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Effect of grain supplementation on digestion of alfalfa-grass hay in goats

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To my father, for his spiritual guidance.

To my mother, for her prayers.

To you, for your unconditional encouragement.

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ABSTRACT

The effect of alfalfa-grass hay supplemented with milo was evaluated in four goats. Four female goats, fitted with ruminal and duodenal cannulae, were fed four diets, consisting of immature alfalfa-grass hay, mature alfalfa-grass hay, a 1:1 mixture of mature hay supplemented with milo-soybean meal concentrate and a 1:1 mixture of immature hay supplemented as above. The milo-based supplement was balanced to be isonitrogenous with the mature hay. Ruminal pH decreased ($P<.05$) when grain was added to the hay diets but there were no significant differences between immature and mature hay. Grain supplementation did not affect the amount or proportion of DM digested in the rumen. The digestibility coefficient of DM in the total tract was increased ($P<.02$) by supplementing the hay diets with milo. Although the amounts of NDF and ADF digested in the rumen ($P<.06$) and total tract ($P<.05$) were lower in goats fed the milo supplemented diets, NDF and ADF digestibility coefficients in the rumen and total tract did not differ between unsupplemented and supplemented diets. Total VFA concentrations were higher ($P<.05$) in diets containing immature hay than mature hay. Hay maturity did not affect molar proportions of acetic and propionic or butyric acids. Grain supplementation decreased molar proportions of acetic and propionic acids ($P<.01$) and increased butyric acid ($P<.01$). The acetate:propionate ratio was greater ($P<.05$) in goats fed supplemented than unsupplemented diets at 0 and 2 h post-feeding. Goats fed immature hay had higher ($P<.05$) ruminal ammonia concentrations than those fed mature hay. Grain supplementation did not affect ruminal ammonia concentration. Neither maturity nor grain supplementation affected duodenal flow of ammonia, bacterial or undegraded-N, proportions of feed-N flowing from the rumen as bacterial or undegraded-N or the microbial efficiency. It was concluded that supplementation with as much as 50% milo to an alfalfa-grass hay had no negative associative effects on fiber digestion.

INTRODUCTION

The rate and extent of digestion as well as the nature of fermentation are primarily influenced by the chemical and physical nature of the diet. Dry matter digestibility may be depressed under conditions in which rate of passage is increased or when fiber digestion is depressed as in high starch diets (Ferrell, 1988).

Concentrates may be used to increase dietary energy, protein, mineral and vitamin concentration. Concentrates must also balance energy sources (starch or fermentable carbohydrates, easily digestible fiber and fats) and protein sources (non-protein nitrogen, fermentable or unfermentable proteins) to optimize efficiency of feed utilization for growth, gestation or milk production (Morhand-Ferh and Sauvant, 1987). However, many workers have demonstrated that concentrate supplementation reduces digestibility of fiber in forage-containing diets (Hoover, 1986; Mould et al., 1983; Moore et al., 1990; Merchen, 1988; Galyean and Owens, 1991).The depressing effect on digestibility of fiber in forage-containing diets, when readily fermentable carbohydrates are added, is related to a preference by rumen microbes for readily fermentable carbohydrates rather than fiber components, a decrease in ruminal pH and competition for essential nutrients (Merchen et al., 1986; Hoover, 1986; Mould et al., 1983; Harrison and McAllan, 1980). The extent of the effect of concentrate on forage fiber digestion also depends on forage species and maturity (Rust, 1983).

Although associative feed effects between forages and grains have been extensively investigated in cattle, few studies have been conducted in goats.

The objective of this study was to evaluate the effects of a milo-based concentrate on the digestibility of alfalfa-grass hay fed to goats in mixed diets.

REVIEW OF LITERATURE

Physiology of Carbohydrate Digestion.

Carbohydrates may be digested by two different processes, fermentation and hydrolysis. Hydrolytic digestion by the ruminant animal occurs in the abomasum and intestine. Certain glycosidic bonds, as in fructosans, are cleaved by gastric acid in the abomasum. However, the majority of the hydrolytic digestion occurs in the small intestine by specific enzymes (Rust, 1983).

Starch Digestion

The general sequence of starch digestion involves hydrolysis of starch into oligosaccharides which are further degraded to hexoses. Amylase is the enzyme responsible for hydrolysis of starch into oligosaccharides.Two types of amylase have been isolated. Beta-amylase is found in plants and hydrolyzes alpha (1-4) glucosidic linkages. Alpha amylase is an animal enzyme which can hydrolyze alpha (1-4)linkages. The hydrolysis of alpha (1-6) linkages is on the other hand performed by alpha-limited dextrinases.

There are four sources of alpha amylase in animal systems: 1) salivary, 2) pancreatic, 3) intestinal, and 4) microbial. Non-ruminant animals generally obtain amylase from all four sources, but ruminants lack salivary amylase.

Ruminal digestion. Ruminal starch digestion begins with solubilization through bacterial extracellular alpha amylase hydrolysis (French, 1973). The soluble oligosaccharides and dextrins are further degraded to hexoses and pentoses by maltase and other oligosaccharidases. The oligosaccharidases may be extracellular or attached to microbial cell wall membranes. Hexoses and pentoses are subsequently used by the microbial population either to supply essential carbon skeletons for the synthesis of microbial biomass or to provide

energy for both microbial maintenance and growth requirements. Volatile fatty acids, CO_2, and methane are the major products of fermentation.

Intestinal digestion. Carbohydrates may be solubilized by the action of hydrochloric acid in the abomasum or rendered more accessible through the action of proteolytic enzymes. Pancreatic alpha amylase may act intraluminally or when bound to the mucosal cells of the small intestine. Intestinal mucosa glycoamylase and bacterial amylases also contribute to intestinal starch digestion.

It is important to emphasize that about 79 to 90% of starch is digested in the rumen (Van Soest, 1994; Beever, 1993; Orskov, 1986) and about half of the remainder is digested in the small intestine. Therefore, most soluble carbohydrates are degraded in the rumen and indeed do not reach the lower gastrointestinal tract. This factor may account for the lower quantities of amylolytic enzymes secreted by the ruminant pancreas. Moreover, it has been speculated that the capacity of amylolytic digestion of starch is limited in cattle and other grazing species (Van Soest, 1994).

The microbes of the cecum and large intestine also digest carbohydrates; this is particularly important when sheep and cattle are fed high-concentrates and potentially fermentable carbohydrates pass to the distal colon.

Fiber Digestion

Mammals lack enzymes to degrade complex polymers such as cellulose, hemicellulose and pectin so they must be degraded by microorganisms found in the rumen, ileum, cecum and colon.

Cellulose digestion is commonly regarded as the principal function of certain rumen microbes, especially in browsers such as the goat that receive a diet sufficiently high in cellulose (Van Soest, 1994).

Structural carbohydrates are degraded by enzymes secreted by bacteria into the gastrointestinal medium. Further hydrolysis or phosphorylative cleavage occurs inside bacterial cells. Certain protozoa also can engulf and attach to fibers and etch pits into cell walls. Many cellulolytic bacteria are encased in a gelatinous coat of glycoprotein which aids in attachment. The cellulases and hemicellulases are found in close proximity to the glycoprotein layer of the cellulolytic microbes. Adhesion of bacteria to cell walls is greatly enhanced by mechanical damage to the wall. This damage could occur with physical processing of feeds or with mastication.

Structural carbohydrates which escape digestion in the rumen may be degraded in the lower gastrointestinal tract. In fact, cellulose and hemicellulose digestion is substantial in the large intestine and cecum. However, the cecum and colon may play a larger role in hemicellulose than cellulose digestion. Organic matter fermented in the cecum and colon has been reported to be of limited value since volatile fatty acid (VFA) absorption is limited and bacteria are excreted in the feces (Rust, 1983).

Effects of Forage Maturity on Digestion

Stage of maturity affects the digestibility of forages because of the changes in chemical composition. Grasses and legumes become more lignified as plants mature, and digestibility is inversely proportional to the amount of lignin present (Van Soest, 1994). Three theories have been proposed to describe the effects of lignin on cell wall digestibility: 1) physical encrustation and entrapment, 2) enzyme inhibition and 3) linkage to carbohydrates.

In addition, a decrease in total digestible nutrients as plants mature has been reported (Schneider and Flatt, 1975). The digestibility of protein was affected to a great extent as maturity advanced, whereas the digestibility of dry matter, nitrogen-free extract, ether extract and fiber were reduced only slightly when late-cut hay was compared with early-cut hay.

