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LACTATIONAL AND CHEMICAL EVALUATION OF SOYBEAN MEALS HEAT-TREATED BY TWO METHODS

BY

TILAHUN SAHLU

A thesis submitted in partial fulfillment of the requirements for the degree Doctor of Philosophy, Major in Animal Science, South Dakota State University 1983

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LACTATIONAL AND CHEMICAL EVALUATION OF SOYBEAN MEALS HEAT-TREATED BY TWO METHODS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

> Dr. David J. Schingoeihe Thesis Giviser

Date

Dr. John G. Parsons Head, Dairy Science Dept. Date

LACTATIONAL AND CHEMICAL EVALUATION

OF SOYBEAN MEALS HEAT-TREATED BY TWO METHODS

Abstract

TILAHUN SAHLU

A series of experiments were conducted to evaluate regular, commercially available solvent extracted soybean meal (SBM), and SBM subjected to additional heat either during desolventizing (HSBM), or by extrusion (ESBM). Soluble nitrogen (14.8, 9.3, and 7.0% of crude protein for SBM, HSBM, and ESBM) and degradable protein (71.0, 68.7, and 58.7% of crude protein) were reduced by heattreating soybean meal. Nonessential amino acids in soybean meals were more soluble and degradable than essential amino acids. The first five limiting amino acids (methionine, lysine, valine, leucine, and isoleucine) for milk production were the same, although relative order was altered by heat treatment, for all fractions of the three soybean meals, except that threonine replaced leucine in the insoluble fraction of ESBM and the undegradable fraction of HSBM and ESBM. Completely mixed rations were made of (dry matter basis) 40% corn silage, 10% chopped alfalfa hay, and 50% concentrate mix containing the respective protein sources (SBM, HSBM, and ESBM). Milk production (33.8, 34.9, and 35.3 kg/day) was increased when heattreated soybean meals were fed to high producing cows, with most of the increased production occurring during the first 4 wk on the experiment (wk 4 through 7 postpartum). Four percent fat-corrected

milk was 30.9, 32.6, and 33.4 kg/day. Increases in milk production were modest when heated soybean meals were fed to lower producing cows. Concentration of milk fat, protein, and solids as well as rumen ammonia, and blood ammonia were similar.

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ACKNOWLEDGMENTS

The author wishes to express sincere thanks to Dr. David J. Schingoethe for his continual guidance, support, and constructive criticism throughout writing of this thesis. Appreciation is extended to Dr. Andy Clark, and Dr. L. Tucker for assistance in statistical analyses.

Thanks goes to my fellow graduate students and members of the farm crew for their cooperation in the experiments. The author is truly grateful to Marlys Moberg for typing of this thesis.

Deep gratitude is extended to my wife, Shetaye, to our children, Jonathan and Sahlu, for their never ending encouragement, patience, and understanding throughout my graduate school.

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INTRODUCTION

High producing cows require more protein than can be synthesized in the rumen by microbes from nonprotein nitrogen and/ or lower quality feed proteins. This is especially true during early lactation, since cows reach peak production during 6 to 8 wk postpartum while maximum feed intake occurs between 12 to 15 wk postpartum. Therefore, cows reach maximum milk production at a time when they are unable to consume sufficient nutrients to support maximum production. This nutritional deficiency can be overcome by mobilizing energy and protein from their body reserve. Cows can mobilize proportionally high amounts of energy from their body reserve, but the amount of body protein that can be mobilized for milk production is small.

Some natural feed protein escapes degradation in the rumen, but to meet protein requirements of high producers during early lactation, it is advantageous to increase the amount of protein, especially high quality protein like soybean meal, escaping degradation in the rumen. Heat-treating soybean meal decreases the solubility and degradation rate in the rumen, which should increase the amount of protein reaching the lower digestive tract for digestion and absorption. The objective of this research was to evaluate soybean meal subjected to additional heat treatment by two methods. Evaluation methods included in vitro determination of protein solubility, degradability, and amino acid content, as well as the lactational response of high producing cows during early lactation

to diets containing heat-treated soybean meals.

LITERATURE REVIEW

Synthesis of Microbial Protein in the Rumen

Nitrogen and Carbohydrate Sources for Microbial Protein. The amino acids presented to the small intestine of the ruminant animal are derived mainly from microbial protein synthesized in the rumen and dietary protein that has escaped degradation in the rumen. The quantity of microbial cells formed in the rumen is a nutritional function of supplies of nitrogen, energy, and other growth factors, and is determined by growth rates of rumen bacteria.

Rumen microbial protein synthesis requires an adequate supply of nitrogen for maximum efficiency. If the concentration of nitrogen is inadequate, uncoupled fermentation may occur and this will result in fermentation without useful ATP production (32, 112). In contrast, if the nitrogen concentration is excessive, energy may be the limiting factor for efficient nitrogen utilization. Therefore, for maximal efficiency of microbial growth to occur in the rumen there must be a balanced supply of nitrogen and energy.

In a study with sheep fed semi-purified protein-free diets containing urea as the nitrogen source (78), increasing the dietary nitrogen concentration from .95 to 1.82% increased ruminal protein production. There was no further increase in protein synthesis when the dietary nitrogen concentration was raised to 3.29%. Hume et al. (80) reported a depressed microbial protein synthesis when the dietary crude protein was below 11% of the dry matter. Others

(31, 145) suggested 12 to 13% dietary crude protein was needed to get maximum microbial protein synthesis.

There are contrasting views on the concentration of ammonia nitrogen required for maximum microbial growth. 'Many (4, 101, 121, 146) claim the optimum rumen ammonia nitrogen concentration level to be 5 to 8 mg/100 ml, while others (78, 101, 107) have reported the optimum concentration to be from 9 to 29 mg/100 ml.

Several experiments have adequately demonstrated that ruminants can survive on diets containing urea or other nonprotein nitrogen sources as the main form of nitrogen (172, 177, 178). An issue to be resolved is whether diets containing mainly nonprotein nitrogen as the nitrogen source can promote optimum growth and production.

Bryant (28) studied 44 strains of rumen bacteria and reported that 80% utilize ammonia as the sole nitrogen source, 26% require ammonia for growth, and 55% would utilize either ammonia or aminonitrogen. Pilgrim et al. (129), using 15 N, estimated that 73% of bacterial nitrogen passed through the rumen ammonia pool when diets containing 1.6% nitrogen were fed to sheep. When higher amounts of nitrogen (2.9%) were fed, approximately 59% of bacterial nitrogen passed through the rumen ammonia pool. Although ammonia appears to be the primary nitrogen precusor for bacterial protein, nitrogen utilization was improved when preformed protein was substituted for nonprotein nitrogen in a purified diet (122). Hume (78) demonstrated the need for preformed protein sources by rumen microbes

through a reduction in microbial nitrogen yield when urea was the only source of nitrogen. Microbial nitrogen yield increased when the diet was supplemented with casein and branched chain fatty acids.

Apparently other precusors are required for maximum microbial protein synthesis. Hespell and Bryant (75) suggested that amino acids and peptides were required by bacteria for maximum protein synthesis. Deficiencies of these compounds lead to energetic uncoupling and subsequent bacterial fermentation without growth. Moenz and Baldwin (98) reported that amino acid supplemention increased microbial cell yield 32 to 36% in vitro.

Isoacids (isobutyrate, 2-methyl butyrate, 3-methyl butyrate and n-valerate) derived as a result of dietary protein fermentation in the rumen were suggested to be stimulants, or even required factors, for the growth of a number of bacteria, particularly the cellulotic bacteria (38). Recently, Felix et al. (63) confirmed these claims by feeding urea plus isoacids to dairy cows in which they observed improved utilization of nitrogen by cows fed the isoacids. These findings may suggest that dietary protein sources are essential for optimum microbial growth in the rumen.

Microorganisms can use ammonia to synthesize protein only if there is enough energy available for growth and reproduction. Mertens (104) suggested that 50 to 90% of the feed energy consumed by the cow is fermented by rumen microbes. This wide variation in digestibility was caused by the type and physical form of the feed, and the amount of feed intake of the animal. Rapidly fermented feeds will provide more energy to the rumen organisms than slowly fermented feeds.

Previous investigators (11, 93) have demonstrated that the type of available carbohydrate influences both the rate of ammonia utilization and the extent to which rumen microbes incorporate ammonia into protein. Ely et al. (57) demonstrated that starches were better sources of energy than cellulose for microbial protein synthesis in vitro. MacGregor et al. (97) observed greater milk yield, decreased cellulose digestibility, and decreased rumen ammonia in cows fed diets high in nonstructural carbohydrates (32.9%) than cows fed a low level (24.9%) of total nonstructural carbohydrates.

Mertens (104) pointed out that as intake increases, materials are moved through the gastrointestinal tract at a faster rate. This in turn results in less time for microbial utilization of energy. Finely ground feed may pass out of the rumen before microbial degradation resulting in less energy for protein synthesis.

Nitrogen Metabolism by Microbes. Although the nitrogen concentration in diet may appear to be adequate for maximal microbial growth, resistance of protein to ruminal degradation may result in nitrogen deficiency. This has been reviewed and demonstrated by many investigators (8, 12, 17, 18, 31, 79, 80, 144, 155, 156, 158, 166, 167, 170, 174, 175).

Information concerning microorganisms responsible for

proteolysis and the nature of microbial proteases has been summarized (3, 13, 20, 39). The hydrolysis of the peptide bonds has been suggested to provide some energy (166). The major importance of the degradation of protein is to supply nitrogen in the form of ammonia. Some species of rumen bacteria use peptides directly for the synthesis of microbial protein (48). Certain amino acids, notably methionine and cysteine, have been suggested as stimulatory growth factors for some strains of rumen bacteria (4, 29). According to Allison (3) rumen protozoa use proteolytic enzymes to digest bacterial protein and they are able to incorporate amino acids and peptides into their system.

