EFFECT OF PROTEIN AND STARCH SUPPLEMENTATION ON THE UTILIZATION OF LOW QUALITY ROUGHAGES

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CHAPTER I

INTRODUCTION

The world population is increasing so rapidly that food supplies may not be adequate for such a huge mass of people. The most underdeveloped or highly populated countries in the world are currently facing a serious hunger problem and food scarcity is a potential menace even for the more developed nations.

Two major reasons probably account for the above situation: one is of socio-political origin and the other is availability of adequate food supplies. Independently of the importance of either reason, the latter must be emphasized due to the nature of the studies reported herein.

Cereal grains are more efficiently utilized when directly consumed by humans. Therefore, the increasing use of available or potentially available agricultural land to increase cereal production may be logical. Longer term, the intensive and extensive beef production might be displaced to areas less suitable for agricultural purposes, or make greater use of agricultural residues not adequate for human nutrition. Moreover, finished beef production may require shorter finishing periods on high concentrate diets.

Ruminants are able to utilize forages of high fiber content and low nutritive value efficiently, due to their unique physiological characteristics. Thus, there is a growing interest in and necessity for improving the utilization of roughages of low quality. Considerable quantities of

roughages having low feeding value exist for feeding ruminants. Many of these roughages are currently wasted natural resources or improperly utilized for different reasons.

Studies to improve the utilization of low quality roughages, such as crop residues, mature native range forages or hays have been conducted with varying degrees of success. Alkalinization of crop residues has been reported to improve both apparent digestibility and intake of cereal straws.

Supplementation of low quality feedstuffs with nonprotein nitrogen sources (e.g., urea, biuret) has increased utilization in some instances. Moreover, protein supplements of plan origin (e.g., soybean meals, cottonseed meal, etc.) have been shown to improve intake and utilization of low quality forages. Although not well understood, in some cases, energy supplementation of low quality roughages has not improved forage utilization but has decreased intake, possibly by producing a fermentation pattern and rumen environment not beneficial for certain strains of rumen bacteria.

Actually, more research is necessary to determine the effects of different levels of supplementary nitrogen and/or energy on the utilization of different low quality roughages. Protein or nitrogen supplementation has been shown to be beneficial in improving forage intake and fiber digestibility of low quality forages. However, less is known about the effects of different kinds of supplementation programs such as high versus low protein supplements fed at equal daily supplemental protein intakes and the effect of starch level in the supplement on ruminal parameters, intake and utilization of low quality forage. Perhaps, even small starch concentrations in the supplement may be able to alter rumen fermentation, cause changes in rumen population and depress fiber

digestion rate when the nitrogen supply is not adequate. However, at adequate levels such negative effects might be alleviated.

An intake and digestion study, and a dacron bag trial were conducted to determine: the effects of different protein and starch levels in the supplement on intake and fiber digestion of low quality native prairie hay; ruminal parameters, such as pH and ruminal NH_3 concentration; and assessment of the degradability of fiber fractions in fistulated steers fed high or low starch supplements.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Mechanisms of evolution have provided different animal species with particular physiological and anatomical characteristics, permitting them to survive in different environments. Thus, the anatomical uniqueness of the ruminant digestive tract permits the consumption of materials that otherwise would be of little or no value for animals with a simple stomach. However, the existence of other mammals with less important cecal or pregastric fermentation must be mentioned (Hungate, 1966).

The presence of a complex microbial population in ruminants is responsible for the degradation of dietary compounds by enzymatic action, producing fermentation end products such as volatile fatty acids (VFA), which are the main sources of energy for the host animal for maintenance and production. In addition, these microorganisms are able to synthesize their own protein having a quite stable amino acid composition for absorption in the small intestine.

The Reticulo-Rumen Environment

The Reticulo-Rumen Population

The constancy of rumen conditions such as pH, anaerobiosis, and temperature restrict the presence of microorganisms to very distinctive

groups which are best able to grow under the particular environmental conditions (Hungate, 1966).

Rumen bacteria are the predominant group of microorganisms in the rumen (Hungate, 1966; Ørskov, 1982). Strains of cellulolytic bacteria such as Bacteroides succinogenes, Butyrivibrio fibrisolvens, Clostridium locheadii, Ruminococcus flavefaciens and Ruminococcus albus (Hungate, 1966) give the ruminant animal the ability to survive on poor quality fibrous forages (Ørskov, 1982).

A number of cellulolytic bacteria also have amylolytic properties, e.g., Clostridium locheadii and Butyrivibrio fibrisolvens with Streptococcus bovis, Bacteroides amylophilus, Bactereroides ruminicola, Succinomonas amylolytica and Selenomonas ruminantium being strictly amylolytic. Other bacteria have been classified as hemicellulose digesters, proteolytic bacteria, methanogenic bacteria and lypolitic bacteria (Hungate, 1966).

Much less is known about the specific activity of protozoa in the rumen. Protozoa are larger, but present in fewer numbers than the rumen bacteria (Hungate, 1966; Ørskov, 1982). They can be classified in two general categories: Entodiniomorph (starch digesters) and Holotrich (digesters of soluble sugars). Substrate within protozoal cells ferments at a slower rate, which could provide a more stable pattern of rumen fermentation (Ørskov, 1982).

Protozoa are also considered to be highly proteolytic cells. However, proteolytic activity in the rumen has been reported to be almost entirely associated with bacterial cells, but cell-free rumen fluid and protozoa are reported to have little proteolytic activity (Nugent, 1981). In addition, the bacterium Streptococcus bovis has been identified as

actively proteolytic (Russell et al., 1981). Moreover, the bacteria engulfment activity by protozoa reported by Hungate (1966) was confirmed by Nugent (1981). Large numbers of anaerobic phycomycetous fungi were also found to colonize plant fragments within the rumen of cattle and sheep consuming fibrous diets, but the activity of these groups as fiber digesters remains to be investigated (Bauchop, 1979).

Rumen Parameters Affecting Cell Growth and Proliferation

Bacterial anaerobic fermentation in the rumen yields four to five moles of ATP per mol of carbohydrate fermented. These ATP molecules are utilized to incorporate nonprotein nitrogen (NPN) into the cells, producing VFA and CH_4 as end products (Ørskov, 1982).

The VFA, acetic, propionic and butyric, are utilized by the ruminant animal as energy sources. Protein synthesis, by the microorganisms, appears to be the main source of amino acids for the host animal under most conditions, whether dietary N is supplied either from NPN or protein sources (Ibrahim, 1972). For most diets, from 50 to 80 percent of nitrogen which passes from the abomasum to the intestine is likely to be from microorganisms (Hogan, 1975). Thus, it appears evident that the nutrient composition of the diet may directly affect the growth and activity of the ruminal microbial population, which in turn will be reflected in the animal performance.

Ruminal Ammonia Concentration. Urea and biuret, as well as protein of animal and plant origin have been used as supplementary sources of nitrogen with different degrees of success when low quality roughages

were fed to ruminants (Abou-Akkada and el-Shazly, 1958; Wilson et al., 1975; Pendlum et al., 1977; Umunna, 1982). Urea is rapidly hydrolyzed to ammonia and CO_2 (Wallace, 1979), whereas protein is degraded to amino acids, peptides and ammonia (Ørskov, 1982). Ruminal ammonia is a central intermediate in both the degradation and assimilation of dietary N. Ammonia is required by many bacteria; amino acids and peptides also appear to be required but to a lesser extent (Maeng, 1976).

The size of microbial flora and their hydrolytic activity increase when ruminal ammonia concentration increases (Wallace, 1979). Kropp et al. (1976) suggested that the ammonia level in the rumen is a more precise index of nitrogen status for rumen bacteria than that which can be calculated from the feed composition.

Some discrepancy exists in the literature as to the optimal ammonia concentration for supporting maximum rate rumen bacteria growth. Satter and Slyter (1974) did not find an increase in microbial protein production by increasing in vitro ammonia concentration beyond 5 mg/dl of rumen liquor. However, Mehrez et al. (1977) reported a minimal ammonia concentration of 25.5 mg/dl for maximal rate of fermentation in sheep fed whole barley plus urea. Results from another study in vivo support the view that 2 to 5 mg/dl is enough to allow maximum growth of rumen microbes (Slyter et al., 1979). Leng (1982) suggests that 5 to 8 mg/dl is a suitable range for achieving the highest rates of fermentation. Data reported by Hillis et al. (1971) illustrate the fact that both the source of nitrogen and the time post-dosing affect the maximum ammonia concentration in sheep. Moreover, sampling in the rumen (dorsal, midpoint or ventral rumen) as well as method and time of sampling, type of diet and rumen fluid volume have been reported to alter the NH_3 concentration (Wohlt, 1976).

Despite the numerous factors which appear to contribute to the variability in rumen ammonia concentrations, ruminal ammonia is an extensively used parameter to estimate N status in the rumen.

Ruminal pH and Fermentation Products. The reticulo-rumen and the entire physiological makeup of a ruminant animal has evolved to work in the presence of high fiber levels in the diet. Fiber stimulates the processes of mastication and rumination which increase salivary secretion, enhancing buffering capacity of the ruminal liquor. The rumen in a steady state condition on a high forage diet has a pH close to neutral with only slight variations.

Unfortunately, when roughage diets only are fed, cattle are often unable to achieve the high levels of performance needed in the beef industry unless supplements are fed. Thus, protein or protein-energy supplements are often fed to cows or growing animals receiving low quality forages. Thus, rumen etiology may be affected by a number of factors, including a shift in pH from normal range.