Factors Affecting Rate of Digestion

Rate of digestion is the speed at which ingesta is physically and chemically reduced to smaller particle size in preparation for absorption. Major factors which influence rate of digestion include : a) composition of the diet, b) nitrogen or mineral deficiencies, and c) level of intake (Rust, 1983).

Dietary composition generally determines the distribution of the rumen microbial population that digests feed nutrients. High-protein diets favor proteolytic organisms and high-starch diets that are low in fiber are associated with a large population of starch utilizers (Van Soest, 1994).

The type and maturity of the forage also influences the rate of digestion. Legumes have a faster rate of digestion than grasses, while mature plants are digested at less than half the rate of plants at the vegetative stage of growth (Rust, 1983).

The particle size of the roughage also can influence the rate of digestion. An increase in the digestion rate constant was demonstrated when the particle size of alfalfa was reduced from 12 mm to 1 mm (Rust, 1983). Moreover, Reid and Tyrrel (1964) reported that the bulkiness or physical form of long or chopped hay alone prevented animals from eating enough to depress digestibility.

Rate of digestion of various concentrates and mixed diets has been reviewed by several investigators (Mould et al, 1983; Berge and Dulphy, 1985, Hassan et al, 1988). Although the results were variable, it is generally accepted that an increased level of readily fermentable carbohydrates decreases digestibility of roughages when they are added to mixed diets.

Nutrient deficiencies may interfere with the rate of digestion. Addition of urea to low quality forage often increases the rate of digestion, passage and intake. When deficient, supplementation with branch chain fatty acids increases the rate of growth of cellulolytic organisms and the rate of cellulose digestion (Hungate, 1966). Certain inorganic minerals can stimulate digestion rate. Adequate external sources of nitrogen, sulfur, and essential minerals

are required for optimum carbohydrate utilization. Sodium, potassium, phosphate and bicarbonate contribute to the buffering system and are recycled through saliva. Thus, if any essential nutrient is lacking, or the rumen environment is not optimal, the rate of digestion will be slow and either intake or digestibility will decrease (Van Soest, 1994).

Generally, apparent organic matter digestibility decreases as the level of intake increases (Van Soest, 1994). This decrease in digestibility is a result of an altered rate and extent of digestion and passage rate. Intake effects on diet digestibilities are more pronounced with mixed diets than with diets consisting of single feedstuffs. Digestibilities of mixed forage-grain diets fed to lactating dairy cows decrease by approximately 4% for each increase in intake equivalent to maintenance (Tyrrel and Moe, 1975). A decrease in digestibility due to increasing dry matter intake is caused by an increase in the rate of passage through the digestive tract and also depressions of the digestibility of both starch and fiber components of the diet (Merchen, 1988).

Effect of Ruminal pH on Digestibility

The ratio of forage to concentrate has been shown to affect the digestibility of feeds. It is generally recognized that increasing the amount of grain supplementation depresses the digestibility of other dietary components by shifting the microbial population which reduces cellulolytic activity (Santini et al, 1992). There are two reasons for the decrease in rumen pH by increasing the proportion of concentrate in the diet. First, the time spent eating and ruminating when grains are consumed is less than for fibrous feeds, and, since saliva secretion is affected to a large extent by eating and ruminating, there is less saliva to buffer rumen fluid. Second, the fermentability or digestibility of a starchy concentrate is generally greater than for forages. As a result the VFA production per unit weight of concentrate is greater than from fibrous feeds. Ideally, more saliva is required with concentrate than with roughages (Orskov, 1992).

Ecological conditions within the rumen must be kept within limits to maintain normal microbial growth and metabolism and thus the well-being of the host ruminant (Van Soest, 1994). Cellulolytic organisms grow optimally at pH 6.7 and deviations substantially higher or lower than this are inhibitory (Van Soest, 1994; Owens and Goetsch, 1988; Hoover, 1986; Leek, 1993). A moderate depression in pH to approximately 6.0 results in a small decrease in fiber digestion, but numbers of fibrolytic organisms are usually not affected. Further decreases in pH to 5.5 or 5.0 result in depressed growth rates, depressed fibrolytic microbes and fiber digestion may be completely inhibited (Hoover, 1986). In addition, it has been stated that low pH may retard attachment of microbes to cellulose. Scarcity of specific nutrients or growth factors at a low pH also can reduce rate of cell division and growth efficiency (Owens and Goetsch, 1988).

Fermentation and Volatile Fatty Acid Production

The VFAs produced as end products of anaerobic microbial metabolism provide the ruminant with a major source of metabolizable energy. Many microbial species are present in the rumen, and most of these are selective fermenters. Hence, differences between species in fermentation patterns reflect to a certain extent, differences in VFA pattern of the preferred substrate degraded (Van Soest, 1994).

The proportion of the principal fatty acids produced in the rumen can be markedly influenced by diet. Forage diets generally produce an "acetate" fermentation pattern, which is characterized by a high molar proportion of acetate and a low proportion of propionate and butyrate, and a typical molar ratio of acetate:propionate:butyrate would be about 75:15:10. On the other hand, diets containing a high proportion of grain generally produce a "propionate" fermentation pattern with a typical molar ratio 55:25:15 (Leek, 1988). When increasing the proportion of grain in the diet, soluble carbohydrates are partially liberated as cell walls are fractured during mastication. The fermentation of these soluble carbohydrates in the rumen

provides incomplete oxidation products such as VFAs which may then be synthesized into either glucose or lipids for metabolism to CO_2, H_2O and energy (i.e. adenosine triphosphate (ATP) and heat). These acid products lower ruminal pH, resulting in a depression in fiber digestion when mixed with forages.

Protein Digestion

The nitrogenous components of the diet support the protein metabolism of the rumen organisms and their host, but the interactions of diet, microbes and animal host that determine the net supply of protein to the host are complex (Van Soest, 1994).

The saliva of ruminants contains no proteolytic enzymes and the mucosa of the rumen, reticulum , and abomasum possesses no secretory glands. It is therefore evident that digestion of protein in the rumen is due only to proteolytic enzymes produced by microbes (protozoa and bacteria).

The most important source of nitrogen (N) for the rumen microbes is normally dietary protein and non-protein nitrogen (NPN) although recycling of urea from blood and saliva together with small amounts of epithelial N form a substantial contribution to the microbial N requirement. The rumen microflora are highly proteolytic, and thus ensure that most of the protein entering the rumen is degraded to peptides and amino acids, most of which are subsequently deaminated (Orskov, 1992; Van Soest, 1994; Leek, 1993).

Several factors affect the degradation of dietary protein. The type of diet represents an important factor to be considered. The rate at which protein supplements are degraded can differ according to whether the rumen environment can support a high or low rate of cellulose degradation. The degradation of protein supplements of vegetable origin occurs more rapidly when they are fermented in a medium which is highly cellulolytic than if the medium is less favorable for cellulolysis (Orskov, 1992). The extent to which degradation rate is related to proportion of roughage in the diet is not adequately understood, and it is quite possible that the

rate of protein degradation from vegetable sources of protein is only affected when the proportion of grain in the diet is such that the resultant rumen pH inhibits cellulolysis (Owens and Goetsch, 1988; Orskov, 1992). If this is the case, then the effect of pH is not directly due to differences in proteolytic activity.

Other factors related to rumen environment on protein degradation include the concentration of free fatty acids which can reduce proteolysis, while outflow rate can influence the extent of degradation of protein. The extent to which rumen effluent flow rate of liquid and solids can also affect the rate of protein degradation is not known. The degradation of protein to ammonia involves hydrolysis to peptides and some amino acids, followed by further degradation of peptides to amino acids and deamination of amino acids to ammonia. Increasing outflow of amino N could therefore theoretically be affected by factors affecting hydrolysis and by factors influencing peptidases and deaminases (Orskov, 1992).

Rumen Ammonia Concentration and Nitrogen Metabolism

Ruminal ammonia concentrations are frequently quantitated as an indicator of rumen metabolism with particular reference to ruminal protein degradation (Noeck et al, 1987). Ammonia concentration in the rumen is a function of the relative rates of entry and removal of ammonia. Ammonia enters the rumen from a number of sources including fermentation of feed, lysed cell fragments, endogenous proteins, miscellaneous soluble-N compounds (such as endogenous urea, nucleic acids, uric acid, and nitrate) and protozoal excretion. It is removed from rumen fluid in several ways: by incorporation into microbial protein which then passes out of the rumen, by absorption through the rumen wall, and by fluid flowing to the lower digestive tract (Nolan, 1993).