Free amino acids arise as intermediate products in the breakdown of proteins by rumen microorganisms. Several researchers (35, 41, 42, 45, 145, 147) indicated that 40 to 80% of the dietary proteins were degraded in the rumen and transferred into microbial protein. These free amino acids can be assimilated directly by rumen microbes but most are deaminated to yield ammonia and other intermediate products (5, 6, 100, 119, 120, 129). The primary function of deamination may be for the production of branched-chain fatty acids which are the principal growth factors for some strains of rumen bacteria (4, 29). Since deamination occurs even when nonprotein nitrogen is supplied as the major nitrogen source, the purpose of bacterial deamination can be only speculated. On the other hand, if the rumen microorganisms cannot degrade the compound in question to yield free ammonia, the compound is useless as a

nitrogen source to the microorganisms (81). Fixation of ammonia is achieved via a number of systems, the most important of which is suggested to be glutamate dehydrogenase (38).

Nitrogen Metabolism by Ruminants

The two main sources of amino acids supplied to the animal are: 1) microbial protein synthesized in the rumen, and 2) dietary proteins and amino acids which escape ruminal degradation. These facets of nitrogen metabolism are closely interrelated because peptides, amino acids, and ammonia produced by the microbial degradation of dietary proteins serve as nitrogen sources for microbial growth.

Stern and Hoover (162) and Stern (161) stated that microbial protein comprises a substantial part of the protein entering the small intestine under most feeding practices. The amino acid composition of duodenal digesta usually reflects that of microbial protein. The exception to this is when the diet contains large amounts of rumen undegradable protein sources.

In general, metabolism of nitrogen compounds after passing through the abomasum is similar to that in nonruminants. Two exceptions are the higher ribonuclease activity in the pancreatic juice of ruminants than that in nonruminants (15), and the occurrence of maximum proteolysis is the lower jejunem in ruminants while occurring in the duodenum in nonruminants (18, 92).

Microbial Protein to Meet Ruminant Amino Acid Needs

Amino acid requirements of ruminants are not constant, but vary in relation to changing productive or physiological state. If energy is not limiting, rumen microorganisms appear to provide sufficient protein for maintenance, slow growth, and early pregnancy or late lactation.

Following the National Research Council's recommendation (113), a high producing cow weighing 650 kg and producing 30 kg of milk containing 4% fat will need 3.125 kg of crude protein in the diet each day. Based on the summary of experiments by Czerkawski (53) less than 50% of the protein requirements could be supplied from microbial protein synthesis. Thus, except where low levels of production are normal or can be tolerated, protein that escapes rumen degradation is needed to meet the amino acid requirements of the animal. Except for the limitation on quantity, rumen microbes are important sources of high quality protein.

In his review article, Chalupa (36) emphasized the need to change the old concept of simply expressing protein requirement as crude protein (nitrogen x 6.25) in a given diet. He suggested the need to consider the utilizable amino acids available to the animal for maintenance plus production as functions of the amount of rumen microbial protein produced and the amount of dietary protein which is resistant to degradation and bypasses the rumen.

Methods for Measuring Protein Escape From the Rumen

In Vitro Estimates. It is difficult to determine the extent of protein degradability in the rumen because of the many factors involved. The challenge has been to devise a laboratory technique which will satisfactorily mimic the action of the rumen bacteria. Many different solvents and procedures (2, 24, 70, 88, 94, 96, 127, 165, 179) have been used for nitrogen extraction from similar feeds. The variation in solubility has been attributed to degree of agitation, length of extraction time, temperature of extraction, pH, chemical composition, sample size, solvent ion strength, and sample particle size.

In earlier studies (94, 96) .02 N NaOH was used as a solvent to estimate digestibility of protein, but Little et al. (94) reported that protein solubility in basic solvents was poorly correlated (r=.25) with in vitro ammonia release. Their data suggested that .02 N NaOH solubility values for soybean meals were 3 to 4.5 times higher than solubilities in autoclaved rumen fluid. The modified Burroughs' mineral solutions (179) were found to simulate protein solubility in autoclaved rumen fluid (51). Water extraction has been used to assess the solubility of proteins (94, 125); however, since water extracts primarily nonprotein nitrogen and less than 5% of the protein, the solubility value observed was lower than the values reported using Burroughs' mineral solution.

Crooker et al. (51) compared modified Burroughs' mineral

mixture, .15 N sodium chloride solution, McDougal's artificial saliva, and autoclaved rumen fluid as a solvent for soluble nitrogen measurements on seven most common feedstuffs. They observed that the quantity of nitrogen extracted by either a modified Burroughs' mineral mixture or McDougal's artificial saliva differed from that extracted by autoclaved rumen fluid, whereas, that extracted by either Burroughs' mineral mixture or by sodium chloride solution did not. Crawford et al. (49) made comparisons between solubility in several solvents and disappearance of feed protein from dacron bags suspended in the rumen of fistulated steers as an indicator of rumen protein degradation. Burroughs' mineral buffer was most highly correlated (r=.66) to degradation value from the bag technique while autoclaved rumen fluid and .15 N sodium chloride were least correlated.

The major difficulty in estimating ruminal escape is that feeds containing more than one protein fraction degrade in the rumen at more than one rate. Techniques such as nitrogen solubility may be inaccurate because they tend to measure the properties of only the rapidly degraded fractions and not those of the protein as a whole (25). This implies that determination of solubility is adequate only for those sources of protein where the degradable material consists solely of the readily soluble fraction.

Broderick et al. (27) suggested caution in interpreting amount of ammonia release as an estimate of dietary protein degradation when using autoclaved rumen fluid. This is because of the

inability to measure microbial uptake of the protein and to take into account rate of ruminal passage rate of the protein, both of which influence degradability in vivo. Broderick and Lane (27) combined the rate of solubility and rumen liquor turnover rate to study the kinetics of protein degradation. This procedure yielded figures closer to the actual degradation rate in vivo.

Recently, an enzymatic technique for determining ruminal protein degradation has been employed. Commercially available proteolytic enzymes which have optimum activity at pH 5 to 7 and temperatures of 35 to 45°C were used (85, 108, 118, 131, 159). These methods employ an incubation of feed sample with a solution containing an active proteolytic enzyme.

Sniffen et al. (159) utilized a commercially available protease preparation (Streptomyces griseus) in vitro to determine the relative rate of nitrogen release from feedstuffs. This method showed good correlations when compared to direct and in situ methods. Mohadevan et al. (108) used <u>Bacterioides amylephilus</u> protease to demonstrate the difference in degradation potential of soluble and insoluble fractions of specific protein substrates. This work clearly demonstrated that the soluble fraction of feed protein may not be necessarily available for microbial assimilation. Lately, Ficin concentrate, an enzyme extracted from the latex of <u>Ficus</u> <u>glabrata</u> (a tropical fig tree) has been used to evaluate protein degradability in vitro (85, 131, 142). Ficin concentrate exhibits its maximum activity in the temperature and pH range normally

found in the rumen. It is also a nonspecific protease which has activity against a wide variety of substrates. Rock et al. (142) used bacterial protease (Streptomyces griseus), ficin (Ficus glabrata), and fungal protease (Aspergillus oryzae) to predict in vivo protein degradability. Ficin showed the best result with a correlation of .87 when compared with known ruminal bypass values for various feeds. The correlation coefficient was even higher (r=.90) for soybean meals, corn gluten meal, blood meal, wilted dehydrated alfalfa, and distillers dried grains. King et al. (85) used ficin protease assay and buffer solution to characterize protein supplements and feeds that are readily available to livestock farmers. They pointed out that heat processing protein feeds decreases protein solubility and degradability but the extent of each may be different. In general, the limitation of all in vitro methods is that although they may yield estimates of degradability they do not represent actual degradation rate in vivo.

In Vivo Estimate of Degradability. Estimates of protein degradation in vivo can be made either via disappearance from a bag suspended in rumen or animals equipped with cannulae in the abomasum or small intestine so that ingesta can be measured. Direct measurement of protein degradation is achieved by incubating a sample of feed enclosed in a nylon or dacron bag directly in the rumen. With this method, disappearance is measured, assuming disappearance to be equal to degradation. Mohamed and Smith (109)

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cautioned that disappearance may not equal degradation because soluble protein may be washed out without actually being degraded. Moreover, protein in the dacron bags may not be subjected to the digestive process in the rumen (166).

Animals equipped with cannulae in the abomasum or small intestine make it feasible to make an accurate assessment of the quantity of protein undegraded in the rumen. Dietary sources of protein can be differentiated from microbial sources by the use of specific markers such as nucleic acids, diaminopimelic acid (DAPA), aminoethyl-phosphonic acid (AEP), D-alanine, ^{15}N and radioisotopes $(^{35}S, ^{32}P)$ (67, 111, 161, 162). Using one or more markers, the microbial protein is isolated from the sample obtained and feed protein is calculated as the difference between total duodenal nitrogen or abomasum nitrogen and microbial nitrogen. However, when determining by difference, the error in the determination of microbial protein may lead to substantial error in estimation of degradability.

Potter et al. (132) and Nishimuta et al. (117) fed diets similar in all aspects except for protein source to abomasally cannulated cattle to determine protein degradability. Total nitrogen, protein nitrogen, and nonprotein nitrogen were determined from samples taken through the cannula. They interpreted the increase in abomasal flow of protein nitrogen as an increase in escape protein. They assumed that representative samples of digesta were obtained and that microbial protein flow to the abomasum was equal

among diets.

Other researchers (133, 182) used direct measurements to estimate protein escape. This involved the use of animals with proximal duodenal cannula and the use external markers (chromic oxide, acid insoluble ash, lignin and rare earth elements) to measure particulate flow from the rumen to the duodenum. The use of chromic oxide as an ideal marker has been criticized by Faichney (58, 60) because it behaves independently of the particulate phase. The use of lignin as a marker was questioned by Fahey et al. (62) since the marker was partially digested as it moves through the digestive tract. Faichney (60) observed that markers used to measure liquid flow such as polyethylene glycol (PEG), chromium ethylendiamine tetra acetate (Cr-EDTA) are absorbed on the particulate material and Cr-EDTA was shown to be excreted in urine. By using external and internal markers, flow of undegraded protein can be calculated as: flow of nonammonia nitrogen in test protein diet - microbial nitrogen flow - endogenous protein.

Protecting Protein Source from Ruminal Degradation

Recently, the concept of supplying bypass or escape protein to growing or lactating ruminants has received a great deal of attention. The concept of bypass and application of the system has been reviewed by several researchers (23, 35, 40, 149).

Researchers have used two approaches to present more undegraded dietary amino acids to the lower digestive tract. One

approach was to formulate diets from ingredients containing proteins with a natural resistance to ruminal breakdown (2, 22, 87, 102, 135, 163). Aitchison et al. (2) used four high producing cows to evaluate the feeding value of rations differing in nitrogen solubility (31.5 to 48.7%). They observed an increase in milk production and nitrogen retention on the low soluble protein ration. Later, Majdoub et al. (99) formulated rations with different proportions of solubility using natural feed sources in a 2 x 2 factorial design. The two levels of crude protein were 13 and 15% and the levels of nitrogen solubility were 22 and 45%. They observed an increase in milk production with decreased protein solubility at both levels of crude protein. The other approach has been to treat feedstuffs with heat, tannins, or aldehyde encapsulation to protect protein from rumen microbial attack. Attempts have also been made to inhibit deaminase activity of microbial enzymes (37) and to use the esophageal groove reflex (76, 123, 124).

Classical work by Cuthbertson and Chalmers (52) and Chalmers et al. (33) demonstrated that nitrogen retention by ruminants could be enhanced by increasing the amount of protein flowing to the small intestine. Nitrogen retention was improved in ruminants when casein was infused through the abomasum rather than the rumen. Their study (52) suggested that feed proteins that are less degraded in the rumen may promote more efficient nitrogen utilization for growth or milk production. Since then, several reports have confirmed the improvement in nitrogen retention (9, 34, 54, 56, 123, 148) and milk production (26, 47, 151, 152, 169) when casein or other amino acids were infused into the abomasum. Since presentation of amino acids through abomasual cannulae is not practical, researchers have concentrated their efforts on chemical and heat treatment as a means of protecting proteins from microbial degradation in the rumen.

Chemical Agents. In the period from 1965 to 1975 a number of chemicals were used to decrease the solubility and microbial degradation of feed protein in the rumen. The theory behind chemical treatment of protein is to create a reversible pH-dependent chemical modification that will inhibit breakdown of the protein at normal rumen pH, but still enable proteolysis at the much lower abomasal pH. Treating feed with chemicals causes cross linking of peptide chains rendering the protein lightly bound under slightly acidic or neutral rumen condition. As the pH drops to 3 or lower in the abomasum, the bonds are weakened and the protein becomes susceptible to enzymatic digestion in the lower gut. Methods of treatment and their effectiveness have been reviewed by (23, 35, 40). Of all the chemicals evaluated, formaldehyde was most widely investigated. Treatment of casein with formaldehyde resulted in increased nitrogen retention, wool growth, and muscle growth in sheep (35), but formaldehyde treated casein or soybean meal have not improved milk or milk protein yield in cows (35, 44, 66, 82, 84, 173). Rogers et al. (141) observed an increase in milk yield

when cows were fed formaldehyde treated casein. However, Clark et al. (43) found no significant difference in milk production and composition when cows were fed untreated and .9% formaldehyde treated soybean meal.

Losses of individual amino acids due to degradation in the rumen were reduced and levels of plasma amino acids increased when casein or peanut meal was treated with formaldehyde and fed to lambs or sheep (59). Calves fed two levels of protein (13 and 20%) treated with formaldehyde did not show differences in weight gain, feed conversion, digestibility, and blood urea concentration when compared to calves fed the respective control diet (61). Amos et al. (7) treated soybean meal with .5 or .75% formaldehyde and sunflower meal with 2.66 or 3.99% formaldehyde for a nitrogen balance study with wethers. They observed decreased percent dietary nitrogen retained and urinary nitrogen excretion, but increased fecal nitrogen. Reis and Tunks (138) and Reis (137) observed a reduction in post-ruminal enzymatic degradation in lambs when formaldehyde treated soybean meal was fed as compared to normal soybean meal.

Hagemeister (71) reviewed data from several research trials and concluded that formaldehyde application rates below .3% of the treated crude protein gave no response, higher than 1.2% had a negative response, and application of .3 to 1.2% may give positive response. The possible causes of the failure of formaldehyde to be an effective treatment could be:

- 1) A remaining portion of unbound formaldehyde interfers with the bacterial fermentation in the rumen,
- Overprotected protein resulting from an excessive treatment leads to:
 - a) A nitrogen shortage for the rumen microorganisms, or
 - b) Reduced digestibility in the intestine and insuffi
 - cient supply of essential amino acids to the animal.

Heat Treatment. Of the methods used to protect proteins, the application of additional heat either during processing or independently has received more attention and is widely used in feed processing. Many feed processing methods either require or generate heat which decreases protein solubility. Common examples include production of meat meal, blood meal, dehydrated alfalfa, corn gluten meal, and dried distillers grains. These proteins are heated during processing to dry the material to a point suitable for storage and handling. Heat denatures the protein to the extent that microbial proteolysis is reduced, either by decreasing solubility of the protein or by rearranging the protein molecule which inhibits microbial protease attachment (25). While soybean meal protein is regarded as rapidly degradable (71 to 85% degraded in the rumen) (95, 181), a number of researchers (86, 95, 102, 164, 176, 181) have reported a lower rumen degradability for meat meal (23 to 30%), blood meal (18 to 29%), dehydrated alfalfa (34 to 63%), corn gluten meal (38 to 54%), and dried distillers grain (39 to 52%). Processing of soybean meal also involves use of heat during initial flaking of the beans and in the final stage of solvent reclamation. Since the soy flakes contain 35% hexane, 8% water, and 1% oil after oil extraction, additional heat at the range of 63 to 69° C is applied to remove the hexane (110). This heating process has the additional advantage of inactivating trypsin inhibitors that could interfere with protein digestion (19, 83, 107, 110), lipase, and urease.

This primary heating process to remove the solvent is not sufficient to protect soyprotein from ruminal degradation. Therefore, additional heat can be applied to increase crosslinking within and between protein molecules. Broderick (23) suggested that heating causes chemical modification decreasing extent of microbial proteolysis in two ways: 1) The soluble fraction of the protein will be reduced and less protein will be available for microbial hydrolysis. Solubility is generally decreased because large numbers of hydrophobic amino acid groups are exposed to the protein molecule surface. In turn, the increase in hydrophobic groups at the surface causes a decrease in solubility. 2) The enzyme reactive sites on the protein will be blocked by chemical rearrangement of the protein. Mohadevan et al. (108) reported that disulfide crosslinking in proteins was responsible for decreasing ruminal degradation, probably due to blocking enzyme reactive sites.

According to Lehninger (91) heat or thermal denaturation is reversable if conditions are appropriate and denaturation is

not too severe. Heat or thermal denaturation can become a problem in processed feedstuffs since heating of proteins in the presence of carbohydrates results in formation of Schiff bases (23) and Maillard products which have been implicated as being resistant to enzymatic digestion (141). Pepsin insoluble nitrogen (69) and the acid detergent insoluble nitrogen (69, 128) have been suggested as a measure of unavailable nitrogen. These fractions represent nitrogen that is bound to the lignin and cellulose or denatured protein which are resistant to microbial and enzymatic degradation.

Solubility of protein has been positively correlated with rumen degradability (49, 50). Heating has been documented to cause a decrease in solubility (68, 77, 94, 150, 153, 154, 165, 168). Many early studies were in vivo with sheep or with beef cattle. Nishimuta et al. (116) fed soybean meal heated at 149°C for 4 h to sheep and observed greater nitrogen retention and lower plasma urea nitrogen. In another study (117) steers fed unheated, commercially processed soybean meal had less protein flowing into the abomasum compared to steers fed heated soybean meal.

Tagari et al. (165) observed a decrease in rumen ammonia concentration in sheep fed soybean meal heated at 80°C for 10 min or at 120°C for 15 min. Sheep fed the soybean meal heated at the higher temperatures and longer time had improved in nitrogen utilization as measured by greater nitrogen retention, improved nitrogen digestibility, and lower plasma urea nitrogen. Sherrod and Tillman (153) observed greater efficiency and improved gains when they fed

soybean meal autoclaved at 121°C for 45 min versus untreated soybean meal to sheep.

Little et al. (94) did not observe improvements in lamb performance when fed soybean meal heated 110^oC for 4 h at different amounts of crude protein. However, Glimp et al. (49) reported that lambs fed heated soybean meal performed better than lambs fed an unheated soybean meal diet containing 12% crude protein.

Thomas et al. (168) conducted nitrogen balance and growth trials with rats and reported no difference in nitrogen digestibility, nitrogen retention, or growth when rats were fed soybean meal heated at 127, 128, or 149°C for 4 h. Rakes et al. (134) evaluated roasted soybeans with six lactating cows. They reported no difference in apparent digestibility of crude protein and milk production when compared to raw soybeans.

Netemeyer et al. (114) used 22 cows in a switchback trial to evaluate the feeding value of soybean flakes subjected to extensive heating during processing (solubility of 8.1%) and soyflakes processed in a conventional manner (solubility of 24%). Milk yield increased when cows were fed the low soluble diet, but no difference in percent fat and protein in the milk. Grummer and Clark (70) used 30 lactating cows in a 20 wk trial to determine the effect of dietary nitrogen solubility on milk yield, milk composition, and apparent digestibility of diets. The treatments were soybean meal processed by conventional manner and defatted soybean flakes heated at 250°C for 30 min, 250°C for 20 min, 215°C for 20 min, and

180°C for 25 min. Covariate adjusted milk, fat-corrected-milk, crude protein, and fat percent were similar for all treatments, which ranged from 21.7 to 34.4% soluble nitrogen. In a second trial where they used the same protein supplements but reduced the solubility to 18.8 to 30.6%, they observed a lower milk yield by cows fed the higher level of soluble nitrogen. Lately, Ruegseggar et al. (143) used 58 high producing cows during early lactation to evaluate the feeding value of heat-treated whole soybeans. Cows fed the heated soybean ration produced significantly more milk than did the control animals (37.0 vs. 36.2 kg/day). Ahrar and Schingoethe (1) used 18 Holstein cows to evaluate the feeding value of extruded soybean meal in a 16 wk lactation trial. They did not observe a significant difference in milk yield in cows fed either regular or heated soybean meal. The lack of response might have been because the cows averaged 9 wk postpartum at the start of the experiment, thus, being past peak lactation and consuming enough dry matter to meet their protein and energy requirements. Later. a similar trial (105) was conducted to determine the feeding value of regular solvent extracted soybean meal, extruded soybeans, and unheated soybeans in a switchback design using 12 cows. Milk production was similar for all treatments, although numerically highest for cows fed extruded soybeans. The lack of significant response observed in this trial again might be due to the use of cows past peak milk production (7 wk postpartum). Recently, Kung and Huber (89) used 84 cows producing over 26 kg milk/day during

early lactation to evaluate soybean meal heat-treated for 2.5 h at 140° C in forced draft oven. They observed that, when the data was adjusted for pretreatment production, cows fed the heated soybean meal produced 1 to 2 kg more milk/day than those fed unheated soybean meal.

Generally, the lack of response in some trials in milk yield and composition when heat-treated soybean meal was fed may have been due to several factors. These include overheating of the protein supplement, thus, depressing digestibility; underheating the protein causing lower bypass; use of animals with low protein requirement (low level of milk production or in mid or late lactation), insufficient length of experimental period, and insensitive experimental design (45, 50, 166).

MATERIALS AND METHODS

A series of experiments were conducted to evaluate regular, commercially available solvent extracted soybean meal (SBM), and SBM subjected to additional heat by one or two methods. The regular SBM had a protein dispersability index (PDI) of about 40. Heattreated soybean meal (HSBM)¹ was subjected to additional heat during desolventizing as described by Netemeyer et al. (114) and had a PDI of about 10. Extruded soybean meal (ESBM)² was from the same batch as the SBM, and was processed in an extruder as outlined by Schingoethe and Ahrar (150).

Completely mixed rations were made of (dry matter basis) 40% corn silage, 10% chopped alfalfa hay, and 50% concentrate mix containing the respective protein sources (Table 1). Concentrate mixes, alfalfa hay and corn silage were sampled each week. Four weekly samples were composited for proximate analyses (14), acid detergent fiber (69), and neutral detergent fiber using the procedure of Robertson and Van Soest (139).

Portions of the feed samples and soybean meals were also used for determination of protein solubility using the modified Burroughs' mineral buffer solution (30) as described by Crooker et al. (51), and degradability by a Ficin protease assay procedure (130) with the following modification. A sample containing 15 mg

¹Farmland Industries, Inc., St. Joseph, MO.
²Triple F Feeds, Des Moines, IA.

	The second second	Ration	-
Ingredient	SBM	HSBM	ESBM
		%	
Shelled corn	77	77	77
Soybean meal	21		
Heat-treated soybean meal		21	
Extruded soybean meal			21
Dicalcium phosphate	1.5	1.5	1.
Trace mineral salt	.5	. 5	

TABLE 1. Ingredient content of concentrate mixes containing regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

^aMixes contained 8,800 IU added vitamin A and 2,200 IU added vitamin D/kg.

nitrogen was placed in polyethylene tubes. The sample was prewetted with 5 ml distilled water for 12 h before adding 10 ml of a 1:1 v/v solution of in vitro rumen buffer (4 g NH_4HCO_3 and 35 g NaHCO3 per liter of distilled water) and macromineral (5.7 g Na2HPO4, 6.2 g KH2PO4 and .6 g MgSO4.7H20 per liter of distilled water) to each tube. Then 1 ml of 1% sodium azide solution and .5 ml of 1% (v/v) Triton X-100 solution were added. A stopper was placed on the tube and incubated at 39°C for 2 h with frequent swirling. After 2 h of incubation, the tubes were removed, filtered, and Kjeldahl analyses carried out on the filter paper and residue to get an estimate of solubility (initial minus residue). To obtain an estimate of degradability (initial minus residue), 10 ml of prewarmed ficin (enzyme extracted from latex of Ficus glabrata) were added after the 2 h incubation and the incubation continued for another hour. The ficin solution was prepared by dissolving 4.3 g of ficin in liters of solutions containing 200 ml stock phosphate buffer and 6.1 g cysteine hydrochloride. The stock buffer was made by dissolving 10.54 g K2HPO4 and 59.66 g KH2PO4 liter of distilled water. At the end of 1 h the tubes were removed and 2 ml of 80% t-butyl alcohol was added to terminate the reaction. Samples were filtered and washed onto Whatman No. 541 and Kjeldahl analyses was carried out on the filter papers plus the residues. Undegradable protein was used as an estimate of the amount of protein which may be escaping degradation in the rumen. Heat damage and unavailable protein were determined using acid

detergent insoluble nitrogen (ADIN) and acid pepsin insoluble nitrogen (APIN) as outlined by Goering and Van Soest (69).

To prepare samples for amino acids analysis, .2 to .5 g samples of SBM, HSBM, ESBM, as well as samples of the insoluble residue and undegraded portion of the respective soybean meals were hydrolyzed with 6 N HCl under a nitrogen atmosphere at 100°C for 24 h. Hydrolysates were evaporated to dryness, diluted with pH 2.2 citrate buffer, filtered, and analyzed on Beckman 118BL automated ion-exchange amino acid analyzer (90). Amino acids were separated on ion-exchange columns with a sodium citrate buffer gradient ranging in pH from 3.49 to 6.4.

Lactational Evaluation

Thirty multiparous Holstein cows producing at least 27 kg of milk per day during the 3rd wk postpartum were used in a 16 wk lactational trial to evaluate the feeding value of regular, solvent extracted soybean meal (SBM), or soybean meal subjected to additional heat either during desolventizing (HSBM) or by extrusion (ESBM). Cows were grouped into trios based on previous production records and milk yield during the 3rd wk postpartum and then randomly assigned to one of these treatments (soybean meal) for weeks 4 to 19 postpartum.

Cows were housed in a stanchion barn and were individually fed a completely mixed ration once daily between 9:00 a.m. and 10:00 a.m. The three diets were formulated to be isonitrogeneous

at approximately 90% of the protein requirements and isocaloric at 110% of the energy requirements of the cows (113).

Cows were milked twice daily with milk yield recorded at each milking throughout the trial. Composite samples from two consecutive milkings during 24 h were collected once weekly from each cow for analysis of fat by the Milko-Tester MK II¹ (114), total solids by the Mojonnier method (115), and protein by Kjeldahl procedure (14). Alternate week samples were also analyzed for milk protein components by the Rowland procedure (136). Body weights of cows were obtained for 3 consecutive days at the start of the experiment, once every 4 wk, and 3 consecutive days at the end of the experimental period.

Between 8 and 10 wk after each cow was placed on experiment, samples of rumen contents were obtained by applying vacuum to an esophageal tube at 0, 2, 4, and 6 h after a.m. feeding. Samples were collected into 100 ml bottles containing .5 ml saturated mercuric chloride. Samples were analyzed for pH within .5 h of collection using a glass electrode pH meter. Rumen fluid was strained through four layers of cheesecloth. A 10 ml aliquot of rumen fluid was acidified with .5 ml of .1 N HCl, centrifuged, the supernant frozen, and later analyzed for ammonical nitrogen as described by Chaney and Marbach (39). Another 10 ml aliquot was acidified with 2 ml of 25% metaphosphoric acid, centrifuged, and

¹MK II, Foss Electric, Hillerod, Denmark.

the supernatant frozen until analyzed for volatile fatty acids (VFA) by gas liquid chromatography with a neopentylglycol succinate column as described by Baumgardt (16). Samples of jugular vein blood were obtained at the times of rumen sampling. Blood was centrifuged and serum was analyzed for blood urea (39).

Milk production and milk components were adjusted by covariance analysis based on pretreatment milk production and composition during the 3rd wk postpartum. All data were subjected to analysis of variance by the General Linear Model Procedure of the Statistical Analysis System Computational Package (74). Whenever significant differences were detected, the Waller-Duncan procedure (160) was used to compare treatment means.

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RESULTS AND DISCUSSION

Chemical Composition

Chemical composition of the feeds are listed on Tables 2 and 3. The dry matter of all total mixed rations contained similar amounts of crude protein (14.3%), ether extract (2.9, 2.8, 3.0%), acid detergent fiber (15.5, 15.3, 15.5%), and neutral detergent fiber (32.0, 31.7, 31.9%) and ash (5.6, 5.6, 5.3%) for SBM, HSBM, and ESBM, respectively.

Solubilities of nitrogen in the soybean meals (Table 3) were highest for SBM and lowest for ESBM using both solubility procedures, namely dilute Burroughs' buffer or buffer from ficin protease assay. The difference in solubility observed between the two procedures was probably due to difference in incubation time and buffer systems. Nitrogen solubility for the total ration followed the same trends as the soybean meal sources, although the differences were small between diets. This was expected since the soybean meal provided only 10.5% of the total ration dry matter (32% of the total crude protein).

The rates of protein degradability, presented in Table 3, agreed with other reports (146) which stated that 40 to 60% of the proteins in a typical dairy ration bypasses ruminal action. Soybean meal samples analyzed using similar procedures were reported to have degradable protein values of 71, 34 to 51, 48 to 51, and 46 to 75% for regular soybean meal, dry heated soybean meal, roasted soybean meal, and extruded soybean meal, respectively (85).

				Fora	rages	
	Cond	centrate	e mix	Corn	Alfalfa	
easurement	SBM	HSBM	ESBM	silage	hay	
ry matter, %	90.0	91.2	91.1	40.7	89.2	
rude protein, % of DM	17.8	17.5	17.1	8.5	21.3	
Soluble protein, % of CP ^a	16.0	11.1	9.6	54.1	31.7	
Degradable protein, $%$ of CP ^b	56.2	54.9	49.9	45.2	32.6	
Acid detergent insoluble-N, % of CP				16.4	7.2	
ther extract, % of DM	3.1	3.2	3.4	2.7	2.0	
Acid detergent fiber, % of DM	5.7	5.3	5.6	24.9	26.9	
leutral detergent fiber, % of DM	19.7	19.0	19.5	44.8	42.6	
Ash	4.6	4.5	4.1	4.9	8.3	

TABLE 2. Chemical composition of forages and concentrate mixes containing regular soybean meal (SBM), heat-treated soybean meal (HSBM), or extruded soybean meal (ESBM).

^aSolubility in 10% Burroughs' solution, pH 6.5 ().

^bFicin protease assay.

	So	ybean me	eal	Tot	al rat:	ion
	SBM	HSBM	ESBM	SBM	HSBM	ESBM
and some source of the second			(% 0	f N) —		
Solubility in 10% Burrough's solution	14.9	9.3	7.0	30.3	27.9	27.0
Ficin protease assay						
Solubility	24.2	14.3	7.1	24.9	22.6	22.1
Degradability	71.0	68.7	58.7	53.3	50.7	48.4
Acid detergent insol- uble-N (ADIN)	3.2	4.1	6.4	13.5	13.8	14.0
Acid pepsin insoluble-N (APIN)	4.3	4.9	5.2			

TABLE 3. Solubility and degradability estimates for nitrogen (N) in regular soybean meal (SBM), heat-treated soybean meal (HSBM), extruded soybean meal (ESBM), and respective ration mixes.^a

^a50:40:10 (concentration:corn silage:alfalfa hay) (DM basis).

In our in vitro study the degradability value for the HSBM was higher than expected indicating additional heat during desolventizing may not greatly reduce protein degradability in the rumen. Generally, actual solubility values in dilute Burroughs' buffer for the total ration (Table 3) were lower than values derived mathematically from data in Table 2 (30.3, 27.9, and 27 vs 32.8, 30.4, and 29.6) while actual degradability values were higher than the degradability values derived mathematically (53.3, 50.7, and 48.4 vs 49.5, 48.8, and 46.3) for rations containing SBM, HSBM, and ESBM, respectively. This possibly indicated that feed protein may solubilize or degrade at different rates when combined as a complete ration rather than individually. Values for the total ration should be better indicators of both solubility and degradability than individual feedstuffs values.

In our in vitro study, there was a high correlation between solubility and degradability (ficin assay) of soybean meals (r=.90). The correlation was even higher when the complete rations were compared (r=.95). The correlation between protein solubility in dilute Burroughs' buffer and degradability was .83 for soybean meals and .98 for the complete ration. Rock et al. (142) reported a correlation coefficient of .90 for ficin enzymatic laboratory evaluation with amount of protein which actually escaped degradation in the rumen. Poos et al. (131) reported a correlation coefficient of \geq .90 between degradabilities of soybean meal, blood meal, and corn gluten meal proteins estimated by in situ technique versus

the ficin proteolytic enzyme assay procedure. Nocek et al. (118) cautioned that amino acid release during the in vitro protease method and nitrogen disappearance in situ are not synonymous for all feedstuffs and that there are considerable differences in the degradation potential of soluble proteins. In our in vitro studies, we were interested in comparing the same source of protein varying only in the amount or type of heat treatment. The data obtained may be a realistic estimate of amino acid release in vitro; however, these values may not reflect actual animal performance since other limiting factors such as rate of passage and dilution rates cannot be accounted for using these in vitro procedures. These factors are the major determinant of protein degradability in the animal but likewise cannot always be accurately estimated using in situ bag techniques either (49).

Acid pepsin insoluble nitrogen (APIN) and acid detergent insoluble nitrogen (ADIN) were used to measure heat damage or unavailable protein (Table 3). The amount of unavailable N was apparently unaffected by the heat treatment in this study, although tended to be higher in the two heated soybean meals than in SBM.

Amino acid content of the total, insoluble, and undegradable fractions of SBM, HSBM, and ESBM are in Table 4. The essential amino acids from all three soybean meal sources tended to be slightly more insoluble than the nonessential amino acids. Seventyeight percent of the total essential amino acids in SBM were recovered in the insoluble portion, whereas 87.5 and 95.1% from HSBM

Amino Total				nsolub		Undegradable fraction			
acid	SBM	HSBM	ESBM	SBM	HSBM	ESBM	SBM	HSBM	ESBM
			(%	of DM	in tha	t fract	ion) —		
Arg	4.0	3.9	3.6	3.9	4.7	5.0	1.4	1.9	2.4
His	1.4	1.5	1.4	1.7	1.8	1.6	.7	.7	1.0
Ile	2.5	2.3	2.3	2.7	3.1	3.0	1.2	1.4	2.0
Leu	4.6	4.1	3.9	4.8	5.2	5.3	2.3	2.8	3.5
Lys	3.7	3.2	2.7	3.9	4.1	3.4	1.6	1.6	1.7
Met	. 7	.7	.7	.8	.9	. 8	. 4	.4	.5
Phe	3.2	2.7	2.6	2.9	3.4	3.4	1.5	1.7	2.3
Thr	2.2	2.0	1.9	2.3	2.5	2.7	1.1	1.2	1.4
Val	2.9	2.4	2.5	3.0	3.2	3.0	1.4	1.6	2.1
Ala	2.5	2.3	2.2	2.8	3.0	2.8	1.3	1.4	1.6
Asp	6.5	5.6	5.8	5.8	6.9	6.9	2.7	2.6	3.3
Cys	.5	.6	.5	.6	.7	.8	.2	.4	.4
Glu	9.8	9.2	9.1	8.9	10.7	10.9	2.5	3.3	3.7
Gly	2.3	2.2	2.2	2.5	2.8	2.7	1.2	1.3	1.5
Pro	2.6	2.5	2.4	2.7	3.0	3.4	1.1	1.4	1.5
Ser	2.7	2.5	2.5	2.8	3.2	3.3	1.2	1.4	1.8
Iyr	2.2	2.1	2.1	2.4	2.7	2.7	1.0	1.2	1.7

TABLE 4. Amino acid profile in total, insoluble, and undegradable fractions of regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

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and ESBM were insoluble (Table 5). The proportion of essential amino acids recovered in the undegradable fraction as the percent of total amino acid in that fraction was 32.3, 34.8, and 48.1 (41.5, 39.8, 50.6 expressed as percent of the insoluble fraction) for SBM, HSBM, and ESBM, respectively. Nonessential amino acids recovered in the undegradable fraction as percent of total amino acids in that fraction were 25.7, 28.6, and 35.4% (34.7, 33.8, and 38.8 as percent insoluble) for the respective protein sources.

The percent recovery of essential amino acids in the insoluble and undegradable fractions is presented in Table 6. Many amino acids in ESBM were apparently slowly degradable since 88 to 100% of the amino acid was in the insoluble form, but only 39 to 55% was undegradable. This held true for HSBM too, but with lower percentages than in ESBM. However, degradability of the individual amino acids was not consistent with corresponding solubility values. Therefore, it would be difficult to predict the fate of an individual amino acid in terms of degradability using the solubility figures. For example, histidine, isoleucine, and arginine were recovered in the highest amounts in the insoluble fraction of SBM, HSBM, and ESBM, respectively. However, histidine was the 4th, isoleucine the 4th, arginine the 7th most recovered amino acids in the undegradable fraction of the SBM, HSBM, and ESBM, respectively. Considering the five amino acids (Table 6) often cited as first limiting in milk production (40, 46, 54, 152) highest recoveries in the undegradable fraction (as % of total)

		Total ^a		Inso	oluble frag	ction ^b	Undegr	adable fr	action
Amino acid	SBM	HSBM	ESBM	SBM	HSBM	ESBM	SBM	HSBM	ESBM
				— (g/kg	total amin	no acids) -			_
Essential									
Arg	73.0	78.4	73.3	53.8	64.0	74.3	18.5	21.9	29.8
His	26.1	29.2	27.6	23.5	24.1	24.4	8.7	8.3	12.8
Ile	44.6	45.2	46.3	36.8	42.2	44.3	15.3	16.4	25.3
Leu	83.2	82.0	80.2	66.2	70.6	79.2	29.3	33.0	43.7
Lys	66.1	63.4	55.1	52.9	57.1	50.3	20.7	19.3	21.6
Met	13.4	14.2	13.8	11.1	12.0	12.6	4.7	4.8	6.6
Phe	57.6	51.4	53.9	40.1	46.2	51.4	18.6	20.2	28.9
Thr	41.7	39.2	39.2	32.0	34.7	36.6	14.5	13.6	17.3
Val	52.6	47.4	50.8	40.8	43.2	45.4	17.8	19.4	26.0
Subtotal	458.2	450.4	440.0	357.2	394.2	418.5	148.1	156.8	211.8
Nonessentia	1								
Ala	44.8	45.0	45.3	38.1	40.6	41.6	16.1	16.3	20.0
Asp	118.2	111.6	117.3	79.1	93.9	102.6	27.7	30.9	41.5
Cys	9.3	12.1	11.6	9.4	10.5	11.5	3.1	4.4	5.0
Glu	178.3	184.0	185.3	122.4	146.7	163.3	32.5	39.0	46.2
Gly	42.2	43.6	45.5	33.7	37.7	39.7	14.8	14.9	19.3
Pro	46.6	49.4	48.0	37.5	41.4	50.3	13.6	16.3	18.9
Ser	49.1	50.0	51.8	38.6	44.3	49.6	15.2	16.4	21.9
Tyr	39.9	41.0	42.2	32.4	37.4	40.3	12.8	15.0	21.1
Subtotal	528.3	536.7	547.1	391.1	.452.7	498.9	135.6	153.2	193.8
Total	986.5	987.1	987.1	748.3	846.9	917.4	283.7	310.0	405.0

TABLE 5. Amino acids present in total, insoluble, and undegradable fractions of regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM) adjusted for solubility and degradability.

% AA	_				x	1000
Total	AA	in	that	Fraction	~	1000

^b $\begin{bmatrix} \frac{\chi_{AA}}{\text{Total AA in that fraction}} \times 1000 \end{bmatrix} \times \chi$ insoluble ^c $\begin{bmatrix} \frac{\chi_{AA}}{\text{Total AA in that fraction}} \times 1000 \end{bmatrix} \times \chi$ undegradable

ω 8

	-	Insolubl	е	A statement	Undegradable				
Amino acids	SBM	HSBM	ESBM	SBM	HSBM	ESBM			
199		1.10		— (% of total amino a	cid)	1000			
Arg	72.7	81.7	101.4	25.3(34.3) ^a	27.9(34.1)	40.6(40.0)			
His ^b	90.0	82.7	88.6	34.5(37.2)	28.5(34.5)	46.3(52.1)			
Ile	82.6	93.5	95.7	34.3(41.5)	36.3(38.9)	54.5(57.0)			
Leu	79.6	86.1	98.8	35.2(44.2)	40.2(46.7)	54.5(55.2)			
Lys ^b	80.0	90.0	91.3	31.3(39.1)	30.4(33.8)	39.2(43.0)			
Met ^b	82.8	84.6	91.6	35.0(42.2)	33.9(40.1)	48.1(52.5)			
Phe ^b	69.6	89.9	95.4	32.3(46.4)	39.3(43.7)	53.5(56.0)			
Thr ^b	76.8	88.5	93.3	34.8(45.3)	34.8(39.7)	44.0(47.2)			
Val	77.6	91.1	89.3	33.9(43.7)	40.9(44.9)	51.2(57.3)			

TABLE 6. Relative distribution of the essential amino acids recovered in insoluble and undegradable fractions.

^aNumbers in first column are percent of total amino acid, while numbers in parenthesis indicate percent amino acid recovered as percent of insoluble.

^bAmino acids often cited as first limiting in milk production.

were for methionine in SBM, phenylalanine in HSBM, and phenylalanine in ESBM. Lowest recoveries were for lysine, histidine, and lysine, respectively. When expressed as percent of the insoluble fraction, phenylalanine was recovered in the greatest amounts for all three protein sources, while lysine was recovered in the least amounts of HSBM and ESBM and histidine in the least amount for SBM.

Relative distribution of essential amino acids in milk (21) and in the total, insoluble, and undegradable fractions of the respective soybean meal sources is presented in Table 7. In all soybean meals and in all their fractions, methionine remained the first limiting amino acid when compared to the amino acid content of milk protein. Arginine, histidine, and phenylalanine were the least limiting. This was similar to a previous report (150) comparing the total and insoluble portion of regular and heated soybean meal. The first five most limiting amino acids in the total fraction were the same in SBM, HSBM, and ESBM, but their relative ranks varied. Lysine was 4th limiting in SBM, but 3rd and 2nd limiting in HSBM and ESBM, possibly indicating some destruction of lysine by these heat treatments. Broderick (23) indicated that heat treatment can destroy lysine or increase its irreversible binding with sugars. In the insoluble fraction, second limiting amino acid was valine for SBM, and HSBM, but lysine for ESBM.

In the undegradable fraction, methionine and lysine were the first and second limiting amino acids in all soybean meals. Valine and isoleucine were the third and fourth most limiting amino

Amino Acid	Milk ^a	CDM	Total			Insoluble	e	U	ndegradabl	e
Autilo Acid	MIIK	SBM	IISBM	ESBM	SBM	HSBM	ESBM	SBM	HSBM	ESBM
			10-1-1-2	-	(g/100 g	essential	amino acids)	1		_
Essential										
Arg	7.5	15.9(9) ^b	17.4(9)	16.7(9)	15.1(9)	16.2(9)	17.8(9)	12.5(9)	13.9(9)	14.0(9)
llis	4.4	5.7(8)	6.5(8)	6.3(8)	6.6(8)	6.1(8)	5.8(8)	5.9(8)	5.3(8)	6.0(8)
Ile	11.7	9.7(2)	10.0(4)	10.5(5)	10.3(3)	10,7(5)	10.6(4)	10,3(4)	10,5(3)	11,9(5)
Leu	20.3	18,2(5)	18.2(5)	18.2(4)	18.5(5)	17,9(4)	18,9(6)	19.8(5)	21,0(6)	20,7(6)
Lys	16.5	14,4(4)	14.1(3)	12.5(2)	14.8(4)	14.5(3)	12,0(2)	14.0(2)	12,3(2)	10,2(2)
Met	5.2	2.9(1)	3.2(1)	3,1(1)	3,1(1)	3,0(1)	3,0(1)	3,2(1)	3,1(1)	3.1(1)
Phie	10.9	12.6(7)	11.4(7)	12.2(7)	11.2(7)	11,7(7)	12,3(7)	12,6(7)	12,9(7)	13,7(7)
Thr	9.6	9.1(6)	8,7(6)	8.9(6)	9,0(6)	8,8(6)	8,7(5)	9,8(6)	8,7(5)	8,1(3)
Val	13.8	11.5(3)	10.5(2)	11.6(3)	11.4(2)	12,0(2)	10,8(3)	12.1(3)	12,4(4)	12,3(4)

TABLE 7. Relative distribution of essential amino acids in milk and in the total, insoluble, and undegradable fractions of regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

^aCalculated from data reported in (21),

^bNumbers in parenthesis indicate the apparent sequence of limiting amino acids for milk protein synthesis.

acids in SBM, with the order reversed in the HSBM. For ESBM, the third limiting amino acid was threonine while valine was fourth limiting. Several researchers (47, 54, 152, 171) have pointed out that methionine, lysine, and threonine are the most limiting amino acids for milk production when cows are fed mainly corn, soybean meal, and corn silage.

Several conclusions can be drawn from these findings. Even though methionine remained the first limiting amino acid for milk production in all fractions of the soybean meals, there were some changes in the profile of the other amino acids due to heat treatment. The order of the most limiting amino acids changed with various heat treatments. Even if the amino acid profile observed in the various fractions is unchanged, heat treatment increased the quantity of essential amino acids (found in the insoluble and undegradable fractions) effectively protected from excessive ruminal degradation. Since solubility in vitro is correlated with degradation of protein in the rumen (55, 73, 103, 157, 180), heat treatment of soybean meal likely increased the quantity of amino acids escaping degradation in the rumen.

Lactational Evaluation

Milk Production. High producing cows (i.e., > 30 kg/day at 3 wk postpartum) receiving the ESBM and HSBM diets produced more milk (P<.01), 4% fat-corrected milk and solids-corrected milk than cows fed the SBM diet (Table 8). Most of the increased production was observed during the first 4 wk on the experiment (wk 4 through 7 postpartum) (Figures 1 to 3). When the data from all 10 cows per treatment were considered, cows fed ESBM produced more 4% fatcorrected and solids-corrected milk (P<.01) than cows fed HSBM and SBM diets, but actual milk yields were similar (P>.05). When production from all cows was plotted by week (Figures 4 to 6), the increased production during the first 4 wk on experiment when fed HSBM and ESBM was modest, since the cows producing less than 30 kg/day did not respond to the heat-treated protein supplements. Therefore, including data from three lower producers per treatment group which did not respond to heated soybean meals, simply created a "dilution effect" on the production data. The lower producers may not have responded to the heated soybean meals because their protein requirements were not sufficiently greater than what could be supplied by normal rumen microbial protein synthesis plus the amount of bypass protein already available from the control (SBM) diet. The reason for greater decline in production by cows fed SBM near the end of the trial (wk 14 and 15) is not known. The decline was attributed to three cows fed SBM, but no injuries or

Measurement	SBM	HSBM	ESBM	SE
Milk, kg/day	33.8 ^{a,d} (32.6) ^b	34.9 ^c (32.4)	35.3 ^c (33.1)	.34(.28)
4% Fat-corrected milk, kg/day	30.9 ^d (29.9) ^d	32.6 ^c (30.4) ^d	33.4 ^c (31.7) ^c	.40(.34)
Solids-corrected milk, kg/day	29.8 ^e (28.9) ^c	31.0 ^d (29.3) ^c	32.0 ^c (30.4) ^c	.33(.29)
Fat, %	3.52 (3.61)	3.52 (3.60)	3.59 (3.58)	.05(.04)
Fat, kg/day	1.17 ^d (1.14) ^g	1.24 ^c (1.17) ^f ,	³ 1.27 ^c (1.21) ^f	.02(.02)
Solids-not-fat, %	8.17 (8.26)	8.03 (8.13)	8.19 (8.24)	.05(.04)
Solids-not-fat, kg/day	2.79 ^d (2.64) ^g	2.84 ^c (2.68) ^f ,	³ 2.88 ^c (2.72) ^f	.03(.03)
Protein, %	3.02 (3.06)	3.02 (3.09)	3.06 (3.07)	.02(.02)
Protein, kg/day	1.01 ^e (.98) ^g	1.05 ^d (1.00) ^f ,	³ 1.07 ^c (1.01) ^f	.01(.01)
Total solids, %	11.73 ^c (11.90) ^c	11.49 ^d (11.65) ^d	11.84 ^c (11.88) ^c	.06(.05)
Total solids, kg/day	3.90 ^e (3.77) ^c	4.04 ^d (3.82) ^c	4.17 ^c (3.96) ^d	.04(.04)

TABLE 8. Milk yield and composition from cows fed rations containing regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

^aData from seven high producing cows/trt.

^b() Data from all ten cows/trt.

c,d,e_{Means} on the same line with unlike superscript are different, P<.01.

f,g_{Means} on the same line with unlike superscript are different, P<.05.

Figure 1. Average milk production of high producing cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

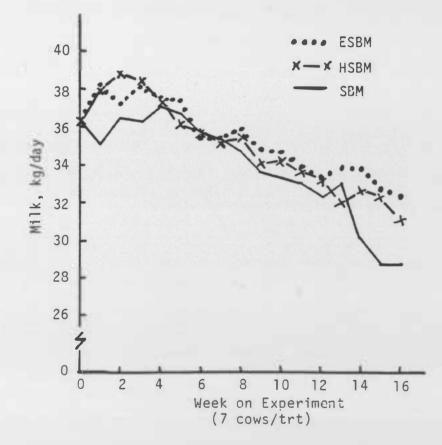


Figure 2. Four percent fat-corrected milk of high producing cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

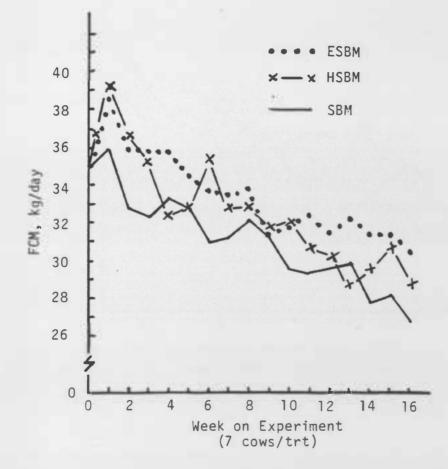


Figure 3. Solids-corrected-milk of high producing cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

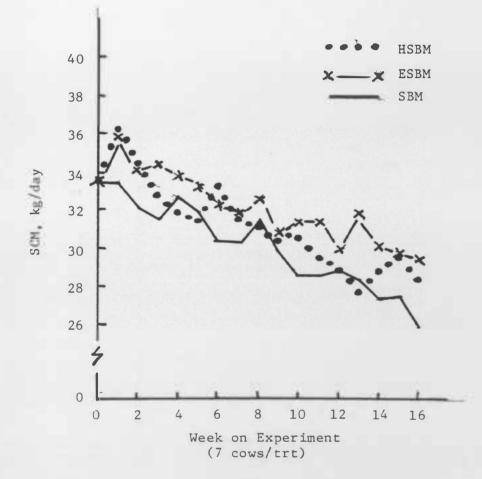


Figure 4. Average milk production of all cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

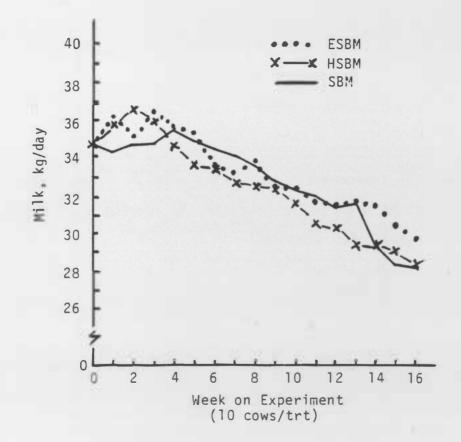


Figure 5. Four percent fat-corrected milk of all cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

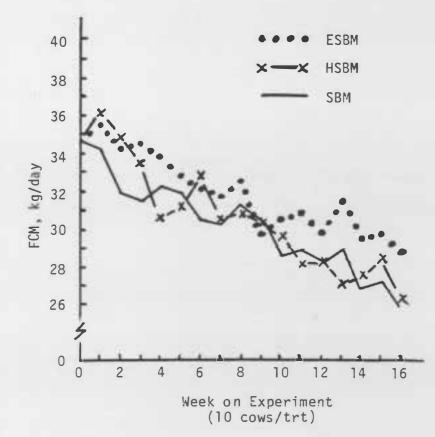
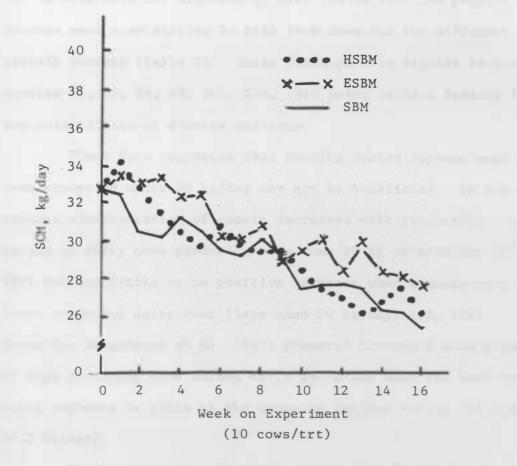


Figure 6. Solids-corrected-milk of all cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).



health disorders were apparent.

Percentage of protein, fat, total solids, and solids-notfat in milk were not affected by diet (Table 8). The protein components were also similar in milk from cows fed the different protein sources (Table 9). These findings were similar to previous studies (1, 65, 89, 99, 105, 126, 134) using various feeding levels and solubilities of dietary proteins.

These data suggested that feeding heated soybean meal to cows producing under 30 kg/day may not be beneficial. In the past, ruminal administration of casein increased milk production 1 to 4 kg/day in dairy cows producing more than 20 kg of milk/day (25, 47, 169) but had little or no positive response when administered to lower producing dairy cows (less than 20 kg/day) (72, 182). Recently, Ruegseggar et al. (143) observed increased milk production by high producing cows during early lactation when fed heat-treated whole soybeans in place of the unheated control ration (37.0 vs. 36.2 kg/day).

The increase in milk yield observed by Netemeyer et al. (114), when cows were fed heat-treated soybean meal was not as dramatic as in the present trial because cows in their trial were in later lactation (14 wk postpartum) and were producing less milk than cows in the present trial. Similarily, Ahrar and Schingoethe (1) were not able to detect a significant increase in milk production when feeding regular or heat-treated (extruded) soybean meal to later lactation (9 wk postpartum) cows producing lower

and the second sec		Ration		
Fraction	SBM	HSBM	ESBM	SE
A Carlot Inter		(%)		
Total nitrogen	.47 ^a (.48) ^b	.47 (.48)	.48 (.48)	.01 (.01)
Protein nitrogen	.44 (.45)	.43 (.45)	,45 (,45)	.01 (.01)
Casein nitrogen	.35 (,36)	.35 (.36)	,35 (,36)	,01 (.01)
Noncasein nitrogen	.12 (.13)	.12 (.13)	.12 (.12)	.01 (.01)
Nonprotein nitrogen	.03 (.03)	.04 (.03)	.03 (.03)	.01 (.01)
Serum protein nitrogen	.09 (.09)	.09 (.09)	.09 (.09)	.01 (.01)

TABLE 9. Nitrogen fractions in milk from cows fed regular soybean meal (SBM), heated soybean meal (HSBM), and extruded soybean meal (ESBM).

^aData from seven high producing cows/trt.

^b() Data from all ten cows/trt.

amounts of milk than the cows in the present study. But, potentially, the greatest response would likely occur in the first 2 mo of lactation that is before the trials (1, 114) were started. Recently, Grummer and Clark (70) reported that varying the solubility of the diet by exposing defatted soybean flakes to various heat treatment did not affect milk production. Although they used high producing cows during early lactation similar to the present trial, they lacked the preciseness of our trial since they used only six cows per treatment and cows were not closely blocked on the basis of expected producing ability. Our observation agreed with the work reported by Kung and Huber (89) who fed a similar diet to cows and observed an increase of 1.2 kg/milk/day from feeding heated soybean meal.

Feed Intake and Efficiency. Cows fed the HSBM diet consumed less (P<.01) feed dry matter and crude protein than cows fed the SBM and ESBM diets (Table 10). However, there were no palatability problems with any diets; even cows fed HSBM consumed 3.5% of their body weight as feed dry matter. The lower dry matter intake of HSBM fed cows was mainly due to the presence of two cows in each of the SBM and ESBM groups which consistently consumed 5% of their body weight. Including feed intake data from these cows inflated the dry matter intakes for SBM and ESBM, which was also reflected in their crude protein intake.

According to Figure 7, none of the cows met protein

Component	SBM	HSBM	ESBM	SE
Dry matter intake, kg/day	22.7 ^{a,d} (23.1) ^{b,c}	21.0 ^e (20.7) ^d	23.8 ^c (23.2) ^c	.36 (.29) ^c
Crude protein intake, kg/day	3.3 ^c (3.3) ^c	3.0 ^d (3.0) ^b	3.3 ^c (3.2) ^c	.05 (.04)
Soluble crude protein intake, kg/day	1.1 ^c (1.1) ^c	.9 ^e (.9) ^e	1.0 ^d (1.0) ^d	.02 (.01)

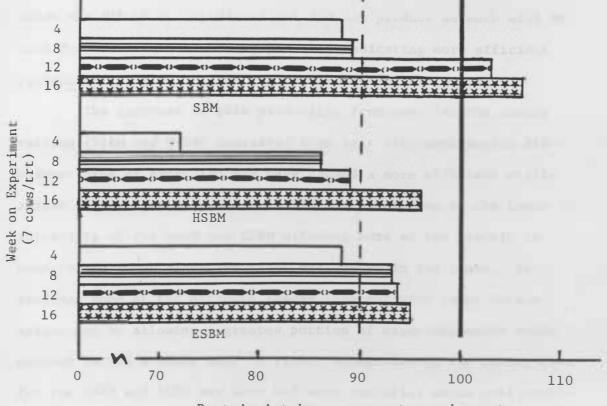
TABLE 10. Average feed consumption from cows fed rations containing regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

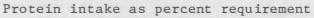
^aData from seven high producing cows/trt.

^b() Data from all ten cows/trt.

 $c,d,e_{Means on the same line and unlike superscript differ, P<.01.$

Figure 7. Protein intake as percent of requirement for high producing cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).





1.6

requirements (113) the first 8 wk on experiment; ESBM and HSBM the first 12 wk on experiment. After that time, all cows were consuming more protein than required, so increased production from heated soybean meals would not have been expected during wk 13 to 16. Cows fed the SBM diet received more protein than they required (113) after the 8th wk on experiment but did not produce as much milk as cows fed HSBM and ESBM diets, possibly indicating more efficient protein utilization by cows fed the heated soybean meals.

The increase in milk production from cows fed the heated rations (ESBM and HSBM) indicated that heat treatment during dissolventizing or extrusion may have caused a more efficient utilization of the dietary protein. This was likely due to the lower solubility of the HSBM and ESBM allowing more of the protein to pass to the lower digestive tract undigested in the rumen. By avoiding some of the nitrogen losses inherent with rumen fermentation and by allowing a greater portion of essential amino acids present in the soybean meal to remain undegraded in the rumen, cows fed the HSBM and ESBM may have had more essential amino acid available for absorption into the blood stream, resulting in increased milk production. Macgregor et al. (97) suggested that the amino acid profile of the undegraded protein which bypasses the rumen may be different from the amino acid profile of the dietary protein as originally ingested. Data reported earlier (Table 5) indicated that the amino acid profile of the rumen undegradable portion may be slightly different than the profile of the total dietary protein.

A number of researchers (1, 68, 94) reported a decrease in loss of fecal nitrogen when feeding heated protein. Others (153, 180) observed a decrease in nitrogen retention as feed proteins were subjected to increased heat. The decrease in nitrogen retention might have been due to overprotection of the protein.

Cows fed HSBM diets were more efficient (P<.05) in converting feed dry matter and feed proteins to milk than were cows fed SBM and ESBM (Table 11). This was mainly accounted for by differences in feed intake and may not reflect an experimental treatment affect. Kung and Huber (89) observed increased feed efficiency when high producing cows were fed heated soybean meal instead of regular soybean meal when cows were provided 17% crude protein in the ration.

<u>Body Weights.</u> Body weights of cows fed the various rations were similar (P>.05) at the start of the experiment and through the first 12 wk of the experiment (Table 12). Cows fed ESBM weighed more (P<.05) than cows fed SBM and HSBM at the end of the experiment. This partially reflected slightly higher initial body weight as well as greater weight gains during the experiment. Some (64, 153, 154) have observed increased weight gains of sheep when protein solubility of diet was decreased. Others (89) reported a decrease in body weight when heated soybean meal was fed to lactating cows without decreasing milk yield. Robinson et al. (140) suggested that at high energy intake, increased protein favors

Component	SBM	HSBM	ESBM	SE
Milk/kg dry matter intake	1.52 ^{a,d} (1.42 ^d) ^b	1,78 ^c (1.68 ^c)	1.52 ^d (1.47 ^d)	.04(.03)
Milk protein/kg crude protein intake	.32 ^d (.30 ^d)	.36 ^c (.34 ^c)	.33 ^d (.32 ^d)	.01(.01)
Milk protein/kg soluble crude protein intake	.99 ^d (.94 ^d)	1.24 ^c (1.17 ^c)	1.11 ^d (1.08 ^d)	.02(.02)

TABLE 11. Feed efficiency for cows fed rations containing regular soybean meal (SBM), heattreated soybean meal (HSBM), and extruded soybean meal (ESBM).

^aData from seven high producing cows/trt.

^b() Data from all ten cows/trt.

 c,d,\bar{e}_{Means} in the same line with unlike superscript differ (P<.01).

		Ration		
Item	SBM	HSBM	ESBM	SE
THE PARTY	19 <u></u>			
Initial	588 ^a (613) ^b	612 (613)	636 (628)	26.2(21.4)
4 weeks	589 (615)	606 (609)	635 (626)	19.6(17.4)
8 weeks	565 (594)	602 (598)	621 (607)	22.0(20.7)
12 weeks	563 (596)	566 (563)	615 (601)	16.3(18.0)
16 weeks (final)	595 ^d (627)	593 ^d (595)	654 ^C (636)	15.7(17.6)
Wt. change	7 (14)	-19 (-18)	18 (8)	

TABLE 12. Body weight of cows fed rations containing regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

^aData from seven high producing cows/trt.

^b() Data from all ten cows/trt.

 $^{c,d}_{Means}$ on the same line with unlike superscript are different, P<.05).

partitioning of energy towards milk rather than tissue stores.

Rumen and Blood Parameters. Volatile fatty acids (VFA), pH, rumen ammonia, and serum urea were not significantly (P>.05) affected by diets during the various sampling hours. Data in Table 13 represents the average values of the four sampling times during the day. Molar percentages of various rumen VFA were similar suggesting that fermentation was similar among rations. This was expected since rumen microorganisms were not subjected to a drastic decrease or increase in the supply of nitrogen. This finding agreed with previous studies (1, 89, 105).

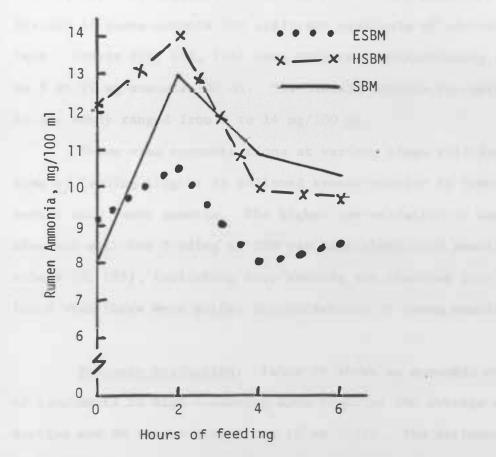
When rumen ammonia concentrations at various times relative to time of feeding were plotted (Figure 8), no significant (P>.05) difference was observed among diets or sampling times, but some trends were apparent. The slightly higher concentration of rumen ammonia in HSBM fed cows prior to feeding was reflected in the higher ammonia concentration 2 h post-feeding in that group. The slightly higher ammonia before feeding in cows fed HSBM may have reflected a slower rate of nitrogen degradation in that diet throughout the day. However, the relative change in ammonia between prefeeding and 2 h post-feeding was greatest for cows fed the SBM diet and lowest for ESBM. Since rumen ammonia may be an indicator of protein degradation rates, the data may indicate that ESEM was more resistant to degradation than HSBM or SB , which agreed with in vitro solubility and degradability studies. This was also

		Ration		SE
Measurement	SBM	HSBM	ESBM	
VFA				
Acetate, mole %	50.2	49.0	50.1	1.44
Propionate, mole %	27.3	26.4	28.4	1.73
Isobutyrate, mole %	1.0	1.2	.9	.12
Butyrate, mole %	15.7	16.8	15.6	.70
Isovalerate, mole %	2.9	2.8	2.5	.20
Valerate, mole %	2.8	3.0	2.4	.34
Total, µm/ml	64.0	66.3	61.7	3.46
pH	6.64	6.59	6.54	.08
Rumen ammonia, mg/100 ml	10.4	10.9	9.1	.93
Serum urea, mg/100 ml	12.8	15.3	12.9	1.34

TABLE 13. Rumen volatile fatty acids (VFA), ammonia, pH, and serum urea in cows fed diets containing regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

Figure 8. Rumen ammonia concentration in cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

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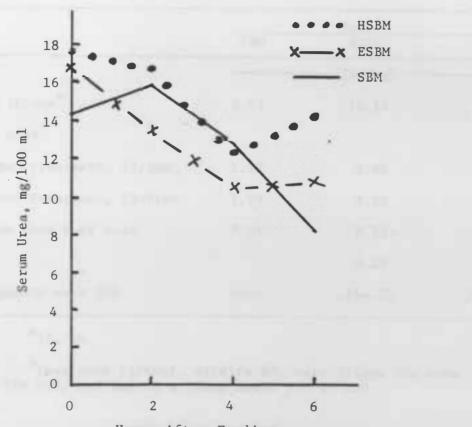
consistent with findings of others (9, 21, 105). Many researchers (4, 10, 17, 145) suggested 5 to 8 mg/100 ml as a maximum concentration of rumen ammonia for efficient synthesis of microbial protein. Others (80, 101, 106) have reported concentrations as high as 9 to 29 mg ammonia/100 ml. The overall ammonia concentration in our study ranged from 8 to 14 mg/100 ml.

Serum urea concentrations at various times relative to time of feeding (Figure 9) followed trends similar to trends observed with rumen ammonia. The higher concentration of serum urea observed with the feeding of SBM was consistent with results of others (1, 105), indicating more ammonia was absorbed into the blood when there were higher concentrations of rumen ammonia.

Economic Evaluation. Table 14 shows an economic evaluation of treatments by high producing cows based on the average milk production and DM intake during the 16 wk trial. The estimated cost of heat treatments were conservative estimates based on limited information available and represents ranges from likely minimum to maximum. Cows fed the ESBM and HSBM diets were more productive and profitable than SBM-fed cows. Cows fed the HSBM diet gave the greatest profits, but this may have partially reflected unusually high feed intake by two cows fed ESBM. The net profit from heated soybean meals instead of regular soybean meal, would be even greater if the calculations were based on the first 4 wk of this experiment, or for the 1st 6 to 10 wk postpartum where the greatest response

Figure 9. Serum urea concentration in cows fed regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM).

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Hours After Feeding

	Ration			
	SBM	HSBM	ESBM	
Milk income ^a	9.55	10.14	10.10	
Feed cost ^b				
Heat treatment, \$5/ton	1.99	1.85	2.10	
Heat treatment, \$30/ton	1.99	1.92	2.18	
Income over feed cost	7.56	8.22-	7.92-	
		8.29	8.00	
Net income over SBM		.6673	.3644	

TABLE 14. Economic evaluation of rations containing regular soybean meal (SBM), heat-treated soybean meal (HSBM), and extruded soybean meal (ESBM) for high producing cows.

a_{13¢/1b}.

^bFeed cost (\$/ton), alfalfa 60, corn silage 20, corn 113, SBM 200, and heated soybean meals 205 or 230. was observed in milk production as a result of feeding the heattreated soybean meals.

CONCLUSIONS

Subjecting soybean meal to additional heat, either during the desolventizing process (HSBM) or by extrusion (ESBM), reduced the solubility and degradability of soybean meal protein as evaluated using several in vitro methods. Nonessential amino acids tended to be more soluble than essential amino acids in soybean meals. Heat treatment tended to increase the percent amino acid in the insoluble and undegradable fraction.

Milk production was increased when heat-treated soybean meals were fed to high producing cows, with most of the increased production occurring the first 4 wk on the experiment (wk 4 through 7 postpartum). The increase was modest with low producers. Concentration of milk fat, protein, protein components, and total solids were similar with all diets. Concentrations of ammonia and volatile fatty acids in the rumen and blood urea were similar in cows fed the various diets. Rumen ammonia peaked at 2 h after feeding in all diets but the relative change in rumen ammonia was highest for cows fed SBM and lowest for ESBM, indicating slower degradation of heated soybean meals in the rumen. Net profits to dairymen would likely be increased by feeding heated soybean meals prepared by either method evaluated to cows capable of high production (>30 kg milk/day) during the first few months of lactation.

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