# INFLUENCE OF ALTERING THE PHYSICAL CHARACTERISTICS OF SOYBEAN MEAL ON PROTEIN UTILIZATION IN THE RUMINANT

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1976

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1979

Thesis
1979D
N469i
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THE RUMINANT

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# ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to Dr. L. J. Bush, Professor of Animal Science, who served as my major adviser for the last five years. His patience, guidance and friendship will long be remembered.

Special thanks is extended to Dr. F. N. Owens, Associate Professor of Animal Science. His research guidance and dedication has made it an honor to work under his council.

Special appreciation is extended to Dr. R. D. Morrison, Professor of Statistics; Dr. E. C. Nelson, Professor of Biochemistry; and Dr. D. L. Garner, Professor of Physiology, for their willingness and knowledgeable help throughout this course of study.

Thanks is extended to Glenden Adams, Herdsman; Dr. S. E. Gilliland, Professor of Animal Sciences; Dr. Jim Williams, Research Associate; and Mr. R. L. VonGunten, Professor of Animal Sciences, for their integrity and friendship during the course of my graduate studies.

Special thanks are due to Steve Bussen, Tom Hoagland, Roger Blair, and Keith Batman for helping collect experimental data at the expense of their own free time and asking nothing in return. Thanks are also due to Ernie Minton, Colin Muncy, Chuck Hibberd, Joycelyn Kapp, Roy Ball, and other graduate students for their friendship throughout the author's graduate program.

A very special recognition and thanks is extended to the author's wife Candy, son Brent, and daughter Cari Jo, for their constant love, support and sacrifice in making this manuscript and life worthwhile.

Also, a special thanks to my parents Mr. and Mrs. Alvin Netemeyer for their guidance through life and encouragement to attain this goal. Thanks also to my wife's mother Elva Slagel for her encouragement and friendship.

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# CHAPTER I

## INTRODUCTION

Since the beginning of recorded time man has relied on animals as a source of high quality food. Among these animals ruminants stand out. Unlike monogastrics (simple stomach animals), ruminants possess a unique ability to digest large quantities of cellulose and hemicellulose. Cellulose contains beta 1-4 glycosidic linkages that are unaffected by any known mammalian enzyme.

Ruminants are characterized by a stomach which is clearly divided into four compartments, namely the rumen, reticulum, omasum and abomasum. The last compartment, the abomasum, is similar to the digestive stomach of monogastrics. Its main digestive function is the secretion of HCl and pepsin. The omasum serves mainly for the removal of water and abrasive activity upon the rumen digesta being passed through it. The reticulum is closely associated with a fold of tissue called the reticulo-rumen fold. The result of this fold joining the reticulum to the rumen allows for transfer to rumen digesta back and forth.

The rumen is inhabited with a multitude of bacteria and ciliated protozoa of many strains and species. It is these microbes that secrete an enzyme, cellulase, which allows for the fermentation of cellulose. Bacterial cellulases effect the hydrolysis of cellulose to form celloboise which in turn is broken down into two molecules of glucose. Glucose is readily fermentated by way of glycolysis to form pyruvic acid. The

pyruvic acid produced is further metabolized into short chain fatty acids, mainly acetic, propionic and butyric acids. These acids (bolatile fatty acids) are absorbed across the rumen wall into the portal blood system (Annison et al., 1957). The absorption rates appear to be enhanced by lower rumen pH (Masson and Phillipson, 1951).

Since the rumen is the first entry point of ingested food, other nutrients are subject to bacterial fermentation as well. Besides carbohydrates, dietary protein is extensively degraded resulting in the formation of ammonia ($NH_3$) and a keto acid. Concurrent with this proteolysis and deamination is a process of microbial protein synthesis. During the normal course of microbial activity digesta is continually moving into the lower gastro-intestinal tract. The digesta passing out of the rumen contains both dietary protein which has escaped microbial degradation and newly synthesized microbial protein. Both forms of protein are subject to enzymatic hydrolysis in the abomasum and the small intestine.

The protein digestion process outlined above has a number of consequences. Many of the rumen bacteria may use ammonia as their sole source of nitrogen for protein biosynthesis. After digestion in the lower tract, the microbial protein provides amino acids for the host animal. Consequently, when the host animal is subject to a poor quality diet, the protein made available to the ruminant is of a much higher quality in terms of the amino acid composition than the protein in the ingested diet. Furthermore, since $NH_3$ is the precursor of much microbial protein, it is of little consequence whether this $NH_3$ comes from non-protein nitrogen (NPN compounds) or from the fermentation of the dietary protein.

The ability to utilize NPN via microbial protein is, however, acquired at a cost. Unlike the situation with cellulose digestion where the ruminant is entirely dependent on microbial activity, the host animal produces enzymes for protein digestion. Due to losses of ammonia the breakdown of dietary protein is not entirely advantageous to the host. An important exception is when, as a result of microbial transformation, the biological value of the digesta mixture reaching the lower tract for digestion is greatly improved over that of the diet.

The microbial conversion of dietary protein to microbial protein breaks down the close relationship between the quality of the diet to that which is actually available to the host. Hungate (1966) found that the composition of the digesta reaching the small intestine consisted of a constant proportion of amino acids. Hogan (1970) reported that the amino acid nitrogen reaching the intestine represented 42 to 127 percent of the nitrogen intake. When expressed in relation to metabolizable energy, the ratio of amino acids released to metabolizable energy ranged from 24 to 40. These data were obtained with herbages ranging in crude protein (CP) from 5 to 32 g/100 g of organic matter and at a level of intake close to _ad libitum_. Thus, neither the nature nor quantity of amino acids presented to the lower tract is closely controlled by dietary intake. Despite this, the ruminant appears to require some amount of protein in a certain amino acid pattern. The attained level of performance is clearly dependent on certain amino acids at the site of anabolism.

Rumen bacteria appear to have the capability of synthesizing all the amino acids that are considered essential to monogastric animals (Virtanen, 1969; Thomas et al., 1951). However, these so called

essential amino acids (EAA) are not synthesized at the same rate (Virtanen, 1969). Thus, many nutritionists have been led to believe that the quality of protein in the ruminant diet is of little consequence. This assumption, along with the fact that animals can utilize NPN as a nitrogen source for protein, has led some to express protein requirements in terms of crude protein (nitrogen x 6.25). Those assumptions appear only to be true at the gastric level of digestion. The quality of protein available at the intestinal and tissue level is one of the critical factors determining the adequacy of nitrogen nutrition of the ruminant. Thus, while ruminants are not likely to experience a lack of any EAA, the problem of insufficiency, particularly at high levels of performance, merits attention.

It has already been pointed out that increasing dietary protein is not a very satisfactory way of elevating the quality of amino acids presented to the lower tract. Nevertheless, the producer is constantly striving for increased levels in milk production and will continue to do so as long as it is economically favorable and not harmful to the cow. Furthermore, with increasing amounts of plant protein being utilized directly for human food consumption, attempts need to be made to improve the utilization of dietary nitrogen in the ruminant.

Recent studies (Hogan, 1975) show that the dairy cow may be deficient in certain amino acids when milk production is very high. The deficiency causes the animal to perform at a suboptimal level below her potential (Clark, 1975). The experiments, presented later in this work, were carried out to determine whether dietary protein can be manipulated to resist rumen degradation, and if so, whether this results in an improved performance by the cow.

# CHAPTER II

# REVIEW OF LITERATURE

## Nitrogen Metabolism

An outline of nitrogen metabolism is presented to illustrate the potential benefit of protecting dietary protein from microbial degradation in the rumen.

### Microbial Degradation of Protein in the Rumen

Ruminants ingest a large variety of nitrogenous compounds, ranging from preformed protein to simpler non-protein nitrogen substances. Such compounds include proteins of varied nature which differ in solubility, amino acid content, purine and pyrimide content, and many non-protein nitrogen compounds such as amino acids, peptides, amides, amines, ammonium salts, nitrates and nitrites, as well as urea and biuret which may be intentionally added by the producer. The seeds of most plants, such as corn, oats, wheat, sorghum grain and barley will have approximately 95 percent of the nitrogen in the form of true protein ($\alpha$ amino nitrogen) and five percent in NPN. However, forages may have as much as 20 percent in alfalfa hay to as high as 50 percent in silages, of their nitrogen in the form of NPN (Waldo, 1968). Nevertheless, true protein is the most common source of nitrogen for the dairy cow.

In the normal diet of the dairy cow, approximately 60 percent of the ingested nitrogen-containing compounds are subject to degradative action by the rumen bacteria and protozoa (Satter and Roffler, 1975). The proteolysis of the ingested protein produces an important end product, namely $NH_3$ (Hogan, 1961). Allison (1970) reported that the main proteolytic bacteria in the rumen were Bacteriodes, Selenomonas and Butyrivibrio, with the main proteolytic protozoa being Endoin, Entodinium, Ophryoscolex, and Eudiplodinium. The products are further metabolized by rumen microbes to produce $NH_3$, $CO_2$ and volatile fatty acids (VFA). The most important $NH_3$-producing bacteria appear to be Bacteriodes ruminicola, Selenomonas ruminantium and Peptostreptococcus elsdenii (Bladen et al., 1961).

The proportion of the diet that is degraded in the rumen depends on several factors. One of the factors contributing to differences in apparent ruminal degradation of proteins is the solubility of the protein in rumen liquid (Wohlt et al., 1976). Soluble and insoluble nitrogen are inherent characteristics of proteins (Chalupa, 1975). The soluble and insoluble protein fractions of feedstuffs can be divided by simply incubating the feed sample in any number of solvents, such as hot or cold water, various buffer solutions or rumen fluid (Owens, 1978). In general, the greater the solubility in rumen fluid, the more readily a feed component is degraded in the rumen. An exception is ovalbumen which is a high quality soluble protein but is not degraded rapidly by rumen microbes in vivo (Mangan, 1972). It was suggested that the cyclic nature of this protein in which there are no terminal amino or carboxyl groups would preclude degradation by exopeptidases. Another exception appears to be fermented feedstuffs which have a high but variable protein

solubility. The increase in solubility through fermentation is attributed to proteolysis by plant enzymes and protein solubilization by acid during storage (Bergen, 1975). Prigge et al. (1978) reported solubilities of 12, 8 and 16 percent for dry, steamed flaked and high moisture corn. Despite this solubility difference, abomasal passage of feed nitrogen, a direct measurement of protein bypass, from these three types of corn was very similar. Apparently, the protein in fermented grain, though solubilized, bypassed rumen digestion. Owens (1978) stated that this finding casts doubt on the value of simple protein solubility as a predictor of rumen bypass with feedstuffs that have been ensiled. Similar results have been observed with distillers grain solubles (Pitchard and Van Soest, 1977).

Considering the wide range in protein solubility which may be achieved by commercial ration formulation, it is clear that more information on soluble nitrogen utilization is needed. In addition, commercial *in vitro* protein solubility measurements which could be related to ruminal protein degradation would be of value. Since rumen ammonia concentrations have been shown to peak within 2 hr post-feeding, it is important to identify the proper solvents for solubility measurements and to measure the soluble protein disappearance during the early stages of protein exposure in the rumen. Kay (1969) reported that highly soluble proteins such as casein or fresh plant cells were rapidly fermented by the rumen proteolytic organisms. When sheep were fed fresh perennial ryegrass the amount of nitrogen reaching the abomasum was markedly reduced as compared to dry grass (Beever et al., 1969). Woods et al. (1957) observed that the solubility of a protein could not fully account for differences in nutritive value. Digestibility appeared to

be a more important factor than solubility. Despite the conflict in results, the general concensus in the literature is that proteolysis is closely related to solubility of the protein.

Retention time is another important factor governing the rumen degradation rate of dietary protein. Retention time may be defined as the length of time a quantity of feed remains in the rumen. The longer the time protein remains in the rumen, the more likely that a greater fraction of it will undergo fermentation. Factors that may influence the retention time are particle size, level of feed intake, and the volume of water consumed (Balch and Campling, 1965).

Anon (1971) ranked feeds according to their solubility or resistance to microbial attack (Table I). It should be noted that the groups of feeds listed in Table I represent a broad classification and large differences may exist within each group. Whitelaw et al. (1964) reported differences in utilization of various types of fish meal by calves attributable to solubility.

McGreggor et al. (1978) listed common feedstuffs according to crude protein, soluble protein and bound protein percentages (Table II). The soluble protein fraction was extracted in a modified version of Burroughs' mineral buffer. Large differences in soluble protein of common feedstuffs are depicted.

It is interesting to note that despite proteolytic activity in the rumen, the concentration of amino acids in the rumen liquor is quite low. Blackburn (1965) observed that the rate of deamination is somewhat slower than proteolysis. If this is true, one should notice an increase in concentration of both short peptides and amino acids in the rumen

TABLE I

RANKING OF FEED ACCORDING TO THEIR EXTENT OF DEGRADATION IN THE RUMEN[a]

| Feed | Degree of Degradation |
|---|---|
| 1. Purified amino acids and soluble protein | 90% resists degradation |
| 2. Proteins from fresh cut material; unprocessed oil proteins | 25-30% resists breakdown |
| 3. Dried fodder (hay) | Slightly greater than 35% resists degradation |
| 4. Extracted oil seed meal | Usually a substantial proportion, but variation according to mode of manufacturing must be considered |
| 5. Fish meal, blood, meat liver and bone meal | Substantially resists microbial attack |

[a]Anonymous. 1971. What Profit from Protected Protein. CSIRO Bulletin. June.

TABLE II

PROTEIN PARTITION OF SEVERAL FEEDSTUFFS[a]

| Ingredient | Crude protein[b] | Soluble protein[c] | Bound protein[cd] |
|---|---|---|---|
| | | (%) | |
| Beet pulp | 9.4 | 2.0 | 10.6 |
| Dried citrus | 6.0 | 25.9 | 10.6 |
| Corn grain | 9.8 | 15.0 | 6.2 |
| Corn distiller's dried grains with solubles | 28.8 | 10.3 | 23.4 |
| Alfalfa grass silage, 2nd cutting | 15.4 | 42.8 | 16.2 |
| Alfalfa grass silage, 1st cutting | 10.3 | 35.9 | 17.1 |
| Oats grain | 12.8 | 31.3 | 6.0 |
| Soybean meal, solvent, extracted, dehulled | 54.9 | 22.4 | 2.5 |
| Soybean mill feed | 15.2 | 22.1 | 5.5 |
| Wheat bran | 17.6 | 43.1 | 3.6 |
| Wheat middlings | 18.0 | 49.2 | 1.1 |

[a]MacGreggor et al. (1978).

[b]Percent of dry matter.

[c]Percent of crude protein.

[cd]Insoluble protein.

immediately after feeding. Deaminase activity, unlike proteolytic, increases with the protein content of the diet.

The products of proteolysis are mainly amino acids and peptides. Peptides are either utilized as such by some bacteria (Wright, 1967) or they are further metabolized to form amino acids. Portugal (1963) studied the degradation of $C^{14}$-glutamic acid and aspartic acid in rumen contents. Most of the labeled carbon was found as $CO_2$, short chain fatty acids and only a small amount in bacterial protein. Under similar conditions protein was being synthesized by incorporation of $C^{14}$ from glucose into the two amino acids of the protein. These results were confirmed by Borchers (1967) who noted that rumen microorganisms have a strong preference for glucose for protein synthesis. This agrees with reports of Hungate (1966) who reported that microbial growth depends on the amount and nature of the dietary constituents and the amount of high energy phosphate compounds (ATP) that can be derived from the substrate.

The essential amino acids are also subject to rumen digestion to form $CO_2$, $NH_3$ and short chain fatty acids. (Stallcup et al., 1966; Williams et al., 1961; Lewis, 1955; Dehority et al., 1958; Menahann and Schultz, 1964; and Lewis and Emery, 1962). For example, El-Shazly (1958) showed that DL-alanine, DL valine and DL leucine were deaminated to the $\alpha$-keto acids and decarboxylated to form $CO_2$ and VFA.

Evidence that dietary protein is extensively altered when microbes convert it to microbial protein has been shown by Smith (1969). Pilgrim et al. (1970) reported that 73 percent of nitrogen of a low protein diet (8.3% CP) and 58 percent in a high protein diet (18% CP) were converted to microbial nitrogen. Similar results were reported by Mathison and

Milligan (1971) who reported that 60-92 percent of the dietary nitrogen intake was transformed into $NH_3$-nitrogen ($NH_3$-N) in the rumen when rations of varying crude protein content were fed. Weller et al. (1958) reported that 63-82 percent of the nitrogen in the rumen was microbial, whereas plant nitrogen constituted only 11-27 percent.

From the information presented above, it is evident that dietary protein is degraded to form $NH_3$ as the main nitrogenous end product. It is this $NH_3$ intermediate that provides the link between dietary protein and NPN compounds. Some of the latter compounds, such as peptides and amino acids undergo the same degradation as dietary protein. The simpler NPN compounds such as urea, biuret, and ammonium compounds, undergo microbial hydrolysis to produce $NH_3$. There is, however, an important difference between the two sources of nitrogen for the host. Urea, for example, can only be utilized by the host after incorporation of its nitrogen into microbial compounds; not withstanding the fact that a small portion of the $NH_3$ may be utilized for synthesis of nonessential amino acids and nucleic acids. On the other hand, dietary amino acids can be utilized directly by the host. Also, after proteolysis and/or deamination in the rumen, this form of nitrogen provides not only $NH_3$, but also branched chain and other fatty acids. These by-products may be absorbed and utilized by the host, or they may be used for cell biosynthesis within the rumen. Carbon skeletons of a specific structure are required for microbial synthesis of some amino acids, and these arise through catabolism of amino acids (Hungate, 1966). Rumen bacteria have been reported to be able to synthesize alanine and glutamate by reductive carboxylation reactions (Allison, 1969).

Besides acting as an energy source for the host, protein supplements may also act as carriers for essential minerals, namely sulfur, phosphorus, manganese, iron and vitamins. Supplying the latter is probably not an important contribution since the rumen microbes are capable of synthesizing all the B complex vitamins (Bryant, 1970). This mineral carrying capacity of proteins is an important aspect when one is considering a feeding regime which is composed of NPN as the main nitrogen source. However, as far as nitrogen metabolism is concerned in general, it is of little consequence whether the $NH_3$ intermediate is from NPN or a dietary plant protein source as long as it is converted into microbial protein.

## Synthesis of Microbial Protein

Concurrent with the process of degrading dietary protein and NPN compounds is the process of biosynthesis of microbial protein. The importance of $NH_3$ as the chief precursor of microbial nitrogen has been reported by many workers. Phillipson et al. (1962) using $^{15}NH_3$ observed that $NH_3$ was a significant source of nitrogen in microbial protein even when preformed exogenous amino acids were present. Later Pilgrim et al. (1970) reported that $NH_3$ was an intermediate in the synthesis of 80 percent of the bacterial nitrogen when the diet contained 10 percent CP and 65 percent when the diet contained 18 percent CP. Since microbial hydrolysis of urea produces only $NH_3$-N, the ability of ruminants to perform at almost maximum levels when fed urea purified diets indicates the importance of ammonia as an intermediate for bacterial protein synthesis (Hume, 1970). Mathison et al. (1971) reported bacterial and protozoal nitrogen values derived from $NH_3$ to be 50-65 and 31-55 percent

of the total nitrogen composition. Approximately 56 percent of the 89 strains of bacteria can be grown with $NH_3$ or protein hydrolzate as the nitrogen source with ammonia being essential for 25 percent of the strains. While it seems almost certain that $NH_3$ is the major source of nitrogen for rumen bacteria, the protozoa in general can very seldom utilize $NH_3$ as their nitrogen source (Smith, 1969). Protozoal nitrogen requirements appear to be related to an absolute quantity of essential amino acids (EAA) and pyrimidine and purine base requirements of the host animal. It is generally believed that the protozoa obtain their nitrogenous nutrient requirements by digesting bacteria or preformed dietary protein particles.

The quantity of bacterial protein that can be synthesized appears to be governed by several factors. The percentage crude protein in the diet, the level of feed intake, the amount of energy available and an optimum mineral supply, especially sulphur and phosphorus. Of all these factors, most researchers appear to agree on the importance of the readily available energy (Satter and Roffler, 1975; Conrad and Hibbs, 1968; Purser, 1970; and Virtanen, 1969). The relationship between microbial protein synthesis and carbohydrate fermentation is a direct one. Addition of starch to the diet of sheep has been shown to improve dietary nitrogen utilization (Lewis and MacDonald, 1958; Dief et al. 1970). Conrad and Hibbs (1968) suggested that for maximum utilization of urea, the quantity of readily fermentable carbohydrates required was about 1 kg per 100 g of urea in dairy cows adapted to urea consumption.

Another important factor is the quantity of nitrogen present in the diet and its physical form. Sater and Roffler (1975) reported that

when the rumen $NH_3$ level reached 5 mg/100 ml of rumen fluid, the microbes became saturated with nitrogen, and adding any further nitrogen to the diet once this concentration is attained, would result in zero utilization of the excess amount added. With this hypothesis Satter and Roffler (1975) further hypothesized that mean ruminal ammonia concentration reached 5 mg $NH_3$-N/100 ml rumen fluid when the ration contains 12.0 percent CP and 81 percent TDN. When TDN is lowered the CP percent allowed before excess $NH_3$ accumulates is also lowered in a linear relationship. When protein requirements are high, there is very little benefit in adding NPN compounds to the diet. Similar predictions were made by Bryant and Robinson (1961) and Allison (1970). All agree that the optimum nitrogen ($NH_3$-N) concentration for maximum microbial growth and efficiency is between 5 and 6 mg $NH_3$-N/100 ml rumen fluid. In contrast Mehrez and Orskov (1977) continuously fed four cannulated sheep a ration composed of varying amounts of ground barley and urea. Rates of barley fermentation in the rumen at various rumen ammonia concentrations were made. Maximum fermentation occurred when the minimum rumen ammonia concentration was 23.5 mg $NH_3$-N/100 ml rumen fluid. Allen and Miller (1972) studied this problem *in vivo* in a similar manner. When allowance was made for 1 gm nitrogen/day as abomasal secretions, the greatest microbial nitrogen flow reaching the abomasum in cannulated sheep was observed when rumen ammonia concentration reached 28.9 mg $NH_3$-N/100 ml rumen fluid. Approximately 70 percent of the $NH_3$-N was converted to microbial nitrogen.

Burroughs et al. (1975) developed a system of equations to predict metabolizable protein that can be synthesized with rations containing NPN compounds. These fermentation potentials are based mainly on the

amount of TDN and amount of nitrogen already in the ration before NPN supplementation. Several researchers have successfully fed urea to lactating dairy cows (Conrad and Hibbs, 1975; Virtanen, 1966; Huber and Thomas, 1971; and Huber, 1975).

Criticism of the mathematical formulas that developed was strongly expressed by Conrad (1976) on the basis that:

1. The models were based on TDN and not specific amounts of starch available.
2. There is no provision for incorporating differences in rumen turnover rates.
3. Much of the information used was obtained from dairy cows in which the feed intakes were not known.
4. The model assumes an average feed intake of 18.1 kg per day, whereas successful urea utilization is dependent on maximizing feed intake.
5. The investigators failed to include results from successful schemes for feeding urea when building their model.

The conflicting opinions of different researchers has caused some confusion. The data suggesting that 5 mg $NH_3$-N ml rumen fluid is the upper tolerable limit for efficient microbial synthesis appears rather firm based on _in vitro_ culture systems and live animal evaluation. Failure of cows to decrease performance in some trials when fed purified NPN rations may be due to low protein requirements and larger than average feed intakes. However, one must bear in mind that most of the feeding trials based upon NPN supplementation to lactating dairy cows

resulted in a decrease in milk production (Thomas, 1971; Gardner and Park, 1973; Polan et al., 1976).

At any rate, all researchers do agree that microbial protein synthesis does occur and is an important source of amino acids to the host animal. Quantitative estimates of microbial protein synthesis have been made by several workers. Some of these estimates are given in Table III. The amount of microbial protein estimated varies from 10.2 to 24.4 g per 100 g of organic matter digested in the rumen. It is surprising that the range is as narrow as it is since these are estimates under a variety of experimental conditions and each has its own assumptions built in. Some of the variable factors encountered in comparing these estimates are: animals used, type of diet, level of intake and procedure used in estimating synthesized microbial protein. In any case, there is a relative constancy of microbial protein production as a function of organic matter fermented in the rumen. This supports the hypothesis that the prevailing anaerobiosis in the rumen limits the extent to which food can be synthesized into cellular protoplasm (Hungate, 1966).

Gray et al. (1958) and Leibholz and Hartman (1972) reported that 50-80 percent of the dietary nitrogen entering the duodenum was altered by microbial activity in the rumen when diets consisted of hay or concentrates or both.

Experiments have been designed to study the effects of feed intake upon non-ammonia nitrogen reaching the lower tract in sheep (Coelho da Silva et al., 1972; Hogan and Weston, 1967; Miller, 1972). It appeared that an increase in feed intake resulted in a proportional increase in the amount of non-ammonia nitrogen reaching the abomasum,

TABLE III

QUANTITATIVE ESTIMATES OF MICROBIAL PROTEIN SYNTHESIZED IN THE RUMEN

| Source | Microbial crude protein g/100 g organic matter fermented |
|---|---|
| Conrad and Hibbs (1968) | 10.2[a] |
| Hogan and Weston (1967) | 15-15.6[b] |
| Hogan and Weston (1970) | 24.4[c] |
| Hume (1970) | 17.1-23.3[d] |
| Hume, Moir and Somers (1970) | 13.3[e] |
| Lindsay and Hogan (1972) | 23[f] (defaunated sheep) |
| Mathison and Milligan (1971) | 13-17[g] |
| Noland and Leng (1972) | 15.0[h] |
| Orskov, Fraser and MacDonald (1971) | 15.6[i] |
| Walker and Nader (1970) | 14.4[j] |
| Zinn et al. (1978) | 15.0[k] |

[a]Per 100 g digestible dry matter. Estimate based on synthesis of methionine from sulfide.

[b]Per 100 g organic matter digested in the rumen. Organic matter taken as the sum of microbial and dietary.

[c]Bacterial crude protein/100 g organic matter digested in the rumen.

[d]Per 100 g organic matter digested in the rumen.

[e]As in foot note d.

[f]Bacterial CP/100 g plant OM digested in the four stomachs.

[g]Per 100 g OM fermented in the rumen, corrected for microbial OM.

[h]Per 100 g OM fermented in the rumen.

[i]Bacterial CP/100 dietary OM fermented in the rumen (bacterial OMT dietary OM disappearing in the rumen.)

[j]Per 100 g OM digested in the rumen. They used the ATP-UFA-microbial protein interrelationships.

[k]Per 100 g OM digested in the rumen.

though measurements to distinguish dietary protein from bacterial protein were not taken. It is conceivable that this increase was a result of an increase in the amount of dietary protein in relation to bacterial protein. The point is that increasing feed intake may reduce carbohydrate fermentation in the rumen thereby reducing microbial protein synthesis (Satter and Roffler, 1975). The question to be asked is: If optimum conditions are employed, will the quantity and quality of protein synthesized by the rumen microbes, plus that fraction of ingested protein which escapes degradation in the rumen be adequate to meet the amino acid requirements of highly productive ruminants?

The quality of protein refers to its amino acid composition. A protein referred to as high quality protein should contain a large number of EAA in a distinct combination. It is generally assumed that the tissues of ruminants do not differ quantitatively from tissues of monogastric animals in relation to nitrogen metabolism. Thus, given sufficient ammonia nitrogen and other nutrients, ruminant tissues can synthesize several amino acids metabolically (Black et al., 1952; Downes, 1961). Schinogoethe et al. (1967) found that bovine mammary gland cells responded to all amino acids considered essential to rat tissue, by actively synthesizing protein. It appears, therefore, that any amino acid indispendable to the rat is also essential for the ruminant. However, the quantity of essential amino acids required by the ruminant need not bear any relationship to that of the rat. For example, wool follicles have a large demand for sulfur containing amino acids. Wool contains approximately 10-16 percent cystine and 14 percent nitrogen (Reis, 1965; Thomas et al., 1951), while milk has a high content of lysine and leucine (Jacobson et al., 1970).

Biological value of Protein in Digesta

Various experimental approaches have been used to assess the value of microbial protein. Estimates have been made of the amino acid composition of the microbial contribution to the digesta reaching the lower gut by examining the rumen contents of animals fed urea as the sole source of nitrogen (Schelling and Hatfield 1968). Bergen et al. (1968) studies mixed microorganisms separated from rumen contents and grown in pure culture. Results of these experiments have shown considerable variation in certain amino acid quantities; however, all agree that lysine content in microbial protein is very high. Ely et al. (1967) reported that methionine and histidine were the amino acids in the lowest quantity in microbial protein. These results were confirmed by Lieboltz and Hartman (1972) when duodenal contents of sheep fed five different diets were analyzed.

The true digestibility of rumen bacteria and protozoa have been reported to be 74 and 89 percent with biological values of 91 and 80 percent, respectively (McNaught et al., 1954). Rumen bacteria were considered to be generally less digestible than protozoa. This may be due to an increased amount of non-amino and amino acid nitrogen present in bacteria cell walls. Mason (1971) concluded that 80 percent of bacterial nitrogen is absorbed by rats. Similar results were reported by Bergen et al. (1968). Purser and Buechler (1966) reported that rumen bacteria contain up to 75-80 percent of their nitrogen in the form of protein, peptides and free amino acids. Smith (1969) has shown that rumen bacteria contain about 20 percent nucleic acids. Ellis and Pfander

(1965) reported a value of 15 percent for nucleic acid composition of microbial protein.

The digestibility and biological values cited above refer to studies of digesta fed to rats. While useful for comparative purposes, they only approximate the true values in ruminant digestion. Also, the microbial cells fed were acid hydrolyzed and might not necessarily be a good indicator of the biological value of a protein.

A number of workers have investigated the relationships between the composition of the diet, or of the microbial population in the rumen, and the concentrations of amino acids in the blood plasma of ruminants, but the results are difficult to interpret. Weller (1957) and Bergen et al. (1968) have examined the effect of diet on the amino acid composition of rumen microorganisms. Both concluded that the diet has no effect on microbial protein amino acid composition. Leiboltz and Hartman (1972) concluded that, despite large differences, there was little variation in the amino acid composition of the bacterial fraction among diets. A typical amino acid composition of bacterial protein is listed in Table IV. From the above results it seems reasonable to assume that the type of diet has little effect upon the amino acid composition of rumen microorganisms.

## Metabolizable Protein Concept

In order to relate microbial growth and protein production to ration characteristics, attempts have been made to relate the amount of microbial nitrogen gained from a quantity of feed fermented in the rumen (Burroughs et al., 1973b; Satter and Roffler, 1975; Roy et al., 1977;

TABLE IV

AMINO ACID COMPOSITION OF RUMEN BACTERIA AND PROTOZOA[a]

| Amino Acid | Bacteria | Protozoa |
|---|---|---|
| Aspartic acid | 6.7-6.8 | 7.4-8.4 |
| Threonide | 3.5-3.8 | 3.1-3.7 |
| Serine | 2.5-3.0 | 2.6-3.2 |
| Glutamic acid | 6.6-7.5 | 7.9-8.7 |
| Proline | 2.1-2.8 | 1.9-2.9 |
| Glycine | 5.9-6.3 | 4.7-5.7 |
| Alanine | 6.4-6.5 | 4.1-4.6 |
| Cystine | 0.7-0.8 | 1.1-1.3 |
| Valine | 4.4-4.5 | 3.6-4.1 |
| Methionine | 1.5 | 1.1-1.4 |
| Isoleacine | 3.6-3.8 | 4.3-4.9 |
| Leucine | 4.5-4.7 | 5.0-5.7 |
| Tyrosine | 2.0-2.2 | 2.0-2.4 |
| Phenylalanine | 2.3-2.5 | 2.8-3.3 |
| Histidine | 2.6-3.0 | 2.6-3.4 |
| Lysine | 7.5-7.8 | 10.6-12.2 |
| Arginine | 8.6-9.3 | 8.1-10.6 |

[a]Weller et. al (1958)

Zinn et al., 1978). In the applications of the various systems, it is desirable to relate microbial nitrogen to feed fermented.

Burroughs et al. (1973b) proposed a system for defining protein requirements and the value of various feed ingredients for cattle. The system was devised to overcome the deficiencies of the 6.25 multiplication system. The measurements employed were designated as "Metabolizable Protein" or "Metabolizable Amino Acids". Metabolizable protein or amino acids is the quantity of protein or amino acids that are absorbed in the postruminal portion of the digestive tract of ruminants.

The system deals directly with the ruminant tissue amino acids needs and the fulfillment of these requirements. The metabolizable amino acid supplies to the ruminant tissues arise from 1) absorbable amino acids from the digested ration ingredients that have escaped rumen destruction and 2) digested proteins which arise from rumen microbial synthesis (Satter and Roffler 1975). The digestion in each case takes place in the true stomach and small intestine of cattle, a situation paralleling the measurement of digestible protein in nonruminants.

A factorial system was established for determining the net protein and amino acids deposited in the milk daily, plus the daily net needs of these constituents for maintenance. The net body maintenance needs for protein (g/day) for various sized animals were established by using the formula developed by Smuts (1935): maintenance g = (0.0125) ($70.4\ W_{kg}^{.734}$). The second step in determining requirements was to convert the total net protein needs into total metabolizable needs on the basis that 53 percent of the metabolizable protein (MP) is lost in metabolism for maintenance and five percent is lost in metabolism for milk production. These estimates were derived from growth and

lactational trials with purified (Oltjen et al., 1972) and natural diets in cattle (Burroughs et al., 1973b). The metabolizable amino acid requirements for maintenance and milk production were based on the amino acid composition of cattle body tissues and milk composition respectively (NRC 1971).

One of the greatest advantages of the metabolizable protein system is that one can predict the metabolizable amino acid value of a ration. To accomplish this the undergraded dietary protein reaching the abomasum was first established for each feedstuff. Next, the degraded protein converted to microbial protein which reached the abomasum was based on a nitrogen conversion of 100 percent up to an amount that did not exceed 51.2 g protein/kg of concentrate feed dry matter. A maximum of 25.6 g of microbial protein was used in the case of roughages. The values were then transformed into abomasal protein. The quantities of amino acids derived from protein digestion in the abomasum and small intestine were determined on a basis of an apparent coefficient of digestion of 78 percent for microbial protein and the apparent coefficient of digestion given for the feedstuff in NRC (1964) feed composition tables.

The urea fermentation potential for feeds (UFP) expresses the amount of urea that can be useful in a given cattle ration (Burroughs et al., 1975). A positive UFP value becomes an important segment of the MP system when urea is part of the ration. A positive UFP value of a ration can be the estimated g of urea/kg of dry feed consumed, that can be used for rumen microbial protein synthesis. The formula used in estimating UFP values is: UFP = (1.044 TDN-B)/2.8. The value 1.004 represents the estimated potential net grams of microbial protein that

results from the consumption of 10 g of TDN (10.44 % of TDN). B in the formula is the estimated grams of protein in 1 kg of feed dry matter consumed and degraded in the rumen, contributing ammonia to the total rumen pool. The 2.8 transforms protein to urea nitrogen equivalence.

It appears that where lactation rations consist entirely of natural proteins and where no supplemental NPN is involved, the new MP system has little advantage over present convential systems. However, the MP system suggests that supplemental urea is highly beneficial and can be added to rations containing natural protein supplements, provided the ration has a positive UFP value. Satter and Roffler (1975) developed an alternative method for calculating metabolizable protein with the final expression in terms of crude protein. Both systems agree that urea can be beneficial in typical dairy rations as long as the crude protein does not exceed 12 percent, feed is fed at rates to supply adequate quantities of energy, and when lactation does not exceed 20 kg milk/day. Neither of the two proposed metabolizable protein systems has been widely adopted for ration formulation under practical conditions.

Poos et al. (1977) tested the effectiveness of the UFP system in feeding NPN to lactating dairy cows. When a 13 percent total protein control diet having UFP of - 1.71 g/kg D.M. was supplemented with urea or SBM to 17 percent total protein, milk yield increased approximately 2 kg/day in cows consuming either diet. The use of non-protein nitrogen was equal in its effect with that of natural protein. Similar results were reported by Orskov (1977).

Roy et al. (1977) proposed a system which offers considerable merit. The following conditions and assumptions are used:

1. It is assumed that under optimum conditions 30 g of microbial nitrogen are gained/kg organic matter fermented in the rumen (Zinn et al., 1978).
2. The energy value of fermented organic matter is 3.72 Mcals ME/kg.
3. The nitrogen requirement of the rumen system is equal to 30 g/kg organic matter fermented in the rumen, or 8.06 g/Mcal ME available in the rumen.
4. Of the total digestible organic matter, 65 percent is fermented in the rumen.
5. The energy value of digested organic matter is 4.54 Mcal digestible enerby/kg.
6. The conversion of digestible energy to metabolizable energy is 82 percent.
7. Eighty-two percent of microbial nitrogen is in the form of amino acids.

This results in a calculation of 15 g microbial protein produced per 100 g organic matter digested in the rumen. This value agrees very well with previous estimates (Table III).

The advantages of this system over systems based on TDN (Burroughs et al. 1973), and rumen ammonia concentrations (Satter and Roffler, 1975) are that the founding relationships are based on actual microbial growth parameters rather than total digestive measurements (TDN) or a residue in the case of rumen ammonia. The system proposed by Roy et al. (1977) is presently being adopted in practical dairy cattle feeding systems in Europe.

Ammonia Excretion

As mentioned earlier, production of ruminal $NH_3$ may exceed microbial uptake of this substance, depending on the level of dietary nitrogen and fermentable substrate consumed. Excess ammonia is absorbed across the rumen wall and enters the portal blood (Hogan, 1970). Lewis (1957) showed that the portal blood $NH_3$ concentration, increased as a curvilinear function of the rumen $NH_3$ content. Visek (1968) reported that blood in the portal vein contained ammonia concentrations several times greater than that of peripheral blood and that one passage of portal blood through the kidney removes virtually all the ammonia delivered from the alimentary canal. Pilgrim et al. (1969) found that 3 - 4.6 g of $NH_3$-N was absorbed per day from the rumen of sheep fed high protein hay diets. Mathison et al. (1971) estimated that 1.6 and 7.2 g of nitrogen were absorbed when sheep ingested 10.6 and 16.8 g of nitrogen per day. Later, Nolan and Leng (1972) estimated only 2 g of nitrogen was absorbed when sheep ingested 23.4 g of nitrogen. Though the above estimates do vary considerably, all agree that increases in $NH_3$ absorption is directly related to nitrogen intake.

Besides concentration of $NH_3$, the pH of the rumen contents also influences the rate of absorption. Combe et al. (1960) showed that the unionized form of this compound was absorbed much more rapidly than the charged form. The pKa of the ammonium ion is 9.3 (Schenk et al. 1977). Therefore, the ionized form accounts for more than 99 percent of $NH_3$ at normal rumen pH. At pH of 6.5 it appears that the rate of absorption is dependent on concentration gradient, but not dependent at pH 4.5 (Hogan, 1961). Under normal conditions the liver is capable

of detoxifying all the $NH_3$ delivered to it. When the peripherial $NH_3$ concentration reaches 1.0 - 1.6 mg/100 ml of blood, the capacity of liver is overwhelmed and toxicity occurs (Lewis and McDonald, 1958).

Ammonia absorbed into the blood from the rumen is not all necessarily wasted. Though large amounts are converted to urea, some may be used for the synthesis of dispensable amino acids and other nitrogen compounds. Preston et al. (1965) found blood $NH_3$ levels were proportional to CP intake. When wether lambs were fed rations containing 9.2 to 22 percent CP, the blood urea levels ranged from 2.7 to 32.9 mg/100 ml. The amount of urea in the blood excreted in the urine is proportional to the level of nitrogen intake (McIntyre, 1970). Another portion of the urea in blood is recycled back into the rumen through the epithelium or via saliva. The fact that urea nitrogen can enter the rumen from the blood has been demonstrated by Vercoe (1969) and McIntyre (1971). Vercoe (1969) found that the amount of nitrogen that enters the rumen of a 396 kg Herford steer was approximately 17 - 20 g N/day. The transfer occurred when 32-35 g urea-N per day was infused intravenously and the steer ingested 0.08 g N per day. Waldo (1968) calculated that the amount of urea transferred across the rumen wall was four to six times the parotid secretion. Cocciamano and Leng (1966) showed that the total endogenous urea influx was a function primarily of blood urea concentration which in turn was a function of nitrogen intake.

The importance of nitrogen recycling under practical conditions has been shown by various workers. Hogan and Weston (1967), Hume (1970) and Leibholz and Hartman (1972) reported that at low nitrogen intakes the output of nitrogen from the rumen exceeded intake. This amazing phenomenon allows for reutilization of metabolized nitrogen and allows

for ruminants to exist for short periods of time on a nitrogen deficient diet.

The above discussion has shown that neither quantity nor the quality of the diet has any influence on the amino acid composition of microbial protein. Yet the ruminant depends on the microbial protein as a source of EAA. The general effect of microbial intervention in protein utilization is to alter the amino acid composition of dietary protein to that of microbial protein (Potter et al., 1969). As was previously discussed, this may be a disadvantage when high quality proteins are in the diet. The literature shows that increasing protein intake is not a very satisfactory method of increasing available EAA to the host animal. Clark et al. (1966) varied the amino acid nitrogen intake of sheep from 3.6 to 17.1 g/day but the quantity reaching the small intestine only varied from 6.2 to 12.9 g. This shows that by increasing amino acid nitrogen only a small fraction of the increase reaches the small intestine. Similar results were reported by Topps et al. (1968) and Leibholz (1972).

## Evidence that EAA Supply May Limit Animal Productivity

The question to be discussed deals with the controversy of whether microbial protein, in addition to that fraction of dietary protein that escapes rumen degradation, is sufficient in both quantity and quality to meet amino acid requirements of highly productive ruminants. The sufficiency must be evaluated in terms of amino acids that are absorbed from the lower tract in relation to requirements for maintenance and production. While rumen microorganisms enable the ruminant to utilize

NPN and upgrade the quality of poor dietary protein, the microbial intervention is distinctly wasteful of high quality dietary protein. This is particularly true when the dietary protein is offered in excess of 12-15 percent of dry matter intake (Satter and Roffler, 1975). Despite this, the tissue demands increasing amount of EAA as production increases. If this demand is not met, production cannot be maintained for extended periods of time.

There is evidence that high producing dairy cows may suffer from insufficiency of amino acids. Clark (1975) reported that when all trials were summed up, post ruminal infusion of casein (a high quality protein) increased milk production in lactating cows from 1 to 4 kg per day.

Hogan (1975) listed the amino acid composition of milk, muscle and digesta in a high producing dairy cow on a normal diet (Table V). Based on several assumptions, it was concluded that a 630 kg cow producing 44 kg of four percent milk per day was deficient 230 gm of methionine per day, at the peak of her lactation. This deficit can only be accounted for by assuming that protein catabolism of body tissue occurred. A large amount of the research in this area has been done with sheep. Schelling (1970) reported an increase in nitrogen retention and plasma methionine level when methionine or casein was infused abomasally into growing lambs fed 11 or 14 percent CP diets containing corn and legume hay. When 0, 1.0 and 2.0 g of DL-methione were infused per day, nitrogen retention was 1.46, 2.07 and 3.11 g per day per lamb, respectively. The plasma levels of methionine were 3.7, 6.9 and 11.9 μg/ml, respectively for the three treatments. Schelling and Hatfield (1968) observed that infusing casein and amino acid mixtures into lambs on a purified urea diet improved nitrogen retention and voluntary feed intake.

TABLE V

ESSENTIAL AMINO ACID (EAA) COMPOSITION OF VARIOUS PROTEINS (g/16 g NIT)[a]

| Amino Acid | Milk | Muscle | Wool | Rumen Digesta |
|---|---|---|---|---|
| Valine | 7.0 | 5.5 | 5.7 | 5.9 |
| Cystine | 1.0 | 1.2 | 15.0 | 0.8 |
| Methionine | 3.2 | 3.2 | 1.0 | 1.9 |
| Isoleucine | 7.5 | 6.0 | 4.5 | 4.9 |
| Leucine | 11.0 | 8.0 | 8.0 | 7.9 |
| Phenyalanine | 5.5 | 5.0 | 3.0 | 5.2 |
| Lysine | 8.7 | 10.0 | 3.0 | 5.6 |
| Histidine | 2.6 | 3.3 | 1.0 | 1.8 |
| Arginine | 4.2 | 7.7 | 10.0 | 4.7 |
| Threonine | 4.7 | 5.0 | 7.7 | 4.2 |
| Tryptophane | 1.5 | 1.4 | 1.2 | 1.5 |
| Total EAA | 56.9 | 56.3 | 60.1 | 44.4 |

[a]From Hogan (1975).

Post-ruminal infusion of casein or soybean improved its utilization, as compared to oral administration in growing wethers (Little and Mitchell, 1967). Reis and Tunks (1969) and Colebrook and Reis (1969) have reported significant improvements in the wool growth of sheep when sulfur amino acids were infused into the abomasum as to when these sources were orally administered. Fisher (1972) reported increases in milk protein and feed intake when lactating dairy cows were intravenously infused with L-methionine. Similar results were reported by Broderick et al. (1970). These results indicate that under certain conditions, ruminants may suffer from an insufficiency of EAA.

The fraction of ingested protein that escapes degradation in the rumen varies somewhat depending upon the factors discussed earlier. It represents the fraction that the producer may have some control over and thus influences the quantity of EAA absorbed by the intestine. The possible ways of increasing the amount of dietary protein escaping rumen degradation may be by: a) dietary proteins could be chosen which resist degradation to a greater extent, b) the solubility of the protein in question might be reduced, c) the retention time (time the protein remains in the rumen) might be reduced thereby allowing less time for degradation or d) the protein might be physically altered, and e) gastric groove closure similar to the action in suckling calves.

## Protection of Dietary Protein Against Degradation in the Rumen

### Chemical Treatment

Proteins can be treated so that degradation in the rumen is reduced (Ferguson, 1975). If such treatments are to be used with expectations

of improved performance, the dietary proteins presumably need to be of high quality. However, one can speculate that partial prevention of rumen degradation of a protein that may be considered to be of a low quality as a sole source of protein may improve performances. This speculation might be explained by the supplementing effect of the dietary protein upon the microbial protein. Zelter and Leroy (1966) proposed that tannins could be used to decrease rumen degradation. They treated five peanut and soybean meal samples with aqueous solutions of tannins (13-15%) and dried them at high temperatures. In *in vitro* studies the untreated proteins were rapidly degraded by the rumen microorganisms, whereas the tannin-treated protein escaped degradation. Six fistulated sheep were used to study ammonia production, volatile fatty acid production and changes in blood urea levels in animals consuming the two diets. Over two g of ammonia per hour were produced from the animals consuming the untreated protein whereas animals consuming tannin-treated protein produced only one g ammonia per hour. The blood urea levels were at maximum two hours post feeding. Later Driedger and Hatfield (1972) reported improvements in average daily gains and feed efficiencies when steers were fed tannin treated soybean meal.

Ferguson et al. (1967) reported that casein treated with formaldehyde had significantly lower solubility (8% vs 83%) as compared to untreated casein. Seventy-four percent of untreated and none of the treated casein was degraded after six hours of *in vitro* incubation in rumen fluid. After 24 hours incubation, 89 percent of the untreated and four percent of the treated casein were degraded. The formaldehyde-treated casein also increased wool growth by 70 percent when growing lambs were fed either treated or untreated casein.

Sibbald et al. (1968) reported that a dietary product consisting of a core of methionine, kaolin and triglycerides, enveloped in a continuous film of triglycerides, reduced rumen degradation and increased methionine concentration in blood plasma. When this product was fed to steers, it raised free methionine in the blood plasma and increased methionine to valine ratios. Grass and Unangst (1972) confirmed that coating methionine with tristearin would provide protection from ruminal degradation, but the release of encapsulated methionine was poor in the lower digestive tract. These workers subsequently discovered that a combination of tristearin plus a liquid unsaturated fatty acid or oil would overcome the problem of poor release.

When the above preparation was fed in a 14 percent crude protein diet to sheep, nitrogen retention increased. When two g methionine was included in a 10 g preparation plasma methionine increased, whereas further supplementation with four g methionine in 20 g preparation or six g methionine in 50 g preparations exceeded the animal's requirement and showed no additional benefit above the 10 g preparation. Broderick et al. (1970) found no improvement in milk production when lactating cows were fed 5, 15, or 45 g/day of encapsulated methionine. Increases in plasma methionine: valine ratios suggest that some protection did occur but the cows failed to respond to increases in methionine.

Structural manipulation of amino acids to create resistance to ruminal degradation has received some attention. Griel et al. (1968) reported significant increases in the production of four percent fat-corrected milk when 40 g and 80 g of methionine hydroxy analog (m-analog) were fed to 42 lactating cows. These results were confirmed by Bishop and Murphy (1972) and Polan et al. (1970). In contrast, Bauchard and

Conrad (1973), Fuquay et al. (1974) and Hutjens and Schultz (1971) all reported a decrease in production of milk and milkfat when m-analog was fed to lactating dairy cows. Belasco (1972) reported that m-analog has showed no more resistance to ruminal degradation than free methionine. Other investigators of in vitro rumen fermentation systems (Gil et al., 1973; Emery, 1971; Hall et al., 1972) concluded that m-analog was completely degraded in the rumen. Chandler et al. (1976) reported no increases in milk production of lactating cows fed m-analog in a large scale feeding trial conducted in several states; however, milkfat percent and conception rates were both significantly improved. Similar findings were reported by Bhargava et al. (1977).

The results have been contradictory, and somewhat confusing. From the standpoint of nutrition, the magnitude of response to supplemental methionine is interrelated with other nutritional factors. The three most apparent factors appear to be total dietary protein, sulfur and energy. The responses observed may have been due to the increase in sulfur intake when m-analog is fed (Bull and Vandersall, 1973). Based on recently reestablished sulfur requirements (Bauchard and Conrad, 1973), it seems feasible that in the later research of Chandler et al. (1976) the responses may have been due to a contribution of methionine to the lower tract for digestion. The author expresses concern of making any conclusive statements until further research has been done.

By far the most research on chemically treating proteins has been done with formaldehyde. Treatment of casein with formaldehyde has been shown to reduce the solubility of protein in the rumen (Ferguson et al. 1967; Isaacs and Owens, 1972). Hemsley et al. (1970) reported that formaldehyde treated clover hay increased the amount of non-ammonia

nitrogen (NAN) reaching the small intestine from 14.4 to 23.3 g per day. Treatment of plant proteins with formaldehyde has been shown to improve growth rates and feed efficiencies (Reis and Tunks, 1969; Faichney, 1972; Faichney, 1971). However, Clark et al. (1974) observed no production increases when formaldehyde treated soybean was fed to lactating dairy cows. Improvements in growth rate and feed efficiency in sheep fed formaldehyde treated proteins have not been as impressive as improvements in wool growth. The fact that no positive response has yet been reported in terms of milk production may mean that methionine is not limiting in the lactating dairy cow. This is further substantiated with the fact that post ruminal infusions of methionine have not increased milk production significantly and the increase of total methionine yield in the milk is very small. Further research is needed to justify any conclusions that may have been made.

Physical Aspects of Reducing Rumen Degradation

There seem to be several alternatives for reducing or preventing the degradation of proteins in the rumen so that they would pass to the abomasum and intestines for subsequent digestion. Feeding a natural protein such as zein, which resists degradation in the rumen is one alternative but it also is poorly digested in the small intestine (Little and Mitchell, 1967). Thus, the use of a protein of this type probably offers no particular advantage.

Perhaps the most promising approach proposed thus far is the modification of good quality protein to markedly reduce its susceptibility to microbial attack in the rumen without reducing its nutritive value in the lower gut. Several experiments have shown that heat treatment

of protein in the presence of carbohydrates will decrease their solubility and rumen degradation. Little et al. (1963) reported that the solubility of SBM nitrogen was reduced by heating. When growing lambs were fed heat treated SBM at 12 percent CP, a significant increase in live weight gain was observed above non-heat treated, but no differences were observed when the rations were raised to 17 percent CP. Glimp et al. (1967) reported that decreasing protein solubility by heat treatment resulted in decreased levels of valeric and isovaleric acids. Since these acids are intermediates of the amino acid catabolism of phenyalanine and valine, it indicates a reduction in proteolysis. Ahrar et al. (1977) reported a five percent improvement in milk production when lactating cows, in early lactation, were fed heat treated SBM as compared to regular SBM. However, total lactational yield did not differ. In a subsequent experiment (Ahrar and Schingoethe, 1978) fed regular and heat treated SBM to 12 Holstein calves. No differences (P>.05) were observed in live weight gain, dry matter intake or digestibility of ration components. However, blood urea nitrogen concentration was lower two hr after feeding in calves fed heat treated SBM, suggesting that the heat treatment may have reduced the rate of protein degradation in the rumen.

The natural rumen bypass method is closure of the gastric groove to provide an extension of the esophagus from the cardia to the reticulo-omasal orfice. Factors thought to influence groove closure include age, temperature of the liquid, posture of the animal while drinking, site of delivery into the esophagus, and chemical composition of the liquid (Chalupa 1975). Rumen bypass of nutrients by closure of the gastric groove has resulted in significant improvements in growth rate and feed efficiency (Orskov et al., 1970). Standaert et al. (1978)

trained 19 Holstein heifers, just prior to freshening (23 mo.), to suckle fluids from a nipple pail. Four animals from two different training groups were used in a crossover experiment to determine the effect of suckling milk on milk and milk protein production. The control group received 500 g casein daily in the concentrate mix. The suckled group was allowed whole milk up to 500 g milk protein daily. Milk, milk protein yields and milk protein percentages were higher ($P<.05$) for the suckled group. The suckling group received only 93 percent of NRC protein requirements as compared to 106 percent for the control group. An average of 20.12 percent of the suckled protein was accounted for by an increase in milk protein yield. Although this procedure would seem to have certain limitations from a practical standpoint, it seems to have considerable advantage for intensive rearing of young animals during the period when amino acid requirements are high and for research purposes in establishing amino acid requirements.

Another approach has been to select feedstuffs for dairy rations based on their solubility characteristics. Majdoub et al. (1978) fed lactating dairy cows high and low protein rations, formulated to contain a high and low protein solubility within each protein level. The protein levels were 13 and 15 percent and the two nitrogen solubilities were 22 and 42 percent. The rations were computer-formulated for different protein solubility from commonly used natural ingredients. Milk yield was five kg higher ($P<.05$) for cows consuming the high protein low soluble ration as compared to high protein high soluble ration. Soluble nitrogen did not affect average daily intake of dry matter, crude protein or net energy of lactation. The author considers the above experiment to be an important break-through for practical dairy

nutrition. However, when selecting or processing feeds for low solubility, one needs to be certain that 1) sufficient soluble protein or NPN is present in the ration to maintain ruminal ammonia and 2) that the insoluble protein is of high quality and is digestible in the lower gastrointestinal tract (Owens, 1978).

Procedures that increase the rate of passage of feed particles through the rumen may effectivley reduce the extent of their fermentation in the rumen. Factors known to influence the rate of passage of digesta include food intake, specific gravity, particle size of diet, concentrate to roughage ration, and rate of rumen digestion (Balch and Campling, 1965). Orskov et al. (1971) reported that as with other nutrients, the amount of protein which reached the small intestine depended upon feed intake. Hemsley (1967) indicates that a high salt intake might induce such a train of events by increasing water consumption and decreasing residence time of digesta in the rumen. Ferguson (1971) reported that water intakes and wool growth rates were approximately doubled when a diet containing 20 percent salt is fed to ruminants. However, the long term effects of high salt intake on animal performance and health are not known. McGilliard (1961) obtained evidence that a low ratio of hay to concentrate in the diet may result in a preferential increase in rate of passage of the concentrate part of the diet from the rumen. In this study, it seemed that passage of the concentrate was considerably more rapid when the hay to concentrate ration was 30:70 than when the ratio was 0:100 or 7-:30. As a consequence, digestion in the lower gut was highest with the 30:70 ratio.

From data of five experiments, Balch and Campling (1965) observed that the rate of passage of hay was primarily related to the particle

size. In trials feeding dairy cattle, ground hay, as a small addition to long hay, was excreted more rapidly than long hay, but completely ground hay was excreted over a longer period than long hay. Digestibility of hay components was not altered by increased rates of passage. In contrast Stielau (1967) reported that feed intake was a more important factor affecting rate of passage than degree of fineness. O'Dell et al. (1963) observed that peleted hay fed to dairy cows had a faster rate of passage than either ground or long hay. Weakley et al. (1977) observed that the particle size of SBM was related to ruminal protein disappearance. Soybean meal of two different particle sizes was suspended by way of nylon bags in the rumen of fistulated steers. The amount of SBM retained in the nylon bags after a specified time was greater with the SMB of a large particle size than that of fine. Limited research has been done in this area. Rates of passage from the rumen are significantly increased as particle sizes and specific gravity decrease (Hungate, 1966). Percent ruminal digestion may also increase with feedstuffs of a smaller particle size. There is need for more information on the extent to which the particle size of SBM is related to ruminal degradation and production criteria in lactating dairy cows.

Even though the diet has little effect upon the microbial composition, the digesta reaching the lower gut is composed of a microbial fraction and a dietary fraction. Undegraded dietary protein is often considered to be identical to that of the feedstuff. Yet soluble protein generally has higher biological value, lysine content and digestibility than the total feed protein (Owens, 1977). Studies with proteins treated to decrease solubility suggest that the value of protein which by-passes rumen degradation may lie in the ability to complement the amino acid

profile of the microbial protein and not necessarily the biological value. Some sacrifice in digestibility may be necessary to maximize intestinal digestible protein. Wohlt et al. (1973) and MacGregor et al. (1978) have shown that there is a wide range in protein solubilities among feedstuffs commonly used in the animal industry. From these studies the observations of differences in protein quality among common feedstuffs, it is evident that the ratios of feed protein to microbial protein (and thus amino acid profiles) presented to the lower gut for absorption can vary markedly on normal diets. Wohlt et al. (1976) fed wethers four isonitrogenous rations with amino acid profiles resembling either hominy feed or SBM and protein solubilities of either 13 or 35 percent. Water consumption, urine volume, urinary nitrogen and rumen ammonia and butyrate were higher ($P<.05$) for wethers receiving the high soluble protein rations. Sheep receiving the rations with amino acid profiles similar to SBM had a lower dietary ($P<.01$), higher non dietary ($P<.01$) fecal nitrogen being excreted and a higher ($P<.01$) percent true digestible protein. Nitrogen degradation in the rumen and animal nitrogen metabolism can be influenced through diet formulation. Sniffen (1974) found a significant decrease in nitrogen retention with increased dietary protein solubility in diets formulated from common feedstuffs for Holstein heifers. The above discussion does suggest that the profile of amino acids reaching the lower gut from common feedstuffs may be an important criteria when selecting feedstuffs of low solubility and should be considered in further research in ration formulation for ruminants.

# CHAPTER III

## EFFECT OF PARTICLE SIZE OF SOYBEAN MEAL ON PROTEIN UTILIZATION IN STEERS AND LACTATING COWS


### Summary

Particle size of soybean meal effects upon protein utilization were examined in three trials. In experiment 1, four steers with ruminal and abomasal fistulas in a partially replicated 3 x 3 Latin square were fed rations of prairie hay and ground corn, supplemented with either 3.2 percent urea (U), 22.3 percent soybean meal finely ground (F), or 22.3 percent soybean meal of a coarser particle size (C). The protein content of the respective rations were 18.2, 20.0, and 20.1. Abomasal non-ammonia nitrogen (g/day) passage was 160, 228, and 198. Calculated by difference nitrogen bypass for rations F and C were (g/day) 56 and 26. Rumen ammonia concentrations or digestibility of ration components were unchanged by soybean meal particle size. In experiment 2, 12 Holstein cows in a crossover design were fed rations containing 5.3 percent sorghum silage, 8.4 percent prairie hay, 45.2 percent corn, and 20.1 soybean meal in either the F or C form. Rumen ammonia concentrations for cows fed the F and C rations at 3 hr post feeding (mg/100 ml) were 12.2 and 13.9 ($P<.01$). Rumen ammonia concentrations at 5 or 7 hr post feeding did not differ and yield of milk and milk fat percentage were unchanged ($P<.05$). In experiment 3, 24 Holstein cows were fed F or C soybean meal at levels

of 14.5 and 32.8 percent of the concentrate ration. Higher milk yield, rumen ammonia and blood urea were noted for cows consuming the high protein rations but particle size had no effect on these factors.

## Introduction

A wide variety of nitrogenous compound will support growth of rumen bacteria. Ammonia is the common denominator (Hendrickx, 1963; Hungate, 1966). Under optimal conditions, it apparently is of little consequence for most bacteria whether ammonia is derived from the hydrolysis of non-protein nitrogen compounds or from the fermentation of preformed dietary protein.

The optimum concentration of ruminal ammonia required for maximum cell yield has not been established. However, Satter and Roffler (1975) observed that the efficiency with which ammonia was incorporated into microbial protein decreased as ammonia concentrations exceeded 2 to 5 mg/dl. Purser (1970) estimated a microbial cell yield of 18.3 g of digestible protein for each megacalorie of digestible energy. Using this estimate, Chalupa (1972) calculated that a dairy cow entirely dependent upon microbial protein synthesized in the rumen for milk protein synthesis could produce only 10 kg of milk per day, and that protein for higher yields must be coming from dietary protein escaping rumen degradation. Flatt (1967) reported that a cow fed a urea purified diet produced 2,630 kg of milk, while her monozygotic twin fed a natural diet produced about twice as much during 305 days. In a second lactation, production of the cow fed the urea purified diet was about 65 percent of that of her twin.

Chalupa (1975) indicated that ruminal degradation of dietary protein ranges from 40 to 80 percent. Satter and Roffler (1975) used 60 percent as an average value for feeds typically found in dairy rations. Factors affecting the amount of protein degradation in the rumen include the amount of protein ingested, solubility of the protein in rumen fluid, and the length of time the protein is retained within the rumen.

Weakley et al. (1977) observed that particle size of soybean meal (SBM) was inversely related to both *in vitro* protein degradation and the rate of protein disappearance from nylon bags suspended in the rumen of fistulated steers. If ruminal retention time were unchanged, one would expect greater bypass of coarse soybean meal.

The objective of this work was to evaluate the effect of SBM particle size on quantity of protein escaping rumen degradation, rumen ammonia concentration and ration disgestibility of fistulated steers and on production of lactating dairy cows.

## Materials and Methods

*Trial 1*. Four mature Herford steers containing both ruminal and abomasal fistulas were assigned to one of three experimental diets in a 3 x 3 Latin square design with three 1-week periods. During each period, one of the three treatments was replicated with the extra animal. The diets (Table VI) were formulated to contain approximately 19 percent crude protein using SBM or urea as the supplemental nitrogen source. Particle size of the SBM was fine (250 μm geometric mean diameter) in one ration and coarse (2,400 μm) in another. Both SBM diets were compared to the negative control diet consisting mainly of corn and urea. All

TABLE VI

INGREDIENT COMPOSITION OF RATIONS

| Ingredient | IRN[a] | Trial 1[b] | | | Trial 2[b] | Trial 3[bc] | |
|---|---|---|---|---|---|---|---|
| | | SBM-F | SBM-C | Urea-basal | | Low | High |
| Corn, ground | 4-21-018 | 66.7 | 66.7 | 85.8 | 45.2 | 83.2 | 64.9 |
| Soybean meal | 5-21-119 | 22.3 | 22.3 | -- | 20.1 | 14.5 | 32.8 |
| Urea | ------ | -- | -- | 3.2 | -- | -- | -- |
| Prairie hay | 1-07-956 | 10.0 | 10.0 | 10.0 | 8.4 | -- | -- |
| Sorghum silage | 3-04-468 | -- | -- | -- | 25.3 | -- | -- |
| Dicalcium phosphate | 6-01-080 | 0.5 | 0.5 | 0.5 | 0.5 | -- | -- |
| Defluorinated phosphate | 6-01-78 | -- | -- | -- | -- | 1.1 | 1.1 |
| NaCℓ | ----- | 0.5 | 0.5 | 0.5 | 0.5 | 0.6 | 0.6 |
| Sulfur (lab.grade) | ----- | -- | -- | d | -- | -- | -- |
| Limestone | 6-02-632 | -- | -- | -- | -- | 0.6 | 0.6 |
| Total Protein[b] | | 20.0 | 20.0 | 20.0 | 17.5 | 16.5 | 23.5 |

[a]International reference number

[b]Dry matter basis

[c]Concentrate mixture

[d]15 mg/100 kg

three diets were isonitrogenous and isosulfurous. The steers were fed 6.7 kg/daily in two equal feedings for the first four days of each period and at 2 hr intervals for the remainder of each period. Polyethylene glycol (70 g) and chromic oxide (15 g) were included in the diets each day to estimate the ruminal liquid and particulate phase outflow. Samples of rumen fluid, abomasal contents and feces were collected at 1230 and 1830 hr on the last day of each period. The rumen samples were mixed immediately after collection with 7 ml of 3.9 N HCl to retain the ammonia. The abomasal contents were centrifuged for separation into liquid and particulate phase. Feces samples were collected by rectal grab procedure.

Trial 2. Twelve lactating Holstein cows, 7 to 9 wk postpartum, were adjusted to a 60:40 concentrate to forage ration (day matter basis) under ad libitum intake conditions for a period of two wk. Then they were assigned randomly to two ration treatments in a cross-over design consisting of two 4-wk periods. The first two wk of each period were allowed for adjustment to the new rations and comparison data were obtained during the last two wk. The test rations (Table VI) had a 66:34 ratio (dry matter) of concentrate to forage and differed only in particle size of SBM, (1900 μm vs. 251 μm geometric mean diameter). The cows were fed to provide 100 percent of 1971 NRC net energy requirements and 125 percent of the NRC recommendations for total protein. Feed allotments were determined according to milk production. Rumen samples were collected at 3, 5, and 7 hr after feeding on the last day of each test period. Hydrocloric acid (3.9 N) was added to lower the acidity of the samples to approximately pH 5. Samples were frozen until analyses at a later date. Milk production was recorded twice daily, with samples

from four consecutive milkings each week composited for analysis of fat and total solids.

Trial 3. Twenty-four lactating dairy cows (21 Holsteins and 3 Ayrshires) at 7 to 9 wk post-calving were challenge fed a 60:40 concentrate to forage ration for a period of 2 to 4 wk. At the end of this adjustment period cows were assigned randomly to either a high or low protein group. Intake of the high protein group was calculated to meet 100 percent of the 1971 NRC standard. The low protein group was fed to meet 75 percent of their protein requirement. Both groups were fed to meet 100 percent of the NRC standard for net energy. Within each protein group the cows were assigned randomly to two treatment sequences for three 4-wk periods in a switchback design (Brandt, 1938, Lucas 1956). The first 2 wk of each period were allowed for adjustment to new rations to minimize carry-over effects. The concentrate mixture (Table VI) contained SBM of two different particle sizes, (1,500 μm and 250 μm). The forage consisted of sorghum silage and sudan hay. The concentrate to forage ratio was altered each period to attain the protein intake criteria set at the beginning of the experiment.

The cows were fed twice daily 2 hr prior to milking. Milk weights were recorded at each milking and samples from four consecutive milkings each week were composited for determination of milkfat and total solids percentage. Feed refusals were recorded daily. Rumen and blood samples were collected on the last day of each period at 2 and 4 hr after feeding. Rumen samples were collected by stomach tube, treated with 3.9 N HCl and frozen until analyzed for ammonia by a micro-ammonia technique Chaney and Marbach, 1962). Venous blood samples were treated with a .5 M oxalic acid and centrifuged. The supernatant solution was frozen

until analyzed for urea concentration using a spectrophotometric technique (Chaney and Marback, 1962).

## Results and Discussion

Trial 1. Soybean meal from a common source constituted 22.3 percent of the total diet for the steers consuming the finely ground and coarsely ground SBM (Table VI). The SBM differed only in particle size. As a percent of total nitrogen intake, SBM provided 62 and 57 percent.

The control diet (Table VI) contained no SBM but had 3.2 percent urea to maximize microbial growth. Abomasal protein passage with the control diet served to estimate the basal yield of microbial protein from the non-SBM portion of the ration. The total non-ammonia nótrogen (NAN) reaching the abomasum was 160 g/day for steers consuming the control ration and 228 g/day and 198 g/day for the steers consuming the finely and coarsely ground SBM rations, respectively (Table VII). By difference from the control ration, bypass of nitrogen from finely and coarsely ground SBM was 56 g and 26 g nitrogen per day or 51.7 and 24.2 percent respectively. Since a higher amount of corn grain was fed in the control diet, the amount of corn nitrogen passing to the abomasum of steers consuming the control may be higher than for steers fed the finely and coarsely ground SBM diets. Consequently, estimated bypass should be an underestimate. In contrast, if addition of SBM to a ration increases amount or efficiency of microbial protein synthesis in the rumen (Maeng and Baldwin, 1976), a portion of the increased bypass with SBM could be bacterial. This would lead to an overestimate in passage as calculated by difference. Relative bypass of SBM nitrogen from the fine and course SBM should remain valid since both the values

TABLE VII

EFFECT OF PARTICLE SIZE OF SOYBEAN MEAL ON DEGRADATION IN RUMEN OF STEERS (TRIAL 1)

| Item | Treatment | | | SE |
|---|---|---|---|---|
| | Urea-basal | SBM-C | SBM-F | |
| Dry matter intake, g/day | 5881.0 | 5917.0 | 6003.0 | |
| PEG intake, g/day | 70.0 | 70.0 | 70.0 | |
| $Cr_2O_3$ intake, g/day | 15.0 | 15.0 | 15.0 | |
| Nitrogen intake, g/day | 189.2 | 188.9 | 175.2 | |
| SBM nitrogen intake, g/day | | 107.7 | 109.2 | |
| Urea nitrogen intake, g/day | 84.7 | | | |
| Abomasal contents | | | | |
| Liquids | | | | |
| Nitrogen, mg/dl | 99.3 | 93.8 | 91.0 | 4.94 |
| $NH_2$-N, mg/dl | 8.1 | 6.0 | 5.6 | 0.53 |
| PEG, mg/dl | 113.0 | 93.0 | 80.0 | 8.47 |
| Solids | | | | |
| Nitrogen, mg/g DM | 29.5 | 34.6 | 37.6 | 1.66 |
| Cr, mg/g DM | 2.9 | 2.7 | 2.5 | 0.16 |
| Total abomasal non-$NH_3$ nitrogen, g/day | $160.2^{ac}$ | $197.8^{bce}$ | $228.1^{bde}$ | 11.54 |
| SBM nitrogen by-pass, g/day | | 26.1 | 56.4 | |
| SBM nitrogen by-pass, % | | 24.2 | 51.7 | |
| Rumen ammonia concentration, mg/dl | 11.9 | 11.6 | 12.4 | 1.79 |
| Apparent Digestibility | | | | |
| Dry Matter | | | | |
| Ruminal | 40.2 | 35.7 | 32.0 | 4.1 |
| Total tract | 68.3 | 71.9 | 69.1 | 2.0 |
| Protein, Total tract | 66.4 | 65.1 | 60.7 | 4.2 |

[ab]Values in same row having different superscripts are different (P<.05)

[cde]Values in same row having different superscripts are different (P<.01)

were calculated by a difference. Results suggest that more intact protein nitrogen was bypassed from finely ground SBM than from coarsely ground SBM.

In previous work at this station (Weakley et al., 1977) incubation of coarse and fine particle SBM *in vitro* or in nylon bags suspended in the rumen resulted in a greater rate of digestion of fine than of coarse SBM. However, rumen retention time was not considered with the nylon bag or *in vitro* techniques. The differences observed in this trial may be attributable to a greater rate of passage of the finely ground SBM particles from the rumen and reduced time for ruminal degradation.

The percent SBM degradation that occurred in the rumen was 44 and 74 for steers consuming the finely and coarsely ground SBM diets. Similar estimates of 77 percent (Kropp et al., 1977) and 39 percent (Hume, 1974) were reported by other researchers.

The concentrations of rumen ammonia (Table VII) at 1 hr post feeding were not different ($P>.05$) among treatment groups. Ruminal digestibility of ration dry matter, and a total apparent digestibility of dry matter and protein also were not different among treatment groups ($P>.05$).

*Trial 2*. The cows consuming the two experimental rations consumed equal amounts of dry matter (Table VIII). Consequently there were no differences in total protein or grain protein intakes. Particle size of the SBM did not alter the acceptability of the rations.

Mean ruminal ammonia concentration at 3 hr after feeding was higher ($P<.01$) in cows fed the coarsely ground SBM treatment than in cows fed the finely ground SBM (Table VIII). Differences in ammonia concentrations in cows at 5 or 7 hr after feeding were not different ($P>.05$). If the

TABLE VIII

INTAKE, RUMINAL AMMONIA AND PRODUCTION OF COWS FED SOYBEAN MEAL (TRIAL 2)

| Item | Treatment | | |
| --- | --- | --- | --- |
| | SBM-C | SBM-F | SE |
| Intake | | | |
| Dry matter, kg/day | 18.8 | 18.8 | |
| Total protein, kg/day | 3.3 | 3.3 | |
| Soybean protein, kg/day | 2.0 | 2.0 | |
| Ruminal ammonia | | | |
| 3 hr post-feeding, mg/dl | 13.9[a] | 12.2[b] | 0.27 |
| 5 hr post-feeding, mg/dl | 11.7 | 11.2 | 0.27 |
| 7 hr post-feeding, mg/dl | 11.0 | 10.9 | 0.27 |
| Production | | | |
| Milk, kg/day | 25.7 | 25.7 | 0.78 |
| SCM, kg/day | 23.5 | 24.2 | 0.75 |
| SNF, % | 8.7 | 9.0 | 0.26 |
| Fat, % | 3.5 | 3.5 | 0.21 |

[ab]Values in same row having different superscripts are different ($P<.01$).

increase in rumen ammonia 3 hr post feeding represents an increase in proteolysis of the coarse SBM and not reduced microbial use of liberated ammonia, the data are in agreement with trial 1 suggesting that more of the finely than the coarsely ground SBM is passing intact to the lower tract.

Yield of total milk or solids corrected milk and percentages of milkfat and non-fat solids were not altered (P>.05) by treatments (Table VIII). Since the cows in this trial were fed in excess of their protein and energy requirements, differences in production responses due to treatments were not anticipated. It is of interest to note that milkfat percentage is maintained at a normal level even though roughage made up only 34 percent of the total diet.

Trial 3. Dietary protein concentration influenced rumen ammonia concentrations both at 2 and 4 hr post feeding (Table IX). Concentrations were approximately 3 mg/dl higher with the higher protein concentration. Ruminal ammonia levels were not altered (P>.05) by SBM particle size. The rumen ammonia concentrations at 4 hr were considerably lower than at 2 hr for both the high and low protein groups. There were no differences (P>.05) in the magnitude of decrease for the coarsely and finely ground SBM treatments within the low or high protein group in contrast to results from trial 2. Failure to observe these differences in trial 3 may be due to difference in particle size used in the two trials, higher roughage concentration in the diet or sampling time differences (3 hr vs. 2 hr post feeding). In trials 1 and 2 the coarsely ground SBM had a geometric mean diameter of 2.4 and 1.9 mm respectively as compared to 1.5 mm in trial 3.

Blood urea nitrogen concentrations were not different (P>.05) between the cows receiving fine SBM and those receiving coarsely ground

TABLE IX

INTAKE, RUMEN AMMONIA, BLOOD UREA AND PRODUCTION OF COWS FED SOYBEAN MEAL (TRIAL 3)

| Soybean meal, particle size | Protein level: Low, Coarse | Low, Fine | SE | High, Coarse | High, Fine | SE |
|---|---|---|---|---|---|---|
| Daily intake | | | | | | |
| Feed dry matter, kg/day | 18.1 | 18.3 | 0.29 | 20.8 | 20.4 | 0.27 |
| Protein, kg/day | | | | | | |
| Total | 2.45 | 2.51 | 0.05 | 3.42 | 3.44 | 0.08 |
| Soybean meal | 0.89 | 0.93 | 0.27 | 1.02 | 1.08 | 0.91 |
| Ruminal ammonia, mg/dl | | | | | | |
| 2 h post-feeding | 7.5 | 7.6 | 0.79 | 11.0 | 10.2 | 0.55 |
| 4 h post-feeding | 4.9 | 3.3 | 1.02 | 7.8 | 9.8 | 0.62 |
| Blood urea nitrogen, mg/dl | | | | | | |
| 2 h post-feeding | 8.7 | 8.9 | 0.77 | 11.5 | 12.4 | 1.28 |
| 4 h post-feeding | 7.1 | 8.2 | 0.74 | 11.0 | 11.1 | 0.92 |
| Production | | | | | | |
| Milk, kg/day | 26.5 | 26.6 | 0.25 | 30.6 | 31.1 | 0.29 |
| SCM, kg/day | 26.0 | 25.7 | 0.44 | 29.9 | 30.1 | 0.52 |
| Fat, % | 4.0 | 3.9 | 0.12 | 3.9 | 3.8 | 0.17 |
| SNF, % | 8.7 | 8.7 | 0.09 | 8.7 | 8.9 | 0.07 |

[ab] values in same row having different superscripts are different ($P<.05$)

SBM in either the high or low protein group Table IX). Time had little effect on blood urea concentrations within protein groups. Cows consuming the high protein rations had higher blood urea concentrations at both 2 and 4 hr after feeding than did cows consuming the low protein ration.

Milk yield of cows in the high protein group was higher than that of cows in the low protein group (Table IX). Dietary protein concentration had little effect on milkfat or non-fat solids percent. Effect of SBM particle size on milk yield was small (P>.05) but tended to favor those fed the fine SBM. Percentage of fat in milk from cows consuming the coarsely ground SBM was slightly higher than in that of cows fed the finely ground SBM.

Cows in the high protein group had higher intake of dry matter, grain protein, and total protein (Table IX). Since feed allotments were made according to milk production and estimated net energy requirements, this reflects a greater persistency of lactation for cows in the high protein group. There were no differences (P>.05) in dry matter intake between cows consuming the coarse or finely ground SBM treatments. SBM protein as a percentage of the total protein for the low protein and high protein groups was approximately 29.5 and 54.5 percent, respectively. The protein intake as a percent of the NRC requirements was 84 percent for the cows in the low protein group as compared to 108 percent for the cows in the high protein group. Failure to obtain a production response may be a result of: 1) lack of an increase in bypass protein, 2) reduced bacterial protein synthesis as more protein bypassed the rumen, or 3) no need for supplemental post-ruminal protein. Results from trial 1 and production response to the higher protein level in

trial 3 would question the first and third explanations. Bypass estimates suggest that particle size of SBM may alter protein bypass but response in milk production remains to be demonstrated.

## CHAPTER IV

## INFLUENCE OF HEAT TREATMENT OF SOYBEAN MEAL ON *IN VITRO* DRY MATTER AND NITROGEN DIGESTIBILITY

### Summary

A modified two-stage *in vitro* digestion procedure was used to estimate the effect of heat treatment of SBM upon microbial and enzymatic digestion. In experiment 1, regular processed SBM was heat treated at 116°C for 0, 5, 10, 20, and 30 minutes, respectively. A 500 mg sample of each SBM was subjected to 24 hr incubation in rumen fluid, with a duplicate 500 mg sample being subjected to 24 hr rumen fluid incubation plus 24 hr incubation in pepsin solution. Percent dry matter disappearance (DMD) and nitrogen disappearance (ND) decreased with each additional heat treatment of SBM. Percent total DMD and total ND also decreased with each additional heat treatment of SBM. Thus, it appears that heat treatment of SBM under these specified conditions would not improve protein nutrition in the ruminant.

In experiment 2, SBM heat treated by the flash desolventizing system and classified according to protein dispersion index (PDI) was obtained from Farmland Industries. Lower PDI values indicate greater heat treatment. The same *in vitro* procedure was used as above. Two trials were

conducted with the difference being the incubation time in pepsin solution. Rumen fluid and pepsin solution incubation times were 12 and 12 hr for trial 1 and 12 and 34 hr for trial 2. In trial 1 ruminal DMD and ruminal ND were consistently lower with each additional heat treatment of SBM. Total DMD and ND of SBM were not affected by heat treatment. Similar results were observed in trial 2; however, differences in microbial DMD and ND were small between the heat treated SBM classified as PDI-20 and -30 or -40 and -50. Total DMD and total ND were lower for heat treated SBM of PDI-10, -20, and -30 as compared to -40 and -50. In summary, heat treatment of SBM by the flash desolventizing system substantially decreased microbial DMD and ruminal ND without greatly altering total DMD and total ND. Heat treated SBM corresponding to PDI-10 appears to be optimum.

## Introduction

In protein nutrition of ruminant animals the goal is to maximize microbial growth, decrease ruminal degradation of high quality plant proteins and maximize the quantity and quality of digestible protein reaching the small intestine. This goal becomes more evident when the protein needs of a high producing dairy cow are considered. The optimum ammonia nitrogen ($NH_3$-N) required in the rumen environment for maximum microbial growth is not agreed upon by different researchers (Satter and Roffler, 1975; Mehrez and Orskov, 1977; Allen and Miller, 1976). However, the principal goal of protein nutrition in the ruminant is not disputed.

Studies on the influence of level of protein solubility on nitrogen metabolism in ruminants generally indicate that the degradation of protein in the rumen increases with protein solubility (Lewis, 1957;

Tagari et al., 1962; Nishimuta et al., 1973; Peter et al., 1971; Chalmers et al., 1954; Glimp et al., 1967). Several different solvent systems have been used to measure the solubility characteristics of a given protein. Solubility of a protein in rumen fluid more closely reflects the short term digestion in the rumen as estimated by ammonia concentrations and protein disappearance than solubility in diluted salt solutions (Little et al., 1963; Hendrickx and Martin, 1963). These scientists have shown that the availability of amino acids for absorption in the lower gastro-intestional tract is influenced by the solubility characteristics of the dietary protein, and that amino acids can be limiting in the ruminant. Based on studies with proteins of different solubilities, it appears that the value of protein which bypasses rumen degradation may lie in its ability to complement the amino acid profile of microbial protein (Sniffen and Hoover, 1978). Wohlt et al. (1973) has shown that there is a wide range of protein solubilities amond common ruminant feedstuffs. Thus it is very important to consider the amino acid profile of the bypassed protein and to consider only high quality digestible proteins when treating to reduce solubility.

There are considerable discrepancies in using solubility of a protein as an index of the extent of protein degradation in the rumen. Based on a study of several measurements in different laboratories, Owens (1978) stated that buffer solubility explains less than one-third of the variability in rumen bypass. Use of a solubility index to predict protein utilization may be inadequate since it is independent of rumen turnover and total degradability. Also the precise role of solubility as a predictor of nitrogen metabolism is unclear (Bull et al., 1979).

Nevertheless solubility may be used for gross estimates of the extent that a given protein will be degraded in the rumen until a better system is developed.

The purpose of this study was to investigate the influence of heat treatment of SBM, at different degrees of treatment, upon its ability to resist degradation using a modified two-stage *in vitro* digestion procedure.

## Materials and Methods

*Experiment 1*. SBM (44% CP) of regular processing was steam heat treated at 116°C for various lengths of time in a closed chamber. A modified two-stage *in vitro* digestion procedure was used to measure the disappearance of dry matter and protein nitrogen (Appendix). The first stage consisted of a 24 hr incubation of SBM in a rumen fluid-buffer solution consisting of 71 percent nutrient buffer solution and 29 percent rumen fluid. Rumen fluid was collected from a ruminal fistulated lactating dairy cow fed a 60:40 concentrate to forage ratio. The rumen fluid was strained four times through double layer cheesecloth and placed in an insulated thermos previously heated to 37°C. The rumen fluid was mixed with a nutrient buffer solution and purged with carbon dioxide ($CO_2$). Twenty-five ml of the rumen fluid nutrient buffer solution (RFBS) was added to prewarmed 50 ml polypropylene centrifuge tubes containing 500 mg of SBM. The samples were incubated for 24 hr, centrifuged and the supernate poured off. At this point half of the tubes were dried and half (replicates) were subjected to 25 ml of pepsin solution of pH 1.0. The pepsin treated samples were incubated for an additional 24 hr. At the end of this second incubation period, the samples were centrifuged, supernate poured off, and dried at 80°C for 48 hr.

Nitrogen content of the residues was determined by the Kjeldahl procedure. The disappearance of dry matter and nitrogen were calculated.

Experiment 2. Soybean flour meal (51% CP), heat treated by a flash desolventizing procedure, was obtained from Farmland Industries. The meal was classified according to a Protein Dispersion Index (PDI) as an indicator of the extent of heat treatment that occurred (Appendix).

A similar two-state *in vitro* digestion procedure was used as in trial 1. Two trials were done with different second stage incubation times. Trial 1 consisted of 12-hour stage 1 and 12-hour stage 2 incubation times as compared to 12-and 24-hour incubation times for the second trial. The disappearance of dry matter and nitrogen was calculated the same way as in experiment 1.

## Results and Discussion

Trial 1. Ruminal dry matter and nitrogen disappearance of SBM, as estimated by incubation of samples in a rumen fluid nutrient buffer solution, were decreased with heat treatment (Table X). Microbial dry matter disappearance (DMD) was lower with each additional increase in heat treatment of SBM. The microbial nitrogen disappearance (ND) from SBM was also reduced with additional SBM heat treatment, except for the difference between the 10 and 20 minute treatments. However, total DMD and ND were reduced in a similar manner as the microbial DMD and ND (Figure 1). The pepsin dry matter and nitrogen disappearance is the difference between the total and microbial DMD and ND. It appears that over protection of SBM protein occurred since total DMD and ND both decreased at almost the same rate as did microbial DMD and ND. Thus, very

TABLE X

MEAN *IN VITRO* DRY MATTER AND NITROGEN DISAPPEARANCE OF SBM AT VARIOUS HEAT TREATMENTS

| Measurement[b] | Treatments[a] | | | | | |
|---|---|---|---|---|---|---|
| | 0 min | 5 min | 10 min | 20 min | 30 min | SE |
| Microbial DMD | 76.53 | 66.52 | 59.63 | 44.91 | 37.78 | 2.14 |
| Microbial ND | 84.18 | 69.68 | 57.66 | 49.85 | 48.30 | 2.60 |
| Total DMD | 84.86 | 71.23 | 68.63 | 64.47 | 57.41 | 0.88 |
| Total ND | 86.92 | 75.61 | 71.87 | 67.17 | 62.08 | 0.79 |

[a]SBM heat treated at 116°C for varying lengths of time. Values represent means of three observations.

[b]DMD, dry matter disappearance; ND, nitrogen disappearance.

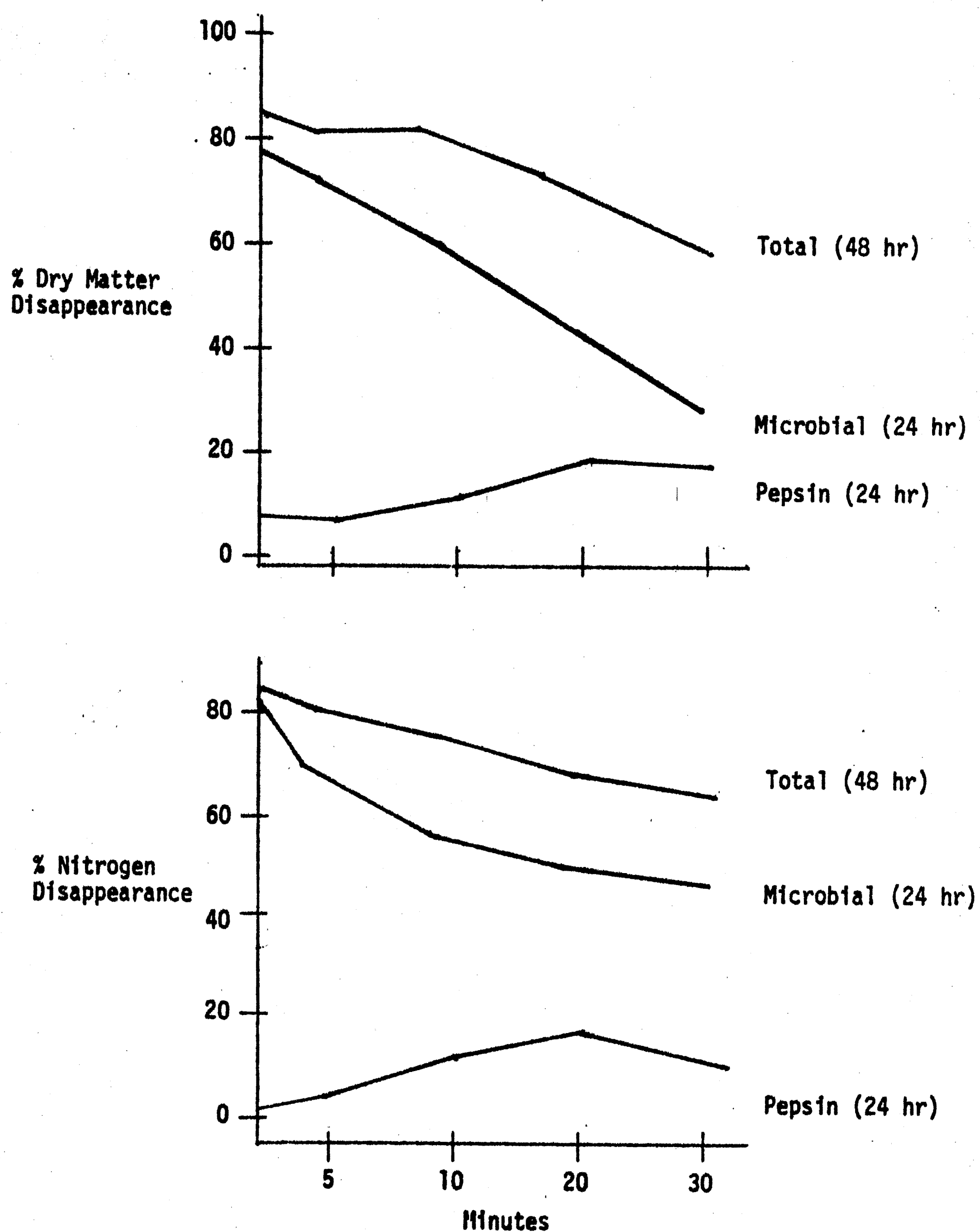


Figure 1. The Influence of Heat Treatment of SBM on *In Vitro* Disappearance of Dry Matter and Nitrogen

little improvement could be expected in animal performance from ruminal protein bypass if ruminants were fed SBM heat treated under the specified conditions in this trial.

Trial 2. There was a substantial decrease in microbial DMD and ND of SBM with increasing heat treatment or lower PDI. Total DMD and ND were not substantially reduced with increased heat treatment of SBM Table XI). It appears that the SBM protein was more protected from a rumen fluid environment than was SBM with less heat treatment, a higher PDI. Based on the fact that total DMD and ND were not different among heat treatments, indicates that the increased undegraded SBM protein from the first stage digestion was digestible when subjected to enzyme hydrolysis (Figure 2). A ruminant being fed SBM of a lower PDI would be expected to have a greater quantity of intact SBM protein reaching the lower tract and this protein would be digestible and available to the animal. However, when making inference to an in vivo situation, one must bear in mind that several factors are not taken into account from in vitro estimates. Such factors as feed and water intake, total ration digestibility, saliva output and rumen turnover rate may greatly alter the in vivo results.

When 12 and 24 hr incubation times were employed for Trial 2, similar differences due to treatment were observed (Table XII). The estimated microbial DMD and ND was less for the PDI-10 than for the SBM heat treatments having a higher PDI. The treatments having a PDI-20 and -30 were less than the SBM treatments having PDI-40 and -50; however, only small differences existed between PDI-20 and -30 or PDI-40 and -50. Similarly as in trial 1 the greater the heat treatment, lower PDI, the greater the SBM resisted degradation in a rumen fluid

TABLE XI

MEAN *IN VITRO*[a] DRY MATTER AND NITROGEN DISAPPEARANCE OF SBM HEAT TREATED AND CLASSIFIED AS PROTEIN DISPERSION INDEX (PDI)

| Measurement[c] | Treatments[b] | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | SE |
| Microbial DMD | 44.44 | 51.26 | 58.40 | 63.86 | 69.98 | 1.20 |
| Microbial ND | 36.97 | 44.31 | 54.62 | 66.69 | 69.02 | 2.04 |
| Total DMD | 79.64 | 82.72 | 83.97 | 82.76 | 83.76 | 2.00 |
| Total ND | 83.27 | 86.85 | 86.30 | 86.24 | 89.47 | 1.08 |

[a]Two stage modified *in vitro* procedure. Stage one, 12 hr incubation in rumen fluid buffer solution; Stage two, 12 hr incubation in pepsin solution.

[b]Heat treatment of SBM and classified according to PDI. The lower the PDI number the greater the heat treatment. Values represent means of three observations.

[c]DMD, dry matter disappearance; ND, nitrogen disappearance; Total results after SBM was subject to both stages of the *in vitro* procedure.

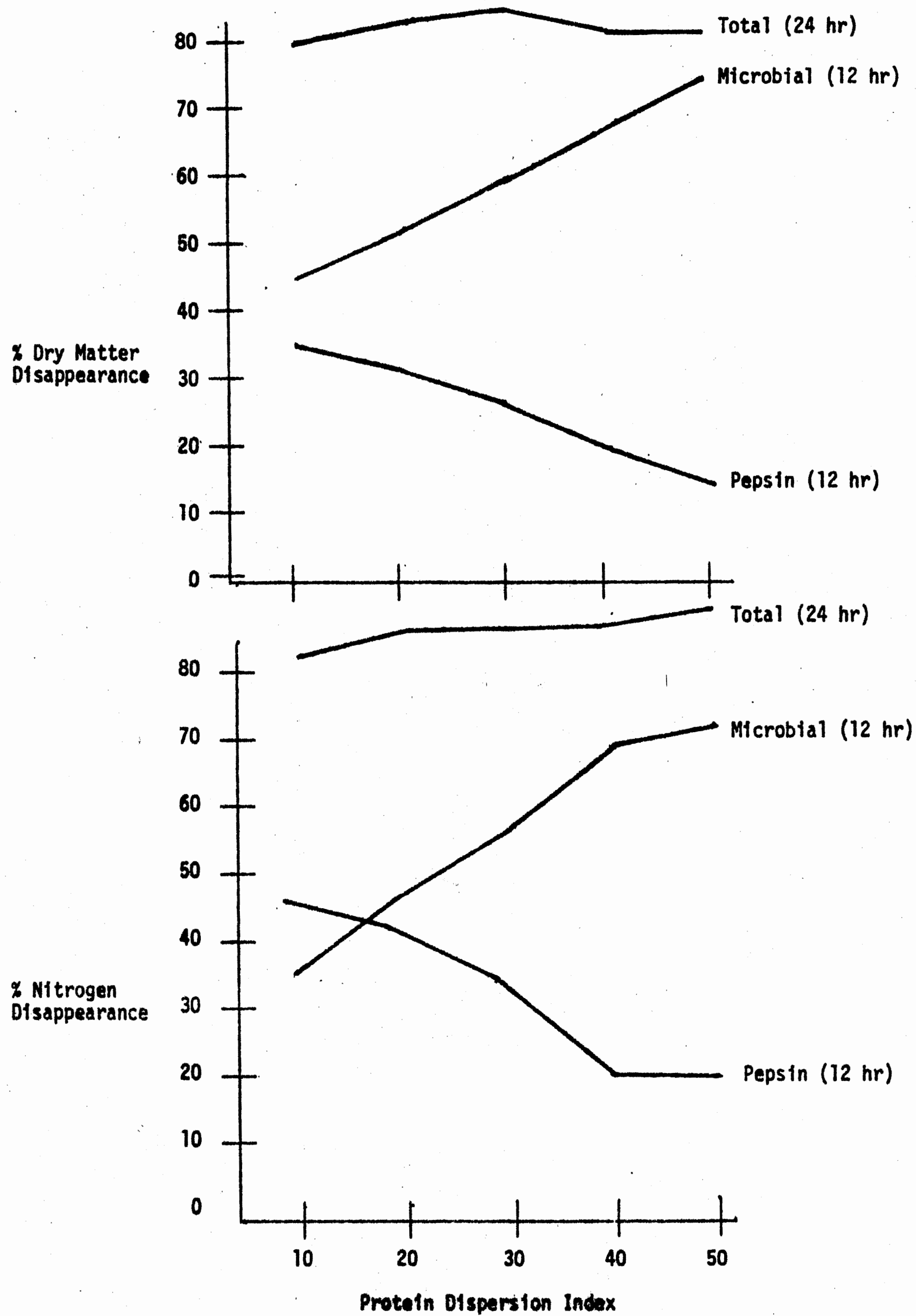


Figure 2. In Vitro Mean Dry Matter and Nitrogen Disappearance from SBM Heat Treated and Classified According to a Protein Dispersion Index (Trial 1)

TABLE XII

MEAN *IN VITRO*[a] DRY MATTER AND NITROGEN DISAPPEARANCE OF SBM HEAT TREATED AND CLASSIFIED AS PROTEIN DISPERSION INDEX (PDI)

| Measurement[c] | Treatment[b] | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | SE |
| Microbial DMD | 24.65 | 34.35 | 35.07 | 50.81 | 55.67 | 2.42 |
| Microbial ND | 19.64 | 36.00 | 38.11 | 55.30 | 63.18 | 1.85 |
| Total DMD | 66.27 | 64.59 | 65.37 | 73.82 | 75.52 | 1.63 |
| Total ND | 68.50 | 67.50 | 68.20 | 75.62 | 78.00 | 0.92 |

[a]Two stage modified *in vitro* procedure. Stage one, 12 hr incubation in rumen fluid buffer solution; stage two, 24 hr incubation pepsin solution.

[b]Heat treatment of SBM and classified according to PDI. The lower the PDI the greater the heat treatment.

[c]DMD, dry matter disappearance; ND, nitrogen disappearance; Total results after SBM was subject to both stages of the *in vitro* procedure.

environment. The total DMD and ND of SBM were not greatly reduced with increasing heat treatment. Nevertheless the SBM heat treatments of PDI-10, -20, and -30 had lower total DMD and ND than did the SBM of PDI-40 and -50. Thus, total digestion was slightly reduced with greater heat treatment. Total DMD and ND was not reduced at the same magnitude as the microbial DMD and ND (Figure 3). Thus when feeding heat treated SBM of a lower PDI to ruminants, total digestion may be sacrificed somewhat, but the total quantity of digestible protein reaching the small intestine may still exceed the digestible protein available to the animal than if SBM of a higher PDI were fed.

Even though a 24 hr pepsin incubation time was used in trial 2 as compared to 12 hour in trial 1, total DMD and ND was lower. Total DMD and ND was also reduced with increased heat treatment of SBM (PDI-30 vs. PDI-40) in trial 2, whereas no treatment differences were observed in total DMD and ND in trial 1. These differences between the two trials cannot be accounted for. Both trials do agree in that heat treatment of SBM by the flash desolventizing system reduces the degradation of SBM in a rumen fluid environment without drastically reducing total digestion. Thus ruminants with a high requirement for protein and essential amino acids would be expected to increase performance when fed SBM of a lower PDI. However caution must be taken in making inference to an *in vivo* situation from data observed under *in vitro* conditions.

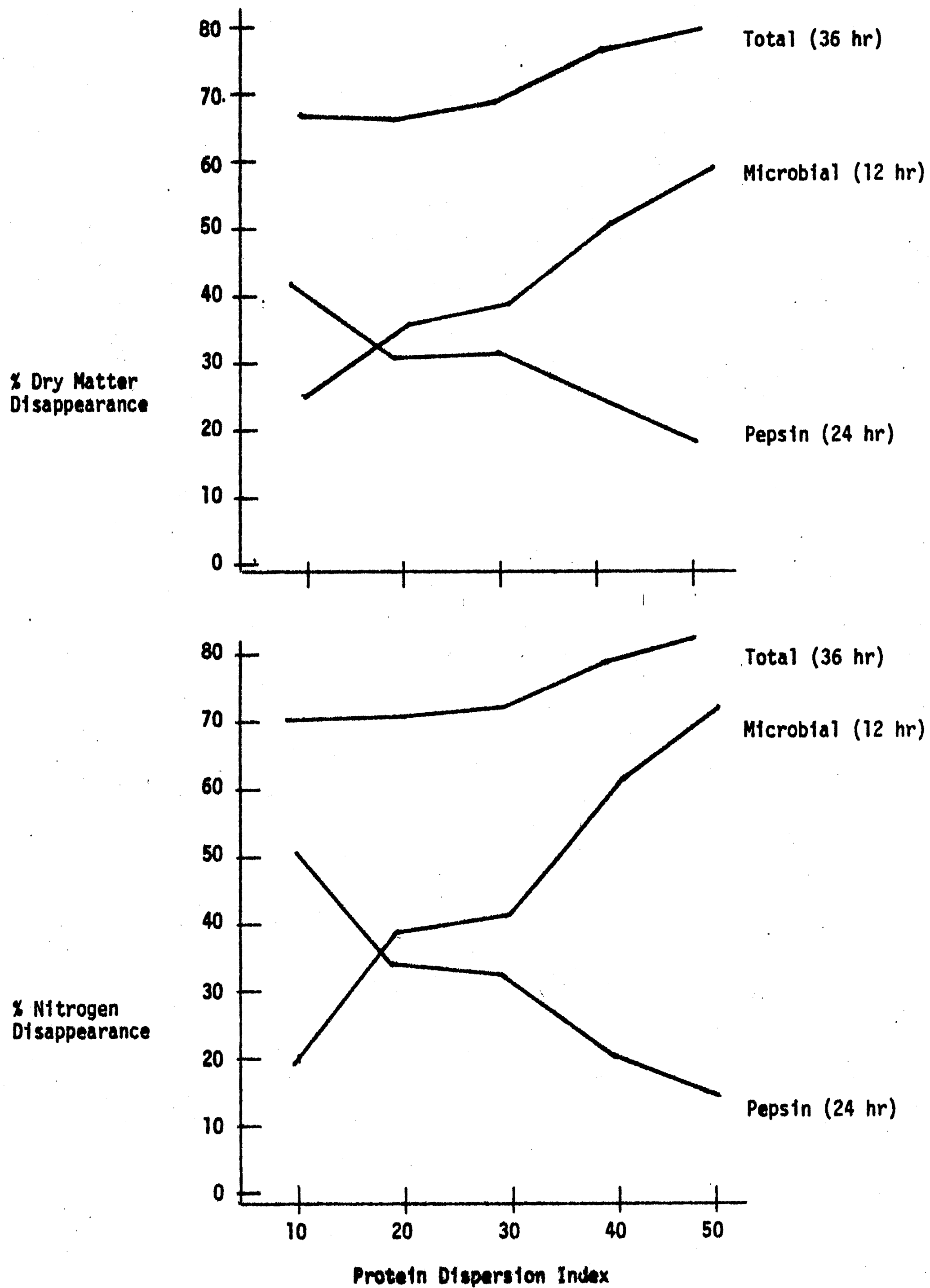


Figure 3. *In Vitro* Mean Dry Matter and Nitrogen Disappearance of SBM Heat Treated and Classified According to a Protein Dispersion Index (Trial 2)

# CHAPTER V

## EFFECT OF HEAT TREATMENT OF SOYBEAN MEAL ON MILK YIELD, MILK COMPOSITION, AND RUMEN AMMONIA IN LACTATING COWS


### Summary

Twenty-two lactating cows, in their 8th to 10th wk of lactation, were randomly assigned to two protein groups. Cows in each protein group were randomly assigned to one of two treatment sequences according to a switchback design. The experiment consisted of three 4-wk periods to determine the effect of heat treatment of SBM on protein utilization. Rations with two levels of protein (17.8 and 10.7%) containing SBM with two levels of heat treatment (PDI-10 and PDI-40) were fed. The rations had a 60:40 concentrate to forage ratio.

Heat treatment of SBM (PDI-10) did not affect dry matter intake, milkfat, milk protein or rumen ammonia concentrations. The heat treated SBM ration (PDI-10) increased milk yield, SCM, milk efficiency and protein intake of cows in the low protein group ($P<.05$). The cows in the high protein group had greater yields of milk, SCM and gained more body weight than cows in the low protein group. No differences in milk yield due to heat treatment were observed in the high protein group.

## Introduction

With higher levels of productivity in ruminants becoming more prevalent and increasing world demand for plant protein in human diets, a large amount of attention has been focused toward increasing the efficiency of protein utilization in the ruminant. Over the last decade substantial improvements in milk yield have been observed with dairy cows. The ability of the lactating cow to convert plant fiber and nitrogen into milk is dependent upon the amount of energy yielding nutrients and amino acids available to the mammary tissues, assuming adequate genetic potential of the cow to incorporate these nutrients into milk. The aspect of nutrient supply is influenced by the amount of feed ingested and the extent of rumen microbial fermentation. Due to high protein requirements in the lactating cow, the producer is forced to feed large amounts of protein to high producing cows to achieve maximum milk production. Consequently, appreciable amounts of dietary nitrogen are lost through rumen fermentation and excreted by way of the urine. If the dairy cow is to continue to be an efficient converter of plant protein to food protein, possible ways of reducing the protein nitrogen losses must be explored.

In recent years there has been considerable research relating the solubility of protein to the extent that the protein is degraded in the rumen (Tagari et al., 1962; Nishimuta et al., 1973; Wohlt et al., 1976). Thus, the amount of protein nitrogen in a given feedstuff that dissolves in a solvent has been used as an index to the amount of nitrogen that is available for microbial use in the rumen. However a solubility index may be too simplified since rumen turnover and total protein degradation are not considered (Bull et al., 1979). Several researchers (Pitchard and

Van Soest, 1977; Prigge et al., 1978; Smith, 1978) have shown that the solubility of a protein feedstuff may bear little relationship to the amount of degradation of that protein in the rumen. Although solubility has its limitations as an index to rumen degradation of a protein, it may be useful for crude estimates of protein bypass of a given protein until a better system is developed. Majdoub et al. (1978) observed a significant improvement in milk yield when rations were formulated for dairy cows with a low solubility index. Other researchers reported feeding proteins of a low solubility increased total solids yield in dairy cows (Hawkins et al., 1977) and reduced urinary nitrogen excretion in sheep (Wohlt et al., 1976) as compared to rations containing high soluble proteins.

Heat treatment of a protein substantially reduces its solubility in a buffer solution (Tagari et al., 1963; Glimp et al., 1967; Danke et al., 1966). Limited data support the concept that heat treated proteins may improve performance. Significant improvements in live weight gains have been reported in sheep (Little et al., 1963) and a five percent improvement in milk yield with dairy cows in early lactation (Ahrar et al., 1977) when fed heat treated SBM as compared to SBM of regular processing.

The purpose of this study was to investigate the effect of lowering the solubility of SBM in the diet by heat treatment upon rumen ammonia concentrations, milk yield and milk composition in high producing dairy cows.

## Materials and Methods

Heat treated soybean flour meal (SBM) with a protein dispersion index of 10 (PDI-10) and regular SBM (PDI-40) was obtained from Farmland Industries. The SBM was stored until the rations were mixed at periodic intervals. Four rations were formulated so that net energy-lactation ($NE_L$), was kept constant (Table III). Two of the rations were high protein (17.8 percent) and two were low protein (12.95 percent). The only difference between the two rations within the high protein and within the low protein groups was the treatment of SBM, being either heat treated (PDI-10) or regular processed SBM (PDI-40). The forage consisted of sorghum silage and sudan hay. All rations were calculated to contain a 60:40 concentrate to forage ratio.

Twenty-two lactating dairy cows (18 Holsteins and 4 Ayrshires) at 8 to 10 weeks post-calving were challenge fed a 60:40 concentrate to forage ration for a period of 2 to 4 wk. Initial mean milk production per cow ranged from 41 to 24 kg/day. At the end of the adjustment period the cows were randomly assigned to either a high or low protein group. Protein intakes for the two groups was calculated to meet 70 and 100 percent of the 1971 NRC standard for the low and high protein groups respectively. Energy intakes were calculated to meet 100 percent of 1971 NRC standard for both groups. Within each protein group the cows were assigned randomly to two treatment sequences for three 4-wk periods in a switchback trial (Brandt, 1938). The data collected during the first two weeks of each period were not used in final calculations to minimize carryover effects. A 60:40 concentrate to forage ratio was fed for the entire trial. Feed allowances were reduced by five percent at the end

TABLE XIII

INGREDIENT COMPOSITION OF RATIONS[a]

| Ingredients | IRN[b] | Treatments | | | |
|---|---|---|---|---|---|
| | | HP-HT[c] | HP-NT | LP-HT | LP-NT |
| | | | % | | |
| Corn, ground | 4-21-018 | 38.3 | 38.3 | 50.8 | 50.8 |
| Soybean meal | PDI-10 | 20.3 | | 7.8 | |
| Soybean meal | PDI-40 | | 20.3 | | 7.8 |
| Dicalcium phosphate | 6-01-080 | 0.9 | 0.9 | 0.9 | 0.9 |
| Limestone | 6-02-632 | 0.3 | 0.3 | 0.3 | 0.3 |
| Salt | - | 0.3 | 0.3 | 0.3 | 0.3 |
| Sorghum silage | 3-04-468 | 23.9 | 23.9 | 23.9 | 23.9 |
| Sorghum - Sudan hay | 3-04-499 | 16.0 | 16.0 | 16.0 | 16.0 |
| Total Protein | | 17.8 | 17.8 | 10.7 | 10.7 |
| $NE_L$ (MCal/kg of DM) | | 1.68 | 1.68 | 1.70 | 1.70 |

[a]Dry matter basis.

[b]International reference number.

[c]HP-HT, high protein with heat treated SBM; HP-NT, high protein with regular SBM; LP-HT, low protein with heat treated SBM; LP-NT low protein with regular SBM.

of each experimental period. Cows were fed in individual stalls twice daily. Feed weigh-backs were recorded daily. Cows were weighed on three consecutive days at the end of the adjustment period and at the end of each experimental period. Representative samples of the concentrate mixtures, sorghum silage and sudan hay were collected each week and analyzed for total protein. Individual cow milk yields were recorded daily. Milk samples were collected from four consecutive milkings each week, composited and analyzed for percents of milk fat and total solids. Milk fat was determined at the Dairy Herd Improvement Laboratory by a Mark II Milk-O-Tester. Milk protein percentage was determined during fourth wk of each period by the Kjeldahl method. Total solids content was determined by placing 3 ml sample of milk in a forced air oven at 100°C for four hours and calculating the weight loss.

Rumen samples were collected during the last three days of each period by stomach tube, treated with 3.9 N HCL and frozen until analyzed for ammonia at a later date. Ammonia concentrations were determined by a micro-ammonia technique (Chaney and Marback, 1962).

## Results and Discussion

The cows in the high protein group had higher intakes of dry matter and total protein (Table XIV). There were no differences in dry matter intakes of cows due to treatment in either the high or low protein groups ($P>.05$). however, the cows in the low protein group consuming the heat treated SBM ration (PDI-10) had greater total protein intakes than those consuming the PDI-40 SBM ration ($P<.05$). Soybean meal protein, as a percent of total protein intake was also higher ($P<.05$). This greater protein intake may be attributed to the slightly greater intake of

TABLE XIV

TOTAL FEED AND TOTAL PROTEIN INTAKE PERCENT CONCENTRATE AND SOYBEAN PROTEIN OF TOTAL DIET, AND RUMEN AMMONIA AND WEIGHT CHANGE, OF COWS FED HEAT TREATED SOYBEAN MEAL

| | Protein Level | | | | | |
|---|---|---|---|---|---|---|
| | Low | | | High | | |
| Item | PDI-10 | PDI-40 | SE | PDI-10 | PDI-40 | SE |
| Feed dry matter intake, kg/day | 18.71 | 18.69 | 0.03 | 19.05 | 19.18 | 0.10 |
| Protein dry matter intake, kg/day | 2.36[a] | 2.31[b] | 0.01 | 3.49 | 3.37 | 0.05 |
| Concentrate intake, % | 60.82 | 57.82 | 0.08 | 57.58 | 58.58 | 0.11 |
| Soybean protein/ total protein intake % | 30.24[a] | 30.90[b] | 0.10 | 54.72[a] | 57.20[b] | 0.45 |
| Rumen ammonia, mg/dl | 5.19 | 5.62 | 0.63 | 6.26 | 8.84 | 0.89 |
| Body weight change, kg/day | -0.35 | -0.04 | 0.47 | 0.51 | 0.10 | 0.28 |

[a,b]Values in same row with different superscripts are different (P<.05).

concentrates by cows consuming the PDI-10 SBM ration as compared to cows consuming the PDI-40 SBM ration.

Dietary protein concentration influenced rumen ammonia concentrations and body weight changes (Table XIV). Rumen ammonia concentrations and body weight changes were not altered (P>.05) by heat treated SBM in either the high or low protein groups.

Milk yield and SCM of cows in the low protein group consuming the PDI-10 SBM ration was higher than that of cows consuming the PDI-40 SBM ration (P<.05). No differences in milk yield, due to treatment, occurred with cows in the high protein group.

Milk yield, SCM and efficiency of milk yield (Table XV) was greater for cows in the low protein group consuming the PDI-10 SBM ration than that of cows consuming the PDI-40 SBM ration (P<.05). No differences occurred due to treatment of cows in the high protein group. Percentage milk fat, milk protein, and non-fat solids of cows consuming the PDI-10 SBM ration were not different from cows consuming the PDI-40 SBM ration in either the high or low protein groups (P>.05).

The cows in the high protein group had greater yields and efficiency of milk and SCM than cows in the low protein group. No differences exist in milk fat, NFS or milk protein percentages between cows in the high and low protein groups.

It appears that heat treatment of SBM, classified as PDI-10, was superior in feeding value for lactating cows than regular processed SBM. A greater yield of milk and SCM was observed in the low protein group with cows consuming the PDI-10 SBM ration than cows consuming the PDI-40 SBM ration (P<.05). However, a greater intake of total protein was also

TABLE XV

MILK YIELD AND MILK COMPOSITION OF COWS FED HEAT TREATED SOYBEAN MEAL CLASSIFIED ACCORDING TO THE PROTEIN DISPERSION INDEX

| | Protein Level | | | | | |
|---|---|---|---|---|---|---|
| | Low | | | High | | |
| Item | PDI-10 | PDI-40 | SE | PDI-10 | PDI-40 | SE |
| Production | | | | | | |
| Milk, kg/day | 22.52[a] | 21.79[b] | 0.20 | 24.78 | 24.21 | 0.29 |
| SCM, kg/day | 21.80[a] | 21.24[b] | 0.18 | 23.95 | 23.92 | 0.38 |
| Fat, % | 3.85 | 3.87 | 0.05 | 3.90 | 3.91 | 0.07 |
| NFS, % | 8.73 | 8.78 | 0.05 | 8.64 | 8.86 | 0.09 |
| Milk Protein, % | 3.23 | 3.21 | 0.05 | 3.29 | 3.32 | 0.06 |
| Milk Efficiency | 1.20[a] | 1.16[b] | 0.01 | 1.25 | 1.27 | 0.01 |
| SCM Efficiency | 1.16 | 1.14 | 0.01 | 1.24 | 1.22 | 0.02 |

[ab]Values in the same row having different superscripts are different (P<.05).

observed ($P<.05$). This difference in total protein intakes among cows in different treatments is very small and probably of no consequence as far as the production responses in this experiment were concerned.

# CHAPTER VI

## SUMMARY

In experiment 1, three trials were conducted to evaluate the effect of altering the particle size of SBM on protein utilization in the rumen. In trial 1, four steers, weighing 567 kg and containing rumen and abomasal fistulas, were fed rations containing SBM of two different particle sizes; namely coarse and fine. The geometric mean diameters of the coarse and fine SBM were 2400 and 250 μm, respectively. The steers consuming the fine SBM ration had greater bypass of non-ammonia nitrogen than steers consuming the coarse SBM ration ($P<.05$). No significant differences were observed in rumen and total digestibility of ration components, or in rumen ammonia concentrations. In trial 2, 12 lactating Holstein cows were fed rations containing SBM of two particle sizes; namely coarse and fine. The particle size of the coarse and fine SBM rations were 1900 μm and 250 μm, respectively. The cows were 7 to 9 wk postpartem, challenge fed a 60:40 concentrate to forage ration for 2 wk, and randomly assigned to one of the two treatment sequences according to a crossover design. The cows consuming the coarse SBM ration had greater 3-hr post-feeding rumen ammonia concentrations than did cows consuming the fine SBM ration ($P<.01$). No differences were observed in rumen ammonia concentrations at 5 hr after feeding or in milk yield and composition. Rumen ammonia concentration was used as an indirect estimate of the amount of protein degradation occurring. Thus

the increase in rumen ammonia at 3 hr after feeding in cows consuming the coarse SBM ration represented an increase in rumen protein digestion as compared to cows consuming the fine SBM ration. It appears that cows fed finely ground SBM would have greater bypass of SBM protein than if cows were fed coarsely ground SBM and should therefore have greater milk yield, if post-ruminal amino acid supply were marginal. This theory was tested in trial 3. Twenty-four lactating dairy cows 7 to 9 wk postpartem were randomly assigned into two protein groups. Two treatment sequences were randomly assigned according to the switchback design (Brandt, 1938; Lucas, 1956). The two treatments in each protein group were SBM of two different particle sizes; namely coarse (1500 μm) and fine (250 μm). No treatment differences were observed in rumen ammonia concentrations, blood urea concentrations, or milk yield and milk composition. However, all of these measurements were higher for cows in the high protein group. It appears that altering the particle size of SBM offers little merit and increasing it may be detrimental to protein utilization when fed to ruminants.

In experiment 2, SBM was heat treated and subject to a modified two-stage _in vitro_ digestion procedure. In trial 1, SBM heat treated at 116°C for various lengths of time, decreased _in vitro_ microbial digestion but also decreased _in vitro_ total digestion. Thus heat treatment of SBM under the above conditions would be expected to contribute very little to protein utilization in the ruminant. In trial 2, soybean flour meal was heat treated by the flash desolventizing system and classified according to a protein dispersion index (PDI). The lower PDI values indicated greater heat treatment. A similar _in vitro_ digestion procedure

was employed as in trial 1. The lower PDI SBM treatments decreased microbial digestion without greatly decreasing total digestion. Thus one would expect that feeding SBM of a low PDI would substantially improve protein efficiency and production responses in ruminants with high protein requirements.

In experiment 3, SBM with a PDI-10 was compared to SBM with a PDI-40 to test the results of experiment 2 in lactating dairy cows. A switch-back experimental design was employed. The cows in the low protein group, consuming the heat treated SBM ration (PDI-10), had greater milk yield and milk efficiency than cows consuming the PDI-40 SBM ration ($P<.05$). A greater protein intake was also observed ($P<.05$). No treatment differences were observed in cows of the high protein group. Cows in the high protein group had greater production responses, rumen ammonia concentrations, and body weight gains than cows in the low protein group. It appears that heat treated SBM of PDI-10 is beneficial in protein utilization and increases production responses in lactating cows, when post-ruminal protein supply may be limiting.

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APPENDIX

TABLE XVI

NITROGEN DETERMINATIONS OF RUMEN FLUID AND BLOOD

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Reagents:

1. Phenol -- 50 g phenol + 0.25 g Na nitroferricyanide. Dilute to 1 liter with deionized water.
2. Sodium hypochlorite -- 25 g NaOH + 16.8 ml Na hypochlorite. Dilute to 1 liter with deinoized water.
3. Urease buffer solution -- 7.11 g $Na_2$ $HPO_4$(anhydrous) + 5.0 g EDTA. Dilute to 1 liter with deionized water.
4. Buffered urease solution -- Add 80 mg urease (sgma type III powder) to 100 ml urease buffer solution.

I. AMMONIA NITROGEN DETERMINATIONS OF RUMEN FLUID

Standards:

1. Dry $NH_4Cl$ in oven overnight.
2. Weigh out 650 mg of dry $NH_4Cl$.
3. Add drop of acid and dilute to 250 ml. Should be 25 mgN/100ml.
4. Dilute to standards of 5, 10, 15 and 25.

Procedure:

1. Collect enough test tubes for triplicates of each sample.
2. Turn on Gilford Spectrophotometer and adjust $H_2O$ bath to 37°C.
3. Pipet 20 μl of sample into each tube.
4. Pipet 1 ml Phenol solution and 1 ml Sodium Hypochlorite into each tube.
5. Stir with Vortex to rinse walls.
6. Incubate in $H_2O$ bath for 15 min. or room temperature for 30 min.
7. Add 8 ml of deionized water and stir with Vortex. Solution is stable for 4 hr.
8. Set spectrophotometer at 630 nm, and adjust to zero from blank solution by altering the silt width. Absorbance range can also be adjusted if desired (500-660 nm).
9. Read absorbance of standards and samples. Periodically recheck standards.

II. UREA NITROGEN DETERMINATIONS

Standards:

1. Dissolve 214.4 mg dry urea in 25 ml deionized water. Add 1 ml concentrated HCl and dilute to 100 ml with deionized water. Concentration of urea nitrogen should be 100 mg/100 mls.
2. Dilute urea solution to standards of 5, 10, 15 and 25 mg/100 mls.

TABLE XVI (Continued)

Procedure:

1. Collect sufficient number of tubes for triplicates as in ammonia determination.
2. Add 200 μl of buffered urease solution to each tube.
3. Add 20 μl of plasma to each tube except for blanks. Add 20 1 of deionized water to blank tubes.
4. Place tubes in water bath at 37°C for 15 min.
5. Remainder of procedure is similar to ammonia nitrogen determination starting with step 4.

CALCULATION FOR BOTH PROCEDURES:

1) Mg/dl ammonia or urea nitrogen in sample =

$$\left(\frac{\text{absorbance of sample}}{\text{absorbance of standard}}\right)\left(\begin{array}{l}\text{concentration}\\ \text{of standard}\end{array}\right)$$

Source: Chaney and Marbach, 1962, p. 130.

TABLE XVII

*IN VITRO* DRY MATTER DIGESTIBILITY PROCEDURE FOR DETERMINATION OF DRY MATTER AND NITROGEN DISAPPEARANCE

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Reagents:

1. Nutrient Buffer Stock Solution (NBSS)
   - $NaHCO_3$ - 14 g/liter
   - (Anhydrous) $Na_2HPO_4$ - 18.5 g/liter
   - NaCl - 2.35 g/liter
   - KCl - 2.85 g/liter
   - Deionized $H_2O$ - dilute above to 1 liter
2. Check PH of NBSS - should be 6.9-7.1.
3. Rumen fluid strained 4 times through double layer cheese cloth.
4. $CaCl_2$ (anhydrous) - 4 g/100 ml deionized $H_2O$.
5. $MgCL_2$ - 6 g/100 ml deionized water.
6. 0.1 N HCl solution - 8.62 mls of 11.6 N HCl. Dilute to 1 liter with deionized water.
7. Pepsin solution - 2 g pepsin/liter 0.1 N HCl solution.
8. $Na_2CO_2$ (anhydrous) - 15 g/100 ml deionized water.
9. Stock solution - dilute NBSS 1:5 with deionized water.

Procedure:

1. Dry [(# Treatments x 4) + 4] 35 ml centrifuge tubes in oven at 80°C for 24 hr.
2. Weigh out eight, 500 mg samples, of each treatment into dry centrifuge tubes. Cap each tube with gas release rubber stoppers.
3. Weigh out duplicate 1 g samples of each treatment into dry aluminum dishes for dry matter determination.
4. Adjust water bath to 30°C. (Make sure water is clean).
5. Place tubes containing 500 mg sample in $H_2O$ bath 12 hr previous to starting the incubation.
6. Mix stock solution (#9) 12 hr previous to start of incubation period and warm to 39°C until use.
7. Prior to going to the barn, purge stock solution with $CO_2$ and leave while obtaining rumen fluid.

TABLE XVII (Continued)

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8. Fill insulated thermos with warm water. Pour water out of thermos. Obtain rumen contents from ruminal fistulated dairy cow. Strain the rumen fluid through double layer cheesecloth into the prewarmed thermos. Be sure to obtain enough volume (25 ml x #tubes x .286). Cap thermos and go back to laboratory. Strain rumen fluid three more times through doubel layer cheesecloth.
9. Add 2 ml of $CaCl_2$ solution and 2 ml of $MgCl_2$/liter of warm stock solution (39°C).
10. Mix stock solution and rumen fluid into one solution containing 71.4 percent stock solution and 28.6 percent rumen fluid.
11. Place rumen fluid stock solution mixture on previous adjusted heat plate set at 39°C. Purge solution continuously with $CO_2$.
12. Add 25 ml of mixed stock buffer solution into each centrifuge tube containing samples.
13. Cap tubes (rubber with gas release valves) and swirl gently.
14. Allow tubes to incubate for specified amount of time (12 hr).
15. Swirl tubes gently at 6 hr incubation time. Be careful not to get sample particles up into the neck of centrifuge tubes.
16. At 6 hr incubation time mix up pepsin solution and warm to 39°C. Keep at 39°C until use.
17. After 12 hr incubation, remove tubes from water bath. Rinse down bottom of rubber stoppers and neck of centrifuge tubes.
18. Add a few drops of $Na_2CO_3$ solution to aid in precipitation.
19. Centrifuge tubes for 10 minutes at 1000-1400 r.p.m. (Be sure to remove stoppers).
20. Decant supernatant carefully.
21. Place the 8 replicate tubes of each sample together. Place 4 of these replicates of each treatment into oven at 80°C for 48 hr. Add 25 ml of prewarmed pepsin solution to the remaining 4 replicates. Replace the same rubber stoppers on each of the remaining tubes. Put these pepsin solution filled tubes in water bath for additional incubation time (12 or 24 hr).
22. At end of second incubation time (stage 2), the centrifuging and decanting are repeated. The tubes with residues are placed in oven at 80° for 48 hr.
23. At end of drying period all tubes are placed in dessicator to cool and are weighed to 4 decimal places.
24. All tubes are weighed back, blank weight subtracted, and dry matter disappearance calculated.

TABLE XVII (Continued)

25. All tubes are now placed together according to treatment and placed back into oven for additional 12 hr drying. This is to insure a dry sample.

26. At end of drying 4 replicate tubes of each treatment for stage 1 are divided into 2 groups. The contents of 2 of the 4 replicate tubes are removed and weighed out for 1 kjehdahl sample. The same is done for the other 2 tubes of the replicates. Likewise, the same is done to the tubes from the second stage digestion.

27. Percent nitrogen of the 2 tubes making up the Kjeldahl sample is determined. The percent nitrogen times the residue weight gives you grams of nitrogen in residue. Percent nitrogen of initial sample times the sample weight gives you initial grams of nitrogen in sample. Now nitrogen disappearance can be calculated.

28. Average the 2 nitrogen disappearance values obtained from the replicates of each treatment. This done individually for stage 1 and stage 2.

29. Stage 1 is an estimate of ruminal degradation and stage 2 estimates total digestion.

30. Do analysis of variance. If significant, test means according to one of the tests for treatment mean differences (Dunnets, LSD, Turkey's etc.).

Calculations:

1. % Dry Matter $= [1 - (\frac{\text{initial weight-ending weight}}{\text{initial weight}})] \times 100$

2. Dry matter disappearance (% DMD) =

$$[1 - (\frac{\text{initial DM - ending DM}}{\text{initial DM}})] \times 100$$

3. % Nitrogen (Kjehdahl procedure) =

$$[\frac{\text{(mls-blank)(Normality of nitrating acid)(14 mg Nit/MM)}}{\text{mg day sample weight}}] \times 100$$

4. Nitrogen disappearance (% ND) =

$$[1 - (\frac{\text{initial g nit - ending g nit}}{\text{initial g nit.}})] \times 100$$

Source: Tilley and Terry, 1963.

TABLE XVIII

PROTEIN DISPERSION INDEX FOR DETERMINATION OF BOUND AND UNBOUND PROTEIN FRACTIONS OF VEGETABLE PROTEIN SOURCES

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DEFINITION: This method determines the dispersible protein in soybean products under the conditions of the test. In contrast to the alternate slow stir method for Nitrogen Solubility Index (NSI), No. Ba 11-65, the faster stirring technique used in this method will give generally higher results than those obtained by the slow stir method.

SCOPE: Applicable to ground soybeans, whole or ground full-fat or extracted flakes, full-fat and defatted soy flours and grits, and soybean meal.

A. Apparatus:

1. Hamilton Beach Drinkmaster No. 30. Modified to accommodate Waring Blendor blade and cup.
2. Blade Assembly. Cenco-Pinto blades. Central Scientific Company, No. 17251-L55. Use two blades, one horizontal, and one with tips pointing down with the cutting edge in the direction of rotation.
3. Waring Blendor Cup. 1 qt. capacity with bottom sealed with No. 3 stopper.
4. Glassware. 300-ml. volumetric flask, 15 ml. pipet, 600 ml breaker.
5. Centrifuge. International Type SB size, 1, 2,700 rpm, with 50 ml. tubes or any equivalent, capable of delivering 1,400 relative centrifugal force at the tip.
6. Balance. 0.1 g. accuracy, important.
7. Timer. Interval, alarm.
8. Variable Transformer.
9. Standard Kjeldahl Equipment. See A.O.C.S. Method AC 4-41.
10. Tachometer. Range to 10,000 rpm.
11. Voltmeter. (Use optimal).

B Reagents:

1. Distilled Water. Neutral.
2. Standard Reagents as used for protein determination, A.O.C.S. Method Ac 4-41.

C. Preparation of Sample:

1. No preparation necessary; use sample as received.

D. Standardization of Blendor:

TABLE XVIII (Continued)

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1. Measure 300 ml. of distilled water into the Blendor cup, and place in position on the mixer.
2. Remove chrome cap, which covers the top of the drive shaft. Using the proper tip, place tachometer in position on the rotating shaft.
3. With the switch in high position, gradually increase the transformer setting until the shaft shows 8,500 rpm on the tachometer. Note voltmeter reading and transformer setting, and use for blending of sample.
4. Standardization of the Blendor should be done before each series of tests to eliminate errors on account of fluctuation in line voltage.

SAMPLING AND ANALYSIS OF VEGETABLE OIL SOURCE MATERIALS

PROTEIN DISPERSIBILITY INDEX (PDI)

E. Procedure:

1. Weigh 20 ± 0.1 g. of soy product.
2. Fill a 300 ml. volumetric flask with distilled water at 25° ± 1° C. Pour about 50 ml. of the water into the Blendor cup. (Water-dispersible protein is related to temperature so the Blendor cup should be at room temperature). Transfer the weighed sample quantitatively to the Blendor cup. Stir with a spatula to form a paste. Add remainder of water in increments, with stirring, to form a smooth slurry. Use last of water to rinse spatula and Blendor cup walls. Place cup in position for blending.
3. Turn Blendor on with switch in high position, and gradually adjust the variable transformer to the point indicated by the water standard at 8,500 rpm. Blend at this speed for 10 min.
4. Remove the Blendor cup, and pour the slurry into a 600 ml. beaker. After the slurry has separated, decant or pipet a portion into a 50 ml. centrifuge tube, and centrifuge 10 min. at 2,700 rpm.
5. Pipet 15 ml. of supernatant liquid into a Kjeldahl flask, and determine protein by using A.O.C.S. Method AC 4-41 (15 ml. = 1.0 g. sample).

F. Calculation of Results:

$$\%\ \text{Water Dispersible Protein} = \frac{(B-S) \times N \times 0.014 \times 100 \times 6.25}{\text{Wt. of sample}/20}$$

Where B = ml. of alkali back titration of blank.

TABLE XVIII (Continued)

S = ml. of alkali back titration of sample.

N = Normality of alkali.

% Protein Dispersibility Index (PDI) =

$$\frac{\%\ \text{Water dispersible protein} \times 100}{\%\ \text{Total protein}}$$

G. Precision:

Two single determinations performed in one laboratory should not differ by more than 4.375.

Agreement between laboratories: Two single determinations performed in different laboratories should not differ by more than 9.664.

Source: A.O.C.S. Official Method Ba 10-65. Revised 1969.

# VITA

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