Cereal grains ferment at a faster rate in the rumen than roughages, increasing VFA production per unit of feed weight consumed. Thus, more saliva is needed to buffer the rumen contents, but less saliva is produced because of decreased rumination resulting in lower pH of the rumen fluid (Ørskov, 1982).

After a meal, pH of the rumen fluid can drop temporarily due to immediate changes in VFA production. This reduction in pH following eating probably has a greater effect on the metabolism of the existing microorganisms than on changes in the whole microbial population (Esdale and Satter, 1972).

However, long-term changes can undoubtedly take place in the rumen population in response to alterations in pH (Esdale and Satter, 1972). CEClulotytic bacteria have been shown to be very sensitive to rumen pH. Their number and activity sharply decrease when pH drops beyond 6.2 (Ørskov, 1982), and at pH 5.5 growth stops completely (Hungate, 1966). Amylolytic bacteria, however, are less labile to pH, and their activity is practically unaltered between a pH of 5.6 and 7.0 (Ørskov, 1982). As a result of sudden shifts from high roughage to high concentrate diets, lactic acid fermentation is promoted; pH is lowered; and a rumen disorder known as lactic acidosis occurs.

Irwin et al. (1977) found an increase in the concentration of lactic acid in the rumen fluid from 0.2 mg/ml to 8.6 mg/ml with a pH of 7.0 and 4.7, respectively, before and after induction of lactic acidosis in sheep. Helm et al. (1972) studied the variations in ruminal lactate, VFA and pH in a diet of 40 percent coastal bermuda grass to 60 percent concentrate (mainly reconstituted sorghum grain), resulting in a reduction of pH and acetate: propionate ratio and an increase in lactic acid levels. Streptococcus bovis in the rumen appears to be responsible for initiating the events that cause lactic acidosis (Hungate, 1966). Thus, it is clear that a severe reduction in the ruminal pH will precipitate the inactivity and cessation of growth of cellulolytic bacteria, causing a depression in total fiber digestibility, dry matter intake, regardless of other physiological implicances.

Bacterial Requirements for Protein Synthesis

Physical-chemical characteristics of the rumen, such as pH, temperature, oxidation-reduction potential and osmotic pressure have been found

to affect bacterial growth. However, nutritional factors more frequently change the size and activity of the ruminal population (Hespell, 1979). Ruminal bacteria, similar to other living organisms, have specific requirements for nutrients and environmental conditions to achieve their optimal growth rate. All nutrients required for cell growth must be present simultaneously and at an adequate concentration for uptake and utilization, since low cell growth rates and nutrient deficiencies represent the major sources of low cell yields (Hespell, 1979).

Dietary carbohydrates are fermented to produce heat, CH_4, acetate, propionate, butyrate and metabolically useful energy in the form of ATP, which is used as a driving force by bacteria to synthesize bacterial protein (Smith, 1979). Frequently, the ATP yield will vary according to the pathway utilized to ferment hexoses (Hogan, 1975). Actually, rumen bacteria as a whole have a low requirement of energy for maintenance even with high cell yields (Hespell, 1979). The efficiency of the overall process is dependent upon the rate and extent of degradation of carbohydrate and nitrogen sources (Stern and Hoover, 1979). An analysis of several studies suggests that 16.9 g of microbial protein are synthesized per 100 g of organic matter apparently digested in the rumen (Stern and Hoover, 1979).

The availability of plant protein is usually limited by the extent to which the fiber fraction is degradable, low pH values (less than 6.2) depress the cellulolytic activity. Then proteolysis may also occur to a lesser extent, affecting rumen ammonia concentration and N uptake (Ørksov, 1982).

NPN sources are readily degradable. Ruminal ammonia concentration increases rapidly after ingestion of NPN. Absorption of ammonia and

reconversion to urea permits urea to be recycled in the blood, part of which can be returned to the rumen via saliva. Passage of urea from the blood through the rumen wall increases once the ammonia concentration falls, suggesting potentially high efficiency of utilization of degradable nitrogen (Ørskov, 1982).

Ammonia (NH_3 and/or NH_4^+) appears to be incorporated rapidly into the rumen bacteria in the form of amide groups and used for synthesis, first of glutamate, aspartate, alanine and other amino acids (Smith, 1979).

Allyson and Bryant (1958) found that branched chain fatty acids, probably produced from amino acids by certain strains, were required by other bacteria to grow. These molecules are largely utilized by cellulolytic organisms to synthesize branched chain amino acids. Sulfur concentration in diet as well as the frequency of feeding also affect the efficiency of microbial protein synthesis (Stern and Hoover, 1979).

A decrease in rumen degradation rate, feed intake and digestibility can be expected from inadequate N consumption, resulting in a reduced protein supply to the animal with a corresponding decrease in overall performance.

Rumen Turnover and Fractions of Ingesta

Rumen turnover can be defined as the length of time that the digesta remains in the rumen. Obviously, turnover time varies with the fraction of the digesta considered, particle size, gravity of feed particles and level of feed intake (Church, 1979).

The rumen content can be physically divided into solid and liquid phases. The liquid phase is composed by water, soluble feed components and nutrients made soluble by microorganisms, whereas the solid fraction

is formed by undegraded and indigestible material (Evans, 1981). Bacteria in the rumen are associated with both liquid and solid fractions but also with the epithelium of the rumen wall. The latter group contains most of the urease-producing kind of bacteria in bovine (Cheng et al., 1979). Thus, it could be expected that the turnover rate of bacteria will be that of the fraction to which they are associated, influencing the rate and amount of microbial protein which moves to the small intestine.

Water intake and saliva production make the liquid volume entering to the rumen much larger than the solid fraction, and may leave the rumen at a faster rate than that exhibited by the solid fraction. Thus, it is reasonable to expect that soluble nutrients will be removed at a higher rate than those which are insoluble (Evans, 1981). Varga and Prigge (1982) have studied the influence of forage species and level of intake on ruminal turnover rates in sheep. Alfalfa (20.6% CP) and orchard grass (13.5% CP) were compared at two levels intake. Forage species did not affect solid turnover rate, but the liquid turnover rate increased two-fold. Also, a tendency was noted for solid turnover rate to increase with the high intake level. Processing of feedstuffs also appears to affect the liquid turnover rate, which in turn will affect the soluble nutrient and bacterial protein removal from the rumen to the lower tract (Evans, 1981).

The most complete digestion of fibrous forages would be achieved with the slowest turnover rate (Hungate, 1966), since the material will be exposed for a longer time to the degradative bacterial action. However, if fibrous materials stay for a longer period of time, ruminal distention will probably affect voluntary consumption. Thus, determining

the optimum turnover rate for different roughages and conditions is very complex.

Factors Affecting the Nutritional Value of Forages

Activity and growth of the rumen microbial population appear to be greatly affected by the quality of the forages consumed. The classical structural components of the plant cell walls--cellulose, hemicellulose and lignin--and their degree of association influence the nutritive value of forages (Burns, 1978). Cellulose and hemicellulose vary in their extent of degradation in the rumen, and lignin was believed to be virtually unaltered. However, large variations in lignin disappearance have been reported in lambs fed either low or high quality forages (Fahey et al., 1979). Consequently, the validity of using lignin as a marker probably needs to be reevaluated. Possibly, analytical problems may be involved.

The proportion of the fibrous fraction increases as plant maturity advances, and inversely the protein decreases, being the main reasons for a decline in quality characteristics. Figure 1, based on NRC (1976) values, illustrates variations in fiber and protein with maturity in the wheat plant. Besides protein, the total energy content, minerals, vitamins and water also decrease, altering irreversibly the nutritive value of a forage.

In addition, several antiquality factors such as terpenoids, flavonoids and aromatic compounds have been reported to be present in various plant species which may be of concern since these factors cause appreciable economic losses by affecting the quality of grazed plants and hays (Burns, 1978).

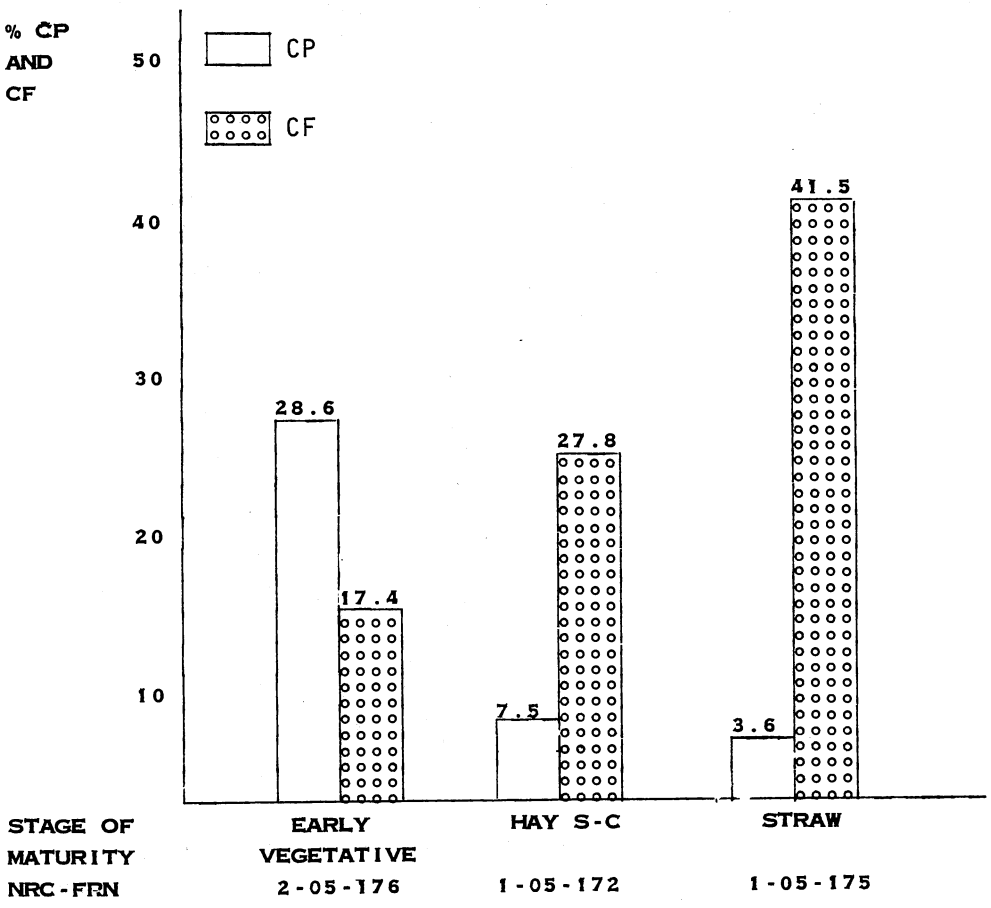

Figure 1. Crude Protein and Crude Fiber Proportions of Wheat at Different Stages (Values NRC [1976])