As an important precursor of microbial protein, ammonia is essential for the growth of certain bacteria (Harrison and McAllan, 1980). While protozoa do not use ammonia, the majority of bacterial species in the rumen utilize it for growth, and for some species it is

essential. Ammonia is the only N source required by three species that probably contribute most of the cellulolytic activity in the rumen, i.e. Ruminococcus albus, R. flavofaciens and Fibrobacter succinogenes.

The rate and extent of dietary degradation in the rumen varies between different protein sources. It also depends on the level of proteolytic activity, which is highly variable, and on the time the protein spends in the rumen as well as other factors such as pH and microbial species present (Nolan, 1993). The kind of diet influences proteolytic activity. Fresh forage diets, usually high in protein and soluble carbohydrate, promote growth of proteolytic bacteria, leading to specific activities in the rumen which are greater than those found in animals given low-protein, hay based diets. The proteins of cereal grain, on the other hand, are less degradable. Moreover, changes in the rumen environment or microbial population could influence the rate at which ammonia nitrogen (NH3 N) is taken up by microbes, thus affecting microbial production at a given ammonia concentration (Hoover, 1986). As an example, Shirley (1986) reported that the amount of fermentable energy sources available influences the growth of rumen microbes and the quantity of ammonia converted to protein.

In contrast, at extremes in ruminal pH as a result of a high grain intake, efficiency of microbial protein synthesis tends to remain relatively unchanged (Weakley and Owens, 1983).

The level of rumen NH_3 N concentration required for maximal microbial protein synthesis seems to range from 1.2 to 22.1 mg/dl (Weakley and Owens, 1983). Orskov (1992) reported that no increase in microbial yield occurs as a result of increasing ammonia concentration in the rumen to more than 5 mg/dl, whereas Van Soest (1994) stated that the optimal level of rumen ammonia is thought to be 10 mg/dl.

Supply of Protein to the Small Intestine

The extent of dietary protein degradation in the rumen and subsequent synthesis of microbial protein can greatly alter amounts and kinds of amino acids available for absorption in the small intestine of ruminants (Theurer, 1980). The arrival of proteins in the small intestine of ruminants is determined by the amount of dietary protein which escapes ruminal degradation and the quantity of microbial protein synthesized in the rumen. Endogenous protein in the form of abomasal secretions and desquamated epithelial cells also reach the small intestine.

Feed intake is a factor that promotes protein escape. Owens and Goetsch, (1988) reported that each 10% increase in feed intake of a high-concentrate diet increased ruminal escape of plant protein by 6.5% in dairy cows. This is probably due both to a decrease in the amount of time the protein spends in the rumen and to changes in ruminal conditions including pH and the microbial population. In addition to level of feed intake, diet type also influences protein escape. The extent of ruminal protein degradation is usually much greater with forage diets than with concentrate diets.

Ruminal pH is involved because the optimum pH of most proteolytic and deaminase enzymes in the rumen is thought to be between 6 and 7. The percentage of a feed protein which is water soluble often is greater at a neutral pH than an acid pH. Also at a neutral pH proteolytic bacteria may be more prevalent or degrade cellulose and cell walls to expose more protein for microbial digestion and subsequent microbial synthesis (Owens and Goetsch, 1988).

Measurement of Microbial Yield

The development of techniques to measure microbial protein is essential to clarify the effects of dietary regimens in altering the proportion of microbial protein and ruminally undegraded dietary protein reaching the intestines (Theurer, 1980). The need to be able to accurately measure microbial protein synthesis in the rumen has been recognized for many years (Schelling, 1980). While several different approaches have been investigated, none of

those procedures meet, or even approach the criteria of an ideal methodology, but they have helped to provide an idea of the quantities involved (Orskov, 1992).

The majority of techniques used to estimate microbial contribution to total protein flow are based on determination of a single chemical marker believed to characterize microbial components. Compounds proportional to microbial crude protein (MCP), which are located only within microbes include ribonucleic acid (RNA), diaminopimelic acid (DAP), deoxyribonucleic acid (DNA), D-alanine and total purines, have been used to estimate both MCP and organic matter flow from the rumen (Owens and Zinn 1988, Siddons et al, 1982). Studies have supported the general potential of using the amount of microbial nucleic acid passing from the rumen as an indicator of microbial protein. The use of RNA as a marker depends on the assumption that all of this component, isolated at the duodenum, is of microbial origin. Pure RNA has been shown to be rapidly and almost totally degraded within the rumen (Ling and Buttery, 1978). Moreover, compared with certain other markers, nucleic acids are present in both bacteria and protozoa (Owens and Zinn, 1988; Orskov, 1992).

Collection of Digesta Postruminally to Determine Degradability of Feed Nitrogen

Several methods such as collection of digesta postruminally, utilization of nylon bags and rate of outflow of protein supplements are devoted to measure protein degradability. The collection of duodenal contents from duodenal cannulae gives an accurate assessment of the quantity of N which passes into the small intestine. Indigestible markers such as chromium oxide are also included in the diet to determine digesta flow and samples obtained are corrected for 100% recovery of the indigestible marker.

The microbial protein is isolated from the duodenal samples, and the feed protein is calculated as the difference between total duodenal N and microbial N. Usually the N found as

ammonia is subtracted, although sometimes an estimate is made of the endogenous contribution (Orskov, 1992).

MATERIALS AND METHODS

Materials

Forages. A hay field, containing a mixture of alfalfa (Medicago sativa L.) and smooth bromegrass (Bromus inermus L.), was divided into two plots. Second harvest forage was mowed at two maturities, sun-cured and baled in small bales. Immature hay was harvested from one plot when the first flower was observed on the alfalfa. Mature hay from the other plot was harvested four weeks later. Bales of hay were ground in a forage grinder with a 2.5 cm screen prior to feeding trials.

Animals and Diets. Four French alpine crossbred female goats (BW= 42 kg) were surgically fitted with 2.5 cm i.d. rubber cannulae in the rumen (Hecker, 1969) and 1.5 cm i.d. t-type cannulae in the proximal duodenum, 5 cm posterior to the pyloric sphincter (Komarek, 1981). Goats were placed in individual pens and allowed to recover for approximately three weeks. After recovery, goats were utilized in a 4x4 Latin Square digestion trial with 14-day adjustment and 6-day collection phases. Treatments consisted of diets containing: mature alfalfa-grass hay; immature alfalfa-grass hay; a 1:1 mixture of mature alfalfa-grass hay with a milo-soybean meal grain mixture balanced to be isonitrogenous to the hay; and a 1:1 mixture of immature alfalfa-grass hay and the same grain mixture as fed with the mature hay (Table 1). Diets were fed twice daily at 0900 and 1900 h at a level 10% above *ad libitum* intake during adjustment. During collection, diets were offered at 90% of the *ad libitum* intake. As a digestibility marker, fiber was extracted from the ground hay and mordanted with 2% sodium dichromate and ascorbic acid (Russell et al., 1993). Chromium mordanted fiber (1.5 g) in a gelatin capsule was inserted through the rumen cannulae at 0900 and 1900 h from d 8 to d 20 of each period. Goats had continuous access to clean drinking water and trace-mineralized salt blocks (Table 1).

Table 1. Ingredients and chemical composition of the diets fed to goats.

	Hay maturity and concentrate percentage			
	immature		mature	
Item	0	50	0	50
Ingredient, % DM				
Immature alfalfa-grass hay[a]	100	50	-------	-------
Mature alfalfa-grass hay	-------	-------	100	50
Ground milo	-------	45.5	-------	45.5
Soybean meal	-------	4.5	-------	4.5
Trace mineral salt[b]				
Chemical composition				
DM, %	87.2	88.2	89.2	88.9
% of DM				
CP	16.1	15.0	13.2	13.4
NDF	54.0	34.5	62.5	39.3
ADF	36.3	20.4	43.3	23.9

[a]The alfalfa-gass hay was ground at 2.5 cm length.

[b]All diets were supplemented free choice with trace mineral salt containing 96% sodiun chloride; .40% zinc oxide; .16% iron oxide; .12% manganous oxide; .0033% copper oxide; .01% calcium iodate; .004% cobalt carbonate.

