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The effect of synchronization of protein and starch degradation in the rumen on nutrient utilization and milk production in dairy cows

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THE EFFECT OF SYNCHRONIZATION OF PROTEIN AND STARCH DEGRADATION IN THE RUMEN ON NUTRIENT UTILIZATION AND MILK PRODUCTION IN DAIRY COWS

By

Rolando Ernesto Herrera y Saldana

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Rolando E. Herrera-Saldana entitled THE EFFECT OF SYNCHRONIZATION OF PROTEIN AND STARCH DEGRADATION IN THE RUMEN ON NUTRIENT UTILIZATION AND MILK PRODUCTION IN DAIRY COWS.

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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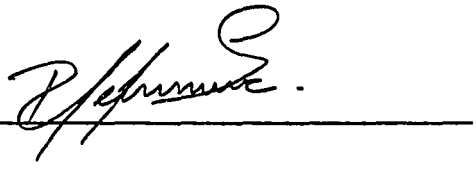
SIGNED: 

DEDICATION

To God, our Lord Who made this possible.

To my beloved wife Patricia for her patience and constant encouragement throughout my studies, and to our children Andres, Alejandra, Dulce Maria and Daniel, for making our life brighter and full of expectations.

To my father, my mother and my brother and sisters for their guidance and help.

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ABSTRACT

Four studies were conducted to determine the effect of synchronization of protein and starch degradation on nutrient utilization, microbial protein synthesis and milk production in dairy cows.

In Experiment 1, five cereal grains and five protein supplements were compared for extent of solubility and degradability of their starch and nitrogen fractions. Results indicated large differences which permitted their ranking from high to low degradability as follows:
grains, oats > wheat > barley > corn > milo
protein supplements, soybean meal > cottonseed meal, (CSM) > corn gluten meal > brewer dried grains, (BDG) > blood meal

In Experiment 2, the five grains were incubated for varying times in vitro (with added amylase) or in situ to determine rate and extent of degradation of dry matter, crude protein and starch. Results showed that rate of starch degradation followed a similar, but slightly different trend than in trial 1 (wheat > barley > oats > corn > milo). Rates for DM and CP degradation were similar to those for starch.

In experiment 3, high (barley, HS) and a low (milo, LS) degradable starch sources were combined with a high (CSM, HP) and a low (BDG, LP) degradable protein sources to formulate four diets; HSHP, HSLP, LSHP and LSLP. Diets were fed to 32 cows, starting two to four weeks postpartum, for a

60-d milk production and digestibility study. Apparent digestibility was calculated using chromium oxide. Organic matter digestibility was higher ($P<.05$) for diets containing CSM and starch digestibility was higher ($P<.05$) for diets containing barley. Milk production was higher for the HSHP diet, but milk fat percent was depressed on the barley diets. Thus, 3.5% FCM did not differ between the HSHP and milo diets.

A fourth study was conducted to determine the effect of the diets used in experiment 3 on rumen nutrient utilization and microbial protein synthesis. Four duodenally fistulated cows were used in a 4 x 4 Latin square. Chromium oxide estimated digestibility and rumen output of nutrients and nucleic acids estimated microbial protein synthesis duodenal contents. Barley diets had higher ($P<.05$) apparent and corrected rumen digestibility of DM, OM, CP and starch than milo diets. No difference ($P>.05$) was found in nutrient output to the small intestine among diets and microbial CP synthesis was higher ($P<.05$) for barley diets.

CHAPTER 1

INTRODUCTION

Ruminants fulfill a key role in converting plant resources humans cannot, or choose not to consume, into desirable high quality human food. The plant resourcs used include cereal grains, forage and silage crops, pasture and range forage, crop residues, and a wide range of byproducts from food processing and other industries.

Cereal grains are not necessary for ruminant production; however, their use improves gross efficiency and productivity. Approximately one-sixth of the energy requirements for all livestock products consumed by humans are derived from ceral grains. Ruminants (dairy and beef cattle, sheep and goats) consume 37% of grains fed to livestock and produce 61% of the human food energy derived from livestock (Wheeler et al., 1981).

The primary objective of research in ruminant digestion and metabolism is improvement of the efficiency of ruminant meat and milk production. The development of superior feeding standards, based upon the present knowledge of digestion and metabolism, has resulted in significant advances in ruminant nutrition. New protein systems have been proposed to classify proteins according to their rumen degradable fractions. Research on rumen degradable and undegradable proteins and rate of protein degradation in the

rumen has permitted further progress in production efficiency. On the other hand, the non-structural carbohydrates (soluble sugars, starches, fructans, galactans, pectins, etc.) lack a satisfactory system of classification, despite their major role as energy-yielding components of feedstuffs. Reasons for this lack of adequate classification are related to their great diversity and to the little basic research into their specific nutritive characteristics (Van Soest, 1987). Starch is the major component in cereal grains and constitutes one of the most important energy-yielding nutrients in high producing ruminants. Rates at which different sources of starch are degraded in the rumen are almost non-existent in the literature. Such information is critical for synchronization of rumen degradation of proteins and starches. The goal of synchronized degradation is to increase efficiency of nutrient utilization and microbial cell synthesis.

The research associated with this dissertation was designed to determine:

1) In vitro solubility and degradability of several common feedstuffs.
2) In vitro and in situ rates of degradation of dry matter, starch and protein of five common cereal grains (corn, milo, barley, wheat and

oats).

3) Effect of varying protein and starch degradabilities in the rumen on performance of high producing dairy cows.

4) Effect of varying protein and starch degradability in the rumen on nutrient utilization and microbial protein synthesis.

CHAPTER 2

LITERATURE REVIEW

STARCH

Next to cellulose, starch is the principal carbohydrate photosynthesized by means of solar energy. It is the reserve substance of most high plants and constitutes an energy source essential for man and for many other organisms. The most important sources of starch are cereal grains, with a total starch content ranging from 40 to 80% of their dry weight. Efforts to increase the efficiency of meat, milk and wool production of ruminants have led to feeding more concentrates and consequently more starch. Starch is subjected initially to fermentation in the rumen resulting in microbial growth and VFA production. Unfermented starch from the rumen is then degraded to glucose by enzymes in the small intestine. Digestion of starch influences the magnitude of nitrogen fixation into microbial cell protein and the efficiency of transforming feed energy into animal products.

Comparisons of cereal grains have been made using in vitro, in situ and in vivo techniques and, even though the structure of starch is practically the same in all cereal grains, their nutritive value for animal feeding varies considerably. Factors identified as responsible for these differences are chemical properties, physical form and

protein-starch interactions. These and other factors related to starch utilization will be further reviewed.

Chemical structure of starch components

Starch is a glucan composed of two major components: amylose and amylopectin. A third component known as "branched amylose" may also be present. The proportion of these components varies from one type to another. Amylose is a linear polymer of -1,4-linked D-glucopyranose units. The proportion of amylose in starch ranges from 0 to 80% depending upon the species and the genetic variations within a species. Most cereal grain starches contain 20 to 30% amylose; however, waxy types of starches contain little or no amylose. The iodine-biding capacity is rather constant for various extracted amyloses (approx. 19.0 mg iodine per 100 mg amylose). The B-amylolysis limit varies from 68 to 95%, confirming the presence of barriers to enzyme action (Guilbot and Mercier, 1985).

Amylopectin is a much larger, branched polymer which is the most abundant component of normal starches. Linear chains of -1,4-linked D-glucopyrase units have -1,6 branch links at every 20 to 25 glucose units. These branching links comprise about 4 to 5% of the total number of linkages in amylopectin. On the basis of methylation and enzymic analysis, Meyer and Bernfeld (1940), as cited by French (1973), proposed the now-famous "tree" model for

amylopectin. This model has been widely accepted s ince it explains many of amylopectin's chemical and biochemical properties. Manners (1985) proposed a model based on "Meyers' tree" and shows alternating crystalline and amorphous regions in amylopectin. The linear chains in the molecule are classed as "A" (nonbranched), "B" (branched) and "C" (the single central chain that carries the free reducing group of the molecule).

Estimates of molecular weight of amylopectin begin at 1.0 million.

Amylopectin comprises 70 to 80% of most cereal starches and is the only starch in waxy genotypes of corn, sorghum, barley, rice and millet. The "A" chains of amylopectin tend to be short and weakly helical, forming a weak, reddish-brown color with iodine. Iodine staining can thus be used to distinguish normal and waxy starch (Rooney and Pflugfelder, 1986).

Intermediate or anomalous amylose and amylopectin, are slightly "branched amyloses" thought to comprise 5 to 7% of ceral grains and potato starch (Banks and Greenwood, 1975).

Physical properties of starch

Solubility. The solubility behavior of starch presents an enigma to scientists. Starch is composed of glucose units, and glucose is water soluble. The starch

oligosaccharides, maltose, maltotriose, etc. are also very soluble in water. However, as the oligosaccharide chains are elongated (more than 10-15 glucose units), the material undergoes a crystallizing transformation and at a range of 70 to 150 glucose units the material becomes water insoluble (French, 1973). Natural starch is difficult or impossible to dissolve without the aid of energy (heat) or chemical agents. Many types of starch solvents are known. The most common are: hot water, aqueous alkali (NaOH or KOH), certain salts ($CaCl_2$, $Ca(NO_3)2$), amines (6 M urea or molten urea), amides (ethylene diamine, dimethyl formamide) and polyols (glycerol or ethylene glycol). Solubility of starch is greatly enhanced by disruption of the regularity of the amylose chain.