Voluntary Intake of Feedstuffs

Eating behavior of ruminants is governed by complex interactions between the proposed physiological mechanisms of regulation and factors associated with the nature of the feedstuffs, the environment, stress conditions, physiological and nutritional status of the animal, age and so forth. The accurate evaluation of voluntary consumption under grazing conditions is quite complex, even though proper techniques appear to have been developed for this purpose. Probably voluntary intake is more precisely assessed under controlled conditions; however, it is very unpredictable as to how imposed restrictions (e.g., animals subjected to immobilization in metabolism stalls) may affect intake. The control of voluntary intake becomes really important when low quality forages are fed. Usually these feedstuffs are not well consumed under practical conditions, most likely because of unpalatability, low nutritive value, slow digestion rate and other factors.

Metabolic Regulatory Mechanisms

Nervous stimulation, hormonal factors or humoral levels of metabolites have been suggested as signals which may stimulate the satiety control center in the hypothalamus for initiation or cessation of eating in various species. In ruminants, VFA have been found to limit intake, but other metabolic regulatory agents may also exist. Heat increment after feeding has also been considered to be a potential intake regulator (Van Soest, 1982).

The gastrointestinal hormones gastrin, secretin and cholecystokinin may play a regulatory role of feed intake, but results of an experiment

with sheep infused intravenously with these compounds were inconsistent (Grovum, 1981). However, a review of metabolic regulatory factors confirms that cholecystokinin is the satiety regulating factor in sheep (Van Soest, 1982). However, when ruminants are consuming high fiber in the diet, rumen fill and distention appear to be the most important limiting factors.

Factors Affecting Voluntary Consumption of Roughages

Distention of the reticulo rumen by bulk fill is the main limiting factor when ruminants are fed roughages as reported by several authors (Campling, 1964; Egan, 1970; Ellis, 1978; Grovum and Phillips, 1978). Van Soest (1982) suggests that grazing and forage-fed ruminants do not reach the limits of satiety since rumen fill and time required for eating and ruminating seems to limit voluntary consumption before satiety is reached. Bulkiness of the digesta, as well as its rate of passage, and the rate at which residues are broken down regulate intakes (Campling, 1964). However, the magnitude of all these factors depends on the volume of reticulo-rumen and ruminal turnover (Ellis, 1978).

Voluntary intake may also be limited by the capacity of the intestinal tract to transport digested materials. However, when sheep were fed alfalfa chaffs to study the effects of distention, flow rate, and intestinal motility, results suggested that if sheep can be induced to eat more than normal quantities of roughages, the intestines could transport the residues, and intake would only be limited by ruminal distention (Grovum and Phillips, 1978).

Reticulum and abomasal distention appears to be of more significance than that of the rumen. Feed intake was depressed by stimulation of the reticulum with negligible volumes, suggesting the presence of mechanoreceptors in the epithelium of this organ, whereas a much larger volume placed in the rumen did not depress intake. Thus, rumen distention may be irrelevant or less important as a satiety signal. Moreover, abomasal distention was less effective than reticular distention to suppress intake. The authors did not note additive effects with distention of both reticulum and abomasum (Grovum, 1979). According to these findings, tactile stimulation of the reticulo-rumen and distention of reticulum and abomasum are the major factors involved in intake regulation. In view of these findings, the concept that intake of poor to medium quality roughage diets is controlled by rumen distention should be reevaluated.

Intake may be regulated to give a relatively constant level of fill to the reticulo-rumen at the end of a voluntarily terminated meal, but this level of fill is not a constant between diets and may be influenced by other nutritional factors, such as the protein concentration in the diet (Egan, 1970). Further fiber level of the ration appears to significantly affect feed intake. In an experiment in which fiber was progressively substituted by soluble carbohydrate, voluntary intake increased when fiber was increased, up to 32 percent of straw (23% ADF) in the diet, and decreased thereafter (Jahan et al., 1970).

Animal species exhibit different eating behaviors. Cattle eat eight to ten times more than sheep, and there is a remarkable variation even if expressed per unit of $w^{.75}$. Voluntary consumption decreases with age and high temperatures (Campling, 1964).

Low Quality Roughages

Utilization

A roughage is a bulky material usually of plant origin with high fiber content. The fiber fraction is more of a biological rather than a chemical entity, and its constituents are the structural components of plant cell wall, mainly cellulose, hemicellulose and lignin (Van Soest, 1982). Low quality roughages are of poor nutritive value due to an increased lignification of cell walls, proportionately higher total fiber and very low protein and soluble carbohydrate contents. However, low quality roughages such as cereal straws, late vegetative stage grasses, cottonseed hulls and other agricultural residues are commonly fed to cattle all around the world. Methods are needed to improve utilization and animal performance on such feeds.

The scientific literature shows a permanent interest of researchers in improving the utilization of low quality roughages. Leng (1982) discussed factors limiting the productivity of cattle fed straw basal diets, suggesting that the low N content, high indigestibility and low mineral and vitamin content are the factors which result in poor utilization.

Hay treated with increasing levels of sodium hydroxide has exhibited a significant increase in utilization of the potentially digestible fiber fraction (Berger et al., 1979). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose digestibilities of wheat straw have been increased by treatment with ammonium hydroxide (Solaiman, 1979). Digestibility and intake of barley straw have been improved by both alkali treatment and additional urea supplementation (Ørskov and Grubb, 1978).

Supplementation of low quality roughages with NPN sources such as biuret (Fick et al., 1973; Martin et al., 1981), urea (Campling et al., 1961; Ørskov and Grubb, 1978; Martin et al., 1981; Umunna et al., 1982; Leng et al., 1982), and plant protein origin concentrates (Rittenhouse et al., 1970; Lyons et al., 1970; Pendlum et al., 1977; Kropp et al., 1977; Lusby et al., 1976; Kartchner, 1981) has also been extensively studied.

Campling (1964) reported no increase in forage intake from grinding or pelleting roughage, but intake was related more to forage quality. But, most likely digestibility and rate of passage of low quality forages may be enhanced by grinding, increasing surface area for microbial attack via a reduction in particle size.

Supplementation

Cubed supplements containing a concentrated source of protein and/or energy are commonly fed to cattle grazing or being fed low quality roughages. Cereal grains or their subproducts often represent the energy source, whereas oil seed meals may contribute much of the protein and possibly energy in these kinds of supplements. Thus, starch and protein concentration can vary widely with the source and proportions of major feed materials used in manufacturing cubes.

Effects of Starch on Ruminant Diets. Starch is a reserve polysaccharide of plants. There are two forms of starch: amylose, with α-1,4 linkages, and amylopectin, with α-1,6 linkages, both polymers of glucose molecules (Stryer, 1981). The relative porportion of amylose and amylopectin,

and characteristics of starch granules in grains varies with plant species (French, 1973).

Starch and other readily soluble carbohydrates have been found to decrease fiber degradability. A depression in the cellulose digestibility of poor quality hay caused by the addition of small amounts of starch was reported by Head (1953).

Corn starch was found to depress NFE, protein and DM digestibility of alfalfa, and the effect was removed when an adaptation period was allowed (Kane et al., 1959). The inhibition of cellulose digestion by dietary starch was attributed to a competition between cellulolytic and amylolitic groups of bacteria, for nutrients, especially nitrogen and nitrogen supplementation have alleviated the inhibition (el-Shazly et al., 1961).

Source of starch was noted to affect intake and ruminal parameters. Feed intake, ruminal pH were decreased and lactate concentration increased by replacing corn by wheat in high concentrate diets (Varner, 1970; Varner and Woods, 1975; Fulton et al., 1979).

In a continuous culture "in vitro" system, ADF digestibility was increased with decreasing starch levels but total DM digestibility was not affected (Stern et al., 1978). In other in vitro study, corn and wheat starch did not differ in their effect upon fiber digestion rate or potential extent of digestion (Mertens and Loften, 1980).

As far as utilization of starch, Waldo (1973) has reviewed 15 previous experiments, calculating that mean starch disappearance along the whole tract is 99 ± 1.2%. Very little starch, if any, escapes digestion, regardless of the degree of processing, origin or animal species, according to the author's view. In ruminants this may be particularly true for

high roughage diets compared with high concentrate diets, where lower starch digestibility may be expected.

When starch is fermented in rumen to VFA, there ia 15 to 20 percent loss as heat and CH_4. A 25 percent energy loss can also be expected by starch fermentation in the cecum. It seems to be that a 100 percent utilization can occur when starch is hydrolized in the small intestine (Leng, 1982).