Methods

Sampling Procedures. Feed samples were collected on d 1 and 2 of each collection phase. During d 3 to 5 of each collection phase, duplicate samples of 100 ml of duodenal digesta were obtained four times daily. Sampling times were advanced 2 h each day such that samples collected over the 3 d represented each 2 h of a 24-h cycle. Samples were frozen (-20 C) for later analysis. Fecal samples were collected on d 3 to 5 of each collection phase and frozen. Ruminal fluid samples (100 ml) were collected from the ventral sac of the rumen through the rumen fistula at 0, 2, 4, and 6 h post-feeding on d 5 of each collection phase. Ruminal pH was measured with a digital pH meter. Rumen samples were acidified with 1 ml 50% sulfuric acid and frozen at -20 C. Five hundred milliliters of rumen fluid were collected 3 h post-feeding on d 6 of each collection phase. Bacteria were isolated by differential centrifugation (Adamu, 1985) and frozen for later analysis.

Chemical Analysis. Dry matter (DM) concentrations of feed and feces were determined in samples dried in a forced air oven at 65 C for 48 h (AOAC, 1975). Samples of duodenal digesta from each animal at each collection time and the bacterial pellet from ruminal fluid for each animal were freeze-dried. Dried feed, duodenal digesta and feces were ground through a 1-mm screen. Dry duodenal digesta and feces were composited on an equal weight basis for each animal in each period. Concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in dry feed, duodenal digesta and feces were determined by the sequential procedure of Van Soest and Robertson (1979). Nitrogen concentrations of feed, freeze-dried duodenal digesta, feces and bacteria pellets were determined with a Kjeltec apparatus (Tecator, Höganäs, Sweden) using selenium as a catalyst. Ammonia-N concentrations of ruminal fluid and wet duodenal contents were determined by the hypochlorite procedure (Van Slyke and Hiller, 1933) and adapted for an autoanalyzer (Technicon, Tarryton, NY). To determine the proportion of total N and ammonia-N in freeze-dried duodenal contents, .5 g freeze-dried

duodenal contents were extracted with 9.5 ml H_2O for 12 h. Ammonia -N concentrations of the extracts were determined by methods similar to those used for ruminal fluid and wet duodenal digesta. Purine concentrations of the rumen bacteria pellet and freeze-dried duodenal samples were determined by the procedure of Zinn and Owens (1986). Bacterial N concentrations of duodenal contents were estimated from the N-to-purine ratios of the bacterial pellets and duodenal digesta. Undegraded feed-N was estimated by substracting ammonia-N and bacterial N concentrations from the total-N concentration of freeze-dried duodenal contents. Dried mordanted fibers, duodenal digesta and feces were ashed at 500 C for 3 h . Chromium was extracted from the ash with a manganese sulfate-potassium bromate-phosphoric acid solution and analyzed by atomic absorption spectrophotometry (Williams et al, 1962). Duodenal and fecal dry matter flow were estimated from the amount of Cr fed and duodenal and fecal Cr concentrations. Rumen fluid samples were centrifuged at 10,000 x g for 10 min and ruminal VFA concentrations were determined by gas chromatography (Varian®) using a packed GC column (10% Ds-1200/1% H_3PO_4 on 80/100 chromosorb WAG, 2 m x 2 mm ID) and a flame ionization detector following the procedure of Lambert and Moss, (1972).

Statistical Analysis. Data were analyzed by analysis of variance (SAS®, 1985) with main effects of goats, periods, hay maturity and concentration level. Only main effects are presented for variables in which interaction between hay maturity and grain supplementation was not significant ($P>.05$).

RESULTS

No significant differences in ruminal pH were found between immature and mature hay diets (Table 2). Ruminal pH, however, decreased ($P < .05$) from 6.5 to 6.2 when the milo-based concentrate was added to the diets.

Similar to ruminal pH, no differences in DM, NDF and ADF intakes were found between diets containing immature and mature hay (Table 3). Addition of concentrate to the diets, however, decreased ($P < .01$) NDF and ADF intake expressed either in g/d or as a percentage of body weight. Hay maturity did not significantly affect the amounts of DM, NDF or ADF digested in the rumen. The proportions of consumed DM and NDF digested in the rumen, however, tended ($P = .26$) to be greater in immature than mature hay. Grain supplementation did not affect the amount of dry matter digested in the rumen, but tended ($P = .23$) to increase the proportion of consumed DM digested in the rumen. Because of lower NDF and ADF intakes, the amounts of NDF and ADF digested in the rumen were lower ($P < .06$) in goats fed the grain-supplemented diets. Ruminal digestion coefficients for NDF and ADF did not differ between unsupplemented and grain supplemented diets. This implies that no associative effects occurred with supplementation of a milo-based grain mixture at 50% of the diets.

The proportion ($P = .09$) of DM digested in the total gastrointestinal tract tended to be greater in goats fed immature hay than when fed mature hay. Hay maturity did not affect the amount or proportion of NDF digested in the total tract. Grain supplementation tended to increase both the amount ($P = .26$) and proportion ($P = .02$) of consumed dry matter that was digested in the total tract. Similar to the rumen, grain supplementation decreased ($P < .05$) the amounts of NDF and ADF digested in the total tract, but did not affect their total tract digestion coefficients.

Apparently because of greater ruminal DM digestion, goats fed diets containing immature hay had greater ($P < .05$) total VFA concentrations in their rumens 2 and 4 h

Table 2. pH in the rumen fluid of goats fed immature and mature alfalfa-grass hay supplemented with milo.

	Hay maturity		Grain supplementation			Significance[a]	
Time, h	immature	mature	0	50	SEM	M	G
0	6.7	6.7	6.9	6.5	.23	.72	.02
2	6.3	6.3	6.5	6.2	.11	.91	<.01
4	6.3	6.3	6.4	6.2	.22	.70	.09
6	6.3	6.3	6.5	6.1	.22	.93	<.01

[a]M = main effect of maturity, G = main effect of grain.

Table 3. Effect of hay maturity and grain supplementation on intake and ruminal digestion by cannulated goats.

	Hay maturity		Grain supplementation			Significance[a]	
Item	immature	mature	0	50	SEM	M	G
Intake, g/d							
DM	1062	1054	1063	1053	134	.90	.88
NDF	477	530	614	392	73	.19	<.01
ADF	304	350	411	243	50	.11	<.01
Intake, % BW							
DM	2.4	2.5	2.4	2.4	.32	.82	.94
NDF	1.1	1.2	1.4	0.9	.18	.17	<.01
ADF	0.7	0.8	0.9	0.5	.12	.09	<.01
Ruminal digestion, g/d							
DM	377.8	303.0	306.8	374.1	144	.34	.38
NDF	224.1	209.3	262.1	171.3	72	.69	.04
ADF	117.6	124.1	150.1	91.2	52	.81	.06
Ruminal digestion, % of intake							
DM	35.6	28.7	28.5	35.7	10	.25	.23
NDF	46.0	39.4	41.4	44.1	10	.26	.62
ADF	34.7	36.3	36.6	34.5	15	.84	.78
Total tract digestion, g/d							
DM	676.6	577.2	558.7	695.0	160	.75	.26
NDF	242.5	220.3	289.2	173.7	80	.60	.02
ADF	131.7	120.7	163.8	88.6	48	.63	.01
Total tract digestion, % of intake							
DM	64.0	55.3	53.5	65.8	8	.09	.02
NDF	43.9	42.9	42.8	44.0	17	.90	.89
ADF	40.8	35.8	40.4	36.1	13	.47	.53

[a]M = main effect of maturity, G = main effect of grain.

post-feeding than when fed mature hay (Table 4). Hay maturity did not significantly affect the molar proportion of acetic, propionic and butyric acids nor the acetate-to-propionate ratio. Supplementation with the milo-based grain mixture did not affect total VFA concentrations at 0, 2 or 4 h post feeding, but tended (P = .11) to increase total VFA concentration 6 h post-feeding. Grain supplementation decreased (P<.01) the molar proportion of acetic acid from 0 to 6 h post-feeding and the molar proportion of propionic acid from 0 to 2 h post feeding while increasing (P<.01) the molar proportion of butyric acid from 0 to 6 h post-feeding. The acetate-to-propionate ratio was greater (P = .02) in goats fed the grain-supplemented diets than unsupplemented diets at 0 and 2 h post-feeding. Grain supplementation did not affect acetate-to-propionate ratio at 4 or 6 h post-feeding. Branched chain VFA concentrations were not affected by the diets in this study.