Starch granule structure.

The structure of starch granules depends on the way in which amylose and amylopectin are associated by intermolecular hydrogen bonds. When these bonds are strong, numerous and regular, the chains associate as crystalline networks, composed mainly of amylopectin; in constrast, when the macromolecules are more independent and relatively unorganized, amorphous zones are apparent (Whister et al., 1984). The crystalline region is resistant to water entry and enzyme attack. The amorphous region (gel phase) is rich in amylose and less dense than the crystalline area. Water

moves freely through it and amylase attack on the granule begins in this region. Around the center of most hydrated granules, there are concentric shells of alternating high and low refractive index. These radial shells correspond to the growth of amylopectin and amylose in the granule (Manners, 1985). The fact that waxy and normal corn starch granules exhibit similar patterns of enzymatic hydrolysis provides strong evidence that amylopectin is the major structural component of starch granules. Amylase attachment begins in the amorphous regions, while hydrolysis of the crystalline regions occurs more slowly. Amylose is preferentially leached from the starch granule during the early stages of gelatinization, suggesting that part is in the amorphous area. Thee subtle differences in granule structure
affect both starch digestibility and processing properties of grains.

Enzymatic degradation of starch

According to Guilbot and Mercier (1984), there are two classes of starch degrading enzymes. The first consists of the glucosidases classified as hydrolases with an irreversible hydrolytic action, which can be written as follows:

$$ROR^1 + HOH \text{ ------ } ROH + R^1OH$$

where R and R^1 are chains of from 1 to n glucose residues.

These glucosidases are subdivided into three groups under the numbering EC 3.2.1-:

1. Enzymes specific for (1 --- 4) - - D linkages
2. Enzymmes specific for (1 --- 6) - - D linkages
3. Enzymes specific for (1 --- 4) - and (1 -- 6) --D linkages.

The second class of starch-degrading enzymes consists of the glycosyl transferases. Their action is to transfer a glycosyl group from starch to an acceptor. The action of most of these enzymes is reversible, and some are involved in starch biosynthesis. The only enzyme from this class involved in starch degradation is the cyclodextrin glycosyl transferase. Table 1 shows a selected group of starch-degrading enzymes, their source and the main end products obtained from their action.

It is interesting to observe that amyloglucosidase is apparently produced only by molds. The enzyme is one of the least specific starch-degrading enzymes and is reported to have the capacity to cleave (1 --- 3) - - D and (1 --- 4) - - D as well as (1 --- 6) - D linkages, but at different rates of hydrolysis (Whistler et al., 1984). In practice, the branched substrates are not completely degraded to glucose by the enzyme. However, complete degradation to glucose is reached with the concomitant action of - amylase, a phenomenon that has been used by

several workers to develop methods of determining starch content (Thivend et al., 1965; MacRae and Armstrong, 1968; Salomonsson et al., 1984).

Factors affecting starch degradability in grains

Ratio amylose:amylopectin. It is now common knowledge that ratio of amylose to amylopectin influences starch digestibility. High amylose grain varieties are less digestible than normal or waxy (less than 5% amylose) varieties. No clear explanation has been found for this phenomenon, but it may be related to amylose's restrictive role in granule swelling, or to orientation of amylose molecules towards the inside of the amylopectin crystallites, causing an increase in intermolecular hydrogen bonding which limits swelling and enzymatic hydrolysis (Rooney and Pflugfelder, 1986).

On the other hand, waxy cereal starches have higher digestibilty values than normal varieties. Sullins and Rooney (1975) compared waxy to normal varieties of sorghum in an in vitro study using porcine pancreatic -amylase. They found waxy starch varieties were more digestible than normal grains.

Hibbard et al. (1982) compared isolated normal starch from corn and sorghum to waxy starch sorghum lines in an in vitro gas production (IVGP) trial. The IVGP was higher for normal sorghum starch than for corn starch.

Among sorghum types, isolated waxy starch gave higher IVGP values than normal starches. They also found differences in IVGP between non-waxy starches, suggesting that other factors, such as granule size or chain length, affect starch degradation.

Little information is available in other cereal grains on the effect of amylose:amylopectin ratio on starch digestibility. Guilbot and Mercier (1985) found that varieties of waxy starch corn were more easily degraded than normal corn varieties.

We can conclude that starch digestibility is inversely proportional to the amount of amylose in the grain.

Protein bodies and protein matrix.

Electron microscope studies (Rooney et al., 1983) have revealed the presence of protein bodies embedded in the mature endosperm of corn, milo, oats and rice grains. These protein bodies, characterized as alcohol-soluble prolamines, are zein in corn and kafrin in sorghum with low digestibilities and globulins in oats and rice with high solubility and degradability. In the cases of sorghum and corn, it has been suggested (Hibberd et al., 1982; Delfino, 1986) that protein bodies retard enzymatic hydrolysis of starch due to the tight packing of starch granules and protein bodies. Another factor is the low degradability of

the protein that forms thee bodies. Protein bodies are particularly abundant throughout sorghum grain, creating problems for the milling industry.

Starch granules are held on a protein matrix that surrounds the endosperm of all grains. The nature of this protein matrix varies according to different grains. Protein matrixes in sorghum and corn are formed mainly by glutelins and prolamines, known to be less digestible than the glutelines, prolamines and globulins that form the matrixes in wheat, barley, and oats, respectively (Sniffen, 1980). Starch granules are almost encapsulated by this matrix and degradation of starch depends in part on degradation of the matrix (Hale, 1973).

Hahn et al. (1982) demonstrated that protein matrix in sorghum grain was a barrier to starch degradability. In this study, availability of sorghum proteins was evaluated by pronase hydrolysis in a single enzyme digestion with automated analysis of free amino acids. When the protein matrix was degraded in sorghum grains, starch degradability was improved.

McNeill et al. (1975) assessed the effect of different processing methods (dry-ground, steam-flaked, reconstituted and micronized) or in vivo digestibility of sorghum grain. They found that any method which produced a disruption of the protein matrix which encapsulated starch

granules in the endosperm increased starch utilization.

Fiber:starch interactions.

Doyle (1978) compared the effectiveness of four methods to determine fiber in barley, wheat and sorghum and found that the NDF method (Robertson and Van Soest, 1977) was unable to remove a large portion of the starch from the cell wall residue. This was especially significant for wheat and sorghum in which 20 and 45% of starch was found in the fiber residue. Using the Fonnesbeck and Harris (1970) method for cell wall determination, the amount of starch associated to the wall ranged from 3.0 to 11%. When the ADF (Goering and Van Soest, 1970) and Baker acid detergent fiber methods were used to determine the lignocellulose fraction in thee grains, the starch content in the residues varied from 1.5 to 5.2%. Even though this information is not sufficient to make a valid conclusion, it leads us to suggest that in minimally processed grains the fiber-starch relationship may interfere with the com. ‹ starch utilizatiron. Further study of this interaction is required in order to determine its importance.

Methods to determine total starch

The literature on the analysis of starch is voluminous, comprising hundreds of referencs. An attempt has been made to briefly describe only those methods which have become standard practice or which may be of practical

value for routine analysis or research in animal nutrition. Most of these methods involve enzyme hydrolysis of starch, so in order to better identify them, they will be called by the name of their main author.

Acid-anthrone calorimetric method. Clegg (1956) developed a method using the affinity of anthrone to bind glucose molecules. In this method, starch is hydrolyzed by perchloric acid to glucose units which are determined by reaction with anthrone to form a green-colored complex. The anthrone-sugar complex is then read in a spectrophotometer at 630 nm.

The method, although widely used in the past, is now used little due in part to the variability and overestimation of the results because of interference by other carbohydrates such as pectic acid, xylose and arabinose which form colored complexes with anthrone. This variation was also reported in a six-laboratory comparison by Topps and Kay (1969).

The MacRae and Armstrong (1968) enzyme method. This has been widely used for the estimation of starch in a variety of feedstuffs. A weighed starch sample is first gelatinized by refluxing in boiling water during 4 h. Samples are then mixed with an acetate buffer containing an amyloglucosidase enzyme (Agidex) obtained from Aspergillus niger, capable of degrading starch completely to glucose.

Flasks are incubated at 60 C for a minimum period of 24 h, after which they are de-proteinized and concentration of glucose is determined using glucose oxidase. The main disadvantages of this method are the number of steps involved which increases risk of mistakes, and the long time required to obtain the results (Kartchner and Theurer, 1981).

The modified Fleming and Reichert (1980) method. Starch-containing samples are extracted three times with hot (60 C) aqueous methanol (80%). Soluble carbohydrates are removed with the supernatant following centrifugation. Calcium chloride solution is added and the mxiture heated to gelatinize starch (125 C, 15 min.). After cooling, potassium hydroxyde and an acetate buffer are added with a mixture of -amyloase and glucoamylase enzymes. Samples are incubated for 3 h (48 C) and glucose is analyzed using either Glucostat or Statzyme reagents. With this method purified wheat starch was found to yield 156.4-162.7% starch when glucose content was determined by the Statzyme reagent. To correct this problem, it was required to add 10 ml of 0.1 M EDTA to samples before glucose analysis. When glucose was determined with the Glucostat reagent, results were more accurate and comparable to those obtained with other methods. The main disadvantage of this method is that only a few samples can be analyzed at any given time.