Associative Effects Resulting From Supplementation. When two or more feedstuffs are given together, it appears to be that there is not a linear response in digestibility and net energy values. This is technically recognized as "associative effects" and influenced by the level of intake, rate of passage and digestion rate (Rust, 1983).

There is some disagreement with respect to this interpretation, and this phenomenon may result from improperly balanced diet, or artifacts of experimental designs (Rust, 1983). In an extensive study of the significance and factors affecting "associative interactions" among feed mixtures, it was demonstrated that associative effects may be present and depend on the physical and chemical composition of the diet, proportion concentrate to roughage, N source, level of intake, and presence of feed additives (Rust, 1983).

The incidence of associative effects may be beneficial or detrimental, as illustrated by Figure 2, in which the dashed line represents the expected digestibility if no associative effects occur, whereas the upper and lower solid curves represent "positive" and "negative" effects, respectively (Van Soest, 1982).

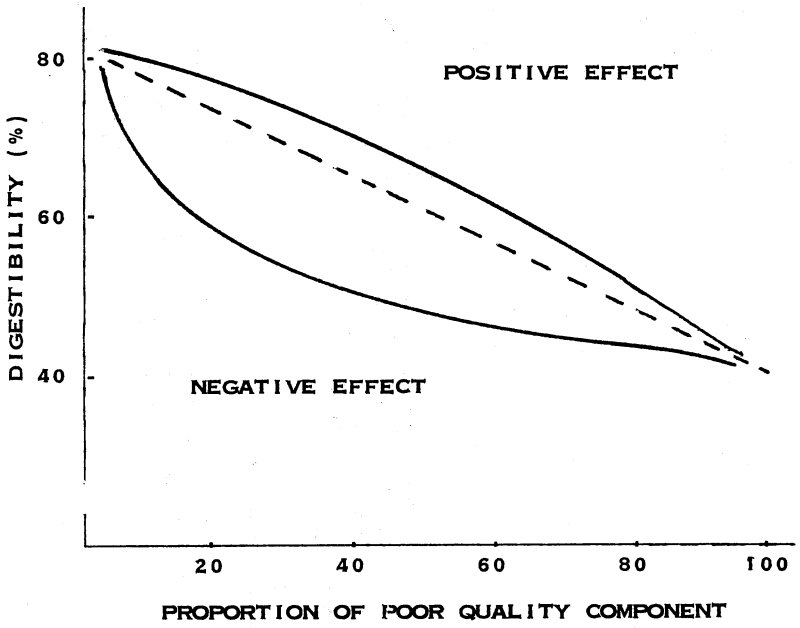

Figure 2. Associative Effects When a Poor Quality Feed is Substituted by a High Quality One (Van Soest, 1982)

Associative effects may be present when low quality roughages are supplemented with energy and N concentrates. Generally, nitrogen supplementation improves the utilization of low quality feedstuffs by increasing their total dry matter digestibility and intake. The addition of large amounts of readily available carbohydrates may, however, depress fiber digestion and intake. Thus, a nitrogen-energy interaction may exist when supplementing low quality feeds.

Meeting the nitrogen needs of the rumen bacteria is essential in order to achieve a higher rate of fermentation. The carbohydrate fraction of a feed appears to be more fermentable when there is adequate nitrogen for the bacterial growth potential available (Ørskov and Grubb, 1978). Abou-Akkada and el-Shazly (1958) did not find any distinct effect on wheat straw digestibility, however, regardless of the level or type of protein supplemented. On the other hand, Campling et al. (1961) demonstrated an increase in mean dry matter digestibility from 41 to 50 percent, and a decrease in rumen retention time from 104 to 83 hours when oat straw fed to cows was supplemented with nitrogen. The NFE and fiber fractions have shown the largest improvement in digestibility. According to Blaxter (1963), a minimum of 8.5 percent crude protein in the total ration is required to achieve maximal intake of forage. On the other hand, Kropp et al. (1977) noted an increase in ruminal ammonia concentration and microbial protein synthesis when the protein level was increased from 8.5 to 10.8 percent and 12.8 percent by supplementation with urea.

An increase in cellulose and total dry matter digestibility was noted by the addition of urea or biuret to a low quality hay (3.6% CP) fed to sheep by Martin et al. (1981). Similar results were observed when native low quality range was supplemented by different methods with urea,

resulting in overall improved performance of the grazing sheep (Ummuna, 1982).

The ability of ruminants to utilize NPN can be altered by the level of urea in the diet. Wilson et al. (1975) reported a feed intake depression with diets containing more than 1 percent of urea. The deterimental effects were attributed by the authors to other physiological parameters rather than undesirable taste.

It appears that once the requirements for rumen degradable N have been met, protein supplementation will not consistently stimulate intake of low quality forages (Sriskandarajah et al., 1982). Previous findings of Williams et al. (1953) showed that requirements of N by bacteria for growth and proliferation were indeed very low.

Protein concentrates of plant origin such as soybean meal (SBM) and cottonseed meal (CSM) are less degradable in both rate and extent than urea or biuret. SBM was found to be more degradable and less variable (82 to 85%) compared with CSM (39 to 76%) under ruminal conditions at the same intake level of a concentrate diet (Zinn et al., 1981). Supplementation of corn silage (7.58% CP) with SBM has had a positive effect on feed efficiency and silage consumption in cattle, but no further improvement was observed by raising the SBM level beyond 0.45 kg/day (Pendlum et al., 1977). The positive effect of protein supplementation on dry matter apparent digestibility is even more remarkable when added to a low quality roughage (Kropp et al., 1977). An increase in feed intake is generally attributed to a increased rate of ruminal digestion of cellulose, but post-ruminal protein supply may also be important (Johnson et al., 1981). Microbial protein is less digestible than dietary protein which escapes fermentative digestion, and may be too low in methionine for live weight

gain in young steers (Hennesey et al., 1981). Thus, bypass of dietary protein from rumen is currently receiving a great deal of attention nowadays.

The effects of supplementation become more complex when a supplemental energy source from concentrates is added to the supplemental nitrogen source. In spite of the studies conducted to date, it is still very difficult to predict with much accuracy the optimal levels of supplemental protein and possibly energy which should be fed with roughages of varying qualities in any given production situation. An early study shows that when low protein diets (14 g/day) were fed to sheep (41 kg average body weight), increments in starch consumption (from 0 to 149 g/day, 8 different levels) led to a reduction in the number and type of microorganisms in the rumen, but when the protein level increased (from 14 to 83.7 g/day, 8 different levels), such effect was not evident. For either medium or high starch contents in the diet the addition of protein has been shown to significantly increase digestibility of DM, protein and rumen counts (Williams et al., 1953).

The apparent digestibility of hays falls with increased intake. Supplementation with concentrates (21.27% CP) increases the apparent digestibility, but when fed at more than one-third of the total ration, concentrates depress voluntary consumption (Blaxter, 1963). Intake in cattle fed Rhodes grass (3.6% CP) was enhanced by increasing dietary protein levels; however, at lower protein levels the intake was depressed by the addition of supplemental energy (Elliot, 1967). Voluntary consumption of hay (6.7% CP) fed to sheep declined linearly when supplemented with a concentrate (19.1% CP), but when oat straw (3.9% CP) was the basal diet, the

same supplement increased straw intake from 242 g to 451 g when added up to 25 percent of the total diet (Crabtree and Williams, 1971).

In an experiment with cattle fed a cereal straw basal diet, Andrews et al. (1972) studied treatments using a factorial combination of several levels of energy and protein. The results were reported as follows: (1) diets containing 8.8 percent CP or more did not differ in performance; (2) 5 to 9 g of concentrate per kg of body weight reduced intake only a little, but increased gains; (3) a low protein-high energy combination was generally refused and gains were low; (4) with a high energy-protein adequate supplement, straw intake was depressed compared with adequate protein-low energy; and (5) long straw (barley or oats) was able to supply only 35 to 45 percent of the daily energy requirement of young growing cattle.

Sheep receiving pangola grass (3.28 to 4.51% CP) have shown an increase in voluntary intake as well as digestibility of cellulose and N, by stimulation with supplemental N; however, 120 to 200 g/day of a 25 percent starch supplement reduced hay consumption; the decrease was greater without supplemental N, probably representing a substitution of a more digestible for a less digestible energy source (Fick et al., 1973). Martin et al. (1981) observed that increasing N levels improved cellulose digestibility and N retention, but a combination with high molasses content depressed voluntary consumption of a low quality hay.

In summary, utilization of low quality roughages appears to be affected by the levels of supplementary energy and protein. Generally, most papers report that high energy depresses voluntary intake and digestibility of fiber and adequate N in diet improves utilization of such roughages.

In Situ Studies

"In situ" methods to determine the degradability of a particular feedstuff or fraction involve the suspension within the rumen, through a fistula, of porous bags containing a measured amount of the material under study. Earlier works have employed nylon bags, but most of the present research has been done employing dacron polyester bags which have the advantage of containing no N, which may interfere when protein degradation is being measured. Bag porosity is critical and bags with 35 to 75 micron pores are generally accepted; the sample size must be related to the surface area of the bag (Broderick, 1980).

This type of study is useful to assess the rate of breakdown of carbohydrates in the rumen, which is an important determinant of voluntary intake, whereas the degradation of protein influences the protein supply to the host animal, and the N available for rumen microorganisms. Besides, it is also a useful technique to relate rumen pH and ammonia with rate of fermentation (Mehrez and Ørskov, 1977; Mehrez et al., 1977). Thus, the nylon bag or dacron bag technique is accepted as a valid method to measure DM, fiber and N disappearance from different feedstuffs and protein sources (Weakley, 1983).