Ruminal ammonia-N concentrations peaked about 2 h post feeding (Table 5). Goats fed diets containing immature hay had higher (P<.01) rumen ammonia-N concentrations than when fed mature hay diets 2 h post-feeding. Goats fed hays supplemented with the milo-based grain mixture had a greater (P =.03) ruminal ammonia-N concentration at feeding. Grain supplementation did not affect mean ruminal ammonia-N concentration from 2 to 6 h post-feeding. Grain supplementation of immature hay decreased ruminal ammonia-N concentrations of goats but increased those of goats fed mature hay (hay maturity x grain supplementation, (P<.06) 2 to 6 h post-feeding.

Goats fed diets containing immature hay consumed greater (P<.01) quantities of N per d than when fed mature hay diets (Table 6). Hay maturity, however, did not affect duodenal flow of ammonia, bacterial or undegraded-N, the proportions of feed N flowing from the rumen as bacterial or undegraded-N or the microbial efficiency. Similar to hay maturity, grain supplementation did not affect flow of ammonia and bacterial-N through the duodenum nor the microbial efficiency. Grain supplementation tended to reduce the amount (P = .12) and proportion of consumed- N (P=.16) flowing as undegraded-N in the duodenum.

Table 4. VFA concentrations in the rumen fluid of goats fed immature alfalfa-grass hay and mature alfalfa-grass hay supplemented with milo.

	Hay maturity		Grain supplementation			Significance[a]	
Time, h	immature	mature	0	50	SEM	M	G
			Total VFA mM/l[b]				
0	51.1	53.7	49.2	55.6	11.4	.67	.30
2	88.7	73.3	81.1	80.9	12.6	.05	.96
4	86.6	71.0	76.8	80.8	13.0	.05	.57
6	66.9	69.1	63.1	72.9	10.6	.69	.11
			Acetic acid, molar %				
0	63.0	64.3	66.1	61.3	2.8	.40	.01
2	63.9	63.8	65.9	61.8	2.2	.92	.01
4	64.2	64.5	67.0	61.7	1.3	.64	<.01
6	64.5	64.8	68.6	60.6	2.2	.80	<.01
			Propionic acid, molar %				
0	14.7	15.5	16.8	13.4	.99	.15	<.01
2	21.1	19.8	22.3	18.7	1.9	.24	.01
4	20.0	18.5	20.2	18.3	1.9	.15	.09
6	18.5	18.2	18.8	17.8	1.6	.26	.97
			Butyric acid, molar %				
0	10.2	9.9	6.0	14.1	1.4	.68	<.01
2	7.5	8.7	4.8	11.4	1.2	.08	<.01
4	8.4	9.5	5.2	12.7	1.4	.18	<.01
6	9.5	9.8	5.7	13.6	1.1	.57	<.01
			Acetic:Propionic molar ratio				
0	4.3	4.1	3.8	4.6	.47	.58	.02
2	3.0	3.2	2.8	3.3	.33	.31	.02
4	3.1	3.4	3.2	3.3	.35	.13	.55
6	3.4	3.5	3.6	3.4	.42	.69	.53

[a]M = main effect of maturity, G = main effect of grain.

[b]Branched chain volatile fatty acids not included in this table.

Table 5. Ammonia concentrations in the rumen fluid of goats fed immature and mature alfalfa-grass hay supplemented with milo. Each value represents the average of four observations.

	Hay maturity and concentrate percentage							
	immature		mature			Significance[a]		
Time, h	0	50	0	50	SEM	M	G	MxG
0	16.7[b]	18.0	14.9	20.1	2.4	.88	.03	.15
2	35.1	31.6	22.3	27.9	2.9	<.01	.48	.02
4	23.8	14.5	14.6	19.2	5.2	.42	.39	.03
6	11.4	8.3	9.9	13.9	3.1	.25	.77	.06

[a]M = main effect of maturity, G = main effect of grain, MxG = interaction of maturity and grain.

[b]mg/dl.

Table 6. Effect of forage maturity and grain supplementation on duodenal flow of N by cannulated goats.

	Hay maturity		Grain supplementation			Significance[a]	
Item	immature	mature	0	50	SEM	M	G
N intake, g/d	162	130	152	140	16	<.01	.17
Duodenal N flow, g/d							
Ammonia-N	7.6	8.3	8.5	7.4	2.7	.64	.43
Bacterial-N	97	102	92	107	31	.79	.36
Undegraded feed-N	43	29	46	26	22	.28	.12
% N intake,							
Bacterial-N	61	77	62	76	21	.20	.22
Undegraded feed-N	27	23	30	19	15	.63	.16
Microbial efficiency[b]	29	37	35	32	17	.38	.76

[a]M = main effect of maturity, G = main effect of grain.

[b] calculated by g bacterial-N/g ruminal digested DM.

DISCUSSION

The effects of feeding mixed grain-roughage on digestibility of the diets are variable. Improvements in DM digestibility, when mixed forage-grain diets are fed to sheep, have been reported (Hassan at al., 1988; Brandt and Klopfenstein, 1986). In contrast, other researchers have found negative associative effects present in animals fed mixed diets (Mould et al 1983; Galloway et al 1993; Antoniou and Hadjipanayiotou, 1985, Berge and Dulphy, 1985). Only 10 to 15% of added readily fermentable carbohydrate can impair fiber digestion, and 30% or more of DM intake causes severe depression in fiber digestion (Hoover, 1986). It is recognized that increasing levels of grain supplementation decreases digestibility by reducing rumen pH and, thereby, shifts the microbial population to reduce cellulolytic activity (Mould et al, 1983; Galloway et al, 1993). Ginger et al, (1988) also reported the reduction of rumen pH in goats fed alfalfa hay supplemented with oat-, barley-, or wheat-based concentrates at a level of 45% of the DM consumed. The depression in cell-wall digestion resulting from grain supplementation of cattle is greater in mature forages than in immature forages (Schneider and Flatt, 1975).

In this study the proportion of consumed DM digested in the rumen and total digestive tract tended to be greater either when immature hay was fed or when diets were supplemented with a milo-based grain mixture. The ruminal digestion coefficients for NDF and ADF, however, were not affected by grain supplementation.

The reduction in fiber digestion observed when grain was supplemented with forages has been associated with ruminal pH values lower than 6.2 (Van Soest, 1994; Leek, 1993). Inasmuch as ruminal pH only decreased to 6.2 when alfalfa-grass hays were supplemented with a milo-based grain mixture at 50% of the dietary dry matter in this study, the microbial population was not affected enough to alter fiber digestion.

When grain is supplemented with a forage, the reduction in fiber digestibility is normally associated with an increase in amylolytic species of bacteria which produce lactic and

propionic acids (Van Soest, 1994). The increase in molar percentage of butyric acid when alfalfa-grass hay diets were supplemented with milo in this study, implies that the microbial population was dominated by butyric acid producing bacteria . These species tend to be cellullolytic and hemicellulolytic bacterial species (Van Soest, 1994). Ginger et al. (1988) also found that goats receiving a diet based on alfalfa hay supplemented with oat-, barley-, or wheat-based concentrates developed increased proportions of butyric acid and decreased proportions of acetic acid.

Supplementation with a milo-based grain mixture decreased ruminal ammonia -N concentrations in goats fed immature alfalfa-grass hay, but increased ruminal ammonia-N concentrations in goats fed mature alfalfa-grass hay. This may be the result of different N concentrations of the diets. The grain supplemented mature hay diet was balanced to be isonitrogenous to the unsupplemented mature hay diet. Since the same supplement was fed with the immature hay, the CP concentration of the diet containing immature hay supplemented with the grain mixturewas 1% less than that of the unsupplemented immature hay. Nevertheless, in the present study, all diets generated ammonia-N concentrations far above the minimum required for optimal microbial growth (Satter, 1974). Ruminal ammonia-N concentration, however, may also reflect differences in the supply of readily fermentable carbohydrates and the degradability of the dietary N source (Hoover, 1986). In the present study, supplementation with a milo-based grain mixture tended to increase the proportion of consumed N that was incorporated into bacterial-N. Shirley (1986) observed that the amount of fermentable energy sources available in the diet influences the growth of rumen microbes, however, Weakley and Owens (1983) and Galloway et al. (1993) demonstrated that the efficiency of microbial protein synthesis tends to remain relatively unchanged at high grain intakes.

On the other hand, incorporation of the milo-based grain mixture decreased the proportion of consumed N which escaped ruminal degradation. This result was unexpected because literature values for the undegradabilities of ground milo (49%) and soybean meal (35%) are considerably lower than alfalfa-grass forages (21%) (NRC, 1989); and because grain supplementation has been reported to reduce protein degradation from plant protein sources (Orskov, 1992).

IMPLICATIONS

The production of meat or milk from goats in developing countries is limited by nutrient intake from low quality roughages. Nutrient intake by goats may be increased by increasing the nutritive value of the forages by harvesting at an immature stage. Improving the nutritive value of forages may not always be possible. In such case, supplementation with grain like milo may be used to improve nutrient intake. Grain supplementation of forages, however, has been reported to result in negative associative feed effects, particularly with low quality roughages. Results of this experiment indicate that supplementation with as much as 50% milo of an alfalfa-grass hay with a ruminal DM digestibility as low as 29% did not adversely affect fiber digestion.

REFERENCES

Adamu, A.M. 1985. Supplemental protein sources for growing cattle fed corn stover silage. Ph.D. Dissertation. Iowa State University. Ames, IA.

Antoniou, T and M. Hadjipanayiotou. 1985. The digestibility by sheep and goats of five roughages offered alone or with concentrates. J. Agric. Sci., Camb. 105:663

AOAC. 1975. Official Methods of Analysis. 13th Ed. Association of Official Analytical Chemists, Washington, DC.

Beever, D.E. Rumen function. 1993. In J.M. Forbes, and J. France (Eds.) Quantitative Aspects of Ruminant Digestion and Metabolism. pp 187-215. CAB International. Wallingford, Oxon. UK.

Berge, P. and J.P. Dulphy. 1985. Etude des interactions entre fourrages et aliment concentré chez le mouton. I. Facteurs de variation du taux de substitution. Ann Zootech. 34:313

Brandt, R.T. and T.J. Klopfestein. 1986. Evaluation of alfalfa-corn cob associative action. II. Comparative tests of alfalfa hay as a source of ruminal degradable protein. J. Anim. Sci. 63:902

Ferrel, C.L. 1988. Energy metabolism. In Church, D.C.(Ed.) The Ruminant Animal. Digestive Physiology and Nutrition. pp 250-268. Prentice Hall. Englewood Cliffs, NJ.

French, D. 1973. Chemical and physical properties of starch. J. Anim. Sci. 37:1048

Galloway, D.L., Sr., A.L. Goestsch, L.A. Foster, Jr., A.C. Brake and Z.B. Johnson. 1993. Digestion, feed intake, and live weight gain by cattle consuming Bermuda grass and supplemented with different grains. J. Anim. Sci. 71:1288

Galyean M.L. and F.N. Owens. 1991. Effects of diet composition and level of feed intake on site and extent of digestion in ruminants. In: T. Tsuda, Y Sasaki and R. Kawashima. (Ed.) Physiological Aspects of Digestion and Metabolism in Ruminants. pp 483-513. Academic Press. San Diego, CA.

Giger, S, D. Sauvant, M. Duran and J. Hervieu. 1988. Influence de la nature de l'aliment concentré sur quelques paramétres de la digestion dans le rumen. Reprod. Nutr. Develop. 28:117.

Harrison, D.G and A.B. McAllan. 1980. Factors affecting microbial growth yields in the metabolism in ruminants. In: Y. Ruckebush and P. Thivend (Ed.) Digestive Physiology and Metabolism in Ruminants. Proceedings of the 5th International Symposium on Ruminant Physiology. pp 205-226. MTP Press. Lancaster, England.

Hassan, A.A., H.D. Woody and A.W. Young. 1988. Associative effects of mixed diets in sheep. Transactions of the Illinois Academy of Science. 81:1

Hecker, J.F. 1969. A simple rapid method for inserting rumen cannulae in sheep. Aust. Vet. J. 45: 293

Hoover W.H. 1986. Chemical factors involved in ruminal fiber digestion. J.Dairy Sci. 69:2755

Hungate, R.E. 1966. The Rumen and its Microbes. Academic Press. New York, NY. 553 pp.

Komarek, R.J. 1981. Intestinal cannulation of cattle and sheep with a T-shaped cannula designed for total digesta collection without externalizing digesta flow. J. Anim. Sci. 53: 796

Lambert , M.A. and C.W. Moss.1972. Gas-liquid chromatography of short-chain fatty acids on Dexsil 300 GC. J. Chromatogr. 74:335

Leek, B.F. 1993. Digestion in the ruminant stomach. In: M. J. Swenson and W.O Reece. (Ed.) Dukes' Physiology of Domestic Animals. pp 387-416. Cornell University Press. Ithaca, NY.

Ling, J.R. and P.J. Buttery. 1978. The simultaneous use of ribonucleic acid, ^{35}S, 2, 6-diaminopimelic acid and 2-aminoethylphosphonic acid as markers of microbial nitrogen entering the duodenum of sheep. Br. J. Nutr. 39:165

Merchen N.R., J.L. Firkins and L.L. Bergen. 1986. Effect of intake and forage level on ruminal turnover rates, bacterial protein synthesis and duodenal amino acid flows in sheep. J. Anim. Sci. 62:216

Merchen, N.R. 1988. Digestion, absorption and excretion in ruminants. In: D.C. Church (Ed). The Ruminant Animal. Digestive Physiology and Nutrition. pp 172-201. Prentice Hall. Englewood Cliffs, NJ.

Morhand-Ferh, P and D. Sauvant. 1987. Feeding strategies in goats. In: O.P. Santana, A.G. da Silva and W.C. Foote (Ed.) Proceedings of the 4th international conference on goats. Vol II. pp 1275-1295. Departamento de Difusao de Tecnologia. Brasilia, Brazil.

Moore, J.A., M.H. Poore and R.S. Swingle. 1990. Influence of roughage source on kinetics of digestion and passage, and on calculated extents of ruminal digestion in beef steers fed 65% concentrate diets. J. Anim. Sci. 68:3412

Mould, F.L., E.R. Orskov and S.O. Mann. 1983. Associative effects of mixed feeds. I. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. Anim. Feed Sci. Technol. 10:15

Noeck, J.E., S.P. Hart and C.E. Polan. 1987. Rumen ammonia concentration as influenced by storage time, freezing and thawing, acid preservative, and method of ammonia determination. J. Dairy Sci. 70:601

Nolan, J.V. 1993. Nitrogen Kinetics. In: J.M. Forbes, and J. France. (Ed.) Quantitative Aspects of Ruminant Digestion and Metabolism. pp 123-143. CAB International. Wallingford, Oxon. UK

NRC. 1989. Nutritional requirements of dairy cattle.(6th Ed.) National Academy Press. Washington, D.C.

Orskov, E.R. 1992. Protein Nutrition in Ruminants.(2nd Ed.) Academic Press. San Diego, CA.175 pp

Orskov, E.R. 1986. Starch digestion and utilization in ruminants. J. Anim. Sci. 63:1624

Owens, F.N. and A.L. Goetsch. 1988 . In D.C. Church (Ed) The Ruminant Animal. Digestive Physiology and Nutrition. pp 145-171. Prentice Hall. Englewood Cliffs, NJ.

Owens, F.N. and R. Zinn. 1988. Protein metabolism of ruminant animals. In D.C. Church, (Ed.) The Ruminant Animal. Digestive Physiology and Nutrition. pp 227-249. Prentice Hall. Englewood Cliffs, NJ.

Reid, J.T. and Tyrrell, H.F. 1964. Effect of level of intake on energetic efficiency of animals. Cornell Nutr. Conf. Proc. 19:25

Russell, J.R., M.R. Brasche, and A.M. Cowen. 1993. Effects of grazing allowance and system on the use of corn-crop residues by gestating beef cows. J. Anim. Sci. 71:1256

Rust S.R. 1983. Associative effects in the ruminant animal. Ph.D. Dissertation. Oklahoma State University. Stillwater, OK.

Santini, F.J., C.D. Lu, M.J. Potchoiba, and J.M. Fernandez. 1992. Dietary fiber and milk yield, mastication, digestion, and rate of passage in goats fed alfalfa hay. J. Dairy Sci. 75:209

SAS. 1985. SAS User's guide: Statistics (Version 5 Ed.). SAS Inst. Inc., Cary, NC.

Satter, L.D. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32:199.

Schelling, G.T. 1980. Nucleic acids and purine or pyrimidine bases as markers for protein synthesis in the rumen. In: F.N. Owens (Ed). Protein Requirements for Cattle. Proceedings of an International Symposium at Oklahoma State University. pp 1-9 Oklahoma State University Press. Stillwater, OK.

Schneider B.H. and W.P. Flatt. 1975. The Evaluation of Feeds Through Digestibility Experiments. The University of Georgia Press, Athens. 423 pp

Sekine, J., R. Oura and Y. Asahida. 1992. Effects of ratio of concentrate to roughage and kinds of hay in a ration on estimating the rumen degradability of protein of formulated concentrate. AJAS 5:19

Shirley, R.L. 1986. Nitrogen and Energy Nutrition of Ruminants. Animal Feeding and Nutrition. Academic Press. Orlando, FL. 358 pp

Siddons, R.C., D.E. Beever and J.V. Nolan. 1982. A comparison of methods for the estimation of microbial nitrogen in duodenal digesta of sheep. Bri. J. Nutr. 48:377

Theurer, C.B. 1980. Microbial protein estimation using DAP, AEP and other amino acids as markers. In: F.N. Owens (Ed). Protein Requirements for Cattle. Proceedings of an International Symposium at Oklahoma State University. pp 10-22 Oklahoma State University Press. Stillwater, OK.

Tyrrel, H.F. and P.W. Moe. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58:1151

Van Slyke, D.D. and A. Hiller. 1933. Determination of ammonia in blood. J. Biol. Chem. 102:499

Van Soest, P.J. Nutritional Ecology of the Ruminant. 1994. Cornell University Press. New York, NY. 476 pp

Van Soest, P.J. and J.B. Robertson. 1979. Systems and analyses for evaluation of fibrous feeds. In: W.J. Pidgen, C.C. Balch, and M. Graham (Ed). Proc. Int. Workshop on Standardization of Analytical Methodology for Feeds. pp 49-60. Int. Dev. Res. Center, Ottawa, Canada.

Weakley D.C. and F.N. Owens. 1983. Influence of ammonia concentration on microbial protein synthesis in the rumen. Okla. Agr. Exp. Sta. Res. Rep. MP-114:39

Williams, C.H., D.J. Davis and O. Iismaa. 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophotometry. J. Agri. Sci. 59:381.

Zinn, R.A., and F.N. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66: 157

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APPENDIX PROCEDURES AND DETERMINATIONS

Table A1. Surgical procedure

1. Fast animals 24 h before surgery.
2. Administration of atropine to inhibit salivation and improve respiration.
3. Induce and maintain anesthesia with halothane.
4. Ruminal fistulation
   a) Place animal in right lateral recumbence.
   b) Make an incision 5 cm long approx. 1.5 cm from last rib.
   c) Separate underlying abdominal muscles and peritoneum by blunt dissection.
   d) Withdraw rumen from beneath the incision.
   e) Place rumen wall between clamp.
   f) Place loose suture over incision.
   g) Remove clamp and place cannula from seven to ten days later.
5. Duodenal fistulation.
   a) Place animal in left lateral recumbence.
   b) Perform transcostal laparotomy; make incision 5-7 cm. length over 12th rib.
   c) Strip periostium from rib.
   d) Remove rib.
   e) Perform incision into peritoneal cavity.
   f) Localize position near pyloric sphincter.
   g) Make longitudinal incision in intestinal stump.
   h) Place cannula inside intestinal lumen carefully.
   i) Suture intestinal serosa with purse string.
   j) Drape cannula with Dracron®.
   k) Make some holes in mesenterium and suture Dacron®.
   l) Make sure intestinal loop is not twisted.
   m) Remove skin to exteriorize cannula.
   n) Use puller handle of trocar to reach abdominal cavity.
   o) Remove point and insert trocar in stem of cannula.

Table A1. (Continued)

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p) Pull trocar to exteriorize cannula stem through the body wall. If strong movement is performed, rupture of intestine will result.

q) Remove trocar.

r) Position cannula as naturally as possible.

s) Drop antibiotic into abdominal cavity.

t) Close abdominal incision.

u) Snug intestinal loop against abdominal wall.

v) Place teflon washer and plug to prevent leaking.

w) Let animal to recover.

Table A2. Isolation of bacteria from rumen fluid

a) Take at least 1000 ml of rumen fluid 2-3 h after feeding from each experimental animal.
b) Filter rumen fluid through two layers of cheese cloth.
c) Mix equal volume of filtered rumen fluid and saline (0.9%NaCl).
d) Centrifuge at 500 x g for 5 min.
e) Decant supernatant fluid.
f) Centrifuge supernatant fluid at 20,000 x g for 20 min.
g) Wash resultant pellet with saline (0.9%) and centrifuge as above.
h) Wash pellet once more with water and centrifuge again.
i) Dry the resultant pellet in a freeze drier or in a forced air oven at 60 C for 48 h.

Table A3. Purine determination (Zinn and Owens, 1986)

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1. Weight .5 g duodenal samples, .2 g bacteria and .3 g corn starch (to complete .5 g), and yeast RNA standard as before into 25 ml screw cap culture tubes provided with teflon lined caps.
2. Add 2.5 ml $HClO_4$ (70%), cap tightly and vortex.
3. Incubate tubes for 60 min in a water bath at 90-95 C.
4. Add 17.5 ml 0.0285 M $NH_4H_2PO_4$, break the pellet, vortex.
5. Incubate again for 30 min in a water bath at 90-95 C.
6. Filter through Whatman # 4 filter paper.
7. Transfer .5 ml filtrate into 15 ml centrifuge, add 0.5 ml $AgNO_3$ (0.4 M) and 9 ml of 0.2 M $NH_4H_2PO_4$ and let stand overnight.
8. Centrifuge tubes for 10 min and decant the supernatant fluid, being carefull to not disturb the pellet.
9. Wash pellet with water adjusted to pH 2.0 and repeat step 8.
10. Add 10 ml of 0.5 N HCl and mix thoroughly.
11. Cover tubes with marble and incubate in 90-95 C water bath.
12. Dilute standards, usually 1 ml standard to 9 ml 0.5 N HCl.
13. Read absorbance at 260 nm in a spectrophotometer using ultra violet light.

Reagents

1. $HClO_4$, 70%
2. 0.2 M $NH_4H_2PO_4$ = 23 g/l distilled water
3. 0.0285 M $NH_4H_2PO_4$ = 143 ml/l of 0.2 M $NH_4H_2PO_4$
4. 0.5 N HCl = 41.85 ml /l
5. pH 2 water (add H_2SO_4)
6. 0.4 M $AgNO_3$ = 6.9 g/ 100 ml distilled water.

Table A3. (Continued)

Preparation of the standard curve and calculation of RNA content of duodenal and bacterial content.

The X axis of the standard curve represented the dry weight of yeast RNA in each tube corrected for dilution before absorbance was read.

The Y axis represented the absorbance readings.

To obtain RNA concentrations of the bacteria and duodenal samples, the absorbance readings were interpolated into the standard curve.

Table A4. Washing fiber for mordant (Russell et al., 1933)

1) Fill 2 double bagged bags with forage, make sure the stitching is secure.

2) Hook up washer.

3) Turn on hot water tap.

4) Place bags into washer.

5) Add detergent.

6) Turn washer on to 'cotton/sturdy' cycle.

7) Let washer go through cycle.

8) Repeat steps 5 through 7 for a total of five washings.

9) When washing is done, repeat steps 6 and 7 to rinse the fiber. Continue rinsing until no soap is seen in the drain water.

10) Rinse washed fiber in acetone 3 times:

    1st rinse-soak 15 minutes, wring, change acetone.

    2nd rinse-soak 15 minutes, wring, change acetone.

    3rd rinse-soak 15 minutes, wring, save acetone for first rinse of next bag.

11) Let acetone drain for approximately 8 h.

12) Dry bags in a drying oven.

13) Check the fiber periodically and break it up to speed drying.

14) Dry fiber can then be stored for future use.

Table A5. Mordanting of fiber (Russell et al., 1993)

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1. Preheat oven to 100 C.
2. Weight 120 g fiber into 4 L beaker.
3. Dissolve 7.0 g sodium dichromate dihydrate for 2% marker in 2000 ml distilled water.
4. Pour sodium dichromate solution into fiber.
5. Rinse sodium dichromate out of beaker with two 750 ml water rinses.
6. Add more water to cover water if necessary.
7. Cover beaker tightly with aluminum foil.
8. Prepare additional beakers according to steps 2 through 6.
9. Place beakers into oven.
10. Boil mordant for 24 h . If necessary add more water..
11. Pour contents of one beaker into cheesecloth lined square strainer.
12. Rinse fiber thoroughly with tap distilled water.
13. Repeat steps 11 and 12 for additional beakers.
14. Combine the rinsed fiber and place into one beaker.
15. Prepare ascorbic acid solution using the ratio of 60 g of ascorbic acid for each 120 g fiber and add to 2.5 L of tap distilled water.
16. Pour ascorbic acid solution over fiber.
17. Add tap distilled water until fiber is covered.
18. Let fiber soak in ascorbic acid for 24 h.
19. Place fiber in cheesecloth lined square and rinse thoroughly.
20. Place rinsed fiber into a drying bag.
21. Place bags in a drying oven until dry.

Table 6A. Determination of chromium in chromium-mordanted fiber, duodenal and fecal samples (Williams et al., 1962) condensed by Russell, J.A.(1992)

Reagents:

1. Phosphoric acid-manganese sulfate solution:
   a. Manganese sulfate solution: 10.0 g $MnSO_4$ H_2O in 100 ml distilled water.
   b. Add 30.0 ml manganese sulfate solution to 1 L of 85% H_3PO_4.
2. Potassium bromate solution: 45.0 g $KBrO_3$ in 1 L distilled water.
3. Calcium chloride solution: 41.6 g $CaCl_2$ in 1 L distilled water.
4. Chromium stock solution: 2.8285 g KCr_2O_7 in 1 L distilled water.

Procedure:

1. Ash 1 g sample (triplicate) in 30 ml crucible at 600 C for 2 h.
2. Cool.
3. Add 1.5 ml phosphoric acid-manganese sulfate solution.
4. Add 2 ml potassium bromate solution.
5. Cover with watch glass and digest on a hot plate until effervescence ceases and purple color appears.
6. Deliver 6.5 ml calcium chloride solution to 30 ml volumetric flask.
7. Wash crucible after digestion is finished with distilled water and pour into 30 ml volumetric with calcium chloride solution.
8. Fill to volume with distilled water.
9. Allow settle at least 18 h.
10. Filter solution from volumetric flask through Whatman No. 1 filter paper into plastic vial.
11. Prepare standards: water

Standard (ppm chromium)	ml stock
1	1.0
2	2.0
3	3.0
5	5.0

Table 6A (Continued)

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12. Prepare standards: samples "zero' time (use feces from animals which were not fed chromium-mordanted fiber).
    a. Combine all "zero" time samples for each animal.
    b. 1 ppm: deliver 0.2 ml sodium dichromate into plastic vial. Add 19.8 ml zero solution.
    c. 2 ppm: 0.4 ml sodium dichromate (100 ppm) in 19.6 zero solution.
    d. 3 ppm: 0.6 ml sodium dichromate (100 ppm) in 19.4 zero solution.
    e. 5 ppm: 1.0 ml sodium dichromate (100 ppm) in 19 zero solution.
13. Read in atomic absorption spectrophotometer.

Determination of % Cr :

Formula: [(Concentration x Volumetric ml) / (Sample Wt x 1 million) x 100]

Calculation of flow:

DM flow to the duodenum was calculated from the amount of chromium marker ingested daily and the concentration of chromium in duodenal digesta.

The flow of any constituent of duodenal digesta was calculated by multiplying DM flow by the concentration of that constituent in duodenal digesta.

Calculation of digestibility:

DM digestibility was calculated from DM ingested and DM flow:

Apparent DM digestion = (DM ingested - DM flow)/(DM ingested).

Digestibilities of the constituents of DM were determined similarly.

Table 7A. Determination of Neutral Detergent Fibers (NDF's) (Van Soest and Robertson, 1979), condensed by Russell, J.R. (1992)

Reagents

Neutral-Detergent Solution	
Distilled water	18.0 L
Sodium Lauryl Sulfate	540.0 g
EDTA, Disodium salt	335.0 g
Sodium borate decahydrate	122.6 g
Sodium phosphate, Dibasic, anhydrous	82.1 g
2-Ethoxyethanol (Ethylene glycol monoethyl ether)	180.0 ml

* Dissolve sodium EDTA and sodium borate in about 5 L of water in a clean carboy used for NDF solution.

* Separately dissolve the disodium phosphate in about 1 L of water over heat. Add to carboy while still warm.

* Weight sodium lauryl sulfate and add to carboy (warning: sodium lauryl sulfate should be weighed in a hood.

* Add remaining 12 L of water and 180.0 ml of Ethoxyethanol.

* Check pH to see it is between 6.9 and 7.1.

* Adjust pH, if necessary, with HCL or NaOH.

Amylase

* Weigh .25 g of bacterial amylase (Sigma A-6380 Bacillus species Type II-A) and dissolve in 90 ml of water.

* Add 10 ml of Ethoxyethanol.

* Filter through Whatman 54 filter paper.

* Properly seal with parafilm and store in a refrigerator when not in use. Prepare solution to be used over two weeks.

Table 7A. (continued)

Procedure:

1. Accurately weight .5 g of sample previously ground through a 1-mm screen onto weighing paper.
2. Place sample in a 600 ml Berzelius beaker.
3. Turn on fiber reflux rack.
4. Turn on cooling water in reflux rack.
5. Add 50 ml of neutral detergent solution to the Berzelius beaker.
6. Carefully place Berzelius beaker onto the rack (sample should boil in 10 min).
7. After 30 min of boiling, remove beaker from the reflux rack.
8. Add 50 ml of neutral detergent solution and add 2 ml of amylase solution.
9. Replace Berzelius beaker onto the rack.
10. After 30 min of boiling, filter sample through a pre weighed dry 50 ml Gooch crucible or pre-weighed 9 cm whatman 54 filter paper.
11. Clean beaker with a rubber policeman and hot water.
12. Wash fiber in the crucible twice with hot water and acetone.
13. After filtration, place crucible with fiber in the oven at 100oC for overnight.
14. Using tongs, crucibles are placed in a dessicator for a period of between 1 and 6 h.
15. Using tongs, weigh crucible.
16. If ADF is to be determined by sequential analysis, then save crucible with fiber to proceed with the ADF procedure.

Table 8A. Determination of ADF: sequential procedure (Van Soest and Robertson, 1976) condensed by Russell, J.R. (1992)

Reagents

Acid detergent solution	
Water	17117.0 ml
Sulfuric acid, Reagent grade	882.72 ml
Cetyltrimethylammonium bromide (CATB)	360.0 ml

* Place all water in a carboy.
* Slowly add sulfuric acid to the carboy.
* Stir in a magnetic stirring.
* Standardize:
  - Weigh out .5 g of dry THAM and dissolve in 15 ml deionized water in a 25 ml Erlenmeyer flask.
  - Add 2 drops of indicator.
  - Place acid solution to be tested in 5 ml buret.
  - Titrate THAM solution.
  - N H_2SO_4 = Wt. THAM / (.121136 x ml H_2SO_4 in titration)
  - To adjust acid to 1.0 normal:

    (18000 x Normality of acid) + 18 (ml of concentrated H_2SO_4 added)

    = 18000 x 1N.
* add Cetyltrimethylammonium bromide to the 18 L 1 N sulfuric acid.

Procedure:

1. Turn on fiber reflux racks and cooling water .
2. Place crucible with NDF on percolator tube and place in 600 ml Berzelius beaker.
3. Add 150 ml of Acid Detergent Solution to the beaker.
4. Place beaker on reflux rack.
5. Remove sample from the reflux rack exactly 1 hour after percolation begins.
6. Rinse out outside of the crucible with hot water into the Berzelius beaker and place crucible in filtration funnel.

Table 8A. (continued)

7. Rinse off percolator tube with hot water into the Berzelius beaker.
8. Filter contents of the beaker through the crucible.
9. Clean the beaker with a rubber policeman and hot water.
10. Wash fiber in the crucible twice with hot water and acetone.
11. After filtration, place crucible with fiber in the oven at 100 C overnight.
12. Place crucibles in desiccator (use tongs) for a period of between 1 and 6 h.
13. Weigh crucible (use tongs).