The Kartchner and Theurer method. This is a modification of the method of MacRae and Armstrong (1968) which overcomes certain disadvantages. The main changes proposed were: 1) Direct determination of the volume of the hydrolyzing solution instead of by weighing; 2) Elimination of the paraffin cover by using a watch glass during the 24 h incubation period; and 3) Simplification of the calculations for starch determination. Results obtained using this modified method are reproducible and acceptable in samples having low or high starch contents. The only practical disadvantage of the method is the long time required to obtain results.

The Herrera-Saldana and Huber (1987) method. This method, which is a modification of that described by Salomonsson et al. (1984) was developed in our laboratory in an attempt to simplify the original method and to analyze a large number of samples in less time. This was achieved by reducing the final dilution volume from 100 to 10 ml and by using enzymes with higher amylolytic activities. Thus, it was possible to analyze about 100 samples per day with results that commpared well with those using similar methods. This method is described completely in Chapter 3 of this dissertation.

Feeding value of starch in cereal grains for ruminants

Grains have become a major feed ingredient and an

important source of starch and protein for high-producing ruminants. Large differences in starch and protein characteristics have been observed among cereal grains. Processing of sorghum and corn have usually improved their digestibility. This situation has stimulated the development of fast and simple assays to determine the magnitude and effect of these differences and to predict in vivo digestibility and animal performance.

In vitro and in situ experiments. Several in vitro and in situ techniques have been developed to detremine starch, crude protein and dry matter digestibility from unprocessed and processed grains. Trei et al. (1970) described a method in which grains were incubated with strained rumen fluid and production of gas indicated dry matter digestibility. High correlations were found between gas production and in vitro dry matter disappearance, total VFA production and in vitro starch digestion. Gas production was significantly ($P<.05$) greater with barley than with milo. When steam processed and flaked milo and barley were incubated, significant increases in gas production were observed but barley was higher ($P<.05$)

Osman et al. (1970) studied the effect of flaking on in vitro enzymatic starch digestion of sorghum grain and barley. These grains were finely ground (80 mesh) prior to a 30-min. incubation with porcine pancreatin. When grains

kg sheep. The percent starch intake from barley varied from 23 to 64% of total DM and was 46% for corn. Starch digestibility was estimated using $Cr_2 O_3$ impregnated paper fed with the diet. Rumen digestibility of starch from barley increased from 91 to 97 as intake of starch increased. Total tract starch digestibility was 100% at all levels of intake. For corn, rumen starch digestibility was 90% and total tract digestibility was 100%. These results indicate that the capacity of the rumen to degrade starch from barley was not lowered by the increasing starch intake. The lower digestibility of barley starch at low intakes was not explained.

In a similar study, Orskov et al. (1969) compared starch digestibility of rolled or ground barley to flaked, ground or cracked corn using 40 kg lambs. Digestibility was calculated using polyethylene glycol as a marker. Barley starch degraded in the rumen was not affected by processing and had mean digestibilities of 93.0%; total tract digestibility was 98%. With corn, rumen starch degradation was improved by processing; values observed were 86, 88 and 95% for cracked, ground and flaked corn, respectively. Total tract digestibility was above 99% for all processed grains.

Waldo (1973), in an excellent review of the available information on starch digestibility prior to 1973,

dry matter and starch disappearance of unprocessed than processed corn.

Delfino (1986) compared the in situ DM disappearance of ground barley, corn, sorghum and wheat grains incubated for 144 h in steers fed three concentrate levels (0, 30, 90% of total diet). At all concentrate levels, DM disappearance was lower for barley than other grains, but was not different among corn, sorghum and wheat at any of the three concentrate levels. Evaluation of residues using an electron microscope showed that the main components of the residues were fiber fractions, but a disrupted protein matrix with protein bodies and starch granules remained through 144 h in ground sorghum. Although this comparison says nothing about the initial rate of digestion of these grains, it illustrates the resistance to degradation of the protein matrix that encapsulates starch granules in sorghum grain.

In vivo experiments.

Most of the studies evaluating the nutritive value of starch in grains have been conducted in vivo. However, few studies compare more than two grains in a single experiment. Thus, papers were selected in which at least two grains have been compared.

MacRae and Armstrong (1969b) compared starch digestibility of dry rolled barley vs. flaked corn using 50

he observed that even after 48 h incubation, starch granules were still present in fractured corn and sorghum residues. These observations suggest that the residual starch in cereal grain NDF reported by Doyle (1978) may not be due to inefficient extraction, but might represent starch that is not accessible to digestive enzymes in the rumen because of its close assocation with fiber. No starch could be detected in barley and wheat grain after 24 h incubation indicating the fast breakdown of the protein matrix and the easy availability of starch granules for enzyme digestion.

No reports comparing in situ starch digestibility between cereal grains could be found in the available literature. Most studies reviewed used the in situ technique to evaluate the effect of different processes on DM and starch digestibility using one grain.

Galyean et al. (1981) compared the effect of different particle sizes of processed or unprocessed corn grain on in situ dry matter and starch disappearance. Dry rolled, steam flaked, dry ground and high moisture corn were sieved to particle sizes ranging from 750 to 6,000 nm. After 8 h incubation, it was found that DM and starch digestibility increased as the particle size was reduced. Processing by steam flaking or high moisture produced additive effects beyond those of particle size alone. The effect of particle size appeared to have more influence on

were steamed but not flaked, starch digestion was reduced in both grains. Increasing flake flatness resulted in improved starch digestion, especially in sorghum grain. This study concluded that the degree of flaking (flake flatness) was the principal factor improving in vitro starch digestibility of barley and sorghum grains.

Frederick et al. (1973) studied the effect of moisture, pressure and temperature on enzymatic starch degradation of barley and sorghum grains. Various combinations of moisture, heat, and pressure were used on grains before starch digestion was determined by incubating ground-processed samples with a buffered homogenate of bovine pancrease for 30 min. They found that there is a critical pressure (4.2 kg/cm^2 cooking, 142 kg/cm^2 hydraulic pressure) at which enzyatic degradation of cereal starches were improved. This critical pressure appears to be affected by moisture content of the grain and temperature of the contact surfaces used to flake the whole grain.

In one of several studies comparing more than 2 grains, Delfino (1986) determined in vitro DM disappearance of cracked barley, corn, milo and wheat and observed residues after 12, 24 and 48 h or incubation under the electron microscope. Significant ($P<.01$) differences in IVMD values of 86.0, 95.6, 95.2 and 93.3% at 48 h for the respective grains were found. Using the elctron microscope,

reported the following mean values for starch degraded in the rumen: barley, 94% ± 2.4, 23 observations; corn, 78% ± 12.5, 30 observations; sorghum, 76% ± 22.4, 8 observations. The high variability observed in corn and sorghum was attributed to: 1) The natural differences among different varieties of these species; 2) The different ability of sheep and cattle used in these studies to digest starch; 3) The different processing methods used in the studies reviewed; and 4) The different proportions of starch in the diets. Total tract digestibility of starch in these experiments averaged 99 %, although significant amounts of corn and sorghum starch fed to cattle at high levels and coarse particle size do escape digestion.

Owens et al. (1986) reviewed research trials from 1975 to 1985 to determine the site and extent of strach digestion of corn and sorghum fed to cattle. Averaged across 40 cattle trials and 183 treatment means, total tract digestibility of starch for both grains was only 92.1 % +_ 5.5 (93.06 for corn and 90 % for sorghum). This is considerably lower than the mean of 99 % +_ 1.2 determined by Waldo (1973). Owever, lower means would be expected from the data obtained by Owens et al. (1986) since cattle have consistently lower starch digestion than sheep and starch digestion from sorghum and corn is considerably lower than for barley, wheat and oats. The amount of starch degraded in

the rumen averaged across 23 trials and 116 treatment means was 72.3% ± 12.8 for both corn and sorghum. This value was again lower than that (87.6%) calculated using the values given by Waldo (1973) for barley, corn and sorghum.

Spicer et al. (1986) compared the utilization of starch from barley-, corn- and sorghum-based diets fed to abomasally-fistulated steers. Rumen starch digestibility was calculated using Cr_2O_3 as a marker and was slowest for sorghum (75%), intermediate for corn (83%) and highest for barley (87.7%). Post ruminal and total tract digestion was increased in all grains but sorghum starch was still significantly lower (87.2, 97.2; 93.8, 99.1; 92.9, 99.2% for sorghum, corn and barley, respectively) than other grains. Although a large proportion of sorghum starch was digested postruminally, these data indicate that maximum total tract starch digestibility is positively related to extent of digestion in the rumen.

PROTEIN

Ruminant nutrition has undergone a continual evolution in understanding mechanisms of nutrient utilization. Up to the 1970s, it was generally believed that cattle rations could be balanced for protein by simply using crude protein values. According to most nutritionists prior to 1970, almost any nitrogen source, be it natural or non-protein nitrogen, could serve as a precursor to protein

as long as adequate carbon-chain compounds were available. However, evidence began to accumulate in the 1950s and 1960s indicating that the crude protein system was not entirely sound. Researchers begin to find that certain protein sources achieved better production in cattle even though the experimental diets were isonitrogenous.

Since then, a number of systems (NRC, 1985) have been developed in an attempt to overcome deficiencies of the previous systems (CP and DCP) and better predict the nutritive value of available nitrogen (N) sources. Most of these systems recognize that N requirements of a ruminant animal are logically divided into two parts, namely a requirement for N by rumen microorganisms and a requirement for protein (amino acids) by the host animal.

Since the rumen microorganisms require a source of degradable N and the host ruminant requires intact protein for digestion in the small intestine, the degradability of proteins in the rumen and the dynamics of the process played a central role in new systems for elucidating protein requirements for ruminants. Thus, protein solubility and degradability have become focal points for extensive research and hundreds of publications are available, some of which will be reviewed.