The percent of DM disappearance from nylon bags has been found to increase with each 24-hour increase in length of time that the material remained in the rumen. Legumes have exhibited a higher rate of dry matter disappearance than grasses during the first 24 hours (Van Keuren and Heinemann, 1962). The dry matter disappearance of different incubated roughages has not been shown to increase, however, by being exposed any longer than 72 hours (Neathery, 1968).

Generally, N disappearance from dacron bags follows a pattern of rapid initial disappearance (Nocek, 1979; Broderick, 1980). However, solubility or short-term N degradation appears invalid to predict ruminal degradation, especially with mixed rations (Nocek, 1979).

The origin of the basal diet affects the rate of disappearance of a given substrate from a bag (Van Keuren and Heineman, 1962). A significantly improved dry matter disappearance with a low quality diet, compared with a better one, may reflect a greater cellulolytic activity (Neathery, 1968). Ganev and Ørskov (1979) suggested that if cellulose digestion is rapid, protein may be exposed at a greater degradation rate, since cellulosic material in plant protein appears to have protective properties when fed concentrate based diets. The extent of SBM dry matter disappearance has been shown to be affected by diet, being lower when an animal was fed high grain diets (Weakley et al., 1983).

In situ methods have been compared with in vivo and in vitro studies by some authors. At various times in vitro dry matter digestibilities have been reported to have a limited relationship to in vivo digestibility coefficients. On the other hand, cellulose disappearance from nylon bags exposed 32 and 48 hours has been shown to be significantly correlated, $r = 0.52$ and 0.54, respectively, with in vitro values (Hopson et al., 1963). Moreover, a highly significant correlation (0.81, $P < .01$) between dry matter disappearance in vitro and in situ, has been reported by Monson et al. (1969). The digestible NFE of bermuda grass was only 3.1 percent lower and fiber 1.45 percent higher when exposed for 72 hours in nylon bags compared to in vivo studies, yielding results which were comparable and highly correlated (Neathery, 1972).

CHAPTER III

THE EFFECT OF PROTEIN AND STARCH SUPPLEMENTATION ON VOLUNTARY CONSUMPTION AND DIGESTIBILITY OF LOW TO MEDIUM QUALITY PRAIRIE HAY BY STEERS

Summary

Sixteen 432 kg Hereford steers were used in four replications of a 4x4 latin square design to determine the effect of different supplemental programs on intake and digestibility of low to medium quality prairie hay (5.45% crude protein [CP] on dry matter basis). Prairie hay was given ad libitum. The four treatments were: (1) control, prairie hay plus mineral-vitamin A mix; (2) high protein (HP), 40 percent CP; (3) 20 percent CP, low starch (LS), 22.8 percent starch; and (4) 20 percent CP, high starch (HS), 49.9 percent starch. Daily dry matter intakes of the supplements were 0.11 kg, 0.9 kg, 1.8 kg and 1.8 kg, respectively, to provide equal levels of supplemental protein on the HP, LS and HS treatments.

All three protein supplementation programs increased ($P < 0.01$) daily prairie hay intake, DM digestibility, apparent CP digestibility and ruminal NH_3 compared with the control. ADF and cellulose digestibility tended to be higher on the HP and LS treatments. Ruminal pH did not differ among the diets. Total daily digestible DM intake was highest on the 20 percent CP-LS and HS treatments. Rumen NH_3 was lower at six to seven hours after

feeding ($P < 0.01$) for LS versus HS, possibly due to the higher concentration of cottonseed meal required to reach the desired CP level in the supplement.

In a second experiment four fistulated steers were used in a cross-over design to evaluate ruminal parameters of the animals fed prairie hay plus either 20 percent CP, LS or HS supplements. Dacron bags containing ground pre-washed prairie hay were incubated in the rumen for 4, 12, 18, 24 and 48 hours. Rumen pH and NH_3 were determined at 2, 4 and 8 hours after the supplement was fed. Starch level did not affect DM or ADF disappearance from the dacron bags. Rumen pH was stable and NH_3 levels differed again ($P < 0.05$) only eight hours after feeding (LS = 3.33 mg/dl and HS = 3.97 mg/dl). Results of the dacron bag experiment supported the findings of the feeding digestion trial.

Introduction

A positive effect of N supplementation on low quality forages utilization has been widely recognized. Usually, N supplementation has been shown to increase voluntary intake and improve the digestibility of the fiber fraction (Campling, 1961; Elliot, 1967; Lyons et al., 1970; Crabtree et al., 1971; Fick et al., 1973; Pendlum et al., 1977; Hennessy et al., 1981; Martin et al., 1981; Umunna, 1982).

Supplements containing from 20 percent (or lower) to 40 percent all natural CP are often fed to beef cattle (stockers, replacement heifers and cows, dry or lactating) grazing or being fed low quality roughages such as range pastures. This is especially true during the winter time when protein supplementation is common. Low quality forages, commonly used include winter range pasture, marginal quality grass hays or cereal

straws. While protein supplementation has been shown to be beneficial in improving forage intake and utilization of low quality roughages, limited data have been reported about the effects of different types of protein supplementation programs (e.g., high versus low protein supplementation programs fed at equal daily supplemental protein intakes, high versus low starch levels in the supplements, etc.).

In addition to the effects of protein, starch content of the supplement might affect the rumen environment, altering activity of the ruminal microbial population. Forage intake, forage utilization and/or animal performance may be altered. Starch inhibition of cellulose or fiber digestion has been demonstrated either in vitro or in vivo (Head, 1953; el-Shazly et al., 1961; Mertens et al., 1980). An increase in ADF digestibility was shown by Stern et al. (1981) by reducing the starch level in continuous culture of rumen contents. However, the quantity or level of starch needed to depress fiber intake and utilization has not been well determined. A few trials have shown that nitrogen supplementation or increasing protein level alleviate some of the inhibitory effects of starch or high energy levels in the diet (el-Shazly et al., 1961; Andrews et al., 1972; Hennessy et al., 1981).

Some feeds, which can be used to formulate low protein cubes (e.g., 20% CP), may be high in starch, such as grains, or low in starch, such as by-product feeds.

The objectives of this study were: (1) to investigate the effects of high protein (40%) or low protein (20%) supplements, fed at equal supplemental protein intakes, on forage intake, digestibility, rumen NH_3 and rumen pH using low to medium quality prairie hay, with the low protein

supplements being formulated to be either low or high in starch; and (2) to assess the effect of high versus low starch levels in the supplement on dry matter and fiber disappearance of ground prairie hay from dacron bags incubated in the rumen.

Materials and Methods

Two experiments were conducted at the Nutrition and Physiology Research Center of the Oklahoma State University to study the influence of different protein and starch supplementation treatments on the intake and/or utilization of low quality prairie hay by steers.

Experiment I

Sixteen mature Hereford steers (average initial weight, 432 kg) were randomly allocated to metabolism stalls. Four sets of four animals each were used in a 4x4 latin square design (Table IX, Appendix).

All animals received the same basal diet which was low to medium quality native prairie hay fed ad libitum once a day. The treatments were the following supplements: (1) trace mineralized salt plus vitamin A (Control); (2) 40 percent crude protein-high protein (HP); (3) 20 percent crude protein-low starch (LS); and (4) 20 percent crude protein-high starch (HS).

The HP, LS and HS supplements were fed at 0.9, 1.8 and 1.8 kg/day on DM basis to equalize supplemental protein intake. The mineral-vitamin supplement was fed to the control animals at 0.11 kg/day. The four supplements were provided twice a day.

The ingredient composition of the supplements is shown in Table I, and the nutrient composition of the hay and the supplements in Table II. Supplements were prepared at the beginning of each period during the four periods of the experiment.

Each period in the latin square was 17 days, with adaptation being days 1 through 7. Rejected prairie hay was weighed daily from days 8 through 14, and total feces were collected on days 10 to 16.

The rumen of each animal was sampled the last day of each period within six to seven hours after the supplement was fed. Body weight was recorded the same day.

The prairie hay offered was sampled daily and composited at the end of each period. A grab sample representing about 10 percent of the total daily refusal for each animal was collected during the seven days of the sampling phase and the seven samples of every animal were composited at the end of each period. Feces were collected in individual metallic pans, weighed daily, sampled and composited by using the same procedures as for the rejected items. All samples were placed in individual plastic bags and refrigerated until processing.

Composited fresh and rejected prairie hay samples were weighed and dried at 60°C during 48 hours, to determine dry matter composition. Samples were later ground through a 1 mm screen, stored in glass containers and frozen.

Composited fecal samples were also weighed and dried at 60°C during 96 hours to ensure complete evaporation. Samples were permitted to stabilize with air humidity during 24 hours and weighed again, to calculate air-dry matter concentration. Samples were also ground through a 1 mm screen, stored in glass containers and frozen. The supplements were

TABLE I. INGREDIENT COMPOSITION OF THE SUPPLEMENTS (DM BASIS)

Ingredient	Control (%)	HP[a] (40% CP) (%)	LS[b] (20% CP) (%)	HS[c] (20% CP) (%)
Ground wheat	---	---	---	69.28
Wheat midds	---	---	84.16	---
Cottonseed meal	---	92.00	9.91	23.60
Molasses	---	2.60	5.00	5.00
KCl	37.81	1.60	---	0.34
Dicalcium phosphate	54.02	2.80	---	1.28
$CaCO_3$	---	---	0.43	---
Trace mineralized salt	7.57	0.93	0.46	0.46
Vit A (30,000 iu/g)	0.60	0.07	0.04	0.04

[a]HP: high protein.

