levels of crude protein and found that there was a linear increase in plasma urea nitrogen due to increases in dietary preformed protein levels showing that alpha amino acids were degraded in the rumen to products of ammonia and carbon skeletons.

Leibholtz (1969) conducted a study in which wethers were fed different diets containing seven to nine g of nitrogen once daily. Results showed a significant increase in the concentration of free alpha amino nitrogen and ammonia in the rumen liquor one hour after feeding as well as a positive correlation between their concentrations in the rumen and the dietary protein intake.

The previous references have shown that proteins containing alpha amino nitrogen can be destroyed in the rumen as indicated by increased levels of ammonia in the rumen and portal blood. The following research reports show that alpha amino nitrogen can be synthesized by the rumen microorganisms if nitrogen (from either alpha amino or nonprotein sources) and carbohydrates are available in the rumen.

McDonald (1954) determined that approximately 40% of the zein fed to sheep was converted into microbial protein. This estimate was based upon analyses of the lysine content of materials passing into the duodenum and assumed that zein contained no lysine.

McDonald and Hall (1957) used ruminal and duodenal fistulated lambs fed semi-purified diets and estimated that at least 90% of the casein in the diet was degraded in the rumen and nearly all was utilized for the synthesis of microbial proteins. This conclusion was based on an estimation of the amount of casein in the mixed ingesta leaving the abomasum as indicated by a differential phosphorous chemical procedure.

Bryant and Robinson (1963) conducted an experiment in which 14 species of bacteria, known to be prominent among the rumen bacteria in cattle, were maintained in a medium containing both ammonia and protein hydrolysate-[^{14}C] as sources of nitrogen. All species incorporated [^{14}C] into

microbial protein which proved that bacteria can degrade protein and later reform it into microbial protein.

Leibholtz (1969) fed diets differing in nitrogen content to lambs and found that the proportions of the individual free amino acids in the rumen were relatively constant regardless of the amino acid composition of the diet. This indicated that the dietary proteins had been degraded and resynthesized. There was a significant overall increase in the concentration of free and alpha amino nitrogen in the blood plasma due to protein feeding but no correlation between dietary amino acid intakes and plasma amino acids. This study offers support to the findings of Purser (1970) who fed rations consisting of mainly alfalfa and corn in equal proportions, alfalfa and 20% corn cobs, or rations containing shelled corn and SBM and found that all presented microbial protein of very similar composition to the abomasum.

Pilgrim et al. (1970) administered a continual infusion of [^{15}N] as $(^{15}NH_4)_2SO_4$ into the rumen of sheep receiving either a low-nitrogen diet (mainly wheaten hay) or a higher nitrogen diet (lucerne hay) and estimated that a minimum of 68% of plant nitrogen was converted to microbial nitrogen for the low-nitrogen diet and 53 to 55% for the higher nitrogen diet.

Al-Rabbat et al. (1971) used [^{15}N]-ammonia in artificial

rumen studies to estimate the quantity of microbial synthesis *in vivo* with sheep. They estimated that the continual feeding of alfalfa pellets to sheep would result in about 9.2 g of ruminal microbial cells synthesized per 100 g digestible organic matter fed, with about 61% of the microbial nitrogen derived from ammonia nitrogen and approximately 39% derived from other nitrogen sources such as amino acid and peptide nitrogen.

Conrad *et al.* (1967) fed lactating cows a basal diet consisting of chopped alfalfa which had been either heat dried or wilted and ensiled. Barium sulfide-[^{35}S] was given orally as a source of sulfur for ruminal methionine synthesis. The milk proteins were separated from skim milk and counted in a liquid scintillation counter. The authors concluded that less methionine was synthesized when heat-treated alfalfa was fed as compared to ensiled alfalfa, indicating that alfalfa protein is degraded in the rumen and then resynthesized into microbial protein.

The previous discussion has shown that dietary alpha amino nitrogen sources can be destroyed by microorganisms in the rumen and that some of the liberated ammonia may be resynthesized into alpha amino nitrogen in the form of microbial protein. Bryant and Robinson (1961) showed that some rumen microbial species use ammonia nitrogen in

preference to preformed amino acids. In a later study Bryant and Robinson (1962) found that 82% of the rumen microbial strains isolated on a relatively nonselective medium grew with ammonia as their principal source of nitrogen. Thirty percent of these strains required ammonia for good growth even though the medium contained casein hydrolysate. This suggests that ammonia arising from sources other than dietary alpha amino nitrogen such as urea may be useful to the rumen microorganisms.

Pearson and Smith (1943a) reported that urea can be enzymatically degraded by rumen ingesta to carbon dioxide and ammonia. In a subsequent study Pearson and Smith (1943b) concluded that urea utilization within the rumen takes place in two steps, first the conversion of urea to ammonia and secondly the conversion of ammonia to protein. Slyter et al. (1968) reported that the hydrolytic enzyme for urea (urease) is produced by certain species of rumen bacteria. van Wyk and Steyn (1975) concluded that the facultatively anaerobic Gram-positive cocci (Staphylococcus saprophyticus and Micrococcus varians) were probably responsible for a large proportion of the urease activity present in rumen fluid.

Wegner et al. (1940) reported an in vitro study involving the addition of urea to rumen fluid inoculum followed by measurement of ammonia nitrogen, urea nitrogen, and total nitrogen. They showed that urea was converted to ammonia

which in turn was converted to microbial protein.

Duncan et al. (1953) analyzed the rumen fluid obtained from calves fed a purified ration containing urea as the only nitrogen source and presented quantitative evidence that rumen microorganisms can utilize urea nitrogen to synthesize amino acids.

Loosli et al. (1949) fed a purified diet containing urea as a nitrogen source to sheep and goats. Rumen contents of the animals contained 9 to 20 times more essential amino acids than the ration fed. All 10 essential amino acids for the rat were synthesized in substantial amounts.

McDonald (1952) conducted a study in which starch was added to a casein diet fed to sheep and compared rumen ammonia levels to those produced by a control diet which did not contain additional starch. The addition of starch resulted in very dramatic reductions in rumen ammonia content, suggesting that the starch provided sufficient energy to stimulate the microorganisms to utilize the rumen ammonia for the synthesis of microbial protein.

The following references indicate that urea can serve as a source of nitrogen in the rumen. However, cattle and sheep performance is inconsistent with respect to urea's ability to fully satisfy their protein requirements. Briggs et al. (1947) fed steers weighing 225 to 275 kg a basal diet of prairie hay supplemented with pellets containing molasses, hominy feed,

and different proportions of cottonseed meal and urea so that urea contributed approximately 25, 50, 75, or 100% of the supplemented nitrogen. Performance of steers fed pellets containing no more than 50% of their nitrogen as urea nearly equalled performance with a cottonseed meal supplement. Urea alone was a poor supplement.

Raleigh and Wallace (1963) fed a pelleted ration containing low-quality meadow hay supplemented with either urea or cottonseed meal to raise the protein content from 5.5 to 12%. Two steers receiving the urea-supplemented pellet died from ammonia toxicity. It was found that by replacing half of the urea nitrogen with cottonseed meal the ammonia toxicity symptoms were avoided. The steers fed the all-cottonseed meal supplement out-performed the steers receiving cottonseed meal plus urea supplements.

Perry et al. (1967) conducted a series of experiments with cattle weighing 202 to 302 kg and fed either a finishing diet (high moisture ground ear corn) or a growing diet (corn stover silage) supplemented with either urea or SBM. In all but one trial, feed efficiency of the animals receiving the all-natural protein supplements was superior to that achieved from the urea supplements.

Brown and Jacobson (1966) fed 275-kg dairy heifers a diet consisting of orchard grass and a concentrate containing either basal (corn and cob meal), basal plus 1.06% urea, basal

plus 7.88% SBM, or basal plus 2.09% urea. No differences were found in regard to feed efficiency and daily gains.

Frietag et al. (1968) conducted a metabolism trial using two steers weighing 325 kg and fed a diet containing 75% corn and supplemented with either SBM or a combination of urea and corn. When urea was used it supplied 30% and 70% of the total nitrogen in the control and test diets respectively. No differences in nitrogen retention occurred. The authors also reported a growth trial in which 275-kg dairy steers were fed a corn cob-based ration supplemented with either SBM or urea plus corn to give either 7% or 11% crude protein. It was found that depressed gains resulted when either the crude protein content was lowered from 11% to 7% or when urea was substituted for SBM.

Robertson and Miller (1971) conducted a growth trial with steers weighing 212 kg and fed a diet of corn silage and .91 kg of high quality hay in addition to a supplement consisting of either SBM or corn plus different levels of urea. The authors concluded that 20% of the nitrogen in the total ration could be in the form of urea with few adverse effects on performance.

Greathouse et al. (1974) conducted two finishing trials with steers fed a grain sorghum basal diet supplemented with either urea (1%) or SBM (protein equivalent to 1% urea). In the first trial the steers (weighing 226 kg) did not

consume enough feed and lost weight. In the second trial (using 305 kg steers) no significant differences were observed in performance or carcass characteristics.

Poos and Bull (1977) conducted two experiments with lactating dairy cows to evaluate the accuracy of urea fermentation potentials (UFP) in predicting urea utilization. Experiment one consisted of an 8.5% protein control diet with a UFP of +5.2 g/kg supplemented to 16% protein with either urea or SBM. Milk production for the respective treatments was 15.9, 20.3 and 25.4 kg per day. Feed intake was 14.4, 15.7 and 20.3 kg per day respectively. Experiment two consisted of a 12% protein control diet with a UFP of -1.17 g/kg supplemented to 15% protein with either urea or SBM. Milk production for the respective treatments was 23.3, 23.5 and 24.7 kg per day. Feed intake was 18.6, 20.3 and 20.1 kg per day respectively.

An extensive review of additional research reports concerning urea usage in ruminant rations can be found in a recent publication by the National Research Council (1976).

Protection of Dietary Proteins Against Rumen Fermentation

Cuthbertson and Chalmers (1950) conducted nitrogen balance experiments using sheep fitted with duodenal and rumen fistulae. When a casein supplement was protected against

rumen fermentation by dripping it directly into the duodenum, higher nitrogen retention values were obtained as compared to those from casein administered directly into the rumen. They suggested that the rate of deamination of casein in the rumen exceeded the capacity to synthesize bacterial protein. The excess ammonia was absorbed from the rumen, converted to urea, and excreted in the urine. This early work stimulated Reis and Schinckel (1961) to by-pass the rumen by administering casein directly into the abomasum. This procedure resulted in higher levels of nitrogen retention and wool growth compared to administering casein by normal feeding with no protection from rumen fermentation. In a later study Reis and Schinckel (1963), administered 2 g cysteine daily into the abomasum of sheep with a resultant 35 to 130% increase in rate of wool growth. Infusion of 60 g casein daily into the duodenum increased the rate of wool growth by 84 to 102%. These findings suggested that the sulfur-containing amino acids were limiting. The response received from abomasal infusion of either casein or sulfur-containing amino acids resulted in an improved quantity or an improved amino acid profile reaching the absorptive site of the gastrointestinal tract. This presumably more completely satisfied the protein requirements of the animal.

The sheep utilized in the three previous experiments

required surgery for purposes of by-passing the rumen in protecting casein against rumen fermentation and thus enhancing its nutritional properties. Therefore, if animals fed in the normal manner were to receive these same benefits without surgery, a different method of protection would be required.

Chalupa (1972) stated that the extent to which a dietary protein is degraded in the rumen is dependent upon its solubility. Treatment of proteins by heating or by the application of formaldehyde and tannins has been shown to reduce the solubility of protein. A brief discussion of each of these methods follows.

Application of heat to proteins results in an unfolding and exposure of hydrophobic groups which decreases solubility (Morgan, 1931; Wolf and Tamura, 1969; and Morrison and Boyd, 1973). Little and Burroughs (1960) reported that heat treatment of SBM at 110°C for 24 hours resulted in decreased nitrogen solubility and that the heated meal was ineffective as a nitrogen source for _in vitro_ digestion by washed suspensions of microorganisms. In a later study, Little _et al._ (1963) stated that heated SBM (110°C for 24 hours) was low in water-soluble nitrogen (11% soluble nitrogen in the heated meal versus 16% in the untreated meal) and that the heated SBM was converted to ammonia very slowly in

in vitro rumen studies.

Broderick *et al.* (1976) heated cottonseed meal in an autoclave at temperatures of 115 to 120°C for various lengths of time and found decreases in *in vitro* rumen degradation and protein solubility in .02 N NaOH. A decrease in the number of protein amino groups was also noted.

Mann and Briggs (1950) attempted to study the nature of the changes in soybean proteins at the molecular level when heat was applied. Using electrophoresis, their results indicated that the nonglobulin proteins were most sensitive to heating in aqueous solutions. When heated for two hours at 75°C these proteins were converted from components with different electrophoretic mobilities into an aggregate migrating as a single electrophoretic peak. When heated longer the globular component also appeared to be incorporated into the aggregate formed by nonglobulins.

Circle *et al.* (1964) showed that heating solutions of commercial preparations of soybean globulins caused gelation and suggested that disulfide cross linkages were possibly formed during heating.

Abusalem *et al.* (1975) reported that the number of free amino groups in soybean protein decreases due to heating. The authors suggested that either denaturation of the protein or formation of Maillard products (an insoluble protein-

carbohydrate complex) could cause the reduction.

Hodge (1953) and Ellis (1959) stated that three broad types of browning reactions (Maillard) are recognized with the most common being the reaction between sugar carbonyl groups and amines, amino acids, peptides or proteins. The formation of Maillard product appears to occur in three stages and begins with an initial sugar-amine condensation which is followed by a rearrangement. Continued heating causes the rearranged product to dehydrate, disassociate, and finally form a brown-colored product.

Van Soest (1965) worked with ground fresh-frozen and air-dry forages and concluded that the damage produced by heating in the presence of retained moisture was far more severe than that observed under drying conditions. This conclusion is supported by Morgan (1931).

Goering et al. (1973) studied heat damage in forages and concluded that plants differ in their susceptibility to heat damage. Samples having moisture contents of 20 to 70% appeared to support more browning than those with 10% moisture or less. Temperatures above 60°C for 24 hours in a convection oven resulted in browning.

Formaldehyde can combine with several different kinds of functional groups found in proteins. Walker (1964) stated that amino groups, guanidyl groups, indole groups, mercaptan radicals, imidazole groups and phenolic nuclei will react

with formaldehyde if the correct conditions of pH and temperature are present.

French and Edsall (1945) outlined the two major reactions known to occur between formaldehyde and protein functional groups. An addition-type reaction occurs initially in which a methylol compound is formed. The hydroxyl group of the methylol compound is usually reactive and may further undergo a condensation reaction with another group containing an active hydrogen atom to form a methylene bridge. French and Edsall (1945) stated that the condensation reaction may take place intramolecularly with the formation of cyclic structures or intermolecularly with the formation of molecular aggregates.

Fraenkel-Conrat and Olcott (1948a) showed that primary and secondary amines react with formaldehyde and primary amides over the pH range of 3 to 8 within 24 to 48 hours at room temperature to give condensation products. The authors also demonstrated crosslinking of amino with guanidyl compounds by formaldehyde over a pH range of 4.2 to 8.5 at room temperature.

Ratner and Clarke (1937) reported that the reaction of formaldehyde with cysteine to give a cross-linked product proceeded very rapidly at pH 5 or higher. The reaction product was very stable in acid conditions.

French and Edsall (1945) suggested that serine and threonine probably undergo a reaction in aqueous formaldehyde solution similar to that described for cysteine, but the products are very unstable and can easily be reversed.

Homer (1912) reported that the indole group of tryptophan would react with formaldehyde when the two reactants were heated in a moderately acid solution. Fraenkel-Conrat and Olcott (1948b) suggested that condensations joining indoles, amines and formaldehyde may occur at the >NH group of the indole ring.

In neutral or slightly acid solution, formaldehyde introduces methylene bridges between amines and the reactive >CH groups of phenolic and imidazole rings. The phenols react to completion within a few days at room temperature with the resulting linkage being resistant to acid hydrolysis (Fraenkel-Conrat and Olcott, 1948b).

Barry (1976) stated that the main reactions occurring at neutral pH and room temperature are probably those involving terminal amino groups, the primary amide groups of asparagine and glutamine and the epsilon-amino and quanidyl groups of lysine and arginine respectively.

A desirable protein-modifying agent must render the protein resistant to microbial degradation while in the rumen, then allow the digestive enzymes in the abomasum and small intestine to digest the protein in the normal manner.

The disassociation of the formaldehyde-protein complex is thought to occur in the acidic conditions of the abomasum.

French and Edsall (1945) found that the reaction of formaldehyde and amino groups was rapid and readily reversible in the presence of acidic conditions. Walker (1964) stated that formaldehyde-gelatin condensation products liberate formaldehyde gradually on treatment with warm water and decompose rapidly on exposure to cold hydrochloric acid.

Mills et al. (1972) fed [^{14}C]-formaldehyde-treated casein to sheep and found that approximately 60 to 80% of the consumed [^{14}C]-formaldehyde was metabolized to carbon dioxide and methane, 11 to 27% was excreted in the feces and 5 to 6% was accounted for in the urine. Very small levels of [^{14}C] were found in the tissues and milk.

The ability of formaldehyde to reduce protein degradation in the rumen by rumen microorganisms has been shown by Blackburn and Hobson (1962), Faichney (1971), Macrae et al. (1972) and Amos et al. (1974).

The fact that tannins react with proteins has been known for a long time since tanning of animal hide is an integral part of the leather industry. Haslam (1966) summarized the chemistry of protein and tannin reactions by stating that the active sites on collagen are probably the peptide bonds. These are not internally bonded to other

groups and are thus able to form similar hydrogen-bonded structures with the phenolic groups of the tannin. It is also probable that other groups (such as amide and amine residues) present in polar side chains are involved in the fixation of vegetable tannins to a limited extent.

Gustavson (1956) and McGilliard (1972) stated that the interaction between proteins and tannins is a result of the formation of hydrogen bonds between the hydroxyl groups of polyphenolic tannins and the carboxyl groups of the protein peptide bonds.

Tagari et al. (1965) found that a linear indirect relationship existed between the amount of tannin and the digestibility of casein. The authors suggested that inhibition of proteolytic activity, as evidenced by a decrease in vitro ammonia, could have resulted from the formation of insoluble complexes with the protein substrate, with proteolytic enzymes, or by blocking the growth and enzyme synthesis of rumen microorganisms. Gustavson (1956) showed that the degree of resistance of the tannin-protein complex to trypsin varies depending upon the kind of tannin used.

Driedger and Hatfield (1972) reported in vitro studies in which the ammonia concentration was reduced by 92% with 10% and 25% Allepo tannin levels. Only 57.6% as much ammonia was produced from SBM treated with 5% tannin as was produced from untreated SBM. There was no effect on in vitro pepsin

digestion of the protein due to the presence of tannins.

The tannin-protein complex is not stable at gastric pH below 3 (van Buren and Robinson, 1969; Gustavson, 1966; and Mejbaum-Katzenellenbogen et al., 1959). This property should enable the tannin-treated protein to become available for digestion and absorption post-ruminally.

Beneficial or Deleterious Effects of Modifying Proteins

Experiments involving modified proteins have shown inconsistent animal performance. This section of the literature review will begin by citing some experiments in which the effects of modified proteins were deleterious to animal performance.

Nishimuta et al. (1973) fed lambs weighing 35 kg a diet containing 20% corn, 61.5% cobs and 17.4% SBM treated with either heat (149°C for four hours), formaldehyde (1 g formaldehyde per 100 g air dry SBM) or tannic acid (9% addition of tannic acid w/w to previously moistened SBM, 5% water w/w). None of the treatments increased nitrogen balance or plasma concentrations of methionine ($P < .05$) when compared to untreated SBM controls. None of the diets contained urea.

Carrico et al. (1970) fed a daily ration of 270 g barley, 680 g chopped low quality hay and 100 g of either

formalin-treated or untreated casein to mature Romney wethers. No significant changes in body weight or wool production occurred although there was an indication of a positive response by the treated casein group.

Johnson and Hatfield (1975) fed diets containing ground corn, cottonseed hulls, SBM, molasses and urea to lambs averaging 27.8 kg. The SBM was treated with either 0%, .2%, .4%, .6% or .8% formaldehyde by weight. Gain to feed ratios for the respective treatments were .159, .148, .129, .153, and .155 which shows that the most efficient conversion of feed to gain occurred with the untreated SBM diets.

Amos et al. (1974) fed a corn, cottonseed hull, and SBM-based diet with the SBM either untreated or treated with 1.1% formaldehyde on a w/w basis. No urea was added to the diet. Nitrogen balance studies showed significant increases in fecal nitrogen and decreases in urinary nitrogen. The percentage of nitrogen retained was decreased by 12.5% compared to controls although the difference was not significant.

Sherrod and Tillman (1964) fed lambs a semi-purified diet containing corn dextrose (17.8%), corn starch (17.8%), corn oil (2%), cottonseed hulls (8.9%), and cottonseed meal (22.7%). Cottonseed meal was the major source of protein

and was produced by cold-hexane extraction with no heat applied during extraction. Portions of the meal were then subjected to the following treatments: no heat, or autoclaved under 15 lb. steam pressure per square inch at 121°C for 20, 60, 120, 180 and 240 minutes. The effects of lengthened autoclaving time showed linear increases in fecal nitrogen losses and linear decreases in urinary nitrogen losses. The 120, 180, and 240-minute autoclaved meal lowered the percentage of intake nitrogen retained below that achieved by the unheated control meal. The 60-minute autoclaved meal improved nitrogen retention slightly over the unheated meal. The authors also reported a lamb growth trial in which the proportions of cottonseed hulls and the purified ingredients of the diets were altered to give concentrate to roughage ratios of either 1:2 or 2:1. Cottonseed meal treated as previously described served as the major source of protein in the diet. Autoclaving the raw cottonseed meal for 60 minutes improved sheep gains and feed efficiencies significantly when compared to the unheated meal and the meals autoclaved for 120 and 240 minutes.

Clark et al. (1974) fed lactating cows a ration consisting of 9 kg corn silage, 3.2 kg alfalfa-grass hay, and concentrate (the concentrate consisted of 84.2% corn and 13% SBM) at a rate of 1 kg per 2.5 kg of milk produced. The SBM was treated at a level of .9% formaldehyde w/w of the

meal. Treatment of the SBM resulted in significant reductions in protein content of the milk and in productive nitrogen (milk nitrogen plus retained nitrogen). Milk production was also decreased as compared to the controls although the difference was not significant.

Satter et al. (1970) fed diets containing supplemental nitrogen sources of either SBM, urea or formaldehyde-treated SBM to lambs. Weight gains for the respective treatments were .25, .24, and .21 kg per day and feed efficiencies were 635, 647, and 741 kg feed per 100 kg gain. Depressed animal performance was also seen in a lactation trial in which the protein supplement was either untreated SBM or formaldehyde-treated SBM. Milk production by the control group was 21.4 kg per day versus 20.5 kg per day for the treated group. The milk protein content remained essentially constant (3.34% from controls versus 3.39% from the treated group).

Clark et al. (1971) fed diets containing either untreated or .9% formaldehyde-treated SBM to lactating cows. They found that crude protein digestibility was decreased from 64.2% to 57.2% and that milk production was also decreased from 17.8 to 17.5 kg per day due to formaldehyde treatment.

Wachira et al. (1973) reported an experiment in which

lactating dairy cows were fed rations containing concentrate which was either untreated or treated with .5 parts formaldehyde per 100 parts protein. There were no significant effects on milk yield or milk composition. In another experiment, lambs were fed an untreated ration or a ration treated with .74 or .5 g formaldehyde per 100 g of crude protein. No effect on growth rate was demonstrated.

Jorgensen et al. (1970) fed a basal corn diet supplemented with either SBM, formaldehyde-treated SBM, urea, or a gelatinized grain-urea product called starea to Hereford steers weighing 374 kg for a period of 56 days. Daily gains and feed per gain ratios for the respective treatments were: 1.64 and 7.94; 1.35 and 9.42; 1.36 and 9.27; and 1.40 and 9.00 respectively. Clearly, the performance of steers fed formaldehyde-treated SBM was inferior to that of steers consuming the untreated diet.

Schmidt et al. (1973) conducted an experiment in which steers weighing 374 kg were fed diets containing 2.5% ground corn cobs, and 90.4% corn supplemented with either SBM, formaldehyde-treated SBM (4 ml of 40% formaldehyde per 100 g soy protein), urea or starea. Average daily gain and gain to feed ratio for the respective treatments were: 1.64 and .126; 1.36 and .106; 1.36 and .108; and 1.40 and .111. The cattle receiving the treated SBM had the slowest gains and

poorest feed conversion of all treatments.

In a subsequent study Schmidt et al. (1974) fed Holstein steers weighing 264 kg a diet containing 20% corn cobs, 7% dehydrated cane molasses, 19.4% corn starch and 9.2% SBM (treated with 0, 1.5, or 3.0 ml of 40% formaldehyde per 100 g soybean protein). Daily weight gains and gain to feed ratios for the respective treatments were: 1.31 and .132; 1.27 and .120; 1.20 and .116. Treatment of SBM with formaldehyde significantly reduced daily weight gains and feed efficiency in this experiment.

This literature review will now focus on those experiments in which modified proteins showed some evidence of being superior to unmodified proteins. Ferguson et al. (1967) was one of the first to report success with modified proteins. Three groups of four wethers were fed 800 g per day of a diet consisting of wheaten and lucerne chaff (50:50) during a 12-week preliminary period. During a subsequent 18-week experimental period one group remained on this ration as a control. A second group was given a supplement of 80 g per day treated casein for 9 weeks followed by 80 g per day untreated casein for 9 weeks. The treated casein increased wool growth by approximately 70%, whereas reversal from treated to untreated casein caused a decline in wool growth rate to approximately 15% above the control group.

Hudson et al. (1969) conducted a 3 x 2 factorially-arranged growth trial experiment to compare 10, 12 and 14% crude protein levels and heated SBM (4 hours at 149°C) or unheated SBM for lambs weighing 16 kg. The diets consisted of ground wheat straw (20%), corn (50 to 62%), SBM (8 to 20%), molasses (8%), and steamed bone meal (1.5%). Although the heated meal did not increase gains, feed efficiency was increased ($P < .05$) by 7.3%.

Hudson et al. (1970) conducted a digestion trial with abomasally-fistulated lambs fed a diet containing 20% corn starch, 20% dextrose, 30% cellulose, 2% steamed bone meal and 22% SBM (either unheated or heated 4 hours at 149°C). Total nitrogen in abomasal contents was significantly higher in the wethers fed heated SBM when compared to those given unheated meal.

Glimp et al. (1967) conducted a 2^3 factorially arranged growth trial in which the treatments consisted of two crude protein levels (12.1 and 17.2%), heated SBM (4 hours at 149°C) or unheated SBM, and 0 or 156 mg neomycin added per kg of diet. The diets consisted of ground wheat straw (10%), alfalfa hay (10%), corn (48 or 60%), SBM (10 or 22%), molasses (8%) and steamed bone meal (1%). Lamb performance was highest when the protein level was 17%, but heating the SBM resulted in improved gains at the 12% protein level to a point where they were comparable to those obtained on the 17% protein

level. Feed efficiency was improved by heat treatment in all cases.

Driedger and Hatfield (1970) infused either untreated or tannic acid-treated SBM (10 g tannic acid per 100 g SBM) into the rumen or abomasum. The following nitrogen retention values (g per day) were obtained: rumen SBM, 5.5; rumen tannic acid SBM, 7.4; abomasum SBM, 6.7; and abomasum tannic acid SBM, 7.1. The authors concluded that tannic acid did offer protection to protein in the rumen and that abomasally-infused tannic acid SBM resulted in an incomplete breakdown of the tannic acid-SBM complex.

Peter et al. (1971) fed a diet containing 88.6% corn, and 9.6% SBM (untreated or treated with .6% formaldehyde or 1.5% glyoxal) to lambs weighing 32 kg. Average daily gains and gain to feed ratios for the control, glyoxal and formaldehyde treatments respectively were: .246 and .201; .280 and .249; and .290 and .239. This showed that performance of lambs receiving SBM treated with either glyoxal or formaldehyde was superior to the control lambs.

Nimrick et al. (1972) conducted a growth trial using lambs fed diets containing: corn (64 to 68%), corn cobs (15%), either SBM (14.3%) or fish meal (11.6%), and molasses (5%). The SBM and fish meal were either untreated or treated with .5% and .6% formaldehyde respectively. No statistically significant differences in daily weight gains or feed

efficiency were found. However, there was an indication of superior performance from the formaldehyde-treated diets.

Driedger and Hatfield (1972) conducted a nitrogen balance trial with lambs fed the following ration: corn (79%), SBM (8%), corn cobs (10.8%), and urea (.48%). The SBM was either untreated or treated with 10% Tara tannin. The nitrogen retained (as a percentage of intake nitrogen) for the untreated and treated SBM treatments was 34.1% and 34.5% during the first trial and 33.7% and 42.3% respectively during the second trial. The results in the second trial were statistically different and favored the treated meal.

Nishimuta et al. (1973) fed lambs a high roughage diet containing 61.5% corn cobs, 20% corn, and 17.4% SBM treated with no heat (controls), heat (149°C for four hours), formaldehyde (1 g per 100 g air-dry SBM), or tannic acid (9% w/w). Crude protein digestibility and the percentage of nitrogen intake retained by the respective treatments was: 75.4 and 20.8, 69.9 and 27.6, 46.4 and 17.4, and 67.5 and 23.5, showing an advantage for heat and tannic acid treatment compared to the controls.

Driedger et al. (1969) fed steers a high concentrate diet containing 10% corn cobs and supplemented with either urea, SBM, or tannic acid-treated SBM in a nitrogen balance trial. Average daily gains (kg) and gain to feed ratios for

the respective treatments were: 1.42 and .163, 1.56 and .167, 1.62 and .179. This shows a slight advantage for treated SBM when compared to untreated meal.

The previous discussion has dealt with experiments involving modified feed proteins. Recent literature contains a few reports of studies in which feed proteins were not purposely modified to make them insoluble but rather their inherent protein solubilities were considered in the formulation of diets. Wohlt et al. (1976) formulated four diets, two of which contained approximately 13% of the total protein as soluble protein while the other two diets contained approximately 35%. At each level of soluble protein, one diet was formulated with an amino acid profile resembling SBM and the other with an amino acid profile resembling hominy. Lambs fed the low soluble protein diets excreted slightly more fecal nitrogen (1.6%) but less urinary nitrogen (6%) with a resultant 4.4% increase in the percentage of intake nitrogen retained. Although rumen ammonia concentrations were 17.4% lower in the low soluble protein diets, part of the difference may have resulted from differences in feed intake.

Hawkins and Strength (1977) supplemented lactating dairy cow rations (17% crude protein) with either SBM, fish meal, or corn distillers dried grains with solubles to achieve crude

protein solubility levels of 30.7, 28.7 and 42.5% respectively. Milk production was not affected.

Majdoub et al. (1977) used natural feedstuffs for formulation of four complete rations (concentrates and sorghum silage) as follows: (a) 15% crude protein, 30% soluble; (b) 15% crude protein, 15% soluble; (c) 12% crude protein, 30% soluble; and (d) 12% crude protein, 15% soluble. Milk production (kg/day) for the respective treatments was 22.77, 27.66, 21.49, and 24.60.

Consideration of the Nutritional Status of the Total Biological System

Proper protein nutrition of ruminants occurs when the nitrogen needs of both the rumen microorganisms and the animal body tissues have been satisfied. Until recently very little effort had been made to design diets for the dual purpose of supporting maximum microbial growth and fulfilling the animal tissue amino acid needs. Pigden (1971), and Satter and Roffler (1975) have developed systems giving guidelines that can be used in evaluating the usefulness of dietary nonprotein nitrogen in ruminant rations. Burroughs et al. (1972, 1973, 1974, 1975) developed a more extensive system in which the amino acid composition of absorbable amino acids relative to the amino acid requirements of the body tissues was also considered. A brief discussion of

these systems will follow.

Pigden (1971) stated that each lignocellulose material has a characteristic digestion ceiling which represents the point at which maximum digestion has occurred. The energy remaining in the lignocellulose material is, for practical purposes, unavailable to the animal. Many factors can influence this ceiling such as the degree of lignification, level and availability of the polysaccharides, particle size, and the quantity of nitrogen and minerals present. In some low quality forages nitrogen appears to be the major factor limiting digestion and if supplied in adequate amounts the digestion coefficient can be increased considerably. He concluded that 1% of nitrogen (forage nitrogen plus supplemental nitrogen) is adequate for efficient breakdown of lignocellulose in the reticulo-rumen for feedstuff materials containing no more than 50% digestible energy. Where digestible levels are higher, as may be the case for chemically-treated lignocellulose, the nitrogen requirement may increase to 1.5%. Where readily available carbohydrate (such as starch or sugar) is included at levels in excess of 20% of the ration, the nitrogen requirement for lignocellulose breakdown will increase to about 2%. Some of this nitrogen must be in the form of ammonia or urea to prevent a depression of cellulose breakdown. Any modification of the lignocellulose

material which increases its digestibility such as grinding, chemical treatment with alkali, irradiation, or steam processing will increase the nitrogen requirement.

The system developed by Burroughs et al. (1972, 1973, 1974, 1975) allows an estimate of the MP content of a feedstuff or a mixture of feedstuffs based on the total protein and TDN content of the diet. The quantity of MP available in a given feedstuff includes the quantity of dietary protein which will pass through the rumen into the abomasum without suffering fermentation degradation. Also, it includes the quantity of dietary protein in the feedstuff which will be degraded in the rumen and converted to rumen microbial protein. The maximum quantity of microbial protein formed from degraded protein is equal to 10.4% of the dietary TDN of the feedstuff. If the quantity of protein degradation of the feedstuff is less than 10.4% of the dietary TDN, then urea supplementation of the feedstuff would result in additional microbial protein as expressed by its positive UFP. On the contrary, if the quantity of degraded protein of the feedstuff exceeds the numerical value of 10.4% of dietary TDN, then excess rumen ammonia will exist and further additions of ammonia in the form of urea would be of no value to the given feedstuff (negative UFP) in forming additional microbial protein.

The system assumes that 15 g of metabolic fecal protein per kilogram of feedstuff dry matter is an expense of digestion inherent to all feeds. For convenience this amount is not included in feedstuff MP table values. The digestion coefficients of undegraded feedstuff protein and microbial protein are assumed to be 90% and 80% respectively. Thus, multiplication of the quantities of undegraded feedstuff protein and microbial protein by their respective digestion coefficients gives an estimate of the quantity of protein absorbed from the intestines as MP. The quantity of metabolizable amino acids (MAA) is calculated by multiplying the quantity of MP from each source (feedstuff or microbial protein) by its respective amino acid fractional composition.

The daily amino acid requirements are expressed in grams per animal per day and are based on body size and rate of growth or milk production.

Roffler and Satter (1975a, 1975b) and Satter and Roffler (1975) determined from continuous in vitro rumen fermenters that microbial protein synthesis could be obtained with ammonia concentrations of 5 mg per 100 ml rumen fluid (5 mg %). This system assumes that .75 kg MP will be obtained from each kilogram of dietary alpha amino protein if the rumen ammonia concentration is below 5 mg % in the rumen fluid. Following attainment of this ammonia

concentration each additional kg of crude protein will provide only .3 kg of MP with the excess ammonia being of no value for microbial protein synthesis. If a rumen ammonia concentration of 5 mg % is not attained from the natural dietary proteins, urea would be useful and could be added to raise the rumen ammonia concentration to the optimum level.

Although the exactness of both systems needs to be verified on many different kinds of diets, these feeding standards provide the framework of a new approach to protein nutrition of ruminants that recognizes the nitrogen needs of the rumen microorganisms as well as the host animal.

EXPERIMENTAL PROCEDURE

Chemical analyses of urease activity and protein solubility were conducted in selecting a representative source of supply of SBM to be used in both laboratory and cattle feeding experiments. Small batches of this selected meal were subjected to varying quantities of formaldehyde, wood molasses (Masonex)[1] and heat treatments for purposes of protein modification. The effectiveness of these modifications was screened in the laboratory by *in vitro* rumen fermentations involving decreased ammonia formation. They were further screened in the laboratory by rat growth and nitrogen balance trials to ascertain whether or not the protein modification had been sufficiently severe to injure its nutritional value when received post-ruminally in the digestive tract of cattle. Following these laboratory screening procedures with small batches of meal, larger batches were prepared with the more promising levels of modifying agents (formaldehyde, Masonex or heat) for usage in cattle feeding experiments. These experiments were designed with negative and positive control diets containing unmodified SBM and urea compared with experimental diets containing modified SBM and urea in testing

[1]The wood molasses used in this research project is a tannin and phenolic containing hemi-cellulose extract by-product of the hardboard (Masonite) industry and is sold under the trade name of Masonex.

the effectiveness of the modification.

Laboratory Studies

Selection of soybean meal

Meals from five different manufacturing plants in Iowa were chemically analyzed for the presence of urease activity and the percentage of water-soluble protein. These measurements were used as indicators of the amount of heat applied during the manufacturing process in selecting a suitable representative meal for experimental modification.

Urease activity was determined using the official method of the American Soybean Association as described by Official and Tentative Methods of the American Oil Chemists' Society (A.O.C.S.) (1959).

This consisted of incubating .2 g of SBM sample and 10 ml of a buffered urea solution in a stoppered test tube at 30°C for 30 minutes. Following incubation the pH of the solution was determined and compared to the pH of a blank which contained only the buffered urea solution without SBM. An increase in pH above the blank is indicative of the conversion of urea to ammonia due to the presence of urease enzyme activity.

Water-soluble SBM protein was determined using a modified version of the procedure described by Paulsen et al. (1960)

which has been adopted as the official method of measuring protein solubility by the American Soybean Association. The modified procedure consisted of blending a mixture of 13.2 g SBM and 200 ml distilled water at medium speed setting in a Virtis Super 30 blender for 10 minutes. After transferring the slurry from the blender to a 600-ml beaker and permitting separation, a portion of the supernatant was decanted into a 50-ml centrifuge tube and centifuged 10 minutes at 2700 x G. Kjeldahl nitrogen analyses were then conducted on 15 ml of the supernatant. Total nitrogen and dry matter determinations were also made on the SBM samples in accordance with standard procedures described by Official Methods of Analysis of the Association of Official Agricultural Chemists (A.O.A.C.) (1975). All analyses were performed in duplicate.

Treating of soybean meal with modifying agents

Formaldehyde, heat and Masonex were chosen as possible protein-protecting agents for this research project and were applied to the commercial SBM at different levels as described below.

Formaldehyde treatment of soybean meal The calculated amount of formaldehyde (in the form of commercial Formalin) was added to a sufficient quantity of water such that the weight of the formaldehyde-water solution equaled

8% of the SBM air-dry weight. For example, when 80 ml of a water-formaldehyde solution containing 69.3 ml of water and 10.7 ml Formalin was added to 1 kg of SBM containing 87% dry matter and 50% crude protein (dry matter basis), a treatment level of 1.0% actual formaldehyde of the crude protein dry matter was achieved.

The water-formaldehyde solution was applied to small batches of SBM during mixing in a Hobart mixer (Model K5-A). Following a four-minute mixing period, the treated SBM was stored in a sealed plastic bag at room temperature for a 48-hour period and then placed in shallow trays to dry. Formaldehyde treatment of SBM in larger batches at the beef nutrition farm involved mixing in a floor model Hobart mixer. The treated meal was then stored two to three days in a sealed plastic container located in the basement of the feed mill before being incorporated into cattle diets. The meal was treated on a weekly basis.

Heat treatment of soybean meal Heat treatment consisted of heating the commercial meal in shallow pans (approximately 5 cm deep) in a forced-air oven for a 4-hour period. The heated meal was then stored in steel barrels with tops until incorporated into cattle diets or subjected to laboratory analyses at which time a sufficient amount of water was added to produce a meal containing 80%

dry matter.

Wood molasses treatment of soybean meal Application of Masonex to SBM consisted of partial blending by hand a mixture of SBM (80% dry matter) and Masonex in a plastic barrel, then transferring the material to a floor-model Hobart mixer to achieve more extensive mixing. The material was then spread on 1.2 x 2.4 m plywood trays for a two to three day period after which the treated meals were either incorporated into cattle diets or subjected to laboratory analyses.

In vitro rumen fermentation technique for testing protein modification

Soybean meal treatments subjected to in vitro fermentation studies included: formaldehyde application at levels of .2, .4, .6, .8, 1.0, 1.2, and 1.4%; heating of the commercial meal at temperatures of 127, 138 or 149°C for a four-hour period; and Masonex applied at levels of either 10.1 or 20.2%. The chemical composition of Masonex, as reported by the Masonite Corporation, includes 65% solids which are comprised of the following sugars: arabinose (4-5%), galactose (8-13%), glucose (14-17%), mannose (27-45%), and xylose (21-46%).

A modified version of the Tilley and Terry (1963) in vitro fermentation technique was employed in which the

in vitro apparatus consisted of 50-ml round bottom centrifuge tubes suspended in a 39°C enclosed water bath. Anaerobic conditions were maintained by bubbling CO_2 into the circulating water of the bath. The incubation mixture consisted of .3 g SBM treated with either water (control) or a modifying agent such as heat, formaldehyde or Masonex, 28 ml of McDougall's phosphate-bicarbonate buffer of pH 6.8, McDougall (1948), and 7 ml rumen fluid previously strained through 8 layers of cheese cloth. The rumen fluid was obtained at 10:00 a.m. from a fistulated cow fed (at 8:00 a.m. and 5:00 p.m.), a daily ration of 4 kg hay, 1.1 kg cracked corn, and .09 kg mineral. Blanks were included which contained only rumen fluid in the absence of SBM substrate. Samples of the individual *in vitro* tube mixtures were obtained via disposable pipettes at .5, 2, 4, 6, 8, and 10-hour incubation time intervals and were stored in sealed acidified Technicon Auto Analyzer cups at 10°C until analyzed for ammonia content. An equal volume of distilled water was returned to each *in vitro* tube following withdrawal of the sample. Ammonia content of the samples was determined according to procedures described by Technicon Corporation (1960). All *in vitro* rumen fermentation analyses were performed in triplicate.

Rat growth and nitrogen balance trials for nutritional evaluation of treated soybean meal

Holtage rat cages (Model 45238) were used for all rat trials with the fecal and urinary collection apparatus removed during the growth trials. The room was lighted from 8:00 a.m. to 5:00 p.m. daily and the temperature controlled at approximately 26°C.

Formaldehyde-treated soybean meal rat trial Weanling 21-day-old Sprague Dawley male rats, weighing approximately 75 g, were fed a semi-purified positive control diet initially for a period of four days upon arrival. They were allotted at this time by blocking according to live weight and randomly assigned to one of five dietary treatments (Table 1). Six rats were assigned to each of the treatments which included positive and negative control diets and 3 formaldehyde-treatment diets. The 3 treatment diets and the positive control diet each contained 16% crude protein. They were of identical composition except for the meal treatment permitting the comparison of rat growth rates, feed efficiency and nitrogen balance as indexes of the nutritional value of the treated and untreated meal. The negative control diet contained 10% crude protein and would be expected to produce rat performance approximately 10/16th as large as the positive control diet containing 16% protein.

Table 1. Feed composition of formaldehyde-treated diets fed to rats during growth and nitrogen balance trials[a]

Item	Positive control %	3 treated diets %	Negative control %
Soybean meal[b]	36.8	34.7	23.0
Corn oil[c]	6.0	6.0	6.0
Salt[d]	4.9	4.9	4.9
Vitamin premix[e]	2.2	2.2	2.2
Solka floc	0.8	0.8	3.4
Corn starch	49.3	49.3	60.5
Water	-	2.1	-
TOTAL	100.0	100.0	100.0

[a]Feed composition expressed on air-dry basis with control SBM and treated SBM D.M. contents of 87.0 and 92.7% respectively.

[b]SBM treated with water (controls) or 0.2, 0.8 or 1.4% formaldehyde.

[c]Mazola oil.

[d]Salt and mineral mixture RH from Nutritional Biochemicals Corporation.

[e]Vitamin diet fortification mixture from ICN Pharmaceuticals.

The semi-purified diets were composed of corn starch, solka floc, corn oil, salt, and mineral and vitamin premixes in addition to 44% crude protein soybean meal treated with either water (controls or formaldehyde at levels of .2%, .8% or 1.4% formaldehyde of the crude protein dry matter. The test diets were equalized with respect to TDN (79%), crude fiber (1%), and fat (8%).

Rats were weighed and feed consumption was determined at weekly intervals during a 35-day growth trial. Following the growth trial, the rats were continued on their respective diets but placed on a restricted daily feed intake for a period of four days prior to initiation of the five-day nitrogen-balance trial. The daily quantity of feed given to each rat during the nitrogen-balance trial was calculated in the following manner. Average individual rat metabolic body weight ($g^{.75}$) and average feed consumption (g per day) were determined for the final 14-day period of the growth trial. The numerical value, representing 75% of the average daily feed consumption during the final 14 days of the growth trial, was then divided by the metabolic body weight of each rat respectively to obtain a ratio of feed consumption to metabolic body weight. The largest ratio was then used to calculate the maximum amount of feed offered to each rat such that each rat received the same quantity of feed in relation to its own metabolic body weight.

The urine from each rat was collected in a plastic

bottle containing 5 ml of 5 N HCl. At the termination of the trial the urine was diluted with distilled water to a volume of 200 ml and frozen until analyzed for nitrogen. The feces, following collection, were weighed and frozen until analyzed. Preparation of the fecal samples for chemical analyses consisted of further freezing in liquid nitrogen, then pulverizing in a Sorvall blender fitted with a stainless steel canister. The pulverized sample was then analyzed for dry matter and nitrogen. All chemical analyses of urine and feces were performed in duplicate.

Heat-treated soybean meal rat trial The growth and nitrogen-balance trials of rats fed diets containing heated SBM were conducted in a manner approximately similar to the formaldehyde rat trials. The duration of the growth and nitrogen-balance trials were 28 days and 6 days respectively. The dietary mixtures were similar to those used in the formaldehyde trial with the exception of the type of SBM treatment (Table 2). The SBM was treated by applying heat at temperatures of 127°, 138°, or 149°C for four-hour periods.

Cattle growth studies

The cattle trials were conducted at the Iowa State University beef nutrition farm located 3 miles northeast of the campus.

Table 2. Feed composition of heat-treated diets fed to rats during growth and nitrogen balance trials[a]

Item	Positive control diet %	3 treated diets %	Negative control diet %
Soybean meal[b]	35.1	30.6	23.0
Corn oil[c]	5.7	5.7	5.7
Salt[d]	4.7	4.7	4.7
Vitamin premix[e]	2.1	2.1	2.1
Solka floc	0.9	0.9	1.7
Corn starch	51.5	51.5	62.8
Water	-	4.5	-
TOTAL	100.0	100.0	100.0

[a]Feed composition expressed on air-dry basis with untreated SBM at 87.0% DM and heat treated SBM at 100% DM.

[b]Heat treatments of no heat (positive and negative controls), or four hours at 127, 138, or 149°C.

[c]Mazola oil.

[d]Salt and mineral mixture RH from Nutritional Biochemicals Corporation.

[e]Vitamin diet fortification mixture from ICN Pharmaceuticals.

Formaldehyde-treated soybean meal The cattle utilized in this experiment were of southern Iowa origin, predominantly of Angus, Hereford, or Angus x Hereford breeding and were purchased in February, 1976. Upon arrival at the I.S.U. beef nutrition farm an adaptation diet consisting of corn, corn cobs, SBM, molasses, vitamins and minerals was fed for a period of approximately three weeks before initiation of a 110-day growth trial. Prior to randomly assigning the cattle to one of 5 dietary treatments, weights were taken on two consecutive days for the purpose of obtaining a more accurate starting weight. The experimental design consisted of 2 pens per treatment and 6 or 7 steers (averaging 197.8 kg) per pen. Maximum feed dry matter intake of all treatments was limited to the highest consumption by the 14 steers receiving one of the three formaldehyde treatment diets and not by either of the two control diets.

Composition of the treatment diets fed prior to daily consumption levels of 5.45 kg per steer is shown in Table 3. Upon achieving this level of consumption, the diets were altered to more nearly coincide with the animals' protein requirements. This was done by reducing the SBM content of the negative control and formaldehyde-treated diets from 8% to 5% along with a similar reduction of 20% to 17.7% in the positive control diet as shown in Table 4. The crude protein

Table 3. Feed composition of diets containing formaldehyde-treated SBM fed the initial 4-week period prior to 5.5 kg daily DM consumption by steers

Feed	Negative control plus 3 treated diets	Positive control diet
	DM basis %	DM basis %
Cobs, gr.	59.41	61.30
Corn, gr.	19.20	7.75
Cane mol.	10.00	10.00
Wet SBM[a]	8.00	20.00
Urea	1.80	-
Dical. phos.	0.72	0.52
Salt	0.25	0.25
Dyna-K (KCl)	0.40	-
T.M. premix	0.02	0.02
Vit. A premix	0.12	0.12
Fl. sulfur	0.04	-
Rumensin	0.04	0.04
TOTAL	100.0	100.0

[a]SBM treated with either water (positive and negative controls) or formaldehyde at levels of 0.2%, 0.4% or 0.8% formaldehyde of crude protein dry matter.

Table 4. Feed composition of steer diets containing formaldehyde-treated SBM fed after initial 4 weeks when 5.5 kg dry matter consumption was reached

Feed	Negative control plus 3 treated diets DM basis %	Positive control diet DM basis %
Cobs, gr.	59.41	61.30
Corn, gr.	19.20	7.05
Cane mol.	13.00	13.00
Wet SBM[a]	5.00	17.70
Urea	1.90	-
Dical. phos.	0.72	0.52
Salt	0.25	0.25
Dyna-K (KCl)	0.30	-
T.M. premix	0.02	0.02
Vit. A premix	0.12	0.12
Fl. sulfur	0.04	-
Rumensin	0.04	0.04
TOTAL	100.0	100.0

[a]SBM treated with either water (positive and negative controls) or formaldehyde at levels of 0.2%, 0.4% or 0.8% formaldehyde of crude protein dry matter.

content (N x 6.25) of these diets was equalized by the addition of urea to the negative control and treatment diets. The estimated chemical composition of the diets is shown in Table 5 and the calculated quantity of MP and metabolizable sulfur amino acids (MSAA) consumed daily at various levels of intake along with the estimated daily animal requirements are listed in Table 6. The negative control diet (containing water-treated SBM) and the formaldehyde-treated diets were designed to be deficient in MP and MSAA unless, as in the case of the treated meal, it was actually protected in the rumen and more of it became available for digestion and absorption post-ruminally. The positive control diet, containing a larger quantity of water-treated SBM, was expected to provide MP in quantities similar to that obtained from the treated diets in which rumen protein protection was achieved. Thus the difference in cattle response between the two control diets was expected to demonstrate the potential benefits from protein modification if the agents utilized were effective.

The dietary treatments as outlined in Table 4 were fed for a period of 56 days at which time it was evident that the performance of cattle receiving the .2% formaldehyde treatment (lots 2 and 7) was greatly inferior to those cattle receiving either of the two higher levels of formaldehyde or the positive control diets. Since the

Table 5. Estimated chemical composition of steer diets containing formaldehyde-treated SBM following the initial 4-week period (DM basis)

Item	Negative control pens 1&6 %	0.2% treated pens 2&7 %	0.4% treated pens 3&8 %	0.8% treated pens 4&9 %	Positive control pens 5&10 %
TDN	61.00	61.00	61.00	61.00	61.00
Soy protein	2.57	2.57	2.57	2.57	9.12
Other natural protein	4.07	4.07	4.07	4.07	2.97
Urea prot. equiv.	5.32	5.32	5.32	5.32	-
Total crude protein	11.96	11.96	11.96	11.96	12.09
Calcium	0.38	0.38	0.38	0.38	0.41
Phosphorus	0.27	0.27	0.27	0.27	0.29
Potassium	1.13	1.13	1.13	1.13	1.34
Sulfur	0.18	0.18	0.18	0.18	0.19
MP[a]	5.59	5.59-6.99	5.59-6.99	5.59-6.99	6.65
MSAA[a]	0.17	0.17-0.21	0.17-0.21	0.17-0.21	0.20

[a]The % metabolizable protein or metabolizable sulfur amino acids actually available will vary depending upon the degree of soy protein protection in the rumen, i.e., if 100% protection occurs then 6.99% metabolizable protein and 0.21% metabolizable sulfur amino acids will be achieved.

Table 6. Estimated daily requirements of steers and amounts of MP and MSAA supplied by different levels of dietary consumption

Daily intake	Daily requirement		Negative control		3 treatment diets				Positive control	
					MP		MSAA			
DM	MP	MSAA	MP	MSAA	Min	Max	Min	Max	MP	MSAA
kg	g	g	g	g	g	g	g	g	g	g
5.45	373	11.5	305	9.3	305	381	9.3	11.4	362	10.9
6.36	406	12.5	355	10.8	355	444	10.8	13.3	423	12.7
7.27	438	13.5	406	12.3	406	508	12.3	15.3	483	14.5
8.18	471	14.5	457	13.9	457	571	13.9	17.2	543	16.3

objective of the trial was to gain knowledge concerning the optimum level of formaldehyde application, the cattle receiving the .2% formaldehyde diet (lots 2 and 7) during the initial 56 days of the trial were switched to a level of 1.2% formaldehyde during the final 54 days of the study.

Two pens of reserve steers which had been receiving the positive control diet during the initial 56 days, were also fed the 1.2% formaldehyde treatment during the final 54 days of the trial. Cattle performance throughout the trial was monitored by live weight measurements taken at 2-week intervals and daily feed consumption by pens.

Formaldehyde-, heat-, and wood molasses-treated soybean meal trial The positive results of the first cattle trial stimulated the testing of additional protein-modifying agents in a second cattle trial. The steers utilized in this experiment were purchased in southern Iowa and were primarily of Angus, Hereford or Angus x Hereford breeding. A ration composed of corn, corn cobs, SBM, molasses, vitamins, and minerals was fed upon arrival of the cattle for a period of approximately 4 weeks. During this time some of the cattle showed symptoms of the disease Infectious Bovine Rhinotracheitis (IBR) and were treated by University veterinarians. The calves were randomly assigned to 1 of 7 dietary treatments (2 pens per treatment, 6 calves per

pen) and then weighed on 2 consecutive days to obtain a more accurate starting weight for the 126-day growth trial. The cattle were weighed at 2-week intervals throughout the test and feed consumption by pens was measured daily. The cattle were full-fed with no restrictions throughout the entire trial.

The dietary treatments (Table 7) included negative and positive control diets in which the SBM was treated only with water and contributed 7.0% and 20.0% of the ration dry matter respectively. Crude protein content (N x 6.25) of these diets was equalized by the addition of urea to the negative control diet. The five treated diets were identical to the negative control diet except for the SBM treatment as described below. The treatments consisted of heating the commercial meal in a forced-air oven at temperatures of either 144°C or 155°C, application of Masonex at levels of either 10.1% or 20.2% of SBM dry matter, or the combination of 10.1% Masonex plus .6% formaldehyde (dry matter basis). The estimated chemical composition of the diets is shown in Table 8 and the calculated quantity of MP and MSAA consumed daily at various levels of intake along with the estimated daily animal requirements are shown in Table 9.

Table 7. Feed composition of diets fed during the formaldehyde-, heat- and Masonex-treated SBM cattle growth trial (DM basis)

Feed	Negative control pens 1&8 %	10.1% Masonex pens 2&9 %	10.1% Masonex & .6% formal. pens 3&10 %	20.2% Masonex pens 4&11 %	144°C heat* pens 5&12 %	155°C heat pens 6&13 %	Positive control pens 7&14 %
Cobs, gr.	59.52	59.52	59.52	59.52	59.52	59.52	60.59
Corn, gr.	15.00	15.00	15.00	15.00	15.00	15.00	3.46
Cane mol.	15.00	14.30	14.30	13.60	15.00	15.00	15.00
Masonex	-	0.70	0.70	1.40	-	-	-
Wet SBM	7.00	7.00	7.00	7.00	7.00	7.00	20.00
Urea	2.00	2.00	2.00	2.00	2.00	2.00	-
Dical. phos.	0.72	0.72	0.72	0.72	0.72	0.72	0.52
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Dyna-K (KCl)	0.30	0.30	0.30	0.30	0.30	0.30	-
TM premix	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vit. A premix	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Sulfur	0.03	0.03	0.03	0.03	0.03	0.03	-
Rumensin	0.04	0.04	0.04	0.04	0.04	0.04	0.04
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 8. Estimated chemical composition of diets fed during the formaldehyde-, heat-, and Masonex-treated SBM cattle growth trial (DM basis)

Item	Negative control pens 1&8 %	10.1% Masonex pens 2&9 %	10.1% Masonex & .6% formald. pens 3&10 %	20.2% Masonex pens 4&11 %	144°C heat pens 5&12 %	155°C heat pens 6&13 %	Positive control pens 7&14 %
TDN	61.00	61.00	61.00	61.00	61.00	61.00	61.00
Soy protein	3.60	3.60	3.60	3.60	3.60	3.60	10.30
Other nat. protein	3.80	3.80	3.80	3.80	3.80	3.80	2.70
Urea prot. equiv.	5.60	5.60	5.60	5.60	5.60	5.60	-
Total crude prot.	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Calcium	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Phosphorous	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Potassium	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Sulfur	0.20	0.20	0.20	0.20	0.20	0.20	0.20
MP[a]	5.70	5.70-7.60	5.70-7.60	5.70-7.60	5.70-7.60	5.70-7.60	6.80
MSAA[a]	0.18	0.18-0.23	0.18-0.23	0.18-0.23	0.18-0.23	0.18-0.23	0.21

[a]The % of metabolizable protein or metabolizable sulfur containing amino acids actually available will vary depending upon the degree of soy protein protection in the rumen, i.e., if 100% protection occurs then 7.60% metabolizable protein and 0.23% metabolizable sulfur amino acids will be achieved.

Table 9. Estimated daily requirements of steers and amounts of MP and MSAA supplied by different levels of dietary consumption

Daily intake DM kg	Daily requirement MP g	Daily requirement MSAA g	Negative control MP g	Negative control MSAA g	5 treatment diets MP Min g	5 treatment diets MP Max g	5 treatment diets MSAA Min g	5 treatment diets MSAA Max g	Positive control MP g	Positive control MSAA g
5.45	373	11.5	309	9.6	309	412	9.6	12.7	370	11.3
6.36	406	12.5	361	11.2	361	481	11.2	14.8	432	13.2
7.27	438	13.5	413	12.8	413	550	12.8	16.9	494	15.0
8.18	471	14.5	464	14.4	464	619	14.4	19.0	556	16.9

Statistical Procedures

The data from this research were analyzed by analysis of variance (Steel and Torrie, 1960). The interaction mean square was used to test for significance of the main effects if a significant interaction existed. To determine treatment differences, either the Neuman Keul method or Dunnett's procedure was used (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Laboratory Results

Selection of soybean meal

Chemical analyses of the SBM samples obtained from different manufacturers in Iowa are summarized in Table 10. Although only small differences existed between the meals, the percentage of water-soluble protein and urease activity were slightly greater in the meal obtained from plant number four. SBM obtained from plants number two and three showed no evidence of urease activity, even though the meal from manufacturer number three contained slightly more soluble protein than the meal from plant number two. Based upon

Table 10. Chemical analyses of soybean meal from five different manufacturers

Manufacturing plant[a]	Dry matter %	Crude[b] protein %	Soluble[c] protein %	Urease[d] activity pH units
1	90.3	49.4	13.2	+.07
2	88.2	50.3	12.1	-
3	88.3	49.3	14.0	-
4	88.9	48.4	16.4	+.15
5	88.6	49.7	14.0	+.06

[a]SBM manufacturing plants in Iowa.

[b]Dry matter basis.

[c]Expressed as a percentage of total protein.

[d]An increase in pH indicates the presence of urease.

these small differences, SBM was purchased from plant number three to be used in subsequent laboratory and cattle trials.

Soluble protein and *in vitro* rumen fermentation techniques for testing protein modification

In vitro rumen fermentation results The formaldehyde-treated SBM *in vitro* rumen fermentation results are graphically presented in Figure 1. Although formaldehyde at the .2% level caused a 77% reduction in the ten-hour ammonia concentration when compared to untreated meal, total inhibition was not achieved until levels of .4% or greater were applied. The negative ammonia values reported for SBM treated at levels of .4% or more formaldehyde are probably indicative of low availability of treated SBM for microbial usage causing them to use the ammonia inherently present in the rumen fluid. Table A1 of the Appendix presents the actual values obtained from the analyses.

The heat-treated *in vitro* rumen fermentation results are presented in Figure 2. Heat treatment of the commercial SBM at temperatures of 127°C or 138°C reduced 10-hour ammonia concentrations by 28% and 93% respectively compared to unheated control meal. Accumulation of ammonia was completely inhibited when a temperature of 149°C was applied. The

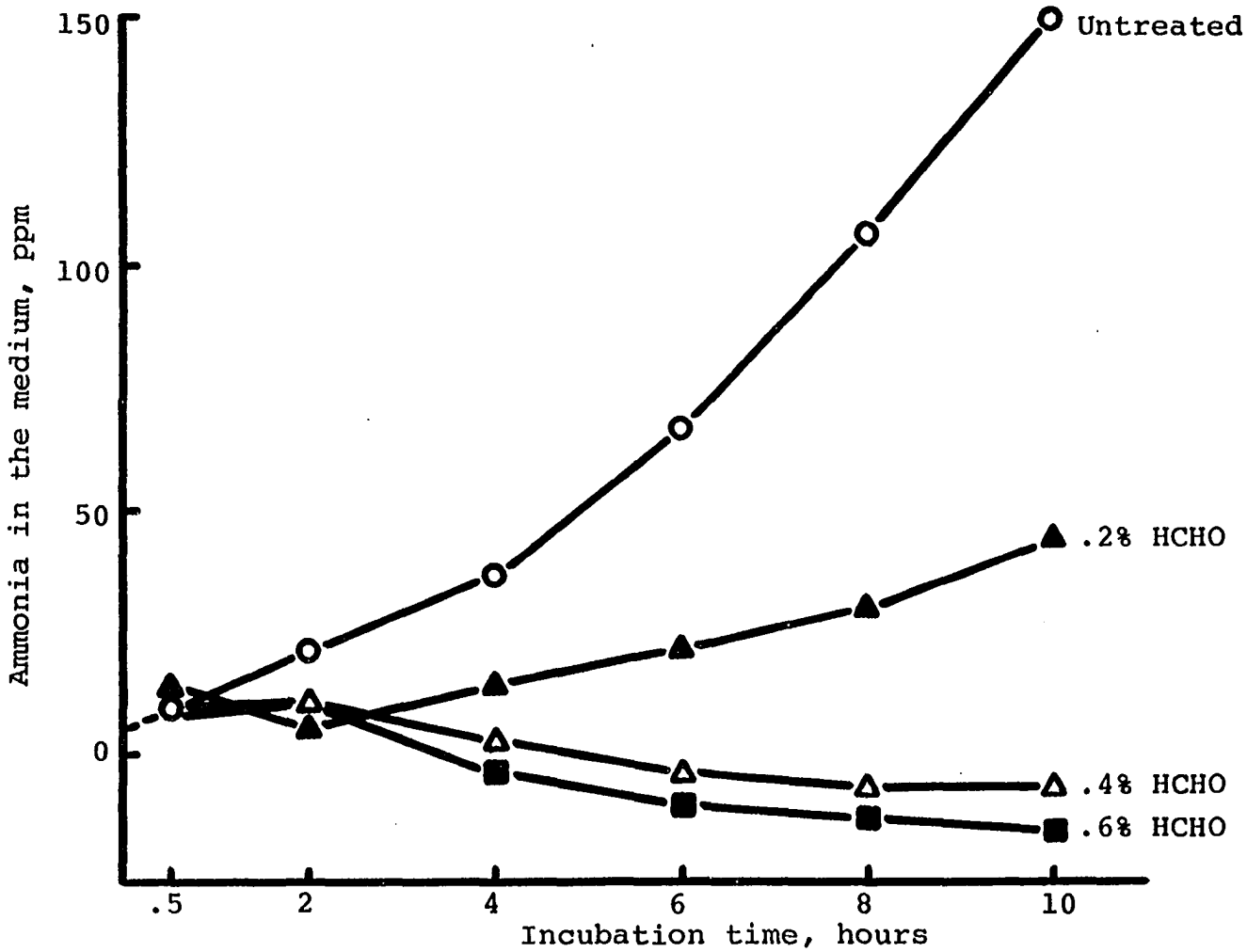

Figure 1. In vitro ammonia concentrations with formaldehyde-treated SBM

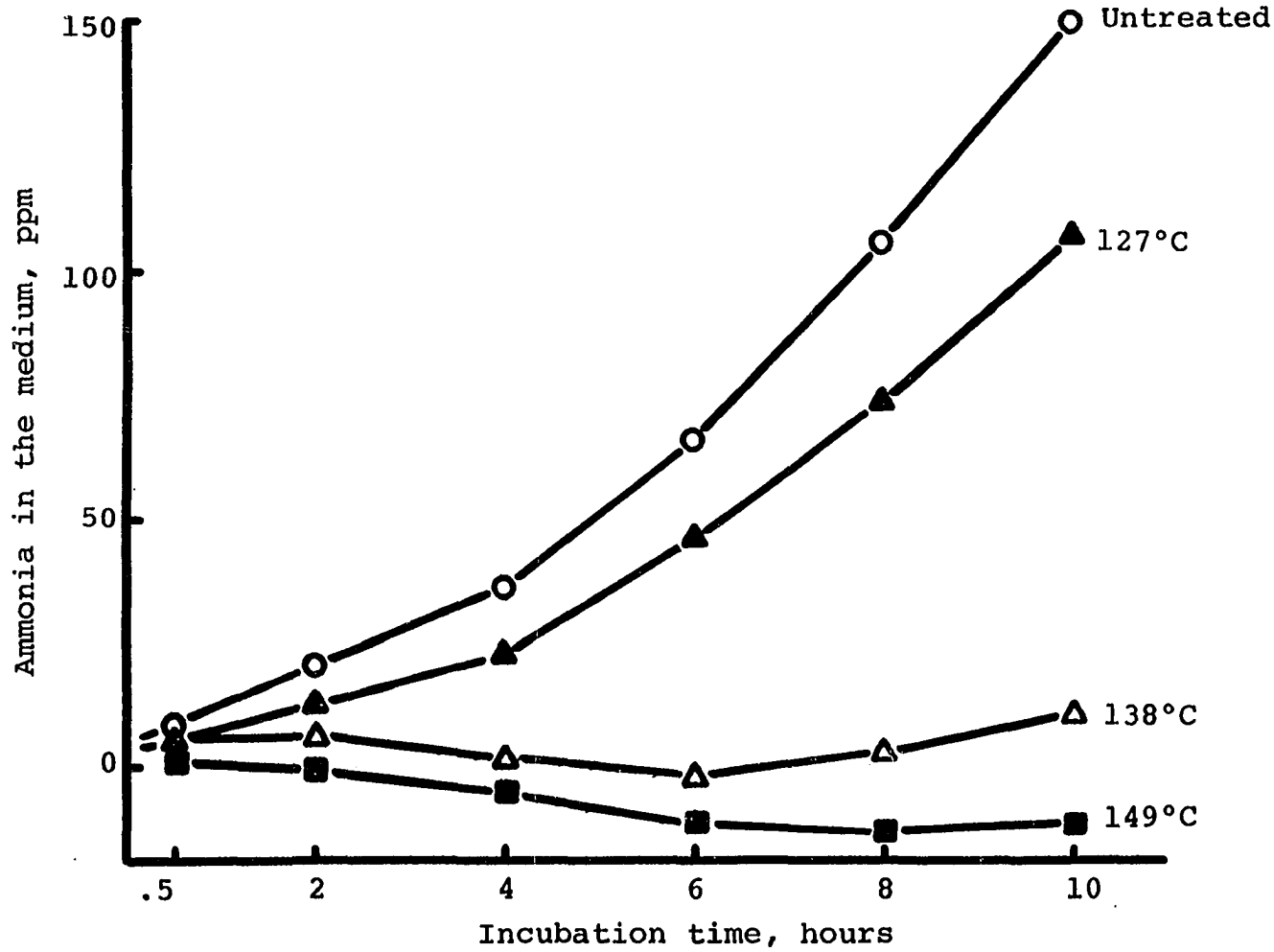

Figure 2. In vitro ammonia concentrations with heat-treated SBM

ammonia concentrations at various incubation times are presented in Table A2 of the Appendix.

In vitro studies with Masonex-treated SBM suggest that Masonex possesses some protein-modifying ability as presented in Figure 3. Application of 10.1% Masonex to the meal decreased the 10-hour in vitro ammonia concentration from 121.0 to 81.5 ppm which is equivalent to a total reduction of 32.6% from the untreated control meal. Treatment of SBM with 20.2% Masonex resulted in a further reduction to 65.6 ppm or a 45.8% reduction from the untreated control meal. Results of the in vitro rumen study with SBM treated with Masonex are reported in Table A3 of the Appendix.

Water-soluble protein determinations Figure 4 shows reductions in water-soluble protein (expressed as a percentage of crude protein) of SBM when treated with different protein-modifying agents. The percentage of soluble protein in untreated meal was 14%. The addition of formaldehyde at levels of .2%, .4%, .6%, .8%, 1.0%, 1.2% and 1.4% reduced ($P < .05$) the percentages of water-soluble protein to 4.4%, 3.3%, 2.9%, 2.5%, 2.4%, 2.4%, and 1.9% respectively. Heat treatment of the meal at temperatures of 127°C, 138°C and 149°C reduced the percentages of soluble protein (from 14% in unheated SBM) to 7.3%, 4.3%, and 3.5%. Treatment of the meal with Maxonex in quantities of 10.1% and 20.2%

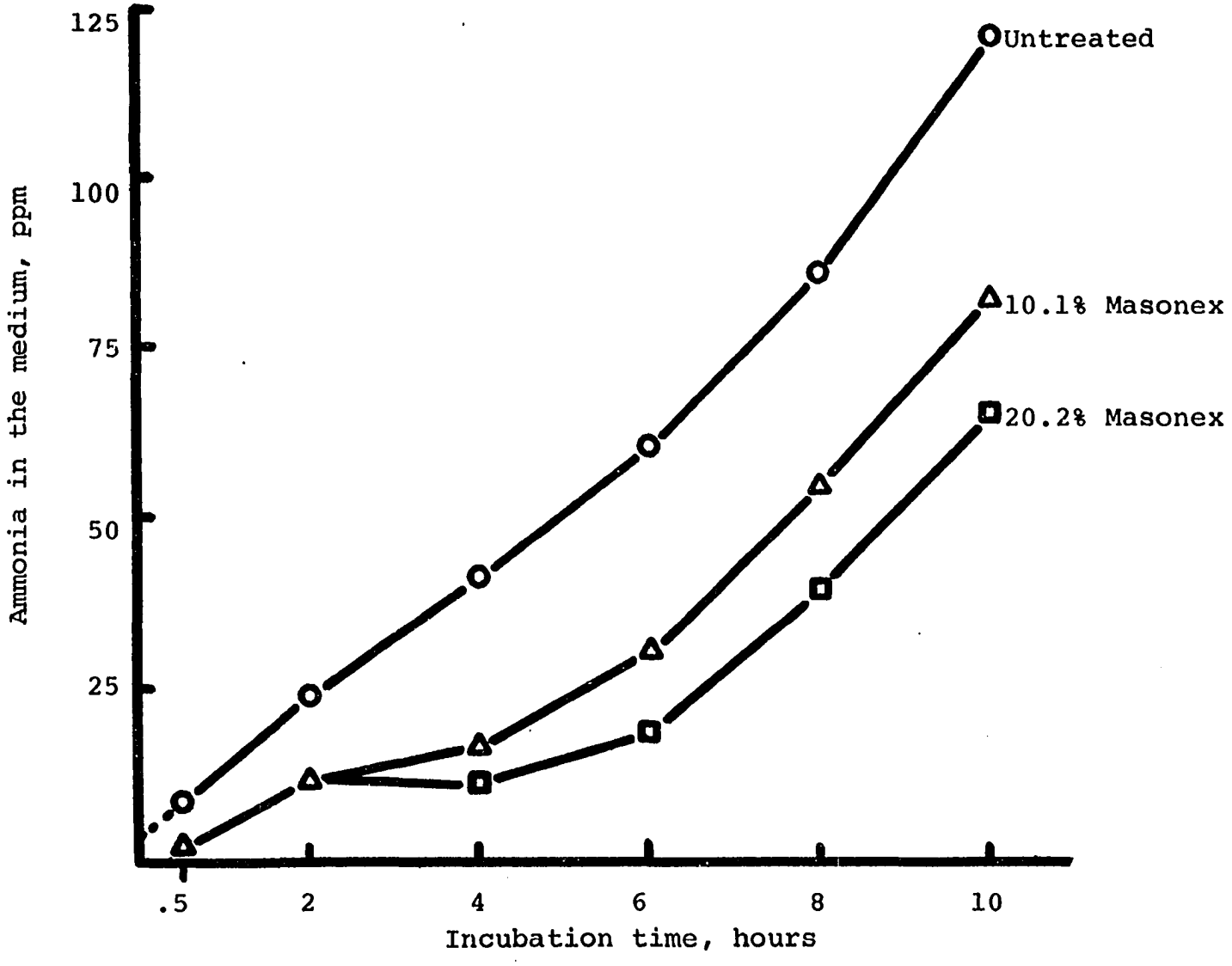

Figure 3. In vitro ammonia concentrations with Masonex-treated SBM

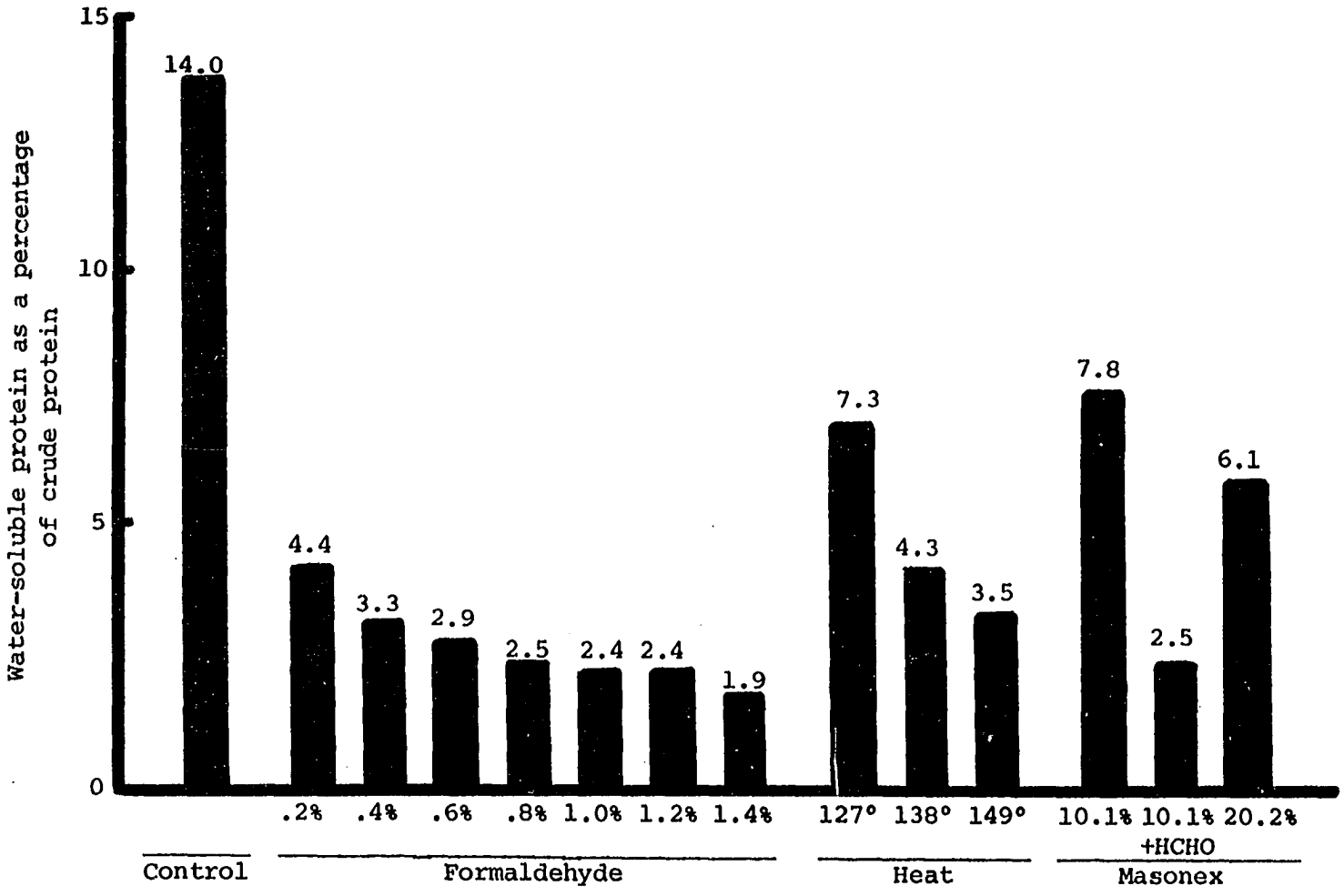

Figure 4. Water soluble protein in SBM treated with formaldehyde, heat, Masonex or Masonex plus formaldehyde (expressed as a percentage of crude protein)

respectively lowered the percentages of water-soluble protein to 7.8% and 6.1%.

The combination of 10.1% Masonex and .6% formaldehyde depressed the percentage of soluble protein to 2.5% which was lower than the 2.9% value achieved with .6% formaldehyde in the absence of Masonex. This difference was not statistically significant.

Rat growth and nitrogen balance trials for nutritional evaluation of treated soybean meal

Formaldehyde-treated soybean meal rat trial Results of the 35-day growth trial are presented in Table 11. Although the addition of formaldehyde to the SBM caused no statistically significant differences in feed intake, the .8% treatment rats consumed the largest quantities of feed (6% more than the positive controls). Feed consumption by the 1.4% formaldehyde and negative control rats was 4.8% and 6.9% less than the positive controls.

Live weight gains of the positive control rats exceeded gains of the .2% and .8% treatment rats by 10.7% and 5.3% although these differences were not significant ($P > .05$). The 1.4% formaldehyde and negative control rats gained less (34% and 32%) than the positive controls ($P < .05$).

Increased levels of formaldehyde application tended to decrease feed efficiency as seen by the 7.6%, 11.5%, and

Table 11. Rat feed intake, gains and feed efficiency with formaldehyde-treated SBM (35-day trial)[a]

Treatment	Daily feed intake g	Daily weight gained g	Feed efficiency g	Reduced feed efficiency[b] %
Positive control	14.5	5.6[c]	2.6[c]	-
.2% formaldehyde	14.1	5.0[c]	2.8[c]	7.6
.8% formaldehyde	15.4	5.3[c]	2.9[c]	11.5
1.4% formaldehyde	13.8	3.7[d]	3.8[d]	46.1
Negative control	13.5	3.8[d]	3.5[d]	

[a]Values represent the mean of 6 individual observations.

[b]Reduced feed efficiency compared to the positive controls.

[c]Means in the same column having the same superscript do not differ statistically ($P<.05$).

[d]Means in the same column having the same superscript do not differ statistically ($P<.05$).

46.1% reductions in feed conversion of the .2%, .8% and 1.4% formaldehyde treatments compared to the positive control group. The 46.1% reduction was significant ($P<.05$) and suggested that a considerable portion of the SBM protein was irreversibly bound with formaldehyde and of little nutritional value to the rats.

Results of the nitrogen balance trial with formaldehyde-

treated SBM are graphically presented in Figure 5 with a complete summary of the data appearing in Table A4 in the Appendix. Differences in daily nitrogen intake occurred ($P<.05$) due to imposed restrictions based upon metabolic size and feed consumption during the latter part of the growth trial.

The daily quantities of fecal nitrogen excreted by the .2% and .8% treatment rats did not differ ($P<.05$) from the positive controls in contrast to the 1.4% treatment rats which excreted 209% more fecal nitrogen ($P<.05$). Although the quantities of fecal nitrogen are of interest, the apparent nitrogen digestibility coefficient is a better indicator of the nutritional value of the treated meal because nitrogen intake is also considered. The digestion coefficients of the untreated positive control meal and the .2% formaldehyde-treated meal were identical (84.3%) in contrast to the .8% treatment which reduced ($P<.05$) digestibility to 77.4%. Application of 1.4% formaldehyde to the meal reduced digestibility ($P<.05$) to 61.8% which is 22.5% lower than the untreated positive control meal.

Quantities of urinary nitrogen excreted daily accounted for 50 to 55% of the nitrogen consumed by rats receiving the positive control, .2% and .8% formaldehyde diets. The 1.4% treatment rats excreted only 35% of the nitrogen consumed in

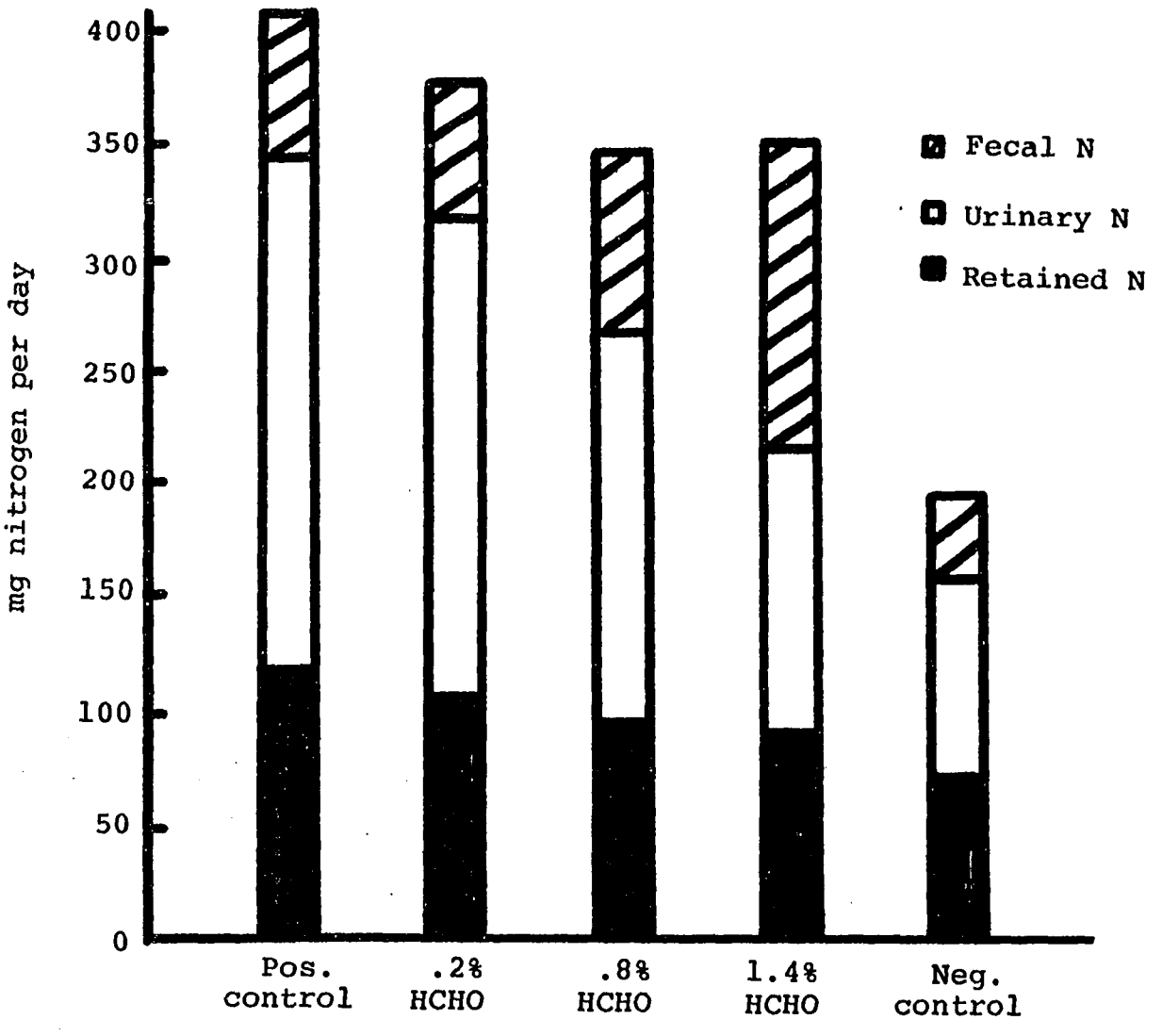

Figure 5. The intake, excretion and retention of nitrogen by rats fed untreated or formaldehyde-treated SBM

the form of urinary nitrogen compared to 44% for the negative controls.

An alternate method of comparing the quantities of urinary nitrogen excreted would be to calculate the amount of urinary nitrogen as a percentage of absorbed nitrogen. This method may be superior to the previous comparison because the digestibility of the nitrogen consumed is considered rather than ignored. When the daily amounts of urinary nitrogen were expressed as a percentage of nitrogen absorbed the following values were determined: positive controls, 65.4%; .2% formaldehyde, 66.3%; .8% formaldehyde, 64.2%; 1.4% formaldehyde, 57.6%; and negative controls, 54.7%. These calculations suggest that only slight differences existed between the positive control, .2% and .8% formaldehyde groups.

The negative control rats received a diet containing 10% crude protein which was protein-deficient according to recommendations of the National Research Council (1970). A comparison of the amounts of urinary nitrogen excreted (expressed as a percentage of nitrogen absorbed) by the positive and negative controls shows that the negative controls, which were receiving a protein-deficient diet, excreted a smaller percentage (10.7% less) of the nitrogen absorbed than did the positive controls. The apparent protein digestibility of the 1.4% treated diet was 22.5% lower than the

positive control diet. This would result in a decreased amount of protein available for absorption. Thus, this could create a protein-deficient state intermediate to that caused by the .8% formaldehyde and negative control diets. If this reasoning is valid, the decreased excretion of absorbed nitrogen by the 1.4% treatment group would appear to be a logical response. On this basis, it offers support to the statement by Mitchell (1962) that the amount of nitrogen excreted in the urine is determined primarily by the magnitude of nitrogen intake by the animal.

Additions of formaldehyde to SBM at levels greater than .2% depressed the quantity (mg/day) of nitrogen retained ($P<.05$) compared to the positive control rats. However, these differences were not significant when expressed as a percentage of nitrogen intake. The percentage of nitrogen retained by the negative controls was 7.8% greater ($P<.05$) than the positive controls.

The rats utilized in this trial started coughing and sneezing immediately upon arrival which may have resulted from either the cold conditions during shipment or the improperly adjusted ventilation system in the rat room. By mechanically adjusting the ventilator to reduce draftiness and increasing the room temperature from 21°C to approximately 26°C, the coughing and sneezing had nearly stopped

by about day 28 of the 35-day growth trial. Very little evidence of respiratory problems was seen during the nitrogen balance trial.

Heat-treated soybean meal rat trial Results of the 28-day rat growth trial with either heated or regular SBM are shown in Table 12. Daily feed intake of rats consuming the 127°C and 149°C heated SBM diets was nearly 10% greater than that of the positive control rats receiving the unheated meal. The 138°C treatment rats consumed quantities of feed similar to the positive controls in contrast to the negative controls which consumed 16% less feed ($P<.05$).

Rats receiving diets containing heated meal grew faster than did the positive control rats. Although daily gains were not statistically different, the 127°C, 138°C and 149°C treatments supported daily rat gains exceeding those of the positive controls by 19%, 4%, and 6% respectively. Negative control rats grew 37% slower than the positive controls, which was approximately the difference in protein present in the two diets.

Feed per gain figures showed that heat treatments of 127°C and 138°C increased feed efficiency over positive controls by 10% and 5% respectively while the 149°C treatment decreased efficiency by 5% although none of these margins were statistically different. Feed efficiency of the negative

Table 12. Rat feed intake, gains and feed efficiency with heat-treated SBM (28 day trial)[a]

Treatment	Daily feed intake g	Daily weight gained g	Feed per gain g
Positive control	16.7[b]	4.6[b]	3.7[b]
127°C	18.4[b]	5.5[b]	3.3[b]
138°C	16.6[b]	4.8[b]	3.5[b]
149°C	18.5[b]	4.9[b]	3.9[b]
Negative control	14.0[c]	2.9[c]	4.8[c]

[a]Values represent the mean of 6 observations.

[b]Means in the same column having the same superscript do not differ significantly (P<.05).

[c]Means in the same column having the same superscript do not differ significantly (P<.05).

control rats was 29% lower (P<.05) than the positive controls.

Comparison of the feed efficiency figures indicate that heating of the meal at 127°C was most desirable followed by the 138°C heat treatment. Explanation of the improvements in feed efficiency from the heated meals compared to the unheated positive control SBM probably involves a more complete destruction of the trypsin inhibitor which would result in an increase in the availability of the SBM protein to the

rats. The decrease in feed efficiency (127°C versus 138°C heat treatments) may be related to the formation of protein-carbohydrate complexes (Maillard reaction) due to heating (Sgarbieri et al., 1973) which decreases the digestibility and availability of protein. The further reduction in feed efficiency occurring when the meal was heated to 149°C was probably due to a continued and more extensive Maillard reaction.

A graphical representation of daily nitrogen consumed, fecal and urinary nitrogen and nitrogen retained when feeding the heat-modified meals is presented in Figure 6.

The significant differences in amounts of nitrogen consumed are a reflection of the feed intake restrictions imposed based on rat metabolic weight and feed consumption ratios occurring late in the growth trial. Compared to the daily nitrogen intake of the positive controls, the consumption of the 127°C, 138°C, 149°C and negative control diets was 7% more ($P<.05$), .5% less, 5% less, and 44% less ($P<.05$) respectively.

The quantities of fecal nitrogen excreted daily by rats receiving heated SBM diets did not differ statistically from the positive controls. The negative control rats excreted 40% less ($P<.05$) fecal nitrogen daily than the positive controls as would be expected due to the 44%

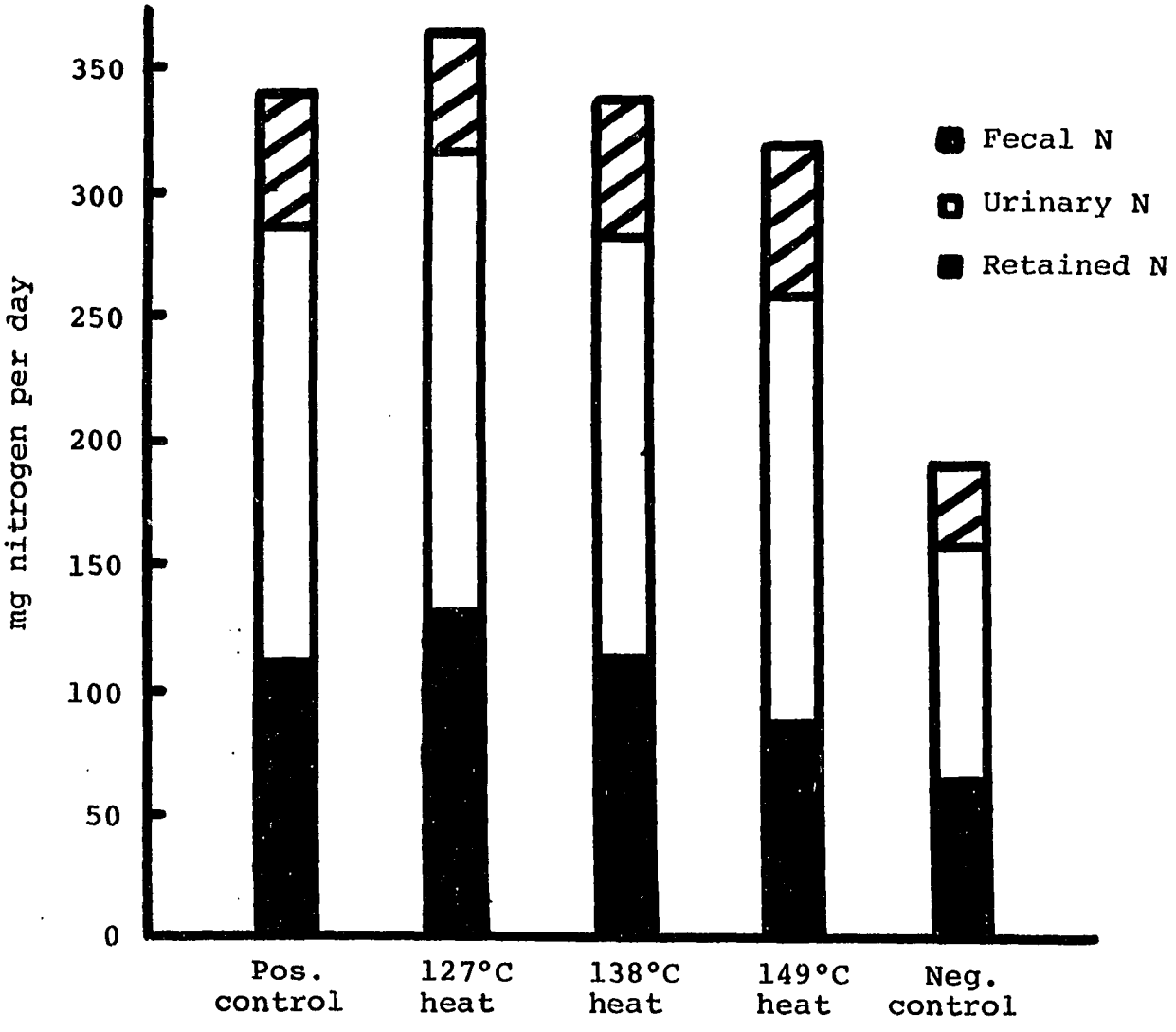

Figure 6. The intake, excretion, and retention of nitrogen by rats fed untreated or heated SBM

reduction in daily nitrogen intake.

The apparent nitrogen digestibility of the 127°C heated SBM was 2% greater than that of the positive control unheated meal. Heat treatments of 138°C and 149°C caused only slight reductions in digestibility, .4% and 3% respectively, which was similar to the 1% reduction manifested by the negative control group. None of these differences were statistically significant ($P<.05$).

The quantities of urinary nitrogen excreted daily from rats receiving the positive control diet and the 127°C and 138°C heated SBM diets did not differ significantly and tended to follow nitrogen consumption patterns. Urinary nitrogen excreted by the negative control rats was 46% less ($P<.05$) than that excreted by the positive controls.

Conversion of urinary nitrogen values (mg/day) to urinary nitrogen as a percentage of absorbed nitrogen for the respective treatments gives the following values: positive control, 61.1%; 127°C, 58.5%; 138°C, 60%; 149°C, 66%; and negative control, 58.8%. It is noted that the 149°C treatment resulted in a greater percentage of the absorbed nitrogen excreted in the urine. In rat studies, Brüggemann and Erbersdobler (1968), Sgarbieri et al. (1973), and Tanaka et al. (1975), used the Maillard products fructose-lysine, fructose-leucine and fructose-tryptophan, and fructose-

tryptophan respectively. They showed that the Maillard products were absorbed mostly in the large intestine (approximately 60% was absorbed) but were excreted in the urine without being metabolized. This research may explain the excretion of a larger percentage of absorbed nitrogen from animals fed the 149°C treated meal although actual measurements of the degree of Maillard product formation and excretion were not determined.

Heat treatments of 127°C and 138°C increased ($P<.05$) the percentage of nitrogen retained over the unheated positive control meal by 3% and .8% respectively which was in contrast to the 5% reduction ($P<.05$) due to the 149°C treatment. The negative controls retained 1.6% more of the nitrogen consumed when compared to the positive controls ($P<.05$). Table A5 of the Appendix shows the nitrogen balance data in tabular form.

Very little coughing and sneezing were observed throughout the growth and nitrogen balance trials which can possibly be attributed to the ventilation and temperature adjustments made during the preceding formaldehyde trial.

Results of the in vitro rumen studies and the rat growth trials predicted that ruminal protein degradation of SBM protein could be stopped by application of formaldehyde at minimum levels of approximately .4% while amounts up to at least .8% could be applied without decreasing post-ruminal

availability. These studies also indicated that heating of the meal at temperatures of 138° to 149°C were needed to stop protein degradation within the rumen and that post-ruminal availability might be slightly reduced by the 149°C heat treatment. Selection of SBM treatments to be used in two cattle growth trials was based upon these predictions.

Cattle Growth Studies

Formaldehyde-treated soybean meal trial

Results of the initial 56 days of the cattle growth trial are shown in Table 13. Daily feed intake of the various

Table 13. Cattle feed intake, live weight gains, and feed efficiency with formaldehyde-treated SBM (initial 56 days)[a]

Treatment	Feed intake kg/d	Daily gain kg	Feed per gain kg
Negative control	5.2	.67[b]	7.8[b]
.2% HCHO	5.3	.76[b]	7.0[b]
.4% HCHO	5.1	.73[b]	7.0[b]
.8% HCHO	5.6	.97[c]	5.8[c]
Positive control	5.5	.98[c]	5.6[c]

[a]Values represent the mean of two pens containing 6-7 steers per pen.

[b]Means in the same column having the same superscripts do not differ statistically from the negative control ($P<.05$).

[c]Means in the same column having the same superscripts do not differ statistically from the negative control ($P<.05$).

treatment groups did not differ statistically although consumption of the .8% formaldehyde-treated diet and the positive control diet were 7.6% and 5.7% higher than the negative control group. Steers receiving the .2% treated diet consumed slightly more feed (1.8%) than the negative controls in contrast to the .4% treatment groups which consumed 1.9% less feed.

Steers fed the diet containing .2% formaldehyde-treated SBM gained 13.4% faster and 10% more efficiently than the negative controls. Feed efficiency of steers given the .4% treatment was identical to the .2% treatment group although daily gains were slightly reduced (8.9% faster gains than the negative controls). Treatment of the SBM with .8% formaldehyde statistically ($P<.05$) improved gains (44.7%) and feed efficiency (25.6%) above the negative controls and approximately equalled the performance of the positive control group which gained 46.2% faster ($P<.05$) than the negative controls. The bar graph in Figure 7 shows that feed utilization in cattle was significantly improved by the addition of formaldehyde to the SBM when compared to performance of the negative control diet containing untreated meal.

At the conclusion of the initial 56 days of the trial it was apparent that the performance of steers receiving the

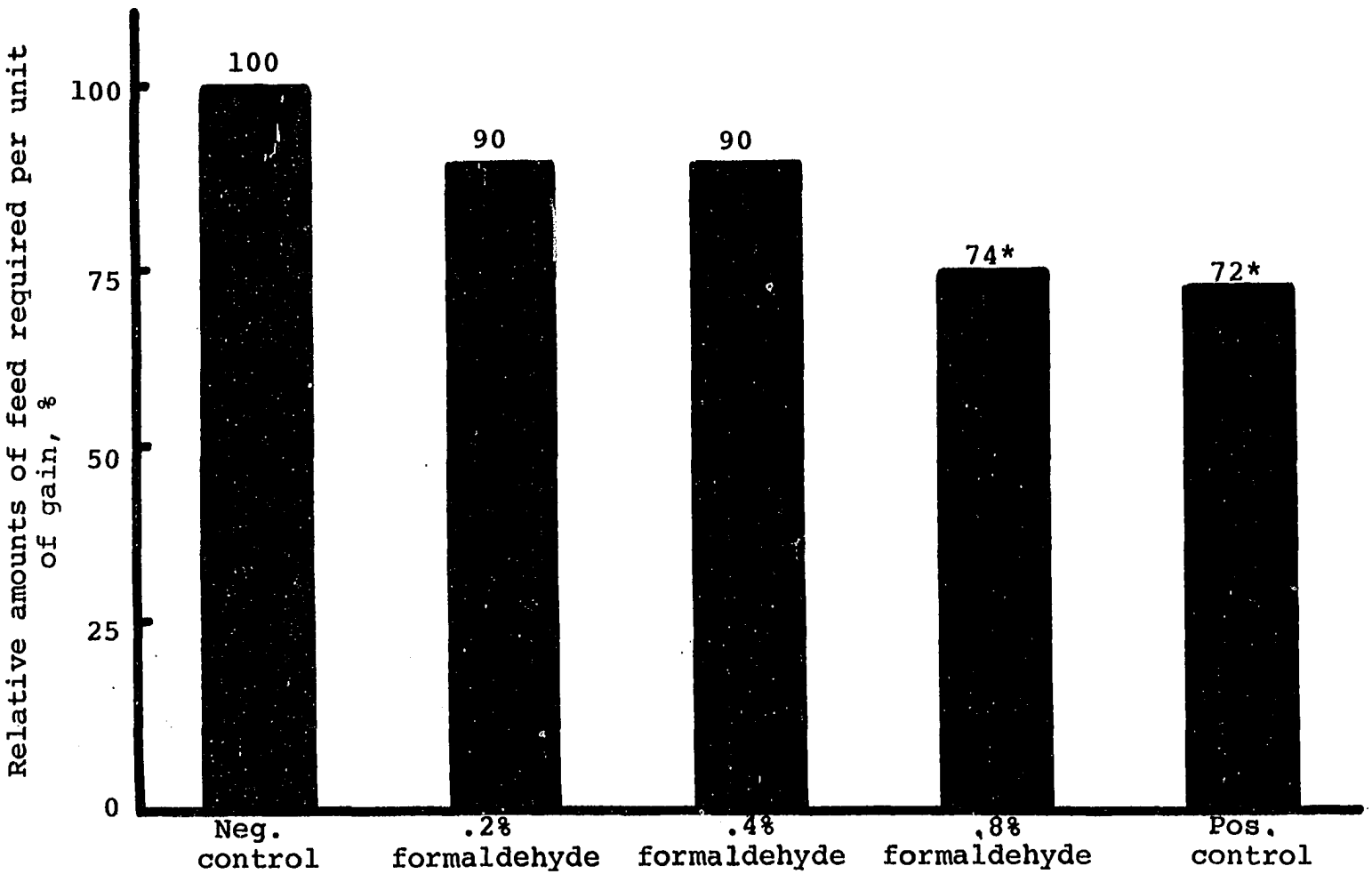

Figure 7. Comparison of cattle feed efficiency during the initial 56 days with diets containing either untreated or formaldehyde-treated SBM (* statistically different from negative controls, P<.05)

.2% formaldehyde-treated SBM (pens 2 and 7) was superior to the negative controls but greatly inferior to the .8% formaldehyde treatment group. Therefore, as explained in the experimental procedure section, pens 2 and 7 plus pen 11 (a pen of reserve cattle fed the positive control diet during the initial 56 days of the trial) received a diet containing SBM treated with 1.2% formaldehyde during the final 54 days of the trial. The results of this final period are shown in Table 14.

Although there were no statistical differences in the parameters measured, daily feed intake by the negative control cattle exceeded that of the .4% and .8% treatment groups by 5.7% and 10%. The 1.2% formaldehyde treatment steers and the positive controls consumed 4% more feed than the negative controls.

Daily gains of the negative control steers were 15% greater and 11% more efficient compared to the .4% formaldehyde treatment group. Steers fed the .8% formaldehyde treatment gained 9.6% faster than negative controls and achieved the best feed efficiency of all treatments showing a 17.8% improvement over the negative controls. Although cattle fed the 1.2% formaldehyde treatment grew 20% faster and 13% more efficiently than the negative controls, feed efficiency appeared to be reduced by 4.8% compared to the .8%

Table 14. Cattle feed intake, live weight gains, and feed efficiency with formaldehyde-treated SBM (final 54 days)[a]

Treatment	Feed intake kg/d	Daily gain kg	Feed per gain kg
Negative control	7.0	.83	8.4
.4% HCHO	6.6	.70	9.4
.8% HCHO	6.3	.91	6.9
1.2% HCHO	7.3	1.00	7.3
Positive control	7.3	1.04	7.0

[a]Values represent the mean of two pens containing 6-7 steers per pen.

treatment group. This may suggest that over-protection or irreversible protein-formaldehyde bonds were formed, rendering the SBM protein less available in the post-ruminal portion of the tract. The positive control steers grew faster than all groups showing a 25% improvement over the negative controls and a 16.6% improvement in feed efficiency which was 1.2% inferior to the feed efficiency achieved by the .8% formaldehyde treatment steers. Figure 8 graphically presents the relative feed efficiency of the various treatment groups which reflect the ability of each diet to satisfy the protein requirements of the steers.

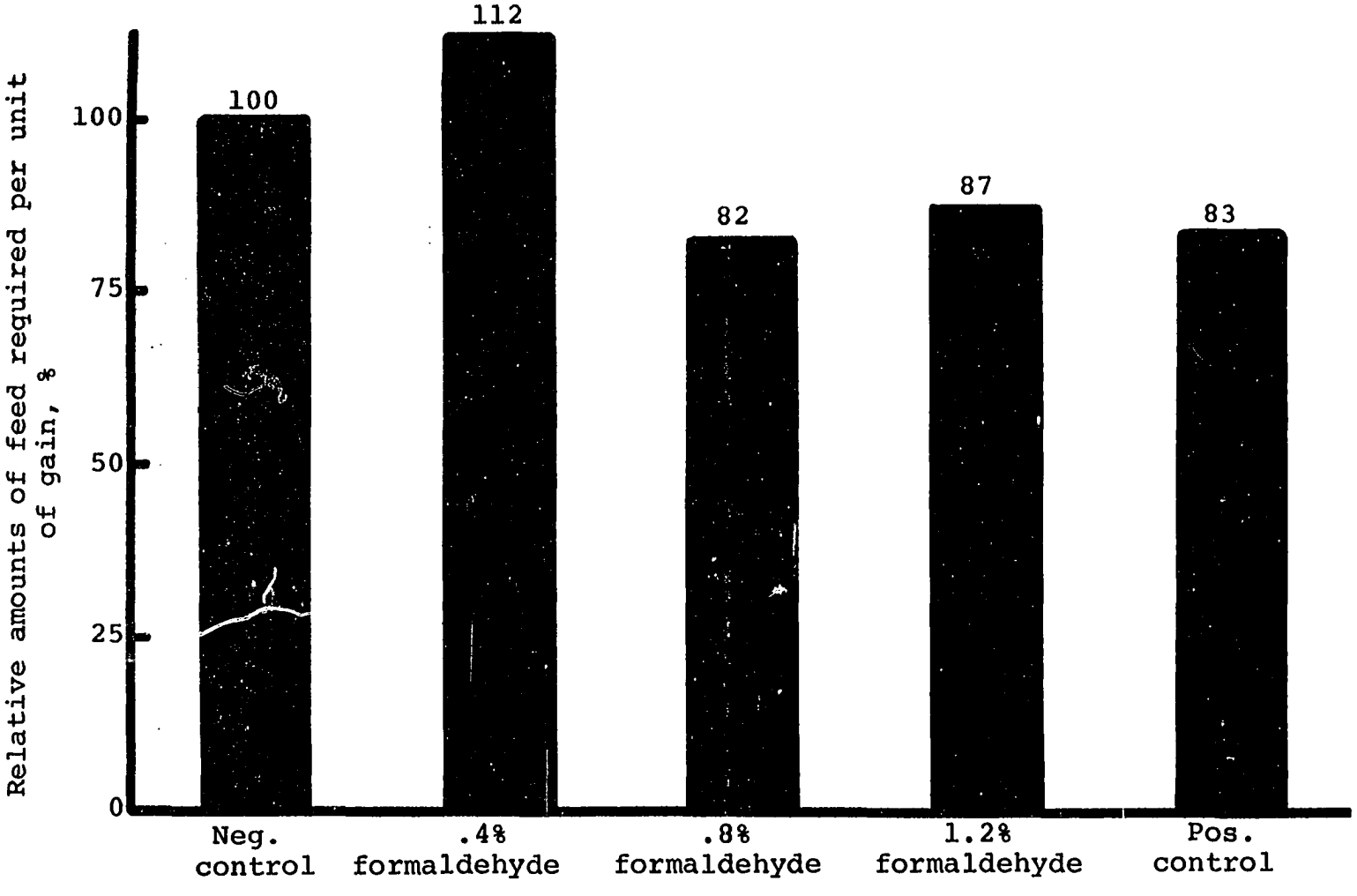

Figure 8. Comparison of feed efficiency during the final 54 days with diets containing either untreated or formaldehyde-treated SBM

Formaldehyde-, heat-, and wood molasses-treated soybean meal cattle trial

Evaluation of the individual performance of all 84 steers after 70 days irrespective of diets fed revealed that 14 animals were gaining rather slowly and were distributed by treatments as follows: negative controls (5 steers); lower level Masonex (1 steer); lower level Masonex plus .6% formaldehyde (1 steer); higher level Masonex (4 steers); 144°C heat (3 steers); 155°C heat and positive control (none).

Although the exact cause of the poor performance was not known, possible explanations included residual effects of IBR disease, vitamin A deficiency resulting from reduced feed intake, or suboptimal protein nutrition. Therefore, on day 72, the 7 poorest steers which had failed to gain weight were removed from their respective pens and placed in a hospital pen, injected with vitamin A and fed the positive control diet for a period of 12 days then returned to their respective pens for the duration of the trial. The other 7 steers, which had achieved only minimal weight gains, were given an injection of vitamin A on day 72 of the trial and allowed to remain in their pens and consume their respective diets throughout the remainder of the trial.

Figure 9 is a graphical representation of the changes in body weight at each weigh period for the two groups of poor

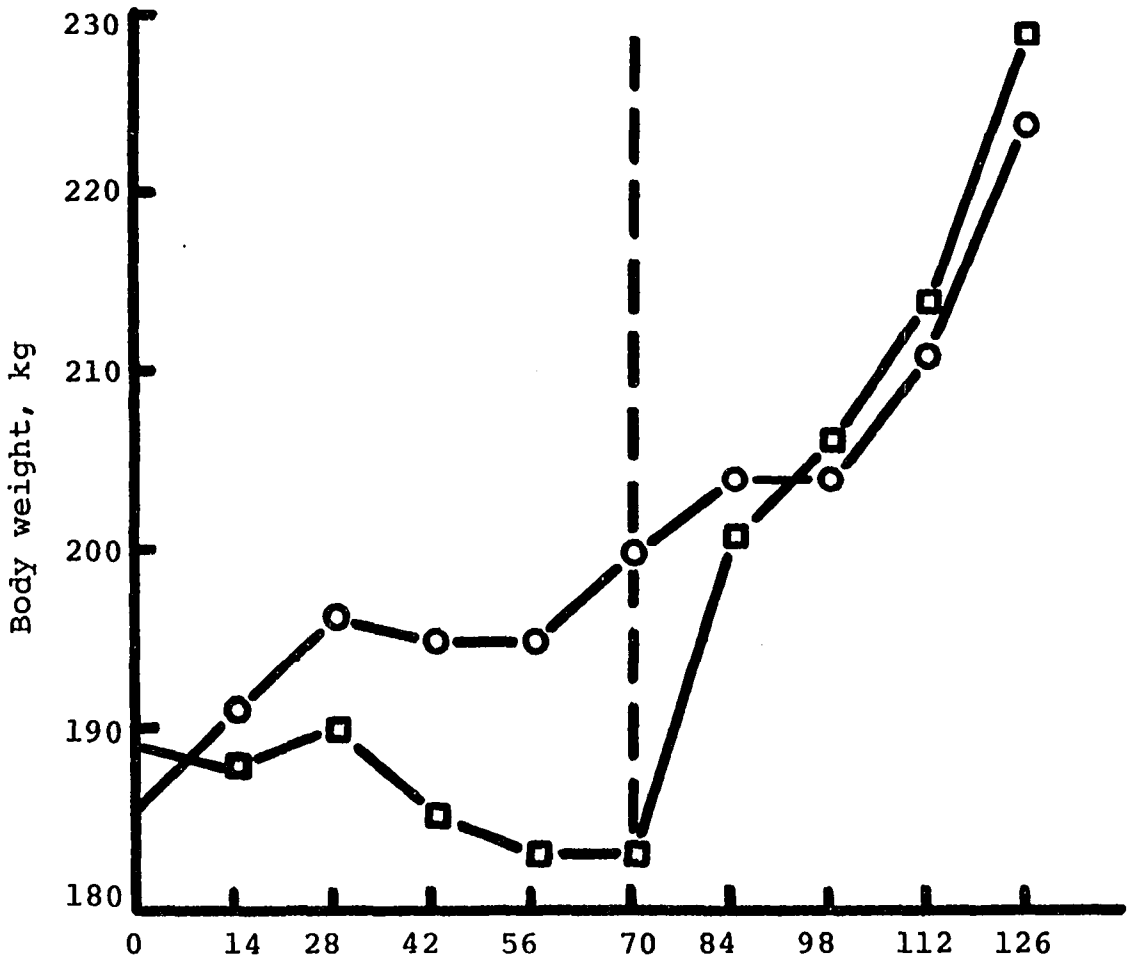

Figure 9. Relative weights of 14 slow-gaining steers during the 126-day trial (○, 7 steers receiving vitamin A; □, 7 steers receiving vitamin A and positive control diet; ——, administration of treatments on day 70)

gaining steers. The vertical dashed line represents animal weights on day 70 prior to initiation of the special treatments on day 72.

Daily gains of the steers which received only the vitamin A treatment averaged .36 kg during the 14-day period preceding treatment (days 56 to 70) and .28 kg during the 14-day period following treatment (days 70 to 84) indicating no response to the vitamin A injection. The 7 steers fed the positive control diet (high SBM) and injected with vitamin A achieved gains of 1.28 kg per day while in the hospital pen (days 72 to 84) which was a dramatic improvement over pre-treatment performance in which the steers lost .08 kg of weight daily. Although daily gains of 1.28 kg did not continue for the duration of the trial, gains of .67 kg per day were achieved during this period. The poor performance prior to treatment might possibly be attributed to the incidence of disease early in the trial resulting in reduced feed intake and thus impaired rumen fermentation and utilization of the dietary nonprotein nitrogen. If the preceding assumption is valid, performance of steers would be expected to improve following consumption of the positive control diet which contained a greater percentage of alpha amino nitrogen compared to the treated diets. This would permit a greater proportion of the metabolizable protein requirement to be

obtained from the SBM with less dependence upon microbial protein synthesis. Although the previous discussion is purely speculative since no measurements of rumen function were obtained, nevertheless, it appears logical that the poor performance of the 14 steers cannot be attributed totally to either disease or diet stress singly. Instead it may have been an interaction of both factors which resulted in a reduced ability of the steers to satisfy their protein needs.

Results of the 126-day growth trial are presented in Table 15. It appears that many of the early difficulties with a few animals during the first half of the experiment were largely overcome during the remaining part of the feeding trial. The addition of 10.1% Masonex to the SBM resulted in increased feed consumption by 9%, increased daily gains by 39%, and improved feed efficiency by 27% compared to the negative controls. Cattle receiving the combination of 10.1% Masonex plus .6% formaldehyde gained 46% faster and 32% more efficiently than the negative controls. A comparison of cattle performance from the two treatments suggests that formaldehyde provided a small amount of protein protection in addition to that achieved from Masonex alone as seen by the 7% differential in gains and 5% advantage in feed efficiency favoring the combination of Masonex plus formaldehyde. Although the steers fed the 20.2% Masonex-

Table 15. Cattle feed intake, live weight gains, and feed efficiency with Masonex-, Masonex-formaldehyde- and heat-treated SBM (126 days)[a]

Treatment	Daily feed intake kg	Daily gain kg	Feed per gain kg
Negative control	5.5	.56[b]	11.5
10.1% Masonex	6.0	.78[b]	8.4
10.1% Masonex plus .6% HCHO	5.9	.82[b]	7.8
20.2% Masonex	5.4	.61[b]	10.3
144°C Heated	6.5	.91[c]	8.6
155°C Heated	6.4	.90[c]	7.2
Positive control	6.4	.92[c]	7.1

[a]Values represent the mean of 2 pens with 6 animals per pen.

[b]Means in the same column having the same superscript do not differ statistically from the negative control ($P<.05$).

[c]Means in the same column having the same superscript do not differ statistically from the negative control ($P<.05$).

treated diet gained faster (8.9%) and more efficiently than the negative controls, their performance was inferior to the lower level Masonex treatment groups. This lower average response to Masonex treatment was confined entirely to one of the two replicate pens of cattle.

Heat treatment of the SBM appeared to provide a high degree of protein protection as seen by the 18% increase in feed consumption, 62% faster gains ($P<.05$) and 25% improvement in feed efficiency of the 144°C heat treatment animals compared to the negative controls. Steers fed the 155°C heat-treated diet showed a 37% advantage in feed conversion over the negative controls and achieved weight gains similar to those attained by the lower heat treatment group.

Performance of steers fed the positive control diet was only slightly superior to the 155°C treatment group as shown by the 64% improvement in gains ($P<.05$) and the 38% improvement in feed efficiency relative to the negative control group. Figure 10 graphically illustrates the dramatic differences in feed utilization achieved by protecting a level of SBM similar to the level fed in the negative control diet.

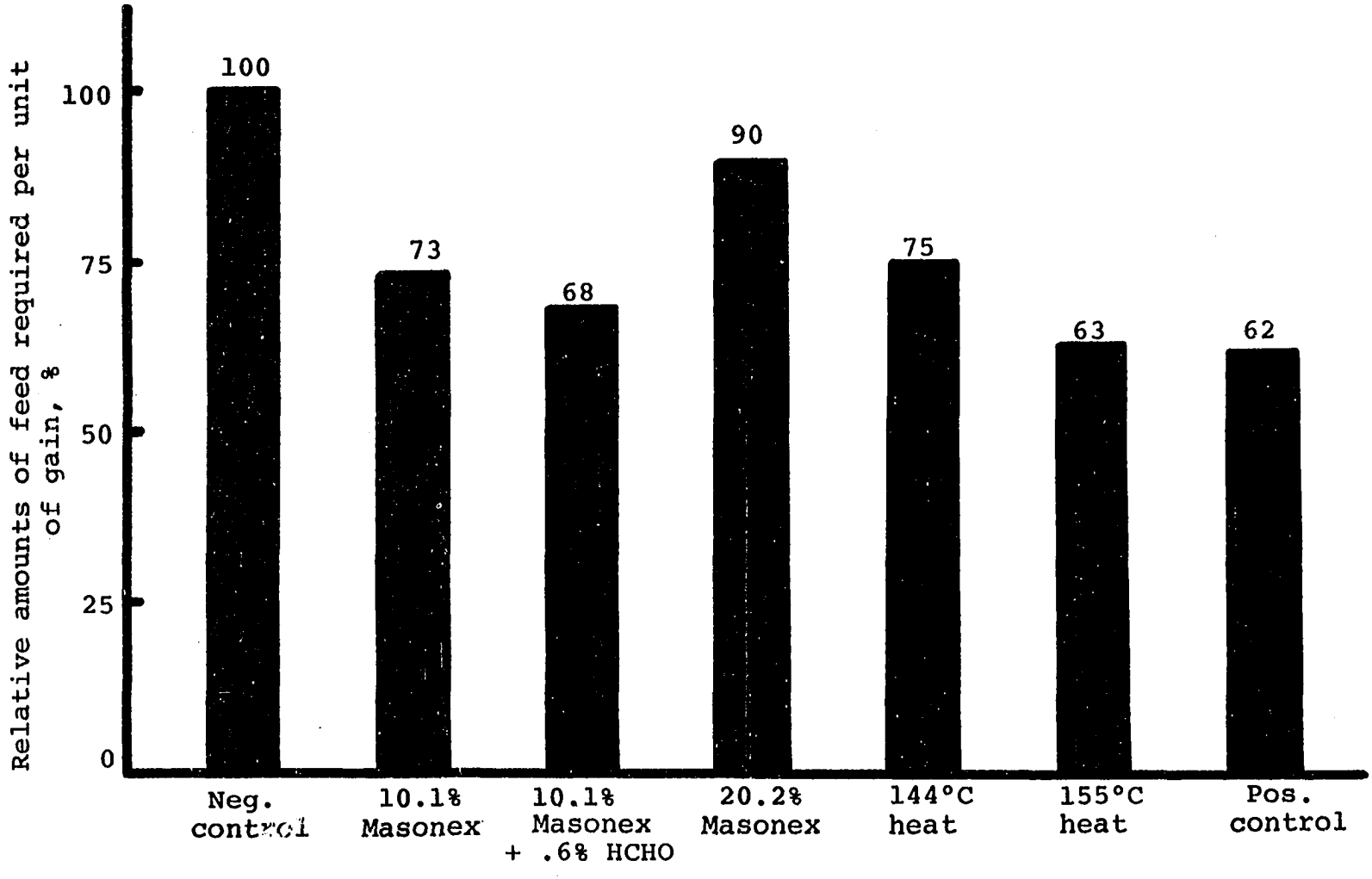

Figure 10. Comparison of feed efficiency during 126-day cattle trial with diets containing either untreated or treated-SBM

GENERAL DISCUSSION

The presence of soluble sugars for rumen microorganisms has been reported by Hungate (1966) to result in more rapid fermentation compared to media containing less soluble substrates. The possibility existed in the *in vitro* fermentation that the presence of soluble sugars in Masonex used in treating SBM might have stimulated microbial growth and ammonia uptake. This method of lowering ammonia concentrations would give a false appearance of protein protection as measured in the present *in vitro* research. To more precisely estimate the true protein protection ability of Masonex, the calculated quantities of sugars and low-molecular weight polymers of sugars contained in the Masonex treatments (10.1 and 20.2% Masonex) were added to the SBM for testing their maximum influence upon the *in vitro* systems. Addition of these sugars at the level contained in 10.1% Masonex treatment decreased the 10-hour *in vitro* ammonia concentration from 121.0 to 99.9 ppm (a 17.4% reduction). By comparison, application of 10.1% Masonex to the meal further lowered the ammonia concentration to 81.5 ppm which is equivalent to a total reduction of 32.6% from the untreated control meal. This data has been interpreted to indicate that the sugar effect may have accounted for as much as 17.4% of the total reduction. Also,

that at least the remaining 15.2% (32.6%-17.4%) reduction was due to the protein protection offered by the nonsugar components found in Masonex. The addition of sugars contained in 20.2% Masonex lowered the ammonia concentration to 76.6 ppm (36.7% lower than control levels). The treatment of SBM with 20.2% Masonex resulted in a further reduction to 65.6 ppm or a 45.8% reduction from the untreated control meal. The minimal net decrease in ammonia concentration that can be attributed to the actual protein-protection effects of 20.2% Masonex (excluding the sugar effects) is estimated to be 24.3% as determined by the calculations (32.6-17.4) + (45.8-36.7). It may be that much more of the decreased ammonia effect from the Masonex treatment is due to nonsugar constituents since the majority of the sugars present in Masonex are not true sugars but are low-molecular weight polymers of simple sugars.

Although the in vitro rumen fermentation method provides an estimate of the quantity of each modifying agent needed to inhibit protein degradation, this estimate may be very crude and subject to gross errors since solubility of the carbohydrate fraction and other influences can alter the fermentation. The water-soluble protein method of analysis has the advantage over the in vitro fermentation method in that the presence of soluble sugars in the substrate does

not influence the protein solubility measurement. The following correlation "r" values were determined when 10-hour *in vitro* rumen ammonia measurements and water-soluble protein values were compared with SBM: formaldehyde treatment, .99; heat treatment, .93; and Masonex treatment, .99. This suggests that with this particular feedstuff the results obtained from *in vitro* rumen fermentation studies and water-soluble protein determinations are highly correlated and that either method of analysis can be used satisfactorily.

In order to partially test the validity of extending the above conclusion to other proteinaceous feedstuffs, an abbreviated *in vitro* fermentation study was conducted with untreated and formaldehyde-treated alfalfa meal. The results of this study showed an absence of ammonia accumulation in both media containing treated and untreated alfalfa meal. This failure of the *in vitro* method was probably due to the lack of readily available energy sources in the alfalfa media to stimulate microbial growth.

Examination of the fermentation patterns exhibited by SBM, SBM plus sugars, and alfalfa leads to the conclusion that the *in vitro* fermentation technique does not provide an adequate measurement of protein vulnerability to rumen destruction for all types of ruminant feedstuffs.

Lyman *et al.* (1953) used .02 N NaOH to measure protein

solubility but Little et al. (1963) suggested that this method gave higher values than were found in the rumen. Wohlt et al. (1973) described a procedure using a 10% dilution of Burroughs' mineral solution that simulated autoclaved rumen fluid. Perhaps the method described by Wohlt et al. (1973) or the water-soluble protein method employed in the selection of the SBM used in this research provides a measure of protein vulnerability to rumen destruction that can best be used to compare the effects of a protein-modifying agent over a variety of feedstuffs. Additional research in this area is needed.

The report by Mills et al. (1972) in which formaldehyde continued to react with protein for several weeks poses a potential problem if the use of formaldehyde-treated SBM becomes accepted commercially. In the present study SBM was treated with formaldehyde at the .2% level and allowed to react for a period of 4 or 248 days. Following these reaction periods the meals were subjected to an in vitro rumen fermentation in testing protein destruction. Ammonia concentrations were reduced 76.6% compared to untreated SBM when a four-day formaldehyde reaction time was permitted. Extension of the reaction time to 248 days resulted in an additional 15.2% reduction. This observation supports the findings of Mills et al. (1972).

It is generally accepted that highly soluble proteins are degraded more extensively by rumen microorganisms than are less soluble proteins with resultant high rumen ammonia concentrations. Although the total mechanism by which formaldehyde, tannins and heat treatments reduce solubility and decrease protein destruction within the rumen is not known, nevertheless it is of interest to discuss some of the known information concerning possible mechanisms.

Heat treatment of proteins results in structural disruption leading to exposure of hydrophobic groups which are normally positioned toward the interior of the protein molecule. Maillard products (protein-carbohydrate complexes) are also formed during heating. Formaldehyde reacts with certain functional groups of proteins to form an initial methylol compound which may condense and form intramolecular and intermolecular bridges. Tannins interact with proteins by forming hydrogen bonds between the hydroxyl groups of the tannin and the carboxyl groups of the protein peptide bonds.

Several different kinds of bacterial proteases are thought to exist in the rumen, some of which possess trypsin-like specificity. Many investigators have demonstrated that these proteases are primarily cell-bound and function extracellularly. Deaminase activity is thought to occur

primarily inside the cell.

Although the physiology of protein degradation by rumen microorganisms is not known, it seems logical that protein modification might offer protection by the following two mechanisms acting either singly or in combination: (1) prevention of proteolysis by the extracellular enzymes as a result of conformational changes of the protein which block or shield the reactive sites; or (2) reduction or inhibition of peptide and amino acid entry to the interior of the cell where deamination is thought to occur.

Post-ruminal availability of proteins treated with formaldehyde or tannins is probably due to removal of these agents by the acidic conditions in the abomasum. Although heat treatment of proteins does not involve a reversible chemical bond, these proteins also appear to be largely available (depending on the extent of heat treatment) for digestion and absorption post-ruminally. Sanger (1952) stated that the relative rate of hydrolysis in acid of any peptide bond is determined mainly by the number of hydrogen ions that approach the bond. Therefore, it appears logical that the acidic conditions in the abomasum also contribute to the availability of heated proteins by partial hydrolysis of the peptides thereby increasing the susceptibility to other proteolytic enzymes.

As mentioned in the preceding sections of this disserta-

tion the two pens of cattle fed the .2% formaldehyde-treated diet and the two pens of reserve cattle which had been receiving the positive control diet during the initial 56 days of the first cattle trial were switched to a 1.2% formaldehyde-treated diet for the final 54 days of the trial. Daily gains of the reserve cattle were .98 kg during the initial 56 days and .97 kg during the final 54 days. Cattle fed the .2% formaldehyde diet gained .75 kg per day during the initial 56 days and .98 kg per day during the final 54 days. The 30% increase in rate of gain manifested by the .2% formaldehyde treatment cattle and the rather consistent gains achieved by the reserve cattle following the switch to the 1.2% formaldehyde-treated diet provides additional support to the theory that protein modification or protection can be beneficial with proper dietary design.

Experience with the MP system indicates that lightweight calves (weighing approximately 180 to 290 kg) have a high protein requirement and should be fed a diet supplemented with preformed protein rather than urea if maximum performance is to be achieved. Performance of lightweight calves fed high urea supplements is usually suboptimal due to the limited amount of high energy feeds that can be consumed by the animal. The limited energy intake is not capable of supporting enough microbial growth to fulfill

the protein requirements of the animal. The results obtained in the present research showed that performance of lightweight calves fed diets containing large amounts of urea and small amounts of modified protein was nearly equal to the performance of calves fed diets supplemented with high levels of untreated SBM. This equality of performance was expected because protection of the supplemented protein from microbial degradation theoretically reduces rumen ammonia concentrations substantially, thereby increasing the positive UFP of the diet and making urea more useful. The combined effect of large quantities of dietary protein and microbial protein entering the abomasum can greatly increase the amount of MP.

Upon examination of the research reports by others in which protein modification attempts were not successful, the following points were frequently observed: (1) the animals employed were too mature to be suitable for testing benefits which could arise from protein modification, (2) an ammonia deficiency was created in the rumen resulting from the protection imposed when no urea or other high-protein feed was included in the diet containing the modified protein, (3) the negative control diet contained excess protein for the animals employed such that the beneficial effects of the modified protein were masked, and (4) in some studies the protein appeared to be "over protected" which decreased post-

ruminal availability.

The sulfur-containing amino acids are generally thought to be first limiting in typical ruminant diets. Since some protein sources contain far more sulfur amino acids than others it seems logical that modification of these proteins would be most beneficial to the ruminant provided vulnerability to protein destruction is similar to or greater than other feedstuff proteins. Calculation of the relative amounts of digestible sulfur-containing amino acids found in one kg of crude protein obtained from different feedstuffs reveals the following (g/kg): alfalfa, 21.0; corn, 21.8; SBM, 23.4; rumen microbes, 27.2; dried blood, 28.8; fish meal, 32.4; meat scraps, 33.1; and casein, 35.7. These figures suggest that some feedstuff proteins contain a larger percentage of sulfur amino acids than is found in rumen microbes. These feedstuffs should be investigated in the future as good candidates for their improvement in ruminant diets by protein modification.

The research employed in this thesis utilized formaldehyde, Masonex and heat as modifying agents. The possible use of other agents or combinations of agents applied in different ways and at various levels should be investigated. The inclusion in feed of sulphur amino acids or analogues which are resistant to microbial degradation should also be

investigated.

The majority of research conducted to this point have utilized feeds in which only the supplemented protein source has been modified. It is of interest to speculate upon the feasibility of protecting all or a substantial amount of the dietary protein in a complete feed.

The basal ration from the first cattle growth trial has been chosen as an example for speculating upon the benefits from protecting all of the dietary protein. If none of the ingredients are protected, 43.9 g MP/kg diet would be expected. In this case, 5.38 g urea/kg diet would be needed to satisfy the positive UFP created by the corn cobs, corn grain and molasses. If only the SBM were protected (100%) and the TDN from the SBM was assumed to be available for microbial use, the quantity of MP would theoretically be 73.3 g/kg diet or an increase in MP of 67% over the non-protected diet. The quantity of urea needed to fulfill the positive UFP would be 12.30 g/kg diet or a 128% increase. The increased quantity of useful urea results from the change in SBM UFP from a negative value to a positive value.

Protection of all dietary protein (100%) would theoretically increase the quantity of MP to 98.0 g/kg of diet or an increase of 123% above the nonprotected diet. The positive UFP of each feedstuff is theoretically increased

which makes more urea useful. The estimated quantity of urea which would be of value becomes 22.7 g/kg of diet or an increase of 322%. The ability of feedstuffs to support microbial protein synthesis following application of protein-modifying agents needs investigation. Although treatment of the entire diet may not be feasible, the theoretical implications are considerable.

Before concluding this discussion, a few statements are offered concerning chief benefits likely to accrue from the present and future research with protein modification applied to future cattle feeding practice. It appears that high-producing lactating cows might benefit more from modified proteins than feedlot cattle because of their higher protein requirements. The young feedlot animal would be expected to be benefitted a moderate amount from modified proteins for a period of approximately 80 to 125 days when their protein requirements are greatest. Inclusion of modified proteins in the diet beyond this point would not be expected to show major advantage over unmodified proteins when protein requirements are reduced. These older cattle would be expected to be benefitted from modified protein only for short periods of time when feed intake was not maximal. On certain occasions wintering pregnant beef cows might also be benefitted a small amount from modified proteins. However, diets containing low quality roughages

and urea will usually satisfy the low protein requirement of the beef cow.

Modified proteins are probably most useful in diets which are low in protein (less than 8% crude protein) yet have sufficient available energy to support microbial growth. Examples of such diets might include corn silage, corn cobs or corn residue silage as the major ingredients with smaller amounts of corn grain and molasses added to provide additional available energy.

In conclusion, it would appear that those agricultural areas containing large numbers of dairy cows would receive largest benefits from use of modified proteins. Four of the leading states in milk production are Wisconsin, New York, California and Minnesota. The beef cattle industry in the midwest as well as Colorado, Oklahoma and Texas would also be expected to be benefitted by moderate amounts.

Finally, it is interesting to speculate upon the possible new usage of future cattle diets which may become feasible to feed as a result of protein modification. It would appear that many crop residues, little utilized today, might be utilized considerably more in the future when made more nutritious by supplementation with molasses and small amounts of modified protein plus urea. These crop residues, such as straws, stovers, and mature grasses [illegible] in

cellulose and absorb molasses in large amounts. However, both molasses and crop residues are extremely low in protein. Urea supplementation alone provides inadequate protein nutrition for cattle. The addition of modified proteins plus urea to these crop residues would appear to hold promise for their greater usage in the future.

SUMMARY

The objective of this research was to modify soybean meal (SBM) protein such that when fed in planned cattle diets it would become more useful to the animal as a result of the modification. The modifying agents used were temperature, formaldehyde and wood molasses (Masonex). They were each applied in amounts to substantially reduce protein vulnerability to fermentation destruction within the rumen without seriously injuring soybean protein digestion and absorption post-ruminally. These modified soybean proteins were fed in cattle diets formulated with sufficient urea or other natural proteins such that ammonia deficiency and lessened microbial protein synthesis would not be created as a result of soybean protein modification. Ammonia levels in *in vitro* rumen fermentations were recorded periodically over a 10-hour interval in estimating the quantity of modifying agent needed to substantially reduce fermentation destruction. Rat growth and nitrogen balance trials were carried out in estimating the quantity of modifying agent that could be used without seriously injuring soybean protein utilization post-ruminally. The more desirable levels of modifying agents predicted by these laboratory methods and tested in two subsequent cattle feedlot trials were: heating the meal at temperatures of 138 to 149°C for four hours, adding

10 to 20% Masonex, or adding 0.4 to 0.8% formaldehyde for several hours prior to incorporation into cattle diets. The cattle diets fed to young growing steers containing modified soybean protein were formulated making use of the MP feeding standard. They were formulated on the basis that they contained sufficient urea so that no deficiency of rumen ammonia would develop if the modified proteins were 100% resistant to rumen fermentation destruction. Cattle diets containing modified SBM were compared with appropriate isonitrogenous and isocaloric negative and positive control diets containing unmodified SBM. The results of the two cattle trials demonstrated that each of the three modifying agents was capable of substantially increasing the usefulness of SBM in selected cattle diets. Increases in feed dry matter utilization were as much as 26% with formaldehyde, 27% with Masonex, and 37% with temperature modification. It would appear, from predictions utilizing the MP feeding standard, that modified proteins in feeding practice might be most beneficial in diets of high producing lactating cows and lightweight growing cattle. Heavier weight cattle likely would be benefitted only during the starting feedlot period of a few weeks before concentrate feeding reached a high level due to their lower protein requirements.

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APPENDIX

Table A1. *In vitro* rumen ammonia concentration with formaldehyde-treated SBM (ppm NH_3)[a]

SBM Treatment	Incubation time, hours						
	.5	2	4	6	8	10	Average
Water	8.3	20.5	36.4	66.5	109.1	148.8	64.9[b]
.2% formaldehyde	9.3	3.7	11.0	16.5	23.4	34.8	17.6[b]
.4% formaldehyde	6.6	8.2	.4	-3.5	-5.8	-5.7	-.5[c]
.6% formaldehyde	6.3	7.2	-2.3	-8.2	-10.1	-12.2	-3.7[c]
.8% formaldehyde	4.8	5.6	-3.1	-9.7	-11.1	-13.1	-4.9[c]
1.0% formaldehyde	5.1	5.8	-3.2	-9.3	-10.8	-12.9	-4.7[c]
1.2% formaldehyde	4.7	6.1	-1.9	-17.3	-9.1	-10.8	-3.8[c]
1.4% formaldehyde	4.9	6.6	-1.3	-7.4	-23.7	-9.6	-3.0[c]

[a]Ammonia values represent the mean of triplicate determinations and are corrected for sample size and residual NH_3 in the blanks.

[b]Means in the same column having the same superscript do not differ statistically from water-treated SBM ($P<.05$).

[c]Means in the same column having the same superscript do not differ statistically from water-treated SBM ($P<.05$).

Table A2. In vitro rumen ammonia concentration with heat-treated SBM (ppm NH_3)[a]

SBM Treatment	Incubation time, hours						
	.5	2	4	6	8	10	Average
No heat	8.3	20.5	36.4	66.5	109.1	148.8	64.9
127°C	5.4	12.9	22.5	45.8	73.8	106.2	44.4
138°C	4.4	5.4	1.0	-1.8	3.3	10.9	3.8
149°C	2.7	.5	-5.0	-11.2	-11.9	-11.4	-6.1

[a]Reported NH_3 (ppm) represents the mean of triplicate determinations and are corrected for sample size and residual NH_3 in the blanks.

Table A3. In vitro rumen ammonia concentration with Masonex- or sugar-treated SBM (ppm)[a,b]

SBM Treatment	Incubation time, hours						
	.5	2	4	6	8	10	Average
None	8.7	24.4	41.9	60.9	85.9	121.0	57.1
Sugars in 10.1% Masonex	8.8	21.3	34.0	44.0	66.0	99.9	25.6
10.1% Masonex	1.7	11.7	16.9	30.2	54.4	81.5	32.7
Sugars in 20.2% Masonex	11.2	22.9	26.3	30.7	47.8	76.6	35.9
20.2% Masonex	-.6	12.3	12.2	19.3	39.3	65.6	45.8
Sugars contained in 40.5% Masonex	13.8	23.6	19.0	13.5	15.5	45.6	21.8

[a]Reported NH_3 (ppm) represents the mean of triplicate determinations and are corrected for sample size and residual NH_3 in the blanks.

[b]Sugar treatment consisted of the addition of the sugars found in Masonex.

Table A4. Nitrogen balance of rats fed SBM treated with three levels of formaldehyde (5-day trial)[a]

Treatment	Intake N mg/d	Fecal N mg/d	Urinary N mg/d	Retained N mg/d	Retained N %	App. Digestibility %
Positive control	408[b]	64[b]	225[b]	118[b]	28.9[b]	84.3[b]
.2% formaldehyde	377[b,c,d]	59[b]	211[b]	107[b,d]	28.4[b]	84.3[b]
.8% formaldehyde	346[c,d]	78[b]	172[c]	96[c,d]	27.7[b]	77.4[c,d]
1.4% formaldehyde	351[c,d]	134[c]	125[d]	92[c,d]	26.6[b]	61.8[e]
Negative control	196[e]	37[d]	87[e]	72[e]	36.7[c]	81.1[b,d]

[a]Values represent the mean of 6 individual observations.

[b]Means in the same column having the same superscript do not differ statistically ($P<.05$).

[c]Means in the same column having the same superscript do not differ statistically ($P<.05$).

[d]Means in the same column having the same superscript do not differ statistically ($P<.05$).

[e]Means in the same column having the same superscript do not differ statistically ($P<.05$).

Table A5. Nitrogen balance of rats fed SBM treated with three levels of heat (6-day trial)[a]

Treatment	Intake N mg/d	Fecal N mg/d	Urinary N mg/d	Retained N mg/d	Retained N %	App. Digestibility %
Positive control	340[b]	54[b]	175[b]	111[b]	32.6[b]	84.1
127°C	365[c]	49[b]	185[b]	131[b]	35.9[c]	86.6
138°C	338[b]	55[b]	170[b]	113[b]	33.4[b]	83.7
149°C	320[b]	61[b]	171[b]	88[c]	27.5[d]	80.9
Negative control	190[d]	32[c]	93[c]	65[d]	34.2[e]	83.1

[a]Values represent the mean of 6 individual observations.

[b]Means in the same column having the same superscript do not differ statistically ($P<.05$).

[c]Means in the same column having the same superscript do not differ statistically ($P<.05$).

[d]Means in the same column having the same superscript do not differ statistically ($P<.05$).

[e]Means in the same column having the same superscript do not differ statistically ($P<.05$).

Table A6. Chemical composition of air-dry Masonex[a]

Item	%
Protein	.5
Fat	.5
Fiber	1.0
Ash	6.0
Solids	65.0
Calcium	.5
Phosphorus	.07
Simple sugars	10.0
Simple sugars (after hydrolysis)	35.0
Distribution of sugars (after hydrolysis)	
Glucose	14.0
Mannose	27.0
Galactose	8.0
Arabinose	5.0
Xylose	46.0
pH	5.5
Metabolizable energy (ruminants)	890

[a]Analyses of Masonex produced in Laurel, Mississippi, as reported by the Masonex Corporation.

Table A7. Analysis of variance of water-soluble protein of treated SBM

	Source of variation	D.F.	Mean square	F
Formaldehyde	Treatment	9	6.25	110.96
	Error	10	.06	
Heat	Treatment	3	11.34	68.85
	Error	4	.16	
Masonex or sugars	Treatment	3	12.54	88.38
	Error	4	.14	

Table A8. Analysis of variance of *in vitro* rumen ammonia

	Source of variation	D.F.	Mean square	F
Formaldehyde	Time of incubation	5	440.68	.41
	Treatment	9	8291.48	7.63
	Time x treatment	45	1087.02	
	Error	120	1.87	
Heat	Time of incubation	5	6165.48	2.43
	Treatment	3	20217.07	7.96
	Time x treatment	15	2539.26	
	Error	48	2.46	
Masonex or sugars	Time of incubation	5	12627.48	29.59
	Treatment	5	3096.84	7.26
	Time x treatment	25	426.76	
	Error	72	12.16	

Table A9. Analysis of variance of formaldehyde-treated SBM rat growth and nitrogen balance trial

	Source of variation	D.F.	Mean square	F
Live weight gains	Weight (blocks)	5	409.25	.63
	Treatments	4	5815.20	9.04
	Error	20	643.12	
Feed intake	Weight (blocks)	5	1989.86	.60
	Treatments	4	3959.68	1.19
	Error	20	3304.78	
Feed efficiency	Weight (blocks)	5	.17	1.64
	Treatments	4	1.61	15.30
	Error	20	.10	
Apparent digestibility	Weight (blocks)	5	22.30	2.45
	Treatments	4	502.50	55.26
	Error	20	9.09	
Nitrogen retention	Weight (blocks)	5	14.63	.74
	Treatments	4	93.23	4.75
	Error	20	19.62	
Fecal volume	Weight (blocks)	5	2.75	2.20
	Treatments	4	8.23	6.60
	Error	20	1.24	
Fecal nitrogen	Weight (blocks)	5	.01	1.73
	Treatments	4	.19	27.38
	Error	20	.007	
Urinary nitrogen	Weight (blocks)	5	.02	2.53
	Treatments	4	.50	69.59
	Error	20	.007	
Feed consumption during nitrogen balance	Weight (blocks)	5	53.03	2.06
	Treatments	4	156.77	6.09
	Error	20	25.70	

Table A10. Analysis of variance of heat-treated SBM rat growth and nitrogen balance trial

	Source of variation	D.F.	Mean square	F
Live weight gains	Weight (blocks)	5	554.30	1.75
	Treatments	4	4539.11	14.40
	Error	20	315.01	
Feed intake	Weight (blocks)	5	4293.15	2.09
	Treatments	4	16005.16	7.80
	Error	20	2051.06	
Feed efficiency	Weight (blocks)	5	1.06	.84
	Treatments	4	4.93	3.91
	Error	20	1.26	
Apparent digestibility	Weight (blocks)	5	.80	.08
	Treatments	4	25.20	2.57
	Error	20	9.77	
Nitrogen retention	Weight (blocks)	5	8.33	.43
	Treatments	4	59.05	3.04
	Error	20	19.37	
Fecal volume	Weight (blocks)	5	.72	.26
	Treatments	4	9.82	3.67
	Error	20	2.67	
Fecal nitrogen	Weight (blocks)	5	.002	.40
	Treatments	4	.025	4.82
	Error	20	.005	
Urinary nitrogen	Weight (blocks)	5	.019	2.69
	Treatments	4	.302	42.88
	Error	20	.007	
Feed intake during nitrogen balance	Weight (blocks)	5	77.68	4.88
	Treatments	4	248.53	15.62
	Error	20	15.91	

Table A11. Analysis of variance of formaldehyde-treated SBM cattle growth trial

	Source of variation	D.F.	Mean square	F
Initial 56 days				
Live weight gains	Pen position (blocks)	1	2.93	19.53
	Treatment	4	1.60	10.67
	Error	4	.15	
Feed consumption	Pen position (blocks)	1	8.42	7.35
	Treatments	4	2.96	2.58
	Error	4	1.14	
Feed efficiency	Pen position (blocks)	1	30.20	7.88
	Treatments	4	28.87	7.54
	Error	4	3.83	
Final 54 days				
Live weight gains	Pen position (blocks)	1	.04	.08
	Treatments	4	1.29	2.58
	Error	6	.50	
Feed consumption	Pen position (blocks)	1	7.82	3.81
	Treatments	4	12.36	6.03
	Error	6	2.05	
Feed efficiency	Pen position (blocks)	1	.185	.04
	Treatments	4	27.44	5.26
	Error	6	5.21	

Table A12. Analysis of variance of heat, Masonex and formaldehyde plus Masonex-treated SBM cattle growth trial

	Source of variation	D.F.	Mean square	F
Live weight gains	Pen position (blocks)	1	.29	4.14
	Treatments	6	.26	3.71
	Error	6	.07	
Feed consumption	Pen position (blocks)	1	9.14	4.27
	Treatments	6	2.40	1.12
	Error	6	2.14	
Feed efficiency	Pen position (blocks)	1	25.48	.41
	Treatments	6	32.09	.52
	Error	6	62.28	