Protein Solubility

Protein solubility became of interest to ruminant

nutritionists when Hendricks and Martin (1963) showed that in vitro protein degradation was highly correlated (r^2=.99) with solubility in a mineral solution. Since this discovery, several methods for determining solubility were proposed using different solvents and techniques. The goal of all studies was to find the ideal solvent that could separate soluble from insoluble fractions of proteins with high precision and repeatability. It was assumed at this time that the soluble fraction would be completely degraded in the rumen. However, it was later demonstrated (Sniffen, 1980; Leng and Nolan, 1984) that solubility was not always a good indicator of protein degradability. Nonetheless, the solubility information has in many cases (Hogue et al., 1978; Olentine, 1982; Wanapat et al., 1982) rendered valuable data to predict protein degradability. Rgresults obtained with three common methods to determine protein solubility will be briefly described as follows.

Wohlt et al. (1972) described two procedures to measure protein solubility in common feedstuffs. They determined percent total soluble nitrogen of casein and soybean protein using either autoclaved rumen fluid or Wise-Burroughs mineral moisture as solvents. Three pH's (5.5, 6.5 and 7.5) over four intervals (30, 60, 90 and 120 min.) at 40 C were used. They found that protein solubility in autoclaved rumen fluid was significantly lower than in

mineral buffer. As pH was increased from 5.5 to 7.5, mean solubility was elevated from 27 to 57%. No differences were observed between pH 6.5 and 7.5 regardless of solvent or protein. Solubility did not differ between solvents at 60 min. Feedstuffs tested were grouped as to major protein fractions and amount of processing. Feeds whose major protein fractions were composed primarily of ablumins and globulins had higher solubilities than those composed primarily of prolamins and glutelins (42 vs. 18%).

Crooker et al. (1978) described the effect of three solvents, modified Burroughs mineral mixture (MBMM), McDougal's artificial saliva (MDA) and sodium chloride solution (NaCl) on protein solubility of seven feedstuffs. Three ionic strengths were used with each solvent (.11, .15 and .19 m Eg). The quantity of nitrogen extracted by either MBMM or MDA was different from that extracted using NaCl. Changing the ionic strength of solvents had no significant effect on extracted nitrogen. Moreover, there were several feed-solvent interactions, suggesting that ionic species contained in the solvent had an important effect on nitrogen extraction. In another trial, Crooker et al. (1978) compared the autoclaved rumen fluid (ARF), the original Burroughs mineral mixture (BMM) and the three solvents mentioned above. Results showed no significant differences in mean soluble nitrogen values between BMM, ARF, or NaCl.

The NaCl and BMM values were highly correlated (r = .93 for all samples), suggesting that either BMM or NaCl may be used for the extraction of nitrogen from most feedstuffs.

Krishnamoorthy et al. (1982) compared N solubility of five feedstuffs using six solvents with either continuous mixing at constant temperature or intermittent mixing at room temperature. Solvents compared included MDA, BMM, NaCl, ARF, bicarbonate-phosphate buffer (BCP) and borate-phosphate buffer (BP). The pH of all solvents was adjusted to 6.8, the time of extraction was 60 min. at 39 C and mixing was by a shaker at 110 to 120 rotations per min. Results obtained demonstrated that N solubility varied among feedstuffs and among solvents confirming findings from other studies (Crooker et al., 1978; Waldo and Goering, 1979; Wohlt, 1973) that the amount of nitrogen extracted from a feedstuff varies over a wide range depending on the solvent used and type of feed.

Under these circumstances, it seems reasonable to use ARF as preferred solvent. However, it has the disadvantage of variable composition, laborious collection and processing, as well as difficult filtratiron. The NaCl and BMM are not desirable because NaCl has no buffering capacity and BMM has a short shelf life. Even though BCP is simple to prepare, it has the disadvantage of unstable pH. It was established earlier that fluctuation in pH of solvent

affects protein solubility in feedstuffs to a considerable extent. Therefore, a solvent with stable pH is essential. Because BP maintains a stable pH over prolonged storage, this buffer appeared the most promising. However, caution should be exercised when comparing protein solubilities of different feedstuffs or when solubilities have been determined in different laboratories.

Solubility may be a poor predictor of protein degradation when dynamics of ruminal passage are not taken into account (Stern and Satter, 1982). Van der Aar et al. (1983) suggested that solubility is not a good predictor of degradation since not all soluble proteins are rapidly degraded in the rumen. Mahaderan et al. (1980) used a protease from Bacteroides amylophilus to incubate buffer soluble and insoluble protein fractions from soybean meal and other protein sources. They found that buffer soluble and insoluble proteins of soybean meal were hydrolyzed at almost identical rates; concluding that buffer solubility of a protein does not indicate susceptibility to degradation in the rumen.

Although these results contradict the solubility concept, soluble proteins are generally vulnerable more to protolysis than insoluble proteins. However, caution should be exercised when solubility information is used to predict degradability of proteins in the rumen.

Protein Degradabiity

Rumen microbes may supply from 60 to 80% of the amino acids (protein) absorbed in the small intestine of most ruminants (Kaufman and Luppin, 1982). However, with fast growing or high yielding milk cows, the amino acid contribution from microbes is not sufficient to cover the requirements (Kung and Huber, 1983). Therefore, interest has focused on true protein that escapes rumen degradation to increase intestinal supply of amino acids for maximization of animal production. However, equilibrium needs to be maintained between the nitrogen requirements of rumen microbes and the host animal for optimum performance. Sufficient degraded N is necessary to maintain a desirable efficiency of energy utilization in the rumen.

An extensive amount of information has been generated in the last 20 years trying to find an adequate solution to this nitrogen "puzzle". One attempt has been characterization of the various protein fractions in some feedstuffs. Pichard and Van Soest (1977) proposed that different feed protein fractions are degraded at different rates. In their scheme, fraction A is a water-soluble non-protein fraction (NPN) that includes nitrate, ammonia, amines and free amino acids, and is degraded rapidly and completely. The insoluble component is true protein and consists of a rapidly degradable fraction, B_1, a more slowly

degradable fraction, B_2, and an unavailable fraction, "C". Fraction C contains unavailable nitrogen indigenous to feed ingredients and that produced by heat damage. Theoretically, each pool or fraction has a degradation rate that is assumed to be fractional; that is, a constant proportion of the residue is degraded per unit of time. Mathers and Miller (1981) developed an equation using degradation rates for each fraction and a rate of passage factor to estimate escaped protein. The fraction degradation rates are: KdA, KdB and KaC. In practice, KdA is usually considered infinite and A is entirely degraded. KdC is usually considered zero and C is entirely passed. Only B is usually considered to be affected by the relative rates of passage and degradability of KpB and KdB at any time. The fraction of B that is degraded will be KdB/(KdC + KpB) and the fraction of B passed will be KpC/(KdC + KpC). The fraction of total protein that is degraded, D = A + KdB/(KdB + KpB), and the fraction of total protein that is passed, P = KpB/(KdB + KpB) + C. These concepts have been accepted by many researchers, but there is divergence on how to determine fractions and their rates of degradation. Important methods to determine protein degradability will be discussed.

In vitro Methods.

Several promising methods have been proposed as predictive

techniques of protein degradation; however, none has been generally accepted. Verite et al. (1979) based their PDI system on values obtained from the rumen undegradable protein (PDIA), but truly digested in the small intestine. Calculation of PDIA used the ammonia accumulation technique or solubility in a salt solution. They commented that in vitro incubation with rumen fluid was superior to solubility for estimating protein degradability, but this procedure was not suitable for routine analysis.

Broderick (1978) also used the protein fraction technique to estimate protein degradability; however, he found that microbial growth and nitrogen uptake occur simultaneously with protein degradation and ammonia release which makes degradability estimates difficult because microbial uptake varies with different feedstuffs. Broderick (1978) attempted to overcome this problem by inhibiting deamination and uptake of amino acids by microorganisms, thus enabling a direct measure of proteolysis. Another problem with this technique is that incubation conditions (substrate, end products, pH) in a batch culture change with time, so rates of both ammonia production and uptake change.

Another in vitro technique consists of the incubation of test proteins with a proteolytic enzyme. Poos et al. (1980) compared the effect of a neutral fungal

protease and the dacron bag suspended in the rumen for degradation of nine protein sources. Results obtained were correlated with undegradable protein values previously determined in beef cattle performance trials. The proteolytic enzyme assay gave higher correlations (r^2 = .93) than the dacron bag technique (r^2 = .73). In another study, Poos et al. (1985) compared five different proteolytic enzymes: Bromelain, Ficin, Papain, Fungal protease and Bacterial protease and results again were correlated with in vivo results obtained previously. Results showed a higher correlation for fungal protease (r^2 = .73) followed by bacterial protease (r^2 = .39) and bromelain (r^2 = .32). Although fungal protease gave a higher correlation, they recommended the use of ficin, since it was easier to handle for routine analysis.

Several other studies using enzymes have been published (Siddons and Beever, 1977; Hahn et al., 1982; Lichtenwalner et al., 1973) but the number of samples and enzymes teseted were small, so they won't be discussed.

In vivo Studies.