[b]LS: low starch.

[c]HS: high starch.

TABLE II. NUTRIENT COMPOSITION OF PRAIRIE HAY AND SUPPLEMENTS (DM BASIS)

Item	Prairie Hay (%)	HP[a] (40% CP) (%)	LS[b] (20% CP) (%)	HS[c] (20% CP) (%)	SE
Dry matter	94.37	89.96	88.76	87.34	0.56
Organic matter	92.55	88.10	93.14	43.94	0.31
Crude protein	5.45	40.53	20.24	22.08	0.41
Acid detergent fiber	54.02	20.28	13.38	9.63	0.44
Lignin	9.11	3.70	3.28	2.17	0.41
Cellulose	32.23	16.24	9.84	7.36	4.16
Starch	0.91	3.68	22.80	49.91	1.10
Ash	7.46	11.90	6.86	6.06	0.31

[a]HP: high protein.

[b]LS: low starch.

[c]HS: high starch.

sampled in every period individually following preparation. Samples were ground and stored under the same conditions as above.

All samples were analyzed for crude protein (N x 6.25) by the Kjeldhal method, as well as dry matter (DM) and ash (A.O.A.C., 1975). Acid detergent fiber (ADF), cellulose and permanganate lignin were determined following the procedures of Goering and Van Soest (1970). Fresh hay, feces and supplements were analyzed for starch content, determined as α-linked glucose polymers (MacRae and Armstrong, 1968). Samples of the rejected prairie hay, selected at random from different periods, were also analyzed for starch content, and the values were found to be negligible.

A stomach tube equipped with a stainless steel strainer was used to sample the rumen. A vacuum pump was used as a source of suction. About 300 ml of ruminal fluid were collected per animal. Rumen pH was determined at the time of sampling and the ruminal fluid acidified by addition of 1 ml of a 20 percent H_2SO_4 solution per 50 ml of fluid and frozen for ammonia determination. The ammonia-N was determined by Mg O distillation (A.O.A.C., 1975).

The data of this experiment were analyzed using the Statistical Analysis System (SAS). An analysis of variance was performed and pre-planned comparisons of the treatment means were conducted by using orthogonal contrasts as shown in Table III.

Experiment II

Dacron polyester bags containing prairie hay were suspended in the rumens of four fistulated steers fed prairie hay plus supplement LS or HS in a crossover design, to determine DM and ADF disappearance of the prairie hay from the bags exposed to the rumen environment at different

TABLE III. ORTHOGONAL CONTRASTS AMONG TREATMENT MEANS

	Control	40%--HP	20%--LS	20%--HS
HP vs LS + HS	0	+2	-1	-1
LS vs HS	0	0	-1	+1
Control vs All	+3	-1	-1	-1

times. Prairie hay was fed twice daily at 4.2 kg/day (DM basis), with the levels of LS and HS supplements being the same as in Experiment I. There were two periods with five days adaptation.

Prairie hay, used for the dacron bags, was ground through a 1 mm screen, washed and dried at 60°C for 72 hours and sifted again through a 0.5 mm sieve to homogenize the particle size, and saved in a glass container. This prairie hay was analyzed for DM (A.O.A.C., 1975) and ADF (Goering and Van Soest, 1970).

Dacron bags of 10 x 6 cm were made of dacron polyester (Poli-Air R-1019; N. Erlanger, Blumgart & Co., Inc.). Pore size ranged between 25 to 75 µm. The bags were constructed from a single piece of material which was folded in half. Two of the open edges were sewn together and glued to prevent particle loss through the needle holes. The top of the bag was left open for sample placement. A hot metal spatula was used to singe all cut edges to avoid fraying in the rumen.

All the bags were identified, dried and weighed. Approximately 1.5 g of the processed prairie hay was placed in each bag. The bags were secured with synthetic thread to nylon lines with one loaded extreme, to prevent free floating in rumen contents. A single line with two bags attached was introduced through the fistula of each one of the four animals at different times. Thus, the bag contents were exposed to the rumen activity at 4, 12, 18, 24 and 48 hours.

After 48 hours all the lines (one per each exposure time per animal) were pulled out. The bags were then separated from the line and washed with tap water until the squeezed liquid was clear. The bags were dried at 60°C during 48 hours, weighed and the dry matter weight of the residue

calculated. The residue was scraped from the bag, re-dried, re-weighed and analyzed for ADF.

During the 48 hours of the experiment, rumen samples were also collected at 2, 4 and 8 hours after the supplement was fed in both periods. The rumen samples were analyzed for N-ammonia and pH, previously described.

The procedure of Brandt (1938) was used to perform the analysis of variance and to determine statistical differences among treatments for the different exposure and sampling times.

Results and Discussion

Experiment I

Daily dry matter intake of prairie hay was higher ($P < 0.01$) on all three supplemental treatments compared to the control (Table IV). However, no significant differences were observed in forage intake among the three supplementation programs, with intakes being very similar on all three treatments. Moreover, there was an increase in digestible DM intake (Table IV) and a substantial increase ($P < 0.01$) in DM digestibility (Table V) for all three supplements over the control treatment. Average total DM intake for the HP, LS and HS supplemented steers was comparable and in agreement with the minimum suggested DM requirement for maintenance of 5.9 to 6.4 kg/day as reported by the NRC for beef cattle (1976).

Total daily consumption of protein and starch is reported in Table VI. Protein intake was very similar for the three protein supplemented treatments, while protein intake on the control hay diet did not meet the daily maintenance requirement of 540 to 600 g (NRC, 1976). Starch intake

TABLE IV. DAILY DM INTAKE OF PRAIRIE HAY, PRAIRIE HAY PLUS SUPPLEMENTS, DIGESTIBLE DM AND INTAKE AS PERCENT OF B.W.

Item	Treatments				
	Control	HP[a] (40% CP)	LS[b] (20% CP)	HS[c] (20% CP)	SE
Prairie hay DM intake, kg[d]	3.54	4.60	4.43	4.58	0.17
Total DM intake, kg	3.65	5.50	6.23	6.38	---
Digestible DM intake, kg	1.80	3.10	3.70	3.80	---
Total DM intake as a percent of body weight, %[e]	0.86	1.29	1.46	1.50	---

[a]HP: high protein.

[b]LS: low starch.

[c]HS: high starch.

[d]Control vs. all supplement treatments ($P < 0.01$).

[e]Average body weight across periods: 426 kg.

TABLE V. TOTAL NUTRIENT DISAPPEARANCE FROM THE DIGESTIVE TRACT AS A PERCENT OF THE INTAKE

Item	Treatments				
	Control	HP (40% CP)	LS (20% CP)	HS (20% CP)	SE
Total DM, %[a]	45.9	53.7	56.2	56.1	2.0
Crude Protein, T[a]	7.2	54.9	54.1	52.9	3.2
ADF, %	47.5	51.8	50.8	48.1	2.2
Cellulose, %[b]	59.6	64.6	63.6	61.8	1.7
Starch, %[a,c]	82.0	87.6	94.6	98.1	1.9
Lignin, %	17.3	17.5	40.5	3.9	16.0

[a]Control vs. all supplement treatments ($P < 0.01$).

[b]Control vs. all supplement treatments ($P = 0.07$).

[c]HP vs. LS and HS average ($P < 0.01$).

TABLE VI. DAILY INTAKE AND CONCENTRATION OF PROTEIN AND STARCH IN THE DIET

Item	Treatments			
	Control	HP (40% CP)	LS (20% CP)	HS (20% CP)
Protein intake, g/day	193	616	605	647
CP as a percent of total daily intake	5.3	11.2	9.7	10.1
Starch intake, g/day	32	75	450	940
Starch as a percent of total daily intake	0.9	1.4	7.2	14.7

for the HS was approximately twice as high as for the LS treatment, as was previously planned.

The total diet DM digestibility (45.9%) was low and the apparent CP digestibility (7.2%) was very low ($P < 0.01$) on the control treatment, with no significant differences among the three protein supplemental treatments (Table V). No significant differences in digestibility of ADF and cellulose were found. However, all protein supplemental treatments tended to improve the digestibility coefficients of ADF and cellulose.

Although there was a slight but not significant trend for decreasing digestibility of ADF and cellulose with increasing starch levels, starch disappearance values were very high for all treatments, with the three supplemental treatments exhibiting higher coefficients ($P < 0.01$) than that of the control. Lignin disappearance coefficients presented the highest variability (SE = 16.0) with no significant differences being detected.

The ruminal NH_3 concentration (Table VII) was very low (0.35 mg/dl; $P < 0.01$) on the control treatment compared with the three supplemental treatments and was significantly different ($P < 0.01$) between LS (2.79 mg/dl) and HS (4.44 mg/dl). Ruminal fluid pH values were almost identical across treatments (Table VII).

A substantial increase in roughage consumption by the supplemented animals may be attributed to the extra protein furnished. There was a 23, 20 and 23 percent increase in forage consumption on the HP, LS and HS treatments, respectively, over the control. Lyons et al. (1970) reported an increased voluntary consumption of barley straw ranging from 23 to 26 percent for cattle fed supplements containing 15.7, 21.9 and 33.3 percent CP over the control (10.3% CP). Moreover, Pendlum et al. (1977) reported intakes of corn silage (7.6% CP) were increased from 3.6 to 4.6 kg/day in

TABLE VII. RUMINAL AMMONIA CONCENTRATION AND pH AT SIX TO SEVEN HOURS AFTER FEEDING THE SUPPLEMENTS

Item	Treatments				
	Control	HP (40% CP)	LS (20% CP)	HS (20% CP)	SE
Rumen NH_3, mg/dl[a,b]	0.35	3.56	2.79	4.44	0.36
Ruminal fluid, pH	6.74	6.68	6.69	6.63	---

[a]Control vs. all supplemental treatments ($P < 0.01$).

[b]LS vs. HS ($P < 0.01$).

steers fed 0.45, 0.68 or 0.91 kg/day of soybean meal. Observed increases in feed intake with nitrogen supplementation may be due to an increased rate of ruminal digestion (Johnson, 1981). This observation can be particularly true in this experiment since the extent of ADF and cellulose digestion were not significantly altered, but the rate might have been increased due to nitrogen availability to the ruminal population. Other studies have shown the effect of supplementation may be quite variable depending on: winter grazing conditions (Rittenhouse et al., 1970); forage quality and availability (Kartchner, 1981); level of protein intake (Lyons et al., 1970); and roughage palatability (Lusby et al., 1976).

Any of the three supplementation programs increased substantially the digestible dry matter intakes to 3.10, 3.70 and 3.80 (HP, LS and HS, respectively) kg/day compared with the control, 1.80 kg/day. In a study by Crabtree and Williams (1971), lambs fed cereal straw (3.9% CP) alone or plus a concentrate supplement (19.1% CP) increased straw intake about 50 percent and also digestible energy intake when the concentrate was fed up to 25 percent of total diet, but at higher levels of supplement intake, consumption of straw started to decrease. In the present study supplement represented 16, 27 and 28 percent of the total dry matter intake on the HP, LS and HS treatments, respectively, and did not depress forage intake.

Wheat starch appears to have a more detrimental effect on fiber digestion when replacing corn in high concentrate diets, by favoring lactic acid production, decreasing pH and reducing voluntary consumption (Varner, 1970; Varner and Woods, 1975; Fulton et al., 1979). Although wheat served as the major source of starch, at the levels of starch intake in the study, the LS and HS supplements did not appear to have detrimental

effects on forage intake. However, a slight but not significant trend was observed on the HS supplement to depress ADF and cellulose digestibility. Thus, it appears that the effect of starch on voluntary intake and fiber digestibility would depend on the level of fiber and starch in the diet as well as the nitrogen availability to ruminal bacteria. Williams et al. (1953) found that, for low protein diets, increases in starch intake reduced ruminal counts as well as type of microorganism present, but when protein intake was increased no such effect was evident. el-Shazly et al. (1961) suggested that the inhibition of cellulose digestion by starch is due to a competition between cellulolytic and amylolytic bacteria for nutrients, with nitrogen being the major one. Then if nitrogen is readily available, the inhibitory starch effect can be cancelled or at least decreased depending upon the starch concentration of the diet.

Although reported, the digestibility coefficients for starch in the control and HP treatment are probably of very little value. Starch is probably not present in prairie hay, and the small amount reported may be due to either experimental error or the present nonstarch glucose. In the HP treatment most of the glucose polymers detected most likely come from the small amount of molasses added to the supplement. Starch disappearance on LS and HS treatments were very similar and almost complete, being 94.6 and 98.1 percent, respectively. These values are very close to the value of 99 ± 1.2 percent reported by Waldo (1973) after a review of 15 digestion experiments.

Lignin disappearance was highly variable and appeared not to be related to the diet. Fahey et al. (1973) reported a large variation on lignin digestibility (from negative to positive values) occurs when

different lignin assays were employed. Changes in lignin undoubtedly occur as it passes through the digestive tract in ruminants.

The higher ruminal NH_3 concentration ($P < 0.01$) on HP, LS and HS versus the control treatment has shown the positive effect of protein supplementation. Kropp et al. (1977) suggested that the NH_3 level is a precise index of nitrogen status for rumen bacteria. Results of an in vivo study from Slyter et al. (1979) support the previous view of Satter and Slyter (1974), that values ranging from 2 to 5 mg of NH_3 - N/dl are sufficient to allow maximum growth of ruminal microbes. According to the above literature reported values, the supplemental treatments in the present study have met the adequacy levels of ruminal NH_3 (Table VII). If NH_3 is indeed an accurate estimate of N status, then the N status was adequate in the supplemental treatments. Most likely this might be the key factor which prevented a substantial inhibitory effect of starch if any may have existed. The lower ruminal NH_3 ($P < 0.01$) on the LS versus HS treatment may be related to the source of protein in that more cottonseed meal was required in the HS to reach the desired level of CP.

A malfunction of the pH meter yielded unreliable pH figures during the first two periods of the study. As a result, pH values of periods 3 and 4 only were averaged, but statistical analysis was not performed. However, pH values appeared very stable in these two periods, suggesting that dramatic changes in fermentation patterns did not occur.

Experiment II

Disappearance of prairie hay DM (Figure 3) and ADF (Figure 4) from the incubated dacron bags was not significantly different between animals receiving either LS or HS supplements for any of the periods of time

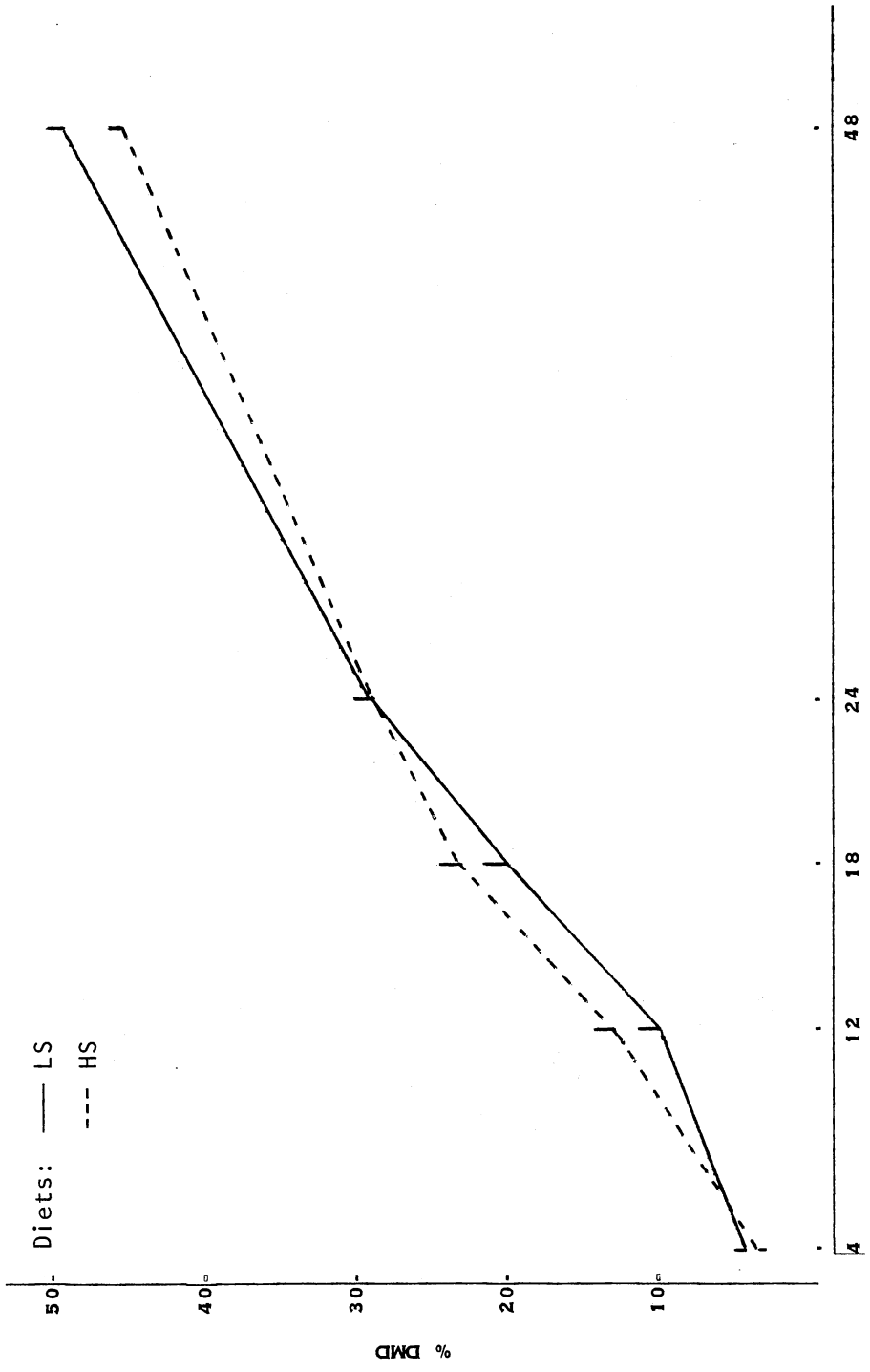

Figure 3. Rate of Prairie Hay DM Disappearance From Dacron Bags

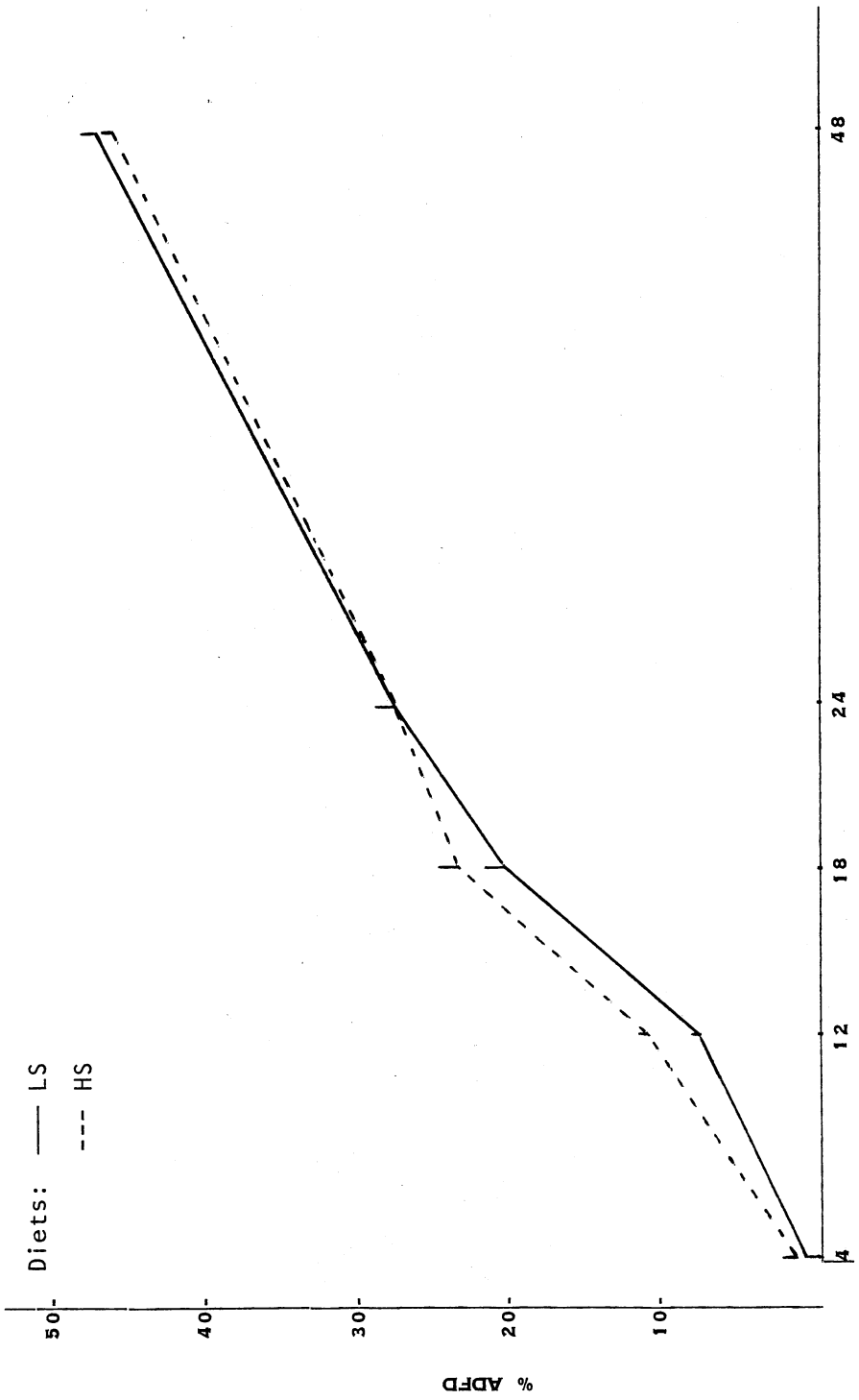

Figure 4. Rate of Prairie Hay ADF Disappearance From Dacron Bags

exposure. DM and DF disappearance increased with exposure time up to 48 hours, as may be expected, regardless of the treatment. The DM and ADF mean disappearance values for the different exposure times are reported in Table XI of the Appendix. The ruminal NH_3 concentration and pH values as determined at 2, 4 and 8 hours after feeding of the supplement are presented in Table VIII. Ruminal NH_3 concentration exhibited a large variability from two to four hours after the supplements were fed for both diets; however, the differences between treatment means were not significant. At 8 hours the mean ruminal NH_3 concentration decreased to the lowest levels for both treatments, being higher ($P < 0.05$) on HS compared with LS.

Ruminal pH values did not change with time or diet, which indicates that the buffering capacity of the ruminal fluid was not altered by the supplement composition.

In general, the rate of DM and ADF disappearance for both diets appears to be faster during the first 24 hours of exposure. For DM the rapid disappearance of easily degradable fractions likely occurs. A faster disappearance of the cellulose fraction in the ADF, not complexed into more insoluble cell wall components during the first 24 hours, may also be assumed. The residue in the bags likely becomes more and more digestible as time goes by, and thus the disappearance rate decreases. Similar disappearance patterns have been reported for cellulose disappearance from alfalfa, brome and timothy when incubated in nylon bags (Hopson et al., 1963), and for DM and nitrogen disappearance from barley incubated for a 24 hour period (Mehrez and Ørskov, 1977).

Although not significant, there is a trend during the first 24 hours of incubation for increased DM and ADF disappearance on the HS treatment.

TABLE VIII. RUMINAL AMMONIA CONCENTRATION AND pH IN FISTULATED STEERS AT 2, 4 AND 8 HOURS POST-FEEDING

Time After Feeding (Hr)	Treatments		
	LS (20% CP)	HS (20% CP)	SE
Ruminal NH_3, mg/dl			
2	8.78	8.95	6.760
4	6.17	5.34	3.190
8[a]	3.33	3.97	0.920
Ruminal pH			
2	6.74	6.76	0.007
4	6.78	6.76	0.007
8	6.78	6.79	0.007

[a]LS vs. HS ($P < 0.05$).

This trend may be explained by the differences in the concentration of ruminal NH_3 at 8 hours. Ruminal NH_3 concentration on HS treatment was again higher ($P < 0.01$) at 8 hours compared to LS, as in the first study. The NH_3 concentration may be more critical at lower levels than at higher ones. Thus, the significant difference observed between LS (3.33 mg/dl) and HS (3.97 mg/dl) may be responsible for the observed trend. This effect is not evident, however, after the first 24 hours. The slight differences observed thereafter in DM and ADF disappearance between diets may be attributed to natural variation.

The results of the second experiment support the evidence found in the first study, that there is a minimal or little inhibitory effect of starch on DM or ADF disappearance at the levels of starch fed in these studies. The effect of starch is probably also linked to the nitrogen concentration of the diet as previously discussed for the first study.

Leng (1982) remarked that maintaining a high NH_3 concentration in rumen is crucial to sustaining a high fermentation rate. As may be expected, the NH_3 concentration decreased in both LS and HS treatments hours after the supplement was fed. However, the NH_3 was still adequate 8 hours thereafter (Table VIII), probably indicating a suitable nitrogen availability during the period of time under study. Moreover, the level of starch in the HS supplements, when expressed as a percent of the total diet (14%), was not very high. Under these conditions starch inhibitory effects are unlikely to be detected.

The lack of much variation in the pH with time and diet suggests minimal alteration of the rumen fermentation patterns as well as adequate buffering capacity of ruminal liquor. Esdale and Satter (1972) reported

that with a variation of the pH between 6.2 and 6.8, there was no measurable alteration in volatile fatty acids production.

Under the circumstances of this study, protein supplementation substantially improved the utilization of prairie hay, but the extent of fiber digestibility did not appear significantly increased, although rate of fiber digestion may have been improved. Moreover, starch content in the supplement did not depress either feed intake nor ADF digestibility, which may be due to the adequate protein supply. The lower protein supplements fed in larger quantities were effective, however, in increasing total energy intake compared to the HP treatment.

Possibly, different results may have been observed with different types or qualities of forages, supplement levels, frequency of feeding of a supplement, feed ingredients or composition of the supplement and other management practices. Further studies are needed to determine the importance of such variables.

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TABLE IX. LATIN SQUARE DESIGN USED IN THE DIGESTION STUDY

Replication No.	Steer ID	Period I	II	III	IV
1	107	A	B	C	D
	110	B	A	D	C
	101	C	D	B	A
	106	D	C	A	B
2	109	A	B	C	D
	111	B	C	D	A
	115	C	D	A	B
	108	D	A	B	C
3	116	A	B	C	D
	105	B	D	A	C
	104	C	A	D	B
	103	D	C	B	A
4	113	A	B	C	D
	112	B	A	D	C
	102	C	D	A	B
	114	D	C	B	A

A = Control.

B = HP--40% CP.

C = LS--20% CP.

D = HS--20% CP.

TABLE X. FECES COMPOSITION FOR THE DIFFERENT DIETS (DM BASIS)

Item	Treatment				
	Control	HP (40% CP)	LS (20% CP)	HS (20% CP)	SE
Total Daily DM Output (g)	1,926	2,529	2,742	2,788	12.70
CP (%)	7.9	10.9	9.8	10.5	0.14
ADF (%)	51.2	50.3	47.6	49.0	0.35
Lignin (%)	12.6	14.2	13.1	13.3	0.33
Cellulose (%)	30.2	28.2	26.9	27.6	0.36
Starch (%)	0.5	0.4	1.0	0.7	0.19
Ash (%)	12.3	13.2	12.3	12.8	0.39

TABLE XI. MEAN DM AND ADF DISAPPEARANCE OF PRAIRIE HAY FOR THE DACRON BAG STUDY

Rumen Exposure Time (Hours)	Treatments LS (20% CP)	HS (20% CP)	SE
DM Disappearance (%)			
4	4.20	3.80	0.55
12	9.90	12.78	1.59
18	19.70	22.21	1.46
24	28.22	28.03	0.70
48	48.65	45.37	0.89
ADF Disappearance (%)			
4	0.89	1.27	0.92
12	7.73	10.90	0.60
18	20.00	23.11	1.53
24	26.94	26.96	1.35
48	48.07	45.94	1.38

VITA

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