EVALUATION OF BY-PRODUCT FEEDSTUFFS USED IN LIQUID SUPPLEMENTS

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CHAPTER I

INTRODUCTION

Inadequate nutrition is an important factor limiting animal production in many areas of the world. Protein and energy sources are often among the nutrients most commonly missing. In this country many livestock producers have attempted to meet the protein and energy needs of livestock grazing weathered forages by feeding molasses-urea based liquid supplements. These supplements have gained appeal by reducing labor and feed expenses. However, it has been shown that nutritional needs have not always been met because animal performance resulting from the feeding of molasses-urea based supplements has been inferior to the performance of animals fed natural protein. Attempts are being made to overcome the protein limitations of these supplements by replacing urea with natural protein supplied by agro-industrial by-products. Their usage is restricted by solubility in the supplement medium. Animal performance resulting from this substitution has not been subjected to extensive experimentation (Lusby and Armbruster, 1977).

Much of the present interest in feeding agri-industrial by-products in this country has been brought about by new anti-pollution regulations which force industries to find alternatives to the dumping of waste by-products, many of which present an enormous untapped potential source of animal feed for other countries as well. Their use

to improve animal nutrition, whether in liquid supplements or in some other form, could reduce waste disposal costs and integrate animal production and allied processing industries, thereby making better use of existing resources (Chenost and Mayes, 1976).

The objectives of the experiments reported herein were: to evaluate the usefulness of several by-product feedstuffs as protein sources for ruminants (1) in vitro using protein solubility, ammonia release, and pepsin-insoluble nitrogen, and (2) in vivo using rumen ammonia levels and cellulose digestibility.

CHAPTER II

REVIEW OF LITERATURE

Protein Solubility

Protein or nitrogen solubility is a physical property of proteins that has been used to evaluate protein nutritional value for ruminants because it has been found that the amount of feed protein hydrolyzed by the action of microbial enzymes depends partially on the solubility of the protein in rumen fluid (Blackburn, 1965). Protein or nitrogen solubilty has been defined by Bull et al. (1977, p. 23) as "the disappearance of nitrogen from the solid phase of a feed when incubated in an inanimate aqueous medium." Ruminal degradation of a protein cannot take place unless the protein has the ability to "solubilize" in the rumen medium.

Ammonia in the ruminal contents of grazing sheep appears to be derived chiefly from the degradation of feed proteins and from urea secreted in the saliva (McDonald, 1948). Study reveals that different proteins produce varying ammonia levels in the rumen. Gelatin and casein which are highly soluble produce much higher concentrations of ruminal ammonia than zein which is highly insoluble in aqueous media (McDonald, 1952). Gelatin and casein are attacked readily by microbial proteinases while zein remains relatively resistant to proteolysis. This was verified by work in which only 40% of the zein in a diet containing 94% zein protein was utilized by rumen microbes of sheep

(McDonald, 1954), whereas in partially purified diets consisting of 87% casein protein, at least 90% of the casein was degraded (McDonald and Hall, 1957).

In other studies by Chalmers et al. (1954), who compared the performance of ewes fed casein and herring meal, and by El-Shazly 1958), who compared casein, beans (Vicia faba), cottonseed cake, linseed cake, and meat meal fed to wethers, evidence was obtained that the solubility of the protein was positively related to the degree of protein degradation as measured by ruminal ammonia concentrations. More recently, Wohlt (1973) formulated rations based on protein solubility and attained a wide difference in solubility (15% vs. 35% of the total nitrogen soluble in a mineral buffer). The difference in solubility resulted in a significant difference in rumen ammonia concentrations, i.e., 5.57 vs. 23.53 mg NH_3 -N/100 ml of rumen fluid.

Attempts have been made to correlate protein solubility with protein degradation as measured by in vitro ruminal ammonia concentrations. A high correlation (0.93) was found to exist between the percentage of total soluble nitrogen of a variety of natural and purified feeds in autoclaved rumen fluid and the level of free ammonia produced after two hours of incubation in viable rumen fluid (Little et al., 1963). The degradation of purified proteins during six hours in vitro incubation in rumen fluid was highly correlated (0.99) with solubility in a dilute Burroughs mineral mixture (Hendrickx and Martin, 1963).

Not all protein sources which ahve been found to be highly soluble are hydrolyzed readily by ruminal microbes. Annison (1956)

compared the degradation rates of casein, arachin, soya protein, bovine albumin, zein, and wheat gluten. He found that washed suspensions of rumen microorganisms failed to degrade appreciably bovine albumin which compared with casein in solubility. Also, Mangan (1972) showed that although ovalbumin is a soluble protein it was not degraded readily by rumen microorganisms. It appeared that proteolytic activity in the rumen had some specificity and that protein solubility may be inadequate to indicate potential degradation.

Protein Solubility and Nitrogen Balance

While it has been recognized that a supply of readily available nitrogen is essential for rumen microbial activity and that insoluble nitrogen sources are generally believed not to be readily available, proteins of low nitrogen solubility have been given higher nutritive values for ruminants primarily because there is much data suggesting that highly soluble nitrogen sources are very rapidly converted into ammonia in the rumen and that part of this ammonia may be lost through ruminal absorption and eventual excretion of the urine. For this reason, Chalmers (1960) proposed that ammonia concentration in the rumen be used as a basis of a technique for comparing the quality of protein to give a result in a shorter time and by using less material than a nitrogen balance trial. If the ammonia concentration resulting from the feeding of one ration was consistently higher for one protein source than that from another source, it was judged to be inferior. More recently, Satter et al. (1977) suggested that as long as there is no ammonia overflow in the rumen, it does not make any difference whether dietary protein escapes microbial degradation or

is synthesized into microbial protein.

In several studies comparing protein solubility, ammonia level (in vitro and in vivo), and nitrogen balance, the soluble nitrogen concentration of rations inversely affected nitrogen retention. Chalmers and Synge (1954) showed that nitrogen retention was greater in ewes fed herring meal than in ewes fed casein which was more soluble and produced higher rumen ammonia levels than herring meal. Heated casein, being less soluble and producing lower ammonia levels than unheated casein, resulted in increased nitrogen retention when compared with unheated casein (Chalmers et al., 1954). Tagari et al. (1962) found that less soluble heated soybean meal produced lower ammonia concentrations (in vitro and in vivo) and higher nitrogen retention than more highly soluble unheated soybean meal. In another experiment with soybean meal, Peter et al. (1971) showed that by reducing the solubility with aldehyde treatments, in vitro ammonia levels were reduced and gains and feed conversion in lambs were improved. The formulation of rations with wide differences in solubility by Wohlt (1973) resulted in greater nitrogen retention by those animals fed the lower soluble nitrogen ration. The difference in utilization among rations was due almost entirely to differential losses of nitrogen in the urine.

While rumen ammonia levels were not measured directly, Woods et al. (1962) who fed heated cottonseed meal to lambs, Nomani and Evans (1971) who fed alfalfa and urea treated corn silage to growing steers, Sniffen (1973) who reported the comparison of nitrogen solubility levels with growing heifers, and Atchison et al. (1976) who measured performance of lactating dairy cows fed urea balanced

rations concluded that animals fed rations of higher nitrogen solubility retained less nitrogen than animals fed rations of lower soluble nitrogen levels. Sniffen (1974) calculated the correlation of total soluble nitrogen intake to nitrogen balance to be -0.77.

There have been other feeding trials in which protein solubility did not appear to influence nitrogen retention. When El Shazly (1958) compared the response to protein sources of varying solubilities, it was observed that, whereas casein was the most soluble, ewes fed casein had higher nitrogen retention than those fed meat meal which was the least soluble. Protein digestibility of these feedstuffs had not been considered. Meat meal was shown to have a digestibility of 47% compared to 70% for casein (Chalmers, 1960). Woods et al. (1957) found that lambs fed heated cottonseed meal of lower solubility than unheated meal (but of comparable digestibility) did not have significantly higher nitrogen retention. Little et al. (1963) carried out lamb growth trials to compare heated soybean meal of reduced solubility with unheated soybean meal. Whereas heated soybean meal produced lower ammonia concentrations in vitro, there was no difference in growth when fed to lambs. Sharma et al. (1977) showed that reducing the solubility of rape seed by aldehyde treatments, while lowering ammonia concentration in vitro, had no significant effect on the nitrogen balance when fed to dairy calves. It was suggested that crude protein digestibility was reduced. More recently, Wohlt et al. (1976) compared the response of yearling wethers fed rations formulated to have 13% and 35% soluble protein. Whereas rumen ammonia levels were four times higher in wethers fed the higher soluble protein ration, there was no significant difference in nitrogen retention because increases

in urine nitrogen were accompanied by decreases in fecal nitrogen.

Protein Solubility and Protein Quality

While much of the interest in protein solubility is concerned with nitrogen loss characterized by high rumen ammonia levels, the quality of the soluble protein fraction which is degraded needs to be compared with the quality of microbial protein synthesized from the hydrolysate products. The biological value of the degraded protein is converted into the biological value of the rumen microbes (Hungate, 1966). This is a beneficial upgrading of quality when low quality protein or non-protein nitrogen is fed to the ruminant. Conversely, degradation and resynthesis of high quality protein may result in a protein of lower biological value (Peter et al., 1971; Hatfield, 1977).

In a study by Little and Mitchell (1967) who compared abomasal versus oral administration of casein and zein, the more highly soluble and higher quality protein, casein, showed higher retention of digested nitrogen when administered to the abomasun than when fed orally, 19.0 vs. -4.2%. In contrast, when the poorer quality protein, zein, was fed to animals, more nitrogen was retained than when abomasal administered, 20.0 vs. 16.2%. The lower quality protein was utilized more effectively when fed than the higher quality protein because of solubility (20.0 vs. -4.2%). For this reason, many attempts have been made to reduce the solubility of protein sources.

Sources of equally degradable nitrogen, either true protein or non-protein nitrogen, have been considered to be equally effective in producing microbial protein (Bull et al., 1977; Hatfield, 1977)

because it has been shown that the quality and digestibility of microbial protein were not affected by changes in the rations (Bergen et al., 1968). Sniffen (1974) suggested that urea be considered a 100% soluble nitrogen source along with the other soluble nitrogen ingredients of a ration. However, Hume (1970b) showed that more microbial protein can be formed when protein is fed than when urea is fed. Microbial protein production was measured in sheep fed urea, gelatin, casein, and zein. While sheep fed gelatin and urea were shown to have similar quantities of microbial protein (90 and 91 g/day), those fed casein, which was as soluble as gelatin and produced comparable rumen ammonia concentrations (suggesting comparable nitrogen losses), were shown to have significantly more microbial protein (101 g/day). Sheep fed zein were determined to have produced the lowest rumen ammonia levels and the highest amount of microbial protein (104 g/day). Hume concluded that differences in protein quality of the degraded nitrogen source, casein> gelatin and urea, accounted for increased microbial growth. More microbial protein is formed and leaves the rumen when a higher quality protein is fed probably because additional supplies of microbial growth factors such as branched-chain fatty acids (which are the result of dietary protein degradation and deamination of branched-chain amino acids), amino acids, and peptides are made available to rumen microbes.

Allison et al. (1962) showed that several species of rumen bacteria have specific requirements for one or more branched-chain fatty acids. Hume (1970a) observed that addition of branched-chain fatty acids to protein-free diets fed to lambs (whith urea as the source of nitrogen) increased microbial protein production from 71 to 81 g/day. When

branched-chain fatty acids were fed to steers consuming diets containing urea nitrogen (Oltjen et al., 1971) urinary nitrogen and ruminal ammonia concentrations decreased and nitrogen retention increased. The concentration of branched-chain fatty acids in the rumen is depressed in the feeding of protein free urea diets (Chalupa, 1972).

While studies conducted by Bryant and Robinson (1962) revealted that a significant percentage of the rumen microbes utilized ammonia as the source of nitrogen for the synthesis of cellular protein, 6% were shown to require amino acids for growth. Furthermore, Nolan and Leng (1972) have estimated that approximately 29% of the nitrogen digested in the rumen is utilized as amino acids by the microbes and 71% is degraded to ammonia. Thus, there is evidence that the amino acid pattern of the soluble dietary protein which is hydrolyzed in the rumen may influence the growth of some microbes.

A greater protion of the total protein reaching the abomasum is microbial protein with little or no by-pass protein when highly degradable protein or urea is the nitrogen source in rations (Chalupa, 1972). This was shown by Hume (1970b) when comparing nitrogen retention in sheep fed urea, gelatin, casein, and zein. It was found that significantly more nitrogen was retained by sheep fed zein than by sheep fed the other nitrogen sources. In addition to the 104 g/day of microbial protein, 44 g. of zein escaped the rumen undegraded. This made a total of 148 g/day of protein entering the abomasum of sheep fed zein compared with 90, 91, and 101 g/day for those fed urea, gelatin, and casein which were completely degraded.

In other studies, Potter et al. (1969) and Tucker and Fontenot (1970) showed that significantly more of the total nitrogen was

recovered in the abomasun of animals fed soybean meal than in animals fed urea. Potter et al. (1969) also determined that a higher percentage of the nitrogen found in the abomasum of sheep fed urea was in the form of pyrimidines and purines. As the percentage of microbial protein increases, the quantity of nucleic acids is increased (Chalupa, 1977). This represents a nitrogen loss because only about 50% of the nucleic acid nitrogen can be utilized by the ruminant (Smith, 1969).

The energy inefficiency of the synthesis of microbial protein from urea and other highly soluble nitrogen sources is another limiting factor (Oltjen, 1969). As the percentage of urea in the diet increases, greater amounts of energy are required to synthesize amino acids from ammonia and carbon precursors. One could therefore never expect the production of microbial protein from urea to be energetically equal to by-pass protein.

Where the solubility of the nitrogen source is relatively low and by-pass or undegraded protein makes a significant contribution to the total protein leaving the rumen, attention should be given to quality of the feed protein (Sniffen and Hoover, 1978). Evans and Biddle (1971) compared the nitrogen retention of animals fed rations of 13% soluble nitrogen containing wheat gluten or soybean meal. They found that animals fed soybean meal retained more nitrogen than those fed wheat gluten (35% vs. 26%). When Wohlt et al. (1976) compared the performance of wethers fed rations of two different protein qualities (amino acid combinations comparable to soybean meal and hominy) at two levels of nitrogen solubility, 13% or 35%, wethers fed the rations of the higher protein quality had higher levels of nitrogen retention at both levels of soluble nitrogen.

MacGregor et al. (1978) showed that knowing the protein quality of the feedstuff may not be adequate for estimating the protein quality of the by-pass protein. After studying the insoluble protein of 19 feedstuffs, they determined that the differences between the amino acid profile of the total protein and the insoluble protein were pronounced in two-thirds of the feedstuffs. This suggests that the protein quality of by-pass protein may be different from that of the protein that was originally consumed.

Measurement of Solubility

No best method for determining protein solubility is universally accepted. As a result, many solvents have been used by different researchers: 0.02 N NaOH (Lyman et al., 1953); a saline buffer at pH 7.0 of 0.2 M NaCl, 0.03 M Na_2HPO_4, and 0.011 M NaH_2PO_4 (Bisset, 1954); 0.1 M phosphate buffer at pH 7.0 (Annison, 1956); 0.01 M NaOH at pH 11.7, 0.5 M Na_2HPO_4 at pH 8.9, 0.5 M NaCl, and 70% ethylalcohol (Smith et al., 1959); artificial saliva of Huhtanen et al. (1954) (Tagari et al., 1962); autoclaved rumen fluid, 0.02 N NaOH, and distilled water (Little et al., 1963); a 10% dilution of Burroughs et al. (1950) mineral mixture (Hendrickx and Martin, 1963); McDougall's phosphate buffer at pH 6.8 (Peter et al., 1971); 3% NaCl solution (Nomani and Evans, 1971); an "Ohio" aqueous buffer solution of Johnson (1969) at pH 5.5 (Prigge et al., 1976).

Smith et al. (1959) compared NaOH, Na_2HPO_4, and NaCl solvents and found that NaOH extracted the highest proportion of protein from the various seeds in most of the cases followed by NaCl and Na_2HOP_4. However, in certain species the NaCl solvent was the most

effective for protein extraction. Little et al. (1963) compared the solubility of nitrogen sources in autoclaved rumen fluid, 0.02 M NaOH, and distilled water. The solubility of nitrogen was highest in NaOH and solubility in one solvent was not necessarily related to solubility in another solvent. In the comparison of autoclaved rumen fluid and 10% Burroughs mineral mixture as solvents for soluble nitrogen of casein and soy protein, Wohlt et al. (1973) found that solubility was higher in the mineral mixture. Although Crooker et al. (1978) found a nigh correlation (0.93) between the soluble nitrogen values of several feedstuffs in 10% Burroughs mineral mixture and a NaCl solvent, certain feedstuffs such as oats and citrus pulp showed great variation of solubility between the solvents.

It has been shown that all methods of measuring protein solubility do not produce the same results, and more importantly, the same relative ranking of all feed ingredients (Bull et al., 1977). The change in the percentage of soluble nitrogen with changing solvents is not simply an additive change but the result of several feed-protein-solvent interactions (Crooker et al., 1978). This is not surprising since protein type determines in what solvent the protein will be soluble (Oser, 1965): albumen--water soluble, globulin--salt soluble, prolamin--alcohol soluble, and glutelin--very dilute acid or alkali soluble. Protein solubility can also be influenced by factors associated with the solvent (Lehninger, 1970) - such as pH, chemical composition, ionic strength, temperature, degree of agitation, and length of extraction time. PH is important because each protein is least soluble at its isoelectric pH or point. At this point the protein has no net charge and therefore, there is not any electrostatic repulsion between neighboring protein molecules. Wohlt et al.

(1973) studied the solubility of casein and soy protein and showed that as pH increased from 5.5 to 7.5 there was an increase in mean solubility from 27 to 57%. The solubility of many proteins is increased ("salting") in salt solutions of low concentrations of such divalent ions as $MgCl_2$ and $(NH_4)_2SO_4$. But as the ionic strength is increased, the solubility decreases or "salting-out" occurs. And finally, the solubility of proteins increases with increases in solvent temperature, degree of agitation, and length of extraction. However, above 40^{o} to 50^{o} C most proteins become increasingly unstable and begin to denature, usually with a loss of solubility at the neutral pH zone.

Factors associated with the nitrogen source such as sample size, processing, and residence time in the rumen, may also affect solubility.

Wohlt et al. (1973) studied the effect of concentration of protein sample upon total nitrogen solubility with soy protein and casein. When increasing quantities of soy protein were added per milliliter of mineral mixture, milligrams of soluble nitrogen increased linearly with the amount added and the percentage of total nitrogen remained constant to 500 mg. With casein the milligrams of soluble nitrogen increased linearly to 250 mg. of soluble nitrogen. Casein continued to dissolve to 500 mg. of nitrogen but at a reduced rate resulting in a lower percentage of total soluble nitrogen.

The processing of feeds may also influence the protein solubility of feedstuffs. Moderate heating tends to reduce the amount of protein in the "albumin" fraction and to increase "residual protein" and the glutelin fraction (which is alkali soluble). Heating of greater severity tends to reduce all protein fractions except "residual protein" (Grau and Carroll, 1958). With heat processed by-product feeds it

is not uncommon to obtain solubilities which differ two to three fold in solubility from one batch to another (Bull et al, 1977).

Increased residence time of protein in the rumen tends to result in increased total soluble nitrogen. Residence time is influenced by feed intake, physical features of the feed particles, and associative effects of other ration ingredients (Satter et al., 1977).

Many of the solvents used ignored the specific properties of rumen fluid as a solvent and researchers have not always considered under what conditions protein solubility must be determined in solvents to compare with rumen fluid (Wohlt, 1973). Hendrickx and Martin (1963) chose a dilution, 10%, of Burroughs mineral mixture because it contained in the concentration of its chief elements the mineral structure of ruminant saliva and the average mineral structure of rumen fluid. There was a high correlation (0.99) between in vitro ammonia concentrations from purified proteins incubated for six hours with protein solubility. Little et al.(1963) calculated a high correlation (0.93) to exist between the percentage of nitrogen soluble in autoclaved rumen fluid and the level of free ammonia after two hours of incubation. The results of Crooker et al. (1978) in comparing a NaCl solution and autoclaved rumen fluid were less conclusive. They found the highest correlation to be 0.67 and 0.58 for NaCl and autoclaved rumen fluid respectively after four hours of incubation. Crooker et al. (1978) suggested that differences in available energy might have affected ammonia level and reduced the correlation.

Crawford et al. (1978) attempted to determine which method for measuring protein solubility is most representative of solubility in the rumen by using the nylon bag technique. They found a correlation

of 0.66 for 10% Burroughs mineral mixture, 0.54 for autoclaved rumen fluid, and 0.47 for 0.15 M NaCl with nitrogen disappearances at two hours from the nylon bags (rather than measuring ammonia levels).

The use of mineral mixtures or sterile rumen fluid to extract nitrogen from feedstuffs has become seen as a measurement of the quality of crude protein because the extracted nitrogen can be divided into two fractions, non-protein nitrogen (NPN) and protein nitrogen (Pichard and Van Soest, 1977). The NPN fraction which includes nitrate, ammonia, amines, and free amino acids, has been shown by solubility and kinetic studies to be more readily soluble and degraded than the protein fraction. This is particularly important for the evaluation of forages and grains at different stages of maturity. Waldo (1968) reported that 10 to 30% of the total nitrogen in fresh forage is NPN and in dry forage for hay or haylage the NPN fraction increases and ranges from 25 to 50% of total nitrogen. Data on corn grain and soybean seed showed a decrease from 30 to 40% of total nitrogen as NPN in an immature stage to 4-5% in the dry seed stage.

Agro-Industrial By-Products

By-products of the sugar industry, paper industry, cheese industry and fermentation processes (distilling, brewing, and wet milling of corn) were selected for evaluation. They consist of condensed molasses solubles, concentrated Steffen's filtrate, lignin sulfonate, condensed whey, yeast slurry, condensed distillers solubles, condensed fermented corn extractives or corn steep liquor, fermentation end liquor, and Dyno-Ferm. The following paragraphs briefly provide information on several of the selected by-products.

Condensed fermented corn extractives or corn steep liquor are obtained by the partial removal of water from the liquid resulting from steeping corn in water and sulfur dioxide solution which is allowed to ferment by the action of lactic acid producing micro-organisms as a process in the wet milling of corn (Inglett, 1970). It consists of the soluble protion of corn kernel removed by the steeping process (Corn Wet-Milled Feed Products, 1975) together with multiple cells of the microorganisms used in the fermentation process. Corn steep liquor is concentrated into a liquid containing 30 to 60% solids (Gutcho, 1973) and is considered to be a high protein liquid feed ingredient because it contains up to 50% crude protein on a 100% dry matter basis (Inglett, 1970).

Whey is the by-product of the manufacture of cheese, casein, quarg, and other sour-milk products. Because it varies with the product from which it is derived and with the manufacturing process, it is not considered to be a product of definite chemical composition. Whey contains half of the total solids, 20% of the total protein, and almost all of the vitamins and minerals of the original milk (Knipschildt, 1977). On an "as-is" basis fresh whey is only 7% solids of which 4.9% is lactose, 0.9% is protein, and 0.5% is ash (Anderson, 1970). Because of the high percentage of sugar, it is comparable to cereal grains as a source of energy (Schingoethe, 1976). But if kept over 35 hours at ambient temperature, it is not readily consumed. To avoid problems with bloat, whey should be fed gradually after a period when it has not been made available. When whey is condensed, 35 to 65% solids, it provides a liquid feed comparable to urea-molasses based liquid protein supplements. However, condensed whey should be mixed

with an equal amount of molasses to be palatable to cattle (Schingoethe, 1976).

Lignin sulfonate is a phenolic compound derived from spent sulfite liquor of the paper pulping industry (Chang et al., 1977). It may be used in animal feeds in either one or a combination of ammonium, calcium, sodium, or magnesium salts (Hathaway, 1973). Lignin sulfonate used in liquid feeds is concentrated to contain 50 to 55% solids which consist of 40 to 50% lignin, 10 to 25% sugars, 12 to 25% NPN compounds, and 5 to 10% acetic acid (Pigden, 1976). While it has been considered as a feed supplement for ruminants because of its high NPN and sugar content (Kromann et al., 1976) lignin sulfonate is limited by the Association of American Feed Control Officials and the Federal Drug Administration to 4% of the finished feed when used as a metabolizable energy source in liquid or dry form (Hathaway, 1973; Pidgen, 1976).

Condensed molasses solubles is an industrial by-product of undefined nutritive value obtained from the organic residue of molasses used in fermentation processes to produce alcohol, bakers yeast, citric acid, mono-sodium glutamate, etc. (Karalazos and Swan, 1977). It is condensed to a liquid containing 65 to 75% solids. On an "as-is" basis condensed molasses solubles consist of 47% organic matter, 2% sugar, 21% ash, 1.1% potassium and 4 to 5% nitrogen. Because of the high nitrogen level, it has been considered a useful ingredient for ruminant feeds (Karalazos and Swan, 1975).

Brewers liquid yeast is the nonfermintative, non-extracted yeast of the botanical classification, Saccharomyces. It is a by-product from the brewing of beer and ale. Brewers liquid yeast

must contain at least 35% crude protein on a dry weight basis (Clarke, 1977) and has been used as a substitute for grain in sheep rations (Zaera et al., 1974; Gomez et al., 1976).

Concentrated Steffen's filtrate is obtained by concentrating the filtrate resulting from the precipitation of calcium sucrate in the treating of beet molasses to recover additional sugar and is used chiefly as a source of amino acids (National Academy of Sciences, 1971).

Distillers solubles are obtained after the removal of ethyl alcohol by distillation from the yeast fermentation of a grain or a grain mixture by condensing the thin stillage fraction (Inglett, 1970). Whole spent stillage contains 5 to 10% solids and depending on the method, centrifuging or screening of large particles, it is eventually evaporated into a syrup of 30 to 55% solids (Chen et al., 1977). Because it contains up to 30% protein and 48% NFE (on a 100% dry matter basis), distillers solubles are considered a medium protein, high energy ration ingredient (Inglett, 1970).

CHAPTER III

IN VITRO EVALUATION OF BY-PRODUCT FEEDSTUFFS

Summary

Nine different agro-industrial by-products used as ingredients in liquid supplements were evaluated by conducting the following procedures: protein/nitrogen solubility in a buffer solution; ammonia release rate estimated by incubation of feedstuff samples in buffered rumen fluid, "as-as" and with sucrose and cane molasses; nitrogen insoluble in a pepsin-0.1 N HCl misture. Urea and soybean meal were used as controls.

Nitrogen solubility ranged from 100% to 16.6% soluble nitrogen of total sample nitrogen. The nitrogen content of lignin sulfonate, Dyno-Ferm, fermentation end liquor, and condensed molasses solubles, was determined to be 100% soluble while the solubility of nitrogen of concentrated Steffen's filtrate, corn steep liquor, condensed whey, distillers solubles, yeast slurry, and soybean meal ranged from 93.2 to 16.5%. Those feedstuffs having the highest percentages of soluble nitrogen also had the highest amounts of non-protein nitrogen.

Samples of lignin sulfonate, Dyno-Ferm, fermentation end liquor, condensed molasses solubles, and urea produced the highest ammonia levels after incubation in buffered rument fluid. With the exception of concentrated Steffen's filtrate, those nitrogen sources which had the highest percentages of soluble nitrogen were more rapidly degraded

and converted into ammonia than those sources that had lesser amounts of soluble nitrogen. In all three trials urea produced the highest ammonia concentrations, higher than other samples which also had 100% soluble nitrogen.

Soybean meal, lignin sulfonate, condensed distillers solubles, and corn steep liquor were determined to have low amounts of pepsin-insoluble nitrogen as percentages of total nigrogen (8.10, 5.80, 4.90, and 3.14%, respectively). The nitrogen content of the other by-product feedstuffs was nearly 100% soluble in the pepsin HCl mixture.

Introduction

The degradation of dietary nitrogen into ammonia by microbes in the rumen and subsequent production of microbial protein from the ammonia are dependent upon the solubility of the nitrogen source in the rumen medium. Because of differences in solubility, the degradation of the dietary nitrogen may vary considerably from one nitrogen source to another (McDonald, 1952; McDonald, 1954; McDonald and Hall, 1957). The higher the solubility of the nitrogen source the greater is the degree of nitrogen degradation as measured by ruminal ammonia concentrations (Chalmers et al., 1954; El-Shazly, 1958; Hendrickx and Martin, 1963; Little et al., 1963; Wohlt, 1973). The measurement of nitrogen solubility in mineral solutions and the determination of ammonia release rates in buffered rumen fluid have been used to evaluate the nutritional value of nitrogen sources for ruminants because it has been shown that the concentration of soluble nitrogen in rations has affected inversely nitrogen retention (Chalmers and Synge, 1954; Chalmers et al., 1954, Tagari et al., 1962; Peter et al., 1971; Wohlt, 1973).

Protein sources that have high solubility are rapidly converted into ammonia in the rumen where part of the ammonia may be lost through ruminal absorption and eventual excretion in the urine because the rate of nitrogen degradation exceeds the capacity of the microbes for conversion of ammonia into microbial protein.

Nitrogen that escapes degradation in the rumen may not be digestible post-ruminally, especially, if heat were involved in the processing. Since heat is used in the condensing of most by-product feedstuffs, it is important to determine whether the digestibility of the nitrogen source has been reduced by heat damage (Goering et al., 1977). This is particularly important in feedstuffs high in insoluble protein rather than non-protein nitrogen, because protein that is rendered indigestible is of no more use to the ruminant than nitrogen that is lost by overflow of ammonia which is known to result from the rapid degradation of non-protein nitrogen.

The objectives of this study were to measure the nitrogen solubility and to determine in vitro the ammonia release rates "as-is" and with sources of excess energy of agro-industrial by-products used as sources of natural protein in liquid supplements. To estimate the post-ruminal digestibility of by-product feedstuffs, samples were also evaluated for pepsin-insoluble nitrogen.

Materials and Methods

Feedstuffs

By-products of the sugar industry, paper industry, cheese industry, and fermentation processes (distilling, brewing, and wet milling of corn) were selected for evaluation. They consist of

yeast slurry (YS), IRN -7-05-521; condensed whey (CW), IRN -4-01-180; condensed distillers solubles (CDS), IRN -5-02-845; condensed fermented corn extractices or corn steep liquor (CSL), IRN -4-02-890; fermentation end liquor (FEL); condensed molasses solubles (CMS), IRN -4-04-697; Dyno-Ferm (DF); lignin sulfonate (LS); and concentrated Steffen's filtrate (CSF). Soybean meal (SBM), IRN -5-04-604, and urea were used as controls.

Laboratory Procedures

Protein/Nitrogen Solubility: The soluble nitrogen was extracted in an "Ohio" aqueous buffer solution (Johnson, 1969) with the pH adjusted to 6.5 and determined by Kjeldahl analysis. The soluble non-protein nitrogen was determined on 50 milliliters of the solution containing the soluble nitrogen fraction by precipitating the protein with 5.0 milliliters of 1.07 N H_2SO_4 and 5.0 milliliters of a 10% Na tungstate solution. This mixture was refrigerated overnight and the precipitate was separated by centrifugation at 10,000 RPM for ten minutes. Kjeldahl analyses were conducted on the supernatant solution and the soluble non-protein nitrogen was calculated as the amount of the nitrogen divided by the amount of sample per 50 milliliters of the solution containing the soluble nitrogen fraction. Soluble protein content represented the difference in nitrogen between the soluble nitrogen and soluble non-protein nitrogen fractions.

In Vitro Ammonia Release: Three in vitro trials were conducted to measure ruminal ammonia release. For each trial, rumen fluid was collected from a fistulated steer fed a ration of 50% alfalfa hay and 50% concentrates. It was strained through four layers of cheese cloth and

transported to the laboratory in an insulated pre-warmed container flushed with CO_2. Equal quantities of rumen fluid and McDougall's buffer (McDougall, 1948) were mixed and 30 milliliters of inoculum were pipetted into 50 milliliter centrifuge tubes containing the sample. Samples of the nitrogen source to be evaluated were weighed into the tubes before rumen fluid collection at 20 milligrams of nitrogen per tube. The tubes were flushed with CO_2, stoppered, and incubated at 39^{o} C in a water bath. Fermentation was stopped by the addition of 0.5 milliliter of 20% HCl solution after one and four hours of incubation. The tubes were centrifuged at 7,600 RPM and ammonia was determined by using the indophenol procedure of Chaney and Marbach (1962) and micro-diffusion.

In in vitro Trial I samples of the feedstuff were incubated "as-is" at 20 milligrams of nitrogen per tube.

In in vitro Trial II sucrose was added to each tube to equalize the soluble carbohydrate content which insured that fermentable energy would not limit the incubation process. To determine how much the feedstuffs vary, they were analyzed for soluble carbohydrate by using the procedure of T. L. Balwani (Johnson et al., 1966). Enough sucrose was then added to each sample so that each tube contained 25% more soluble carbohydrate than the feedstuff which initially contained the highest amount. Since the whey sample had the highest with .420 gram of soluble carbohydrate, each tube contained .525 gram after the addition of sucrose.

In vitro Trial III was identical to Trial II except that molasses was used to equalize the quantity of soluble carbohydrate in each tube instead of sucrose. The molasses was analyzed by the procedure

mentioned previously in Trial II to contain 53% soluble carbohydrate.

The results of the three in vitro trials were analyzed statistically according to the two-factor factorial design procedure (Steel and Torrie, 1960). There were two levels for time (one and four hours of incubation) and eleven for nitrogen sources. The samples of feedstuffs were incubated in duplicate in each trial to measure experimental error. Significant differences between mean ammonia-nitrogen concentration of the different feedstuffs were determined by using Tukey's honestly significant difference test (Steel and Torrie, 1960) (Appendix).

Pepsin-Insoluble Nitrogen: To determine pepsin-insoluble nitrogen, two grams each feedstuff were incubated at 39°C for twenty hours with one gram of pepsin and 100 milliliters of 0.1 N HCl in a 250 milliliter Erlendmeyer flask. The mixture was filtered on 12.5 centimeter Whatman No. 4 filter paper and thoroughly leached with distilled water. The filter paper containing pepsin-insoluble residue was analyzed for nitrogen according to the Kjeldahl procedure. The amount of pepsin-insoluble nitrogen in each sample was calculated by dividing the nitrogen content of the filter paper by the total amount of nitrogen initially in the sample.

Results and Discussion

Protein/Nitrogen Solubility

Nitrogen solubility of the samples (Table I) ranged from 100% for LS, DF, and FEL to 16.6% for SBM. Yeast slurry was the lowest of the by-product nitrogen sources, with 35.71% soluble nitrogen. The relationship between high nitrogen solubility and high percentages of

TABLE I

DRY MATTER, PERCENTAGE OF SOLUBLE NITROGEN, PERCENTAGE OF NPN OF NITROGEN SOURCES[a]

N Source[b]	SBM	CSF	LS	DF	CMS	FEL	CSL	CDS	CW	YS
% Dry Matter	90.58	56.01	49.08	39.05	59.87	41.43	46.53	48.95	33.60	24.84
%N	7.36	5.00	7.17	8.35	7.20	6.76	7.22	1.96	2.38	5.84
% Soluble N	1.23	4.66	7.17	8.75	7.14	7.04	6.40	1.12	1.73	2.05
% Soluble N of Total N	16.61	93.20	100.00	100.00+	99.35	100.00+	88.81	60.56	72.83	35.71
% Soluble NPN	0.16	4.46	7.86	8.04	6.35	7.58	4.71	0.67	0.77	0.76
% Soluble NPN of Total N	0.02	89.31	100.00+	96.37	88.12	100.00+	65.70	33.85	32.65	13.08

[a]All values except % Dry Matter are on a dry matter basis.

[b]Soybean meal - SBM, Concentrated Steffen's filtrate - CSF, Lignin sulfonate - LS, Dyno-Ferm - DF, Condensed molasses solubles - CMS, Fermentation end liquor - FEL, Condensed fermented corn extractives or corn steep liquor - SCL, Condensed distillers solubles - CDS, Condensed whey - CW, and Yeast slurry - YS.

TABLE II

IN VITRO AMMONIA-NITROGEN CONCENTRATIONS AFTER 1 AND 4 HOURS INCUBATION WITH AND WITHOUT THE ADDITION OF SUCROSE AND CANE MOLASSES[a]

Trial I ("As-Is")

N Source	SBM	Urea	CSF	LS	DF	CMS	FEL	CSL	CDS	CW	YS
1 hr.	7.3^{b}	29.8^{efgh}	8.5^{bc}	42.3^{hij}	47.3^{ij}	36.8^{ghi}	40.5^{hij}	21.0^{cdef}	11.8^{bc}	17.8^{bcde}	13.5^{bcd}
4 hr.	15.8^{bcd}	63.3^{k}	13.3^{bcd}	35.3^{ghi}	50.6^{jk}	39.3^{hij}	43.5^{ij}	25.8^{defg}	9.3^{bc}	20.6^{cdef}	8.1^{bc}

Trial II (+Sucrose)

N Source	SBM	Urea	CSF	LS	DF	CMS	FEL	CSL	CDS	CW	YS
1 hr.	7.9^{b}	48.8^{f}	7.9^{b}	38.9^{de}	48.9^{ef}	39.9^{de}	42.4^{ef}	15.4^{bc}	7.9^{b}	13.9^{bc}	12.7^{bc}
4 hr.	10.8^{b}	63.8^{g}	9.8^{b}	40.5^{ef}	45.5^{f}	31.3^{d}	42.3^{ef}	22.3^{c}	12.3^{b}	21.2^{c}	10.8^{b}

Trial III (+Molasses)

N Source	SBM	Urea	CSF	LS	DF	CMS	FEL	CSL	CDS	CW	YS
1 hr.	9.3^{bc}	44.5^{fg}	4.8^{b}	41.5^{efg}	44.5^{fg}	33.3^{def}	47.0^{fg}	10.0^{bc}	4.8^{b}	10.8^{bc}	4.8^{b}
4	5.5^{b}	57.8^{g}	8.8^{bc}	32.0^{def}	36.8^{ef}	24.8^{cde}	37.8^{ef}	16.8^{bcd}	6.8^{b}	17.3^{bcd}	9.3^{bc}

[a] Values expressed as mg. NH_3-H per 100 ml. increase above the blank value.
b,c,d . . . Values with different superscripts differ significantly ($P<.05$) within trials.

NPN agreed with the work of Pichard and Van Soest (1977). The three samples having 100% soluble nitrogen of total nitrogen were determined to contain 100% NPN. As the amount of NPN in the samples decreased, lesser amounts of the total nitrogen were soluble.

The percentage of soluble nitrogen to SBM, 16.6%, was intermediate between the 13% determined by Wohlt et al. (1973) using 10% Burroughs mineral mixture and the solubility of 19% in rumen fluid reported by Little et al. (1963).

The percentage of soluble nitrogen in whey, 72.8%, was within the range determined by Mavropoulou and Kosikowski (1973), 60 to 95%, for various types of whey from several geographical locations. Although they did not determine the amount of NPN in whey by measuring soluble protein, the percentage of NPN in the form of amino acids and soluble peptides was determined to range from 18 to 26% of the total nitrogen. This was less than the 32.6% soluble NPN shown by this solubility study.

In Vitro Ammonia Concentrations

In the in vitro trials to estimate ammonia release rates a significant ($P<.05$) nitrogen source-length of incubation interaction indicated that the two factors, time and nitrogen source, were not independent. There was a trend for nitrogen sources having the highest percentages of soluble nitrogen to produce the highest ammonia concentrations after incubation in buffered rument fluid. This is in agreement with Annison (1956), Hendricks and Martin (1963), and Little et al. (1963). Urea, DF, LS, FEL, and CMS were rapidly converted into ammonia. In contrast, SBM, YS, CW, CDS, CSF, and CSL produced ammonia concentrations which did not accumulate at high levels. DF, FEL, and urea had significantly higher ($P<.05$) ammonia levels after four hours of incubation than

the other feedstuffs tested.

The accumulation of ammonia from the conversion of urea after four hours of incubation exceeded the ammonia levels of the other nitrogen sources. The production of high ammonia concentrations from urea has been shown by earlier research (Stallcup and Looper, 1958; Hume, 1970 b) and, as a result, it has been recommended that urea be considered as 100% soluble nitrogen when calculating the soluble nitrogen of rations from the nitrogen sources.

While LS, DF, and FEL had 100% soluble nitrogen of total nitrogen, they all had lower accumulations of ammonia after four hours of incubation than urea. In Trial I and Trial II LS, which also had 100% NPN, produced significantly lower ($P<.05$) ammonia levels than urea. When T. A. Long at Pennsylvania State University (Hathaway, 1973) compared *in vitro* levels of ammonia produced from ammonium LS, urea, and SBM, it was observed that initial ammonia levels of ammonium LS increased nearly as rapidly as urea but peaked at a lower point, whereas ammonia concentrations from urea continued to increase. The overall ammonia concentration curve produced by ammonium LS placed it intermediate between urea and SBM. The difference in utilization between LS and urea is the result the high soluble carbohydrate content of LS (Pigden, 1976). By using the soluble carbohydrate procedure of T. L. Balwani (Johnson *et al.*, 1966), LS was determined to contain 25.7% soluble carbohydrate (Table III). However, when ammonia LS replaced urea at low levels in finishing rations, animal performance was not significantly altered (Chang *et al.*, 1977).

Sucrose was added to the samples in Trial II to insure that fermentable energy supplies would not limit the incubation process. When the

TABLE III

SOLUBLE CARBOHYDRATE MEASUREMENT[a] AND ADDITIONS OF SUCROSE AND MOLASSES

N Source	SBM	UREA	CSF	LS	DF	CMS	FEL	CSL	CDS	CW	YS
Sample Weight g	0.283	0.044	0.714	0.893	0.613	0.556	0.714	0.585	1.653	2.500	1.538
% Soluble Carbohydrate	12.0	-	10.3	25.7	1.6	8.9	1.8	4.7	14.0	16.8	5.9
+ Sucrose g	0.491	0.525	0.451	0.295	0.515	0.476	0.512	0.498	0.294	0.105	0.434
+Molasses g	0.926	0.991	0.851	0.557	0.972	0.898	0.966	0.940	0.536	0.198	0.819

[a]Percentages of soluble carbohydrate are listed on an "as-is" basis.

mean ammonia concentrations of Trial II were compared with the means of Trial I (samples incubated "as-is"), it was observed that there were no major changes in ammonia level after the addition of sucrose.

These results are not in agreement with earlier experimental work that showed in vivo that the feeding of glucose and sucrose (Annison, 1956; Lewis and McDonald, 1958) and in vitro that the addition of glucose, sucrose, and lactose (Hendrickx and Martin, 1963) reduced rumen ammonia concentrations. The most commonly given reason for these reductions was that increased supplies of readily available energy were utilized by bacteria to increase the synthesis of protein from ammonia (Satter and Roffler, 1975). However, Hendrickx and Martin (1963) reported that the presence of fermentable carbohydrates inhibited protein degradation while at the same time it stimulated protein synthesis.

It was noted that ammonia concentrations in the tubes containing urea continued to increase at high levels after the addition of sucrose and molasses. This is in agreement with Stallcup and Looper (1958) who found that the production of ammonia from urea-molasses based rations was on the same order as rations containing urea fed alone. A possible explanation for this was proposed by Hungate (1966) who suggested that ammonia is probably better utilized by fiber and starch-digesting rumen bacteria than by those which utilize amino acids. He theorized that because large quantities of soluble protein and soluble carbohydrates, which are naturally present together in lush, growing forage, are rapidly degraded and fermented when eaten, certain rumen bacteria utilize available amino acids and fermented sugars simultaneously. Since ammonia accumulates, it is utilized by bacteria which use energy sources that are more slowly made available.

Pepsin-Insoluble Nitrogen

The results of the pepsin-insoluble nitrogen determination are found in Table IV. Of the feedstuff samples that measured 100% soluble nitrogen, only lignin sulfonate (LS) was determined to contain more than 1% pepsin-insoluble nitrogen. Lignin sulfonate has been reported to contain phenolic compounds (Chang et al., 1977) which may have combined with the pepsin to produce insoluble compounds, in a tannin-protein type reaction (Van Soest, 1973). The protein content of by-product feedstuffs which approached a nitrogen solubility of 100% and produced high ammonia concentrations after incubation in buffered rumen fluid (FEL, DF, CMS, and LS) would probably be completely degraded in the rumen, leaving no by-pass protein for post-ruminal digestion. When those by-product feedstuffs which had the highest levels of insoluble nitrogen (CSL, CW, CDS, and YS) were compared, it was noted that the protein of yeast slurry (YS) and condensed whey (CW) was 100% soluble in the pepsin-HCl mixture. In contrast, it was determined that condensed fermented corn extractives or corn steep liquor (CSL) and condensed distillers solubles (CDS) contained 3.14 and 4.90% pepsin-insoluble nitrogen, respectively. The insoluble protein of YS and CW which escaped rumen degradation would probably be completely digested post-ruminally while small amounts of protein of CSL and CDS would remain undigested.

TABLE IV

PEPSIN-INSOLUBLE NITROGEN, PERCENTAGE OF TOTAL NITROGEN AND PROTEIN NITROGEN[a]

N Source	SBM	CSF	LS	DF	CMS	FEL	CSL	CDS	CW	YS
%N	7.36	5.00	4.57	8.35	6.01	7.41	8.21	1.96	2.38	5.23
% Protein N	7.20	0.54	0.00	0.31	0.85	0.00	2.51	1.29	1.68	5.04
% Pepsin - insoluble N	0.60	0.02	0.26	0.03	0.02	0.05	0.26	0.10	0.00	0.00
% Pepsin - insoluble N of Total N	8.10	0.70	5.80	0.31	0.28	0.60	3.14	4.90	0.00	0.00
% Pepsin - insoluble N of Protein N	8.33	3.70	--	9.67	2.35	--	10.36	7.75	0.00	0.00

[a]All values are on a dry matter basis.

CHAPTER IV

EFFECT OF SELECTED BY-PRODUCT FEEDSTUFFS ON IN VIVO RUMEN AMMONIA CONCENTRATION AND CELLULOSE DIGESTION

Summary

Thirty crossbred (Dorsett X Suffolk) wethers were assigned randomly to six different feeding groups for a seven-day preliminary feeding period followed by a six-day period of fecal collection for cellulose digestibility determination. On the sixth day of the collection period, rumen fluid samples were obtained at one and four hours post-feeding for rumen ammonia analysis. The rations of the feeding groups were based on the nitrogen sources to be compared: soybean meal as the positive control, a negative control without a nitrogen supplement, urea, and three liquid feed supplement ingredients, i.e., condensed fermented corn extractives or corn steep liquor, condensed whey, and condensed distillers solubles. Molasses was added to each nitrogen source in amounts to equalize the TDN level to 500 grams of TDN/head/day and mixed with weathered prairie hay to be fed once daily.

Animals fed urea had significantly higher concentrations of rumen ammonia than animals fed the other nitrogen sources. There were no significant differences among treatment groups in ammonia

concentrations in sheep fed the by-product nitrogen sources. Differences in concentrations were not significantly different at one and four hours post-feeding.

There were no significant differences in percentages of cellulose digested among feeding groups receiving urea and the by-product feedstuffs. All groups that were fed the nitrogen sources digested higher percentages of cellulose than the negative control group. Sheep fed condensed distillers solubles digested the highest percentage of cellulose and had the highest cellulose intake.

Introduction

Nitrogen balance trials are conducted to compare the digestion and retention of nitrogen from different nitrogen sources. It has been shown that increasing the concentration of soluble nitrogen in a ration can inversely affect the amount of nitrogen retained (Woods et al., 1962; Nomani and Evans, 1971; Sniffen, 1974; Aitchison et al., 1976). Because increased concentrations of rumen ammonia accompanied the feeding of highly soluble nitrogen sources, ruminal overflow of ammonia has been considered a factor in the reduction of the amount of nitrogen retained (Chalmers and Synge, 1954; Chalmers et al., 1954; Tagari et al., 1971; Wohlt, 1973). Chalmers (1960) proposed that nitrogen sources be compared by measuring the ammonia levels produced when fed to experimental animals. Those nitrogen sources which consistently produced high ammonia concentrations at post-feeding measurement were considered inferior because of the potential for nitrogen loss and reduced nitrogen retention.

While a goal of ruminant nutrition is to produce optimal amounts

of microbial protein from protein of low biological value and non-protein nitrogen sources and to simultaneously increase nitrogen retention by reducing losses from ammonia overflow, another goal is to increase the utilization of cellulose in the form of roughage because of its relative abundance and low cost when compared with other energy sources. Ruminants are unique among domestic animals in their ability to utilize cellulose as an energy source. The practice of feeding molasses-urea based supplements to animals grazing poor quality forage is an attempt to achieve these goals. However, because of the use of urea and other non-protein nitrogen sources which are too rapidly degraded into ammonia for efficient microbial conversion into protein, optimal nitrogen retention is not usually attained (Lusby and Armbruster, 1977). Also, high intake of molasses based supplements has depressed intake and digestion of roughages (Hamilton, 1942, Bohman et al., 1954; Brannon et al., 1954; Martin and Wing, 1966), therefore, countering the goal of increased utilization of cellulose. Agro-industrial by-products are presently being used as alternative nitrogen sources in liquid supplements but their effect on animal performance has not been studied extensively.

The goal of this study was to compare three by-product feedstuffs which represent a range of nitrogen solubilities in a digestion trial using soybean meal and urea as controls. Post-feeding ruminal ammonia concentrations were measured and cellulose digestion was determined for the different feeding groups.

Materials and Methods

Animals and Facilities

Thirty crossbred (Dorsett X Suffolk) wethers averaging 60 pounds were used. All animals were shorn prior to the trial. Each animal was housed in a 3' X 4' raised wooden stall with slatted floor. Canvas diapers were harnessed to each animal to insure that all feces were collected.

Collection

Orts were collected and weighed before each feeding. After each feeding diapers were emptied and fecal collections were weighed. Orts and fecal collections were frozen pending analysis. At the time of analysis the collections for each animal were pooled, mixed, and sampled for determination of cellulose.

Feeding

The thirty wethers were assigned randomly to six different rations. There was a seven-day preliminary feeding period followed by the six-day collection period. The lambs were fed once daily. The nitrogen sources were combined with molasses and mixed with hay at the time of feeding. Water was provided free choice.

Rations

The six rations were based on the nitrogen sources that were compared (Table V). They were soybean meal (SBM) as the positive control, a negative control without a nitrogen supplement (NC), urea,

TABLE V

COMPOSITION OF DIGESTION TRIAL RATIONS (GRAMS/DAY[a])

Feeding Group[b] grams	SBM	Urea	NC	CSL	CDS	CS
Nitrogen Source	104	16	-	218	758	908
Molasses	726	840	840	704	295	363
Dicalcium Phosphate	18.6	27.2	27.2	4.5	22.7	13.6

[a]All weights are on an "as-is" basis.

[b]Soybean meal -SBM, negative control - NC, Condensed fermented corn extractives - CSL, Condensed distillers solubles - CDS, Condensed whey - CW.

and three by-products used as liquid supplement ingredients: condensed fermented corn extractives or corn steep liquor (CSL), condensed whey (CW), and condensed distillers solubles (CDS). Molasses, IRN-4-04-696, was added to each ration to equalize the TDN level to 500 grams of TDN/head/day. National Research Council feed tables were used to determine TDN content of the nitrogen sources and molasses. Each nitrogen source was fed at a level to provide 45 grams of protein/head/day. All rations were balanced with dicalcium phosphate. The nitrogen sources and the necessary quantity of molasses were combined at the time of feeding and mixed with 227 grams of weathered prairie hay harvested in March. An additional 113 grams of hay were fed each afternoon.

Cellulose Analysis

The cellulose content of the weathered prairie hay, orts, and feces was determined by the procedure of Crampton and Maynard (1938).

In Vivo Rumen Ammonia Measurement

Ruminal fluid samples were obtained by using a stomach tube at one and four hours post-feeding on the sixth day of the collection period to compare ammonia release rates. Ruminal fluid samples were analyzed for ammonia-nitrogen by using the indophenol procedure of Chaney and Marbach (1962) and microdiffusion.

Statistical Analysis

The results of the cellulose digestion trial were analyzed using the completely randomized design procedure and the means of the different feeding groups were compared by using Tukey's honestly significant

TABLE VI

IN VIVO AMMONIA CONCENTRATION MEASUREMENT FROM SHEEP USED IN THE DIGESTIBILITY TRIAL AT 1 AND 4 HOURS POST-FEEDING

Feeding Groups[a]	UREA	NC	CW	CDS	CSL
1 Hr[b]	36.9[c]	1.2[d]	8.9[d]	6.4[d]	10.7[d]
4 Hr[b]	37.3[c]	2.0[d]	7.9[d]	13.4[d]	10.6[d]

[a] SBM fed sheep were not sampled because of a failure to uniformly feed the SBM ration.

[b] Values are expressed as mg. NH_3-N per 100 ml.

[c,d] Values with different superscripts differ significantly ($P < .05$).

TABLE VII

CELLULOSE DIGESTIBILITY AND INTAKE OF LAMBS FED WEATHERED PRAIRIE HAY AND DIFFERENT NITROGEN SOURCES

N Source	SBM	UREA	NC	CW	CDS	CSL
Cellulose Intake g/da	103.31	112.16	88.60	113.16	121.83	106.97
Cellulose in Feces g/da	69.27	67.16	71.46	73.75	66.62	64.59
Cellulose Digested g/da	34.04	45.01	17.14	39.41	55.21	42.38
% Cellulose Digested	32.9^{bc}	40.1^{ab}	19.3^{c}	34.8^{abc}	45.3^{ab}	39.6^{ab}

^{a,b,c}Values with different superscripts differ significantly ($P < .05$).

difference test (Steel and Torrie, 1960). The results of the rumen ammonia-nitrogen measurement were analyzed statistically according to the split-plot design procedure and significant differences between the mean rumen ammonia-nitrogen concentrations of the different feeding groups were determined by using Tukey's honestly significant difference test (Steel and Torrie, 1960) (Appendix).

Results and Discussion

In Vivo Ruminal Ammonia Concentrations

The results of the ruminal ammonia analysis that was made from the ruminal fluid samples taken on the sixth day of fecal collection are presented in Table VI. There were no significant differences in ammonia within the different feeding groups at one and four hours post-feeding. This was in agreement with the results of *in vitro* Trial III when molasses was added to feedstuff samples to balance the soluble carbohydrate content. Sheep fed urea had significantly higher ($P<.05$) concentrations of rumen ammonia than sheep fed the other nitrogen sources. Comparisons of the mean rumen ammonia concentrations of the groups fed the by-produce nitrogen sources showed that the differences were not significant ($P>.05$). While CW and CSL produced higher ammonia concentrations than CDS in the *in vitro* trials, *in vivo* ammonia concentrations were similar.

It is possible that these ammonia concentrations were not representative of the ammonia concentrations which were produced daily because the animals were force fed their total daily intake of molasses-nitrogen source mixture in a few minutes. It has been shown that the rate at which feed is ingested can significantly influence ammonia concentrations

and nitrogen retention. Moir and Somers (1957) found that when all the ration was consumed at one time after one daily feeding the ruminal ammonia concentrations were significantly higher and nitrogen retention was significantly lower than when the ration was consumed in smaller units after several daily feedings. In this digestion trial twelve to twenty-four hours were usually necessary for the feed to be eaten rather than a few minutes immediately following feeding.

Because many studies have shown that high ruminal ammonia concentrations are related to ammonia overflow and reduced nitrogen retention, attempts have been made to determine the concentration of rumen ammonia necessary to support maximal bacterial growth without nitrogen loss. By using continuous-culture fermentors charged with rumen fluid from steers, Satter and Slyter (1974) determined that under nitrogen limiting conditions increasing the ammonia concentrations beyond 5 mg. NH_3-N/100 ml had no effect on increasing microbial protein production. All the nitrogen sources in the *in vitro* trials produced ammonia concentrations which exceeded this level. In contrast, when Mehrez and Orskov (1976) measured the *in vivo* ammonia concentrations needed for maximum ruminal digestion of feed contained in Dacron bags, it was observed that the mean concentration of 23 mg. NH_3-N/100 ml corresponded to the highest percentage digested. This is considerably higher than the ruminal ammonia concentrations produced by the by-product feedstuffs in this digestion trial.

Cellulose Digestion

The results of the cellulose digestion trial are found in Table VII. Higher percentages of cellulose were digested by animals fed the nitrogen

sources than those fed the negative control. It has been shown previously that ammonia is an essential nutrient for cellulose digesting bacteria (Little et al., 1963; Chalupa, 1972). When the mean percentages of cellulose digested by the groups of wethers fed the nitrogen sources were compared, there were no significant differences in percentages of cellulose digested. Earlier work by Head (1953), who tested maize protein, casein, white fish meal, peanut meal, urea, and urea+D.L. methionine, and by Akkada and El Shazly (1958), who compared meat and fish meal, beans (Vicia faba), cottonseed meal, casein, and linseed cake, also failed to find any distinct protein source effect on cellulose digestion. In contrast, when Belasco (1954) compared SBM and urea in vitro, he found that higher cellulose digestion was obtained with urea than SBM at equivalent nitrogen levels. Little et al. (1963) observed that in vitro cellulose digestion tended to parallel the ammonia levels produced by the different protein feeds, with large feed-to-feed variation in the quantity of ammonia produced.

While the differences in cellulose digestion among the different groups fed the by-product nitrogen sources were not significant, it was noted that animals fed CDS consumed the highest amount of cellulose and digested the highest percentage. Burroughs et al. (1950) observed that dried distillers solubles (DDS) was among several protein rich feeds which stimulated cellulose digestion in vitro. More recently when Ely et al. (1975) compared cellulose digestion in lambs fed urea, urea+10% DDS, casein, or gelatin, it was found that lambs fed the DDS supplemented ration digested the highest percentages of cellulose. The results of in vitro cellulose digestion trials by Beeson and Chan (1976) and Chan et al. (1976) showed that CDS significantly increased digestion, with

higher percentages being digested with screened CDS than with centrifuged CDS. The proline content of the protein of distillers solubles may be partially responsible for the cellulytic stimulatory property (Potter et al., 1966; Little et al., 1967).

Since the group of wethers fed CDS was also fed the lowest level of molasses of the six feeding groups, reduced molasses intake may have positively influenced cellulose digestion. While the addition of sugar (glucose, sucrose, or cane molasses) at low levels to rations has been shown to result in increased cellulose digestion, both in vivo and in vitro (Hoflund et al., 1948; Aris et al., 1951), the feeding of high levels has produced significant reductions in cellulose digestibility (Hamilton, 1942; Bohman et al., 1954; Brannon et al., 1954). Because the digestion of cellulose was shown to be decreased by increasing the amount of molasses fed from 6% to 18% of the ration (Martin and Wing, 1966), it can be assumed that the feeding of molasses at 50% or higher levels, which was the procedure in four of the six feeding groups in this digestion trial, may have negatively influenced the percentages of cellulose digested.

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APPENDIX

ANALYSIS OF VARIANCE

In Vitro Trial I ("As-Is")

Source of Variation	df	SS	MS	F
Treatments	21	22,291.54	1061.50	108.76
time	1	419.78	419.78	43.01
N source	10	19,570.46	1957.05	200.52
N X time	10	2,301.13	230.11	23.58
Experimental error	22	214.73	9.76	
Total	43	22,506.27		

In Vitro Trial II (+Sucrose)

Source of Variation	df	SS	MS	F
Treatments	21	25,358.74	1207.56	172.02
time	1	121.96	121.96	25.76
N source	10	24,471.82	2447.18	516.83
N X time	10	764.96	76.50	16.16
Experimental error	22	104.18	4.75	
Total	43	25,462.92		

In Vitro Trial III (+Molasses)

Source of Variation	df	SS	MS	F
Treatments	21	24,024.27	1144.02	65.82
time	1	25.10	25.10	1.44
N source	10	22,422.84	2242.28	128.01
N X time	10	1,576.62	157.65	9.07
Experimental error	22	382.25	17.38	
Total	43	24,406.72		

In Vivo Ammonia Concentration

Source of Variation	df	SS	MS	F
N source	4	15,017.49	3754.37	31.50
Animal/N source	20	2,383.24	119.17	
Time	1	2.95	2.95	0.03
Time X N source	4	138.03	34.51	0.32
Animal/Time	20	2,178.53	108.93	

Cellulose Digestion

Source of Variation	df	SS	MS	F
N source	5	2,087.32	417.46	6.39
Experimental error	24	1,566.37	65.27	
Total	29	3,653.69		

VITA

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