In vivo studies usually involve animals equipped with cannulae in the rumen and abomasum or small intestine (duodenum or ileum). Undegraded dietary protein is often determined as the difference between the non-ammonia nitrogen in duodenum and the sum of the endogenous plus

bacterial protein. Determination of digesta flow may be accomplished by total collection of the ingesta (Orskov, 1982) with a reentrant cannula or with an indigestible digesta marker and collection of spot samples (Zinn et al., 1980) using T cannulae. The latter method has been extensively used and results from many studies have accumulated in the last decade. However, it must be recognized that measure of digesta flow to the duodenum is subject to considerable error. Digesta markers do not always reflect the solid or liquid phase they are intended to represent. Wanderley et al. (1986) compared lignin and chromium oxide (Cr_2O_3) derived estimates with automated total collection of digesta on grain and roughage based diets. Estimates with Cr_2O_3 and lignin overestimated bacterial synthesis and rumen undegraded feed protein by 8 to 16%. Moreover, Cr_2O_3 showed great variation in bacterial protein efficiency on the forage diet and lignin gave most variability on the grain diet.

Zinn et al. (1981) studied degradation of several sources of supplemental protein using total collection and Cr_2O_3 as a marker. When casein-, soybean meal-, cottonseed meal- and corn gluten meal supplemented diets were fed at 3,000 g/day, estimates of rumen protein degradation were 103, 85, 76, and 54% respectively. When soybean meal, cottonseed meal, linseed meal, corn gluten meal and meat and

bone meal were fed at 4,000 g/day, rumen protein degradation estimates were 82, 39, 56, 39 and 30%, respectively. A prediction equation was developed for rumen bypass protein on the basis of solubility as well as in situ estimates. Correlations between predicted values and those measured in vivo were high (r=.98). Microbial efficiency (g microbial protein/100 g organic matter fermented) averaged 15.1, with a range of 11 to 19 with soybean the highest and corn gluten meal the lowest. Microbial efficiency was closely related to intake of digestible organic matter and rate of passage of non-microbial organic matter from the rumen.

Kung et al. (1983) mesured flow and digestion of nitrogenous compounds in the digestive tract. They fed 50:50 concentrate to forage diets containing 17% crude protein. Diets were: corn silage-soybean meal (CS-SBM), corn silage-heated soybean meal (CS-HS); and ammonia treated corn silag-heated soybean meal (AS-HS). Lanthanum and chromium-EDTA were used as flow and digestibility markers. Flow or organic matter to the duodenum was overestimated by these markers resulting in low estimates of ruminal digestion of organic matter and high estimates of nitrogen flow. These findings were disturbing and were attributed to a combination of the following reasons: 1) Most studies utilizing rare earth elements as indigestible markers have measured digesta flow in animals consuming smaller amounts

of dry matter than the lactating cows used in this study. Thus, attainment of steady state flow of digesta may be more difficult when intakes are large; 2) A negative correlation between duodenal dry matter content and its rare earth concentration might also be involved; 3) Sampling of liquids and solids from T cannulae may not be representative of true digesta flow.

Due to these reasons, acid detergent lignin was used as an internal marker for estimating dietary nitrogen degraded in the rumen and values obtained for the four rations were: CS-HS, 55.0; AS-HS, 58.8; AS-SBM, 63.3; and CS-SBM, 66.0%. Digestion of non-ammonia nitrogen in the small intestine was equal for all treatments suggesting that the heat treatment of the soybean meal did not alter protein availability in the intestine although ruminal degradability tended to be lower.

The in situ technique has also been suggested as a routine method to determine rumen protein degradability. Meherez and Orskov (1977) made an extensive description of their methods and showed results that had a high correlation with in vivo findings. Orskov and McDonald (1979) developed the following equation to predict the protein degradability in the rumen: $p = a + b\ (1-e^{-ct})$, where p = the amount of protein or nutrient degraded at time (t); a = the soluble or rapidly degraded fraction; b = the slowly degraded fraction;

and c = the rate at which the nutrient will be degraded per hour. The equation is constrained so that a + b cannot exceed 100%. This equation has been modified by several researchers to include rate of passage factors for better prediction of nutrient degraddation. Hence, Orskov and McDonald (1979) described a modified euqation as follows:

$$p = a + \frac{bc}{c + k}\left(1 - e^{-(c+k)t}\right)$$

where k = rate of outfloow.

Mathers and Miller (1981) provided the following method to estimate degradability:

$$A + (B1 \times KBa/(KB1 + Kr)) + (B2 \times KB_2/(KB2 + Kr)),$$

where A = soluble protein; B1 and B2 are protein fractions with degradation rates of KB1 and KB2, and Kr is the rate constant for turnover of ruminal dry matter. Turnover rate will primarily affect the rapidly degraded protein fraction, B1.

Other equations have been described (Uden et al., 1978; Broderick and Craig, 1980; Erdman et al., 1983); however, most of them are modifications of those described above.

Although the in situ technique has many advantages in determining the rate of protein or nutrient degradation in the rumen, it also has several disadvantages (Crawford et al., 1978; Broderick and Craig, 1980; Satter, 1986; Nocek et

al., 1987); the greatest one is related to the pore size of the cloth which may permit efflux of fine, insoluble particles or the influx of particles from the rumen. Another problem consists of the microbial contamination of samples which may seriously affect estimations of protein degradability. Handling and washing of bags after incubation might also be a source of error.

Nevertheless, until a more suitable technique emerges, the in situ technique is quite useful.

Protein Starch Interactions in the Rumen

Microbial protein from the rumen can furnish a large proportion of the amino acids required by ruminants (Orskov, 1982), so microbial growth is of special importance in any system developed to describe protein needs of ruminants.

Russell and Hespell (1981) stated that an optimum balance exists between requirements for microbial growth and substrate availability, which is influenced by utilization of degraded protein and carbohydrate from ingredients present in diets.

Protein degradation in the rumen often exceeds carbohyudrate availability, and protein wastage occurs. In other cases, the reverse is true, and poor digestioon of carbohydrates limits protein synthesis.

Microbial growth in the rumen requires NH_3 derived from dietary crude protein, which may include non-protein

nitrogen, or from endogenous urea present in saliva or diffused across the rumen wall. Microbial protein synthesis is dependent on energy fermented in the rumen and is often expressed as a function of fermented organic matter (Satter, 1986). Therefore, the rate at which dietary protein is degraded in the rumen needs to be matched with the rate of carbohydrate breakdown in an attempt to obtain maximum efficiency in microbial yield.

Although much has been done to maximize the utilization of protein and energy in the rumen, most studies have considered energy as a "whole nutrient" and little importance was given to how the energy was released. Hence, little information is available on rates of carbohydrate (starch) degradation in the rumen and their effect on protein utilization and microbial synthesis.

Few studies have been reported in which a protein source has been matched to a energy source with similar rates of degradation. McCarthy et al. (1987) determined the effect of two proteins (soybean meal, SBM and fish meal, FM) and two carbohydrate (barley, B and ground shelled corn, C) sources, on ruminal fermentation and flow of nutrients to the small intestine of lactating cows. Cows were fed a 45:55 forage top concentrate diets (15% CP, 18 ADF) and treatments were: BSBM, BFM, CSBM and CFM. Diets BSBM and CFM were rapidly and slowly degradable, respectively. Rumen

NH_3-N (mg/dl) and VFA production (mM/l) were as follows: 2.83, 119; 1.39, 123; 3.54, 120; 2.86, 104 for the respective diets. Rumen starch digestibility was higher for barley than corn diets (77.8, 76.3, 46.9 and 51.6%, respectively). No difference was reported on total N flow to the duodenum; however, bacterial-N flow (% of non ammonia-N) was higher for barley than corn. Although barley diets stimulated a higher efficiency in nutrient utilization in the rumen, milk production was lower (32.6, 32.4, 35.2, 35.9 kg/d); but, efficiency in feed conversion to milk was higher for barley diets (1.56, 1.58, 1.45, 1.54 kg milk/kg DMI). There were only four cows per treatment in this study (4 cows in a 4x4 latin square design) so conclusions regarding milk yields should be tentative.

Casper and Schingoethe (1987) reported responses from early lactation dairy cows to diets varying in ruminal solubilities of carbohydrate and protein. In this study, 60 high producing cows were randomly allotted (ion a 3x2 factorial) to three carbohydrates which differed in solubility (corn, barley and 30% dried whole whey, DWW) with two sources of protein (soybean meal, SBM, and 1% urea, U). Total mixed diets (16% CP) contained a 50:50 forage to concentrate ratio. Milk production was higher for corn and lowest for DWW (32.8, 31.5, 31.3 kg/d) while 4% FCM was similar for cows fed corn and DWW, but lowest for barley

(30.0, 27.90, 29.5 kg/d) due to increased milk fat in cows fed DWW (3.37, 3.36, 3.51%). Percentages of milk protein were highest for cows fed corn (3.05, 3.00, 2.98%). Although cows fed corn had better performance, efficiency of conversion of feed to milk (kg milk/kg DMI) and protein utilization (kg milk/kg CPI) were higher for cows fed barley (1.47, 10.0; 1.48, 10.5; 1.43, 9.5, respectively, for cows fed corn, barley and DWW).

Information reviewed in this chapter helped us to better understand the importance of the protein-starch interactions in the rumen, and also gave us support to design the studies presented in this dissertation.

CHAPTER 3

IN VITRO PROTEIN AND STARCH SOLUBILITY AND DEGRADABILITY OF SEVERAL COMMON FEEDSTUFFS

Summary

The solubility and degradability of five cereal grains (corn, milo, wheat, barley and oats) and five protein supplements (soybean meal, SBM; cottonseed meal, CSM; brewers dried grain, BDG; corn gluten meal, CGM; and blood meal, BM) were determined.

Solubility of nitrogen fractions and starch was estimated using a bicarbonate-phosphate buffer (pH 6.8, ionic strengh .13). Total nitrogen was determined by an autoanalyzer; true protein by the Lowry procedure and total starch by the acid-anthrone method. Protein and starch degradabilities were estimated by the ficin and the maltose-glucosidase methods, respectively. Two incubation periods were used in both trials, two hours and overnight. Results from the solubility and degradability trials showed significant differences among grains and protein supplements.

Total nitrogen, true protein and starch solubility were higher ($P<.05$) for oats followed by wheat, barley, corn and milo. Protein degradability with overnight preincubation gave the same ranking for the grains, with results similar to literature values. For protein supplements, SBM had the

highest total nitrogen and true protein solubility and protein degradability followed by CSM, CGM, BDG and BM. Starch solubility was highest for BDG, followed by SBM, CSM and CGM.

Starch degradation as measured by maltose liberated from cereal grains during incubation with pancreatic amylase ranked the grains as follows: barley, wheat, corn, oats and milo. Starch degradation in protein supplements was rapid during the first 15 min, but patterns thereafter were different than for grains which might suggest hydrolysis of maltose to reducing sugars. The large differences in degradability of nitrogen fractions and starch in grains and protein supplements might enable better synchronization of fermentation of protein supplements and grains in diets for ruminants.

Introduction

The rate of rumen degradation of nutrients in a feed feed will depend upon the physical conformation of the nutrients, the chemical bonds among them, the enzymatic ability of rumen microorganisms to break such bonds, the type and amount of available nutrients in the medium and the retention time in the rumen.

The initial rate of degradation of nutrients, especially protein and carbohydrates, will have an important impact on the fermentation in the rumen. Ideally, there

should be a synchronization in the rate of degradation of the nutrients in order to stimulate an increase in the synthesis of microbial protein and volatile fatty acids. Although synchronization may be accidentally obtained in some instances, uncoupling seems to be common in many cases. The effect of uncoupling is variable and may result in depression of ADF digestibility (Stern et al., 1978), increase in ammonia-N loss (Huber and Kung, 1981), a decrease in microbial protein synthesis (Owens and Bergen, 1983), and a decrease in animal performance (Kung and Huber, 1983).

Even though theconsequencesofuncoupling may be significant, few attempts have studied methods to attain synchronization in fermentation of nutrients in the rumen.

In the present study, which is the first in a series a group of cereal grains and protein supplements were selected in order to classify them according to rate of protein and starch solubility and degradation. This information was used to measure the effects of matching protein and energy sources with similar rates of degradation on rumen fermentation and performance of ruminants.

Materials and Methods

Five cereal grains, corn, milo, wheat, barley and oats, and five protein supplements, soybean meal, cottonseed

meal, brewers dried grains, blood meal and corn gluten meal, were obtained from a commercial feed plant in Tucson, Arizona. Chemical composition of these feedstuffs as determined by standard procedures is presented in table 1. Total starch was determined by the acid-anthrone method (Clegg, 1956).

Solubility was determined using two methods. In the first one, described by Krishnamoorthy et al. (1980), samples were ground in a cyclone mill with a mesh of 1 mm. Triplicate 0.5 g samples were mixed with 50 ml of bircarbonate-phosphate buffer (pH 6.8, ionic strength .13 m Eq). Samples were incubated with constant agitation (130 cycles/min.) at 39.0 C for two hours and then filtered through VWR 54 filter paper. Nitrogen solubility was calculated by determining nitrogen in the residue (autoanalyzer) and subtracting from total nitrogen. True protein solubility and starch solubility were determined on the filtrate portion using the Lowry and the acid-anthrone methods, respectively.

In the second method, designed to observe the effect of a larger incubation on solubility of nitrogen and starch, samples were pre-incubated overnight in bicarbonate-phosphate buffer with constant agitation (130 cycles/min.) at 39.0 C. Total nitrogen, true protein, and starch solubilities were determined as described previously.

Protein degradability was estimated following the Ficin method described by Poos-Floyd et al. (1980).

In vitro starch degradability was determined using a method developed in our laboratory. Samples were ground in a cyclone mill to pass a 30 mesh screen. Five grams of sample were placed in 500 ml Erlenmeyer flasks and soaked overnight in 250 ml of bicarbonate-phosphate buffer (pH 6.8, 0.13 m Eq of ionic strength) at room temperature. The next morning, samples were mixed with 2.5 ml of triton X, and spun for one hour using a magnetic stirrer. Flasks were then placed in a water bath at 25.0 C and 10.0 ml of alpha-amylase (Sigma Co.) at a concentration of 2.5 micrograms/ml were added to each flask. Duplicate 1.0 ml aliquots were removed at the time of enzyme addition for the 0 time and every 15 minutes up to 60 minutes. Each aliquot was combined in an assay tube with 1.0 ml of coloring reagent containing 3-5, dinitrosalicilic acid, specific to bind maltose. The tubes were placed in boiling water for exactly five minutes, in ice-water for 2 minutes, and then mixed with 10.0 ml of dionized water. Tubes were then centrifugated at 1,230 x g for 10 minutes and the supernatant was transferred to another tube before being read in a spectrophotometer at 540 nm. Soluble pure starch (Sigma Co.) and cellulose (Solka flock) were used as positive and negative controls, respectively, and maltose

was the standard.

Solubility and degradability values were anlayzed by one-way analysis of variance and means compared using Duncan's multiple range test. In the degradability determinations the slopes from the regression lines were used to make statistical comparisons (Steele and Torrie, 1960).

Results and Discussion

Results from the solubility studies are in tables 2 and 3. Nitrogen solubility was significantly different ($P<.05$) among cereal grains and protein supplements. With regard to their N-solubility values, grains and protein supplements can be classified as follows: for grains, oats > wheat > barley > corn > milo.

for protein supplements: soybean meal > cottonseed meal > corn gluten meal > brewers dried grains > blood meal. The effect of overnight pre-incubation of samples resulted in higher solubility values ($P<.05$) for total nitrogen and true protein than those obtained with two hour solubilization. This may be due to the effect of the buffer in hydrolyzing the bonds between proteins and other nutrients. Results obtained without pre-incubation agree with those reported by Krishnamoorthy et al. (1982) and Sniffen et al. (1979) with a different buffer. Overnight soaking also increased true protein solubility. In this case, however, the values

obtained, even without pre-incubation, were higher than those reported in the literature. No clear explanation is apparent for this discrepancy, but it might be related to the type of grinding used in these grains (cyclone mill). Solubility of the starch was always low for grains with or without pre-incubation and there was no significant difference ($P>.05$) among grains and between methods. In the case of starch solubility of protein supplements, values obtained using the pre-incubation were always smaller ($P<.05$) than those with no pre-incubation. In any case, the solubility of the starch and reducing sugars was always higher than that observed for grains. This may reflect the processing these plant protein sources normally recieve.

Protein degradability, as determined with Ficin, was also obtained with or without pre-incubation. Again, values obtained with pre-incubation were higher ($P>.05$) than those without which may reflect a slight hydrolytic effect of the buffer. Protein degradabilities for pre-incubated grains and protein supplements were significantly different ($P<.05$) and the results were similar to those reported by Poos-Floyd et al. (1985). Values obtained without pre-incubation in our study were generally lower than those reported in the literature (NRC, 1985). The results for pre-incubated grains and protein supplements are shown in figures 1 and 2, respectively.

Starch degradability values are presented in figure 3 for grains and figure 4 for protein supplements. The method used, which is still being tested, showed good reproducibility. Rate of starch degradation in barley and wheat wheat was significantly higher ($P<.05$) than in milo oats, with corn intermediate. The low value in oats was unexpected, since it is known that starch in oats is highly degradable. This may be due to a rapid degradation of starch to glucose by alpha-amylose enzyme and the glucose produced was not recorded by the maltose-glucosidase method. These findings agree with those obtained in animal studies (Theurer, 1985; Spicer et al., 1986).

Most of the starch present in protein supplements was was degraded in the first 15 minutes after which a decline in liberated maltose was observed. No clear explanation can be offered for this observation, but it might be due to a decline in starch available for degradation in the medium, combined with hydrolysis of maltose to reducing sugars.

The results of this study demonstrated significant differences in the solubility and degradability of nitrogen fractions and starch in grains and protein supplements. These differences might enable the matching of grains and protein supplements of similar degradation rates to better synchronize their fermentation in the rumen. Animal effects

of such synchronization will be determined in future studies.

Table 1. Chemical composition of cereal grains used in the study (% of DM).

Item	DM	OM	CP	ADF	STARCH[1]
Cereal grains					
Corn	85.7	98.5	9.1	2.9	82.4
Milo	87.8	98.3	9.8	5.1	80.7
Wheat	92.2	98.3	15.8	4.8	81.1
Barley	92.0	97.0	11.0	9.2	80.2
Oats	91.3	96.7	12.8	20.5	52.1
Protein supplements					
SBM	90.7	93.0	49.7	5.7	27.2
CSM	94.5	92.4	46.2	21.5	12.7
BDG	92.0	98.5	23.1	22.7	22.2
CGM	91.5	98.6	59.2	2.5	21.2
BM	95.0	98.7	82.7	0.0	0.0

[1] As determined with the anthrone method (Clegg, 1956).

Table 2. Effect of preincubation on nitrogen, true protein and starch solubility and protein degradability of cereal grains.[1]

Item	CORN	MILO	WHEAT	BARLEY	OATS	SE
N solubility						
Preincubated	42.2	28.0	46.2	40.9	51.2	3.8
Non-incubated	20.1	11.4	31.2	29.5	40.0	4.9
True protein sol.[2]						
Preincubated	19.4	19.6	19.4	20.0	33.9	2.8
Non-incubated	13.1	10.6	10.1	10.1	18.0	1.5
Starch solubility						
Preincubated	3.7	2.2	3.9	5.3	5.3	0.6
Non-incubated	2.7	1.7	3.2	4.3	4.8	0.5
Protein degrada.						
Preincubated	55.5	45.3	69.3	64.9	73.6	5.1
Non-incubated	22.3	15.6	32.7	33.1	13.2	4.2

[1] Samples were preincubated overnight in buffer solution

[2] As determined by the Lowry procedure

Table 3. Effect of preincubation on nitrogen, true protein and starch solubility and protein degradability in protein supplements.[1]

Item	SBM	CSM	CGM	BDG	BM	SE
N solubility						
Preincubated	36.5	38.8	25.7	27.8	17.8	3.8
Non-incubated	20.3	11.0	7.4	10.4	0.8	3.1
True protein sol.[2]						
Preincubated	20.8	18.5	10.7	10.4	0.8	3.5
Non-incubated	10.6	8.4	5.7	3.2	0.0	1.9
Starch solubility						
Non-incubated	7.3	4.1	8.9	1.9	0.0	1.6
Protein degradability						
Preincubated	71.6	56.6	38.3	46.9	25.7	7.9
Non-incubated	32.8	18.4	13.9	13.5	13.1	3.7

[1] Samples were preincubated overnight in buffer solution

[2] As determined with the Lowry procedure

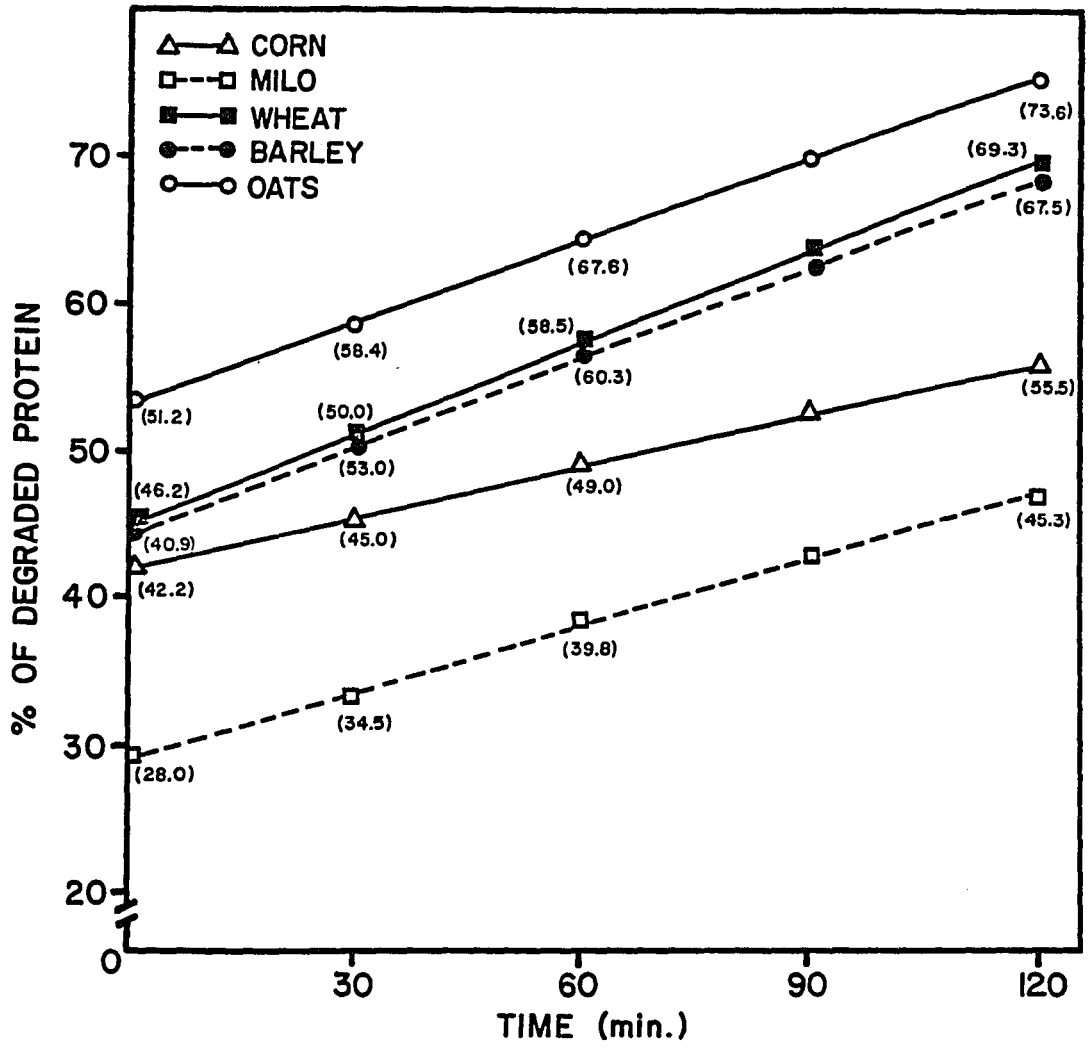

Figure 1. Protein degradability of preincubated cereal grains using Ficin.

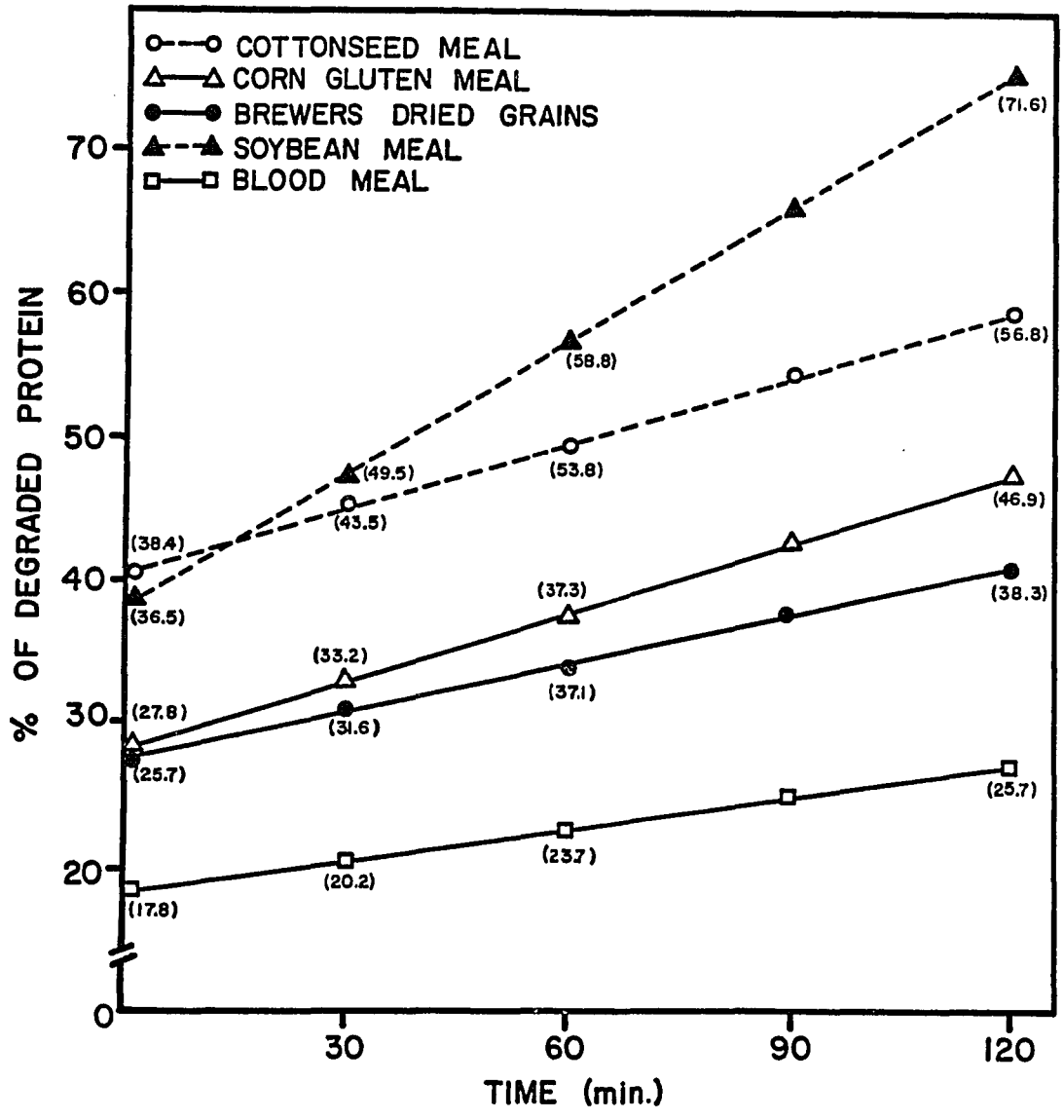

Figure 2. Protein degradability of preincubated protein supplements using Ficin.

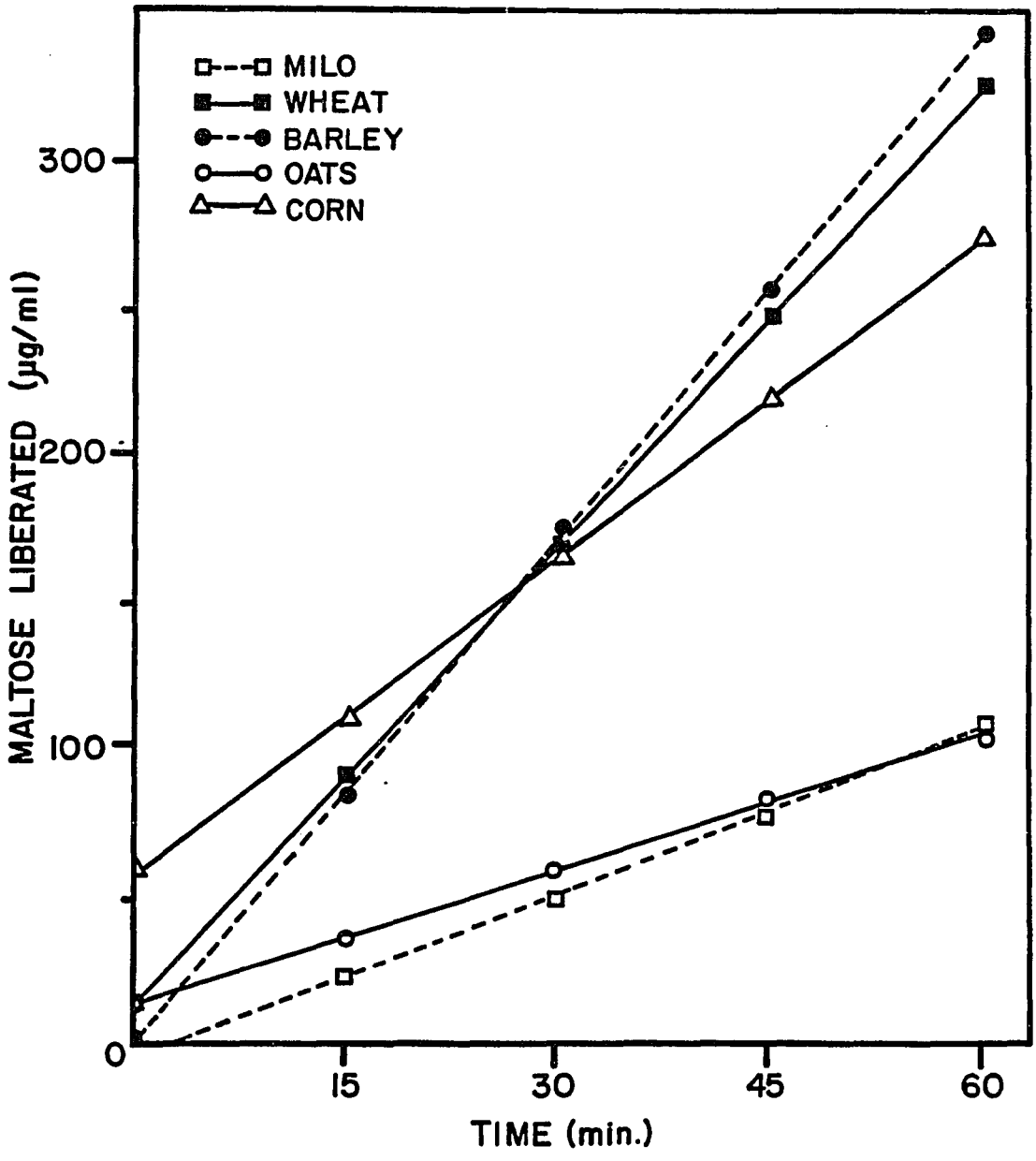

Figure 3. Starch degradability of cereal grains using alpha-amylase.

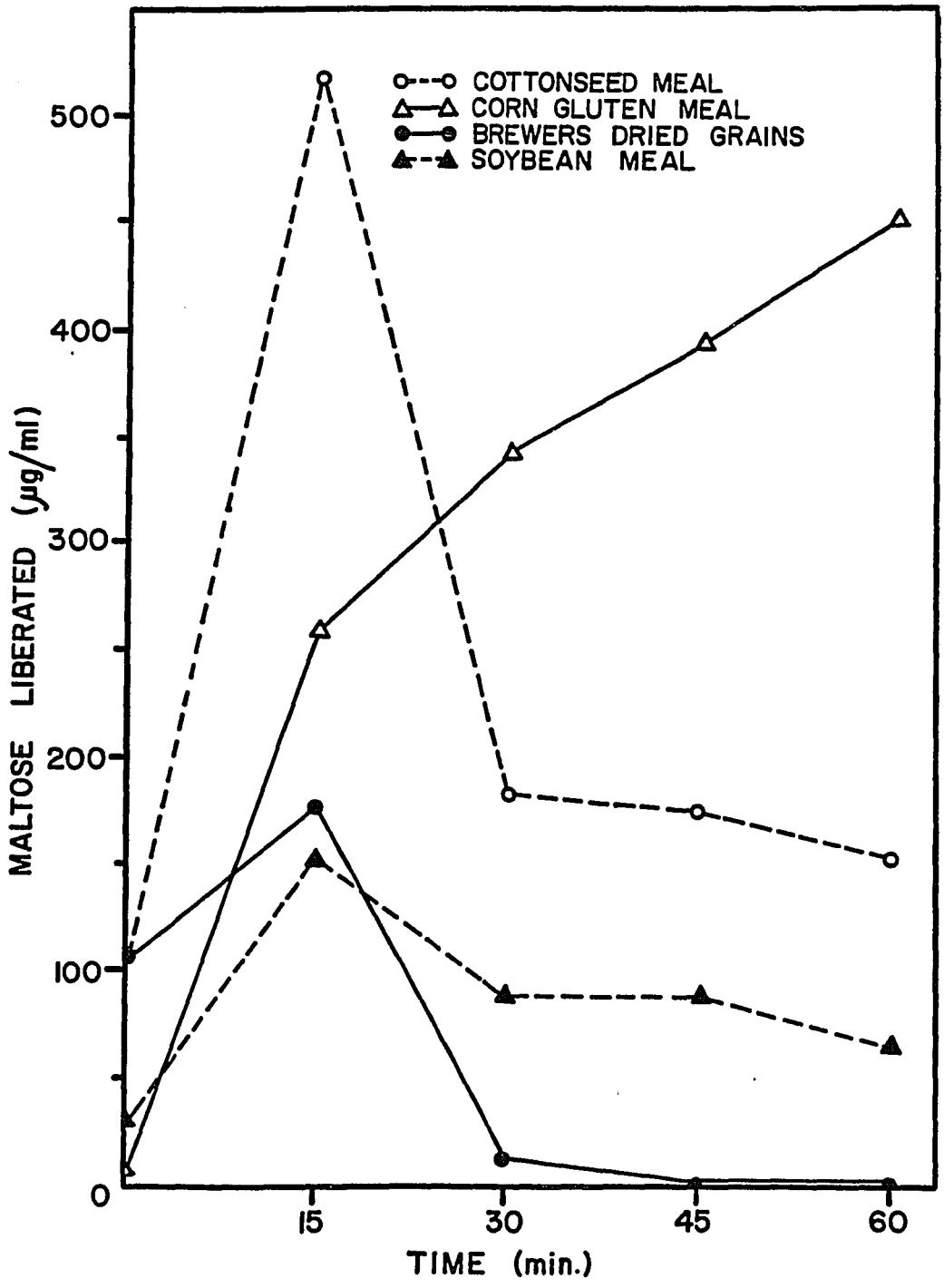

Figure 4. Starch degradability of protein supplements using alpha-amylase.

CHAPTER 4

IN VITRO AND IN SITU DRY MATTER, CRUDE PROTEIN AND STARCH DEGRADABILITY OF FIVE CEREAL GRAINS

Summary

Two trials were conducted to determine extent of degradability of starch and rate of degradation of dry matter (DM), crude protein (CP) and starch of five cereal grains.

In the first trial, rates of degradation of starch for corn, milo, wheat, barley and oats were determined using an in vitro method with a glucoamylase enzyme for 0, 15, 30, 45 and 60 min at 60 C. Differences ($P<.05$) between grains were found in the amount of starch degraded at all times in all grains. From 15 to 60 min, oats had the highest and milo the lowest ($P<.05$) extent of degradation with other grains intermediate. Degradation rates were calculated by transforming observed values to theri natural logarithms and regressing them upon time. Slopes of the lines were used as comparative indicators of the rate of degradation of starch. Wheat had the highest degradation rate followed by oats, barley, corn and milo (39.0, 25.0, 15.0, 11.0 and 5.0 %).

In trial 2, six rumen fistulated steers were used to determine in situ degradabilities and rates of degradation

of DM, CP and starch in the five mentioned grains. Grains were incubated for 2, 4, 6, 8, 12, 24 and 48 h in dacron bags (50 microns). Degradation rates were reported only for the period 2 to 12 h. Wheat had the highest ($P<.05$) degradation rate for DM followed by barley, corn, milo and oats (10.3, 9.7, 4.5, 3.0 and 1.8 % per h, respectively). The slow degradation rate for DM in oats was because practically all the DM was degraded prior to 2 h. Protein and starch degradability had a trend similar to that for DM, with the exception of oats. Based on the in situ degradation rates observed for protein and starch in grains, they might be ranked as follows: wheat > barley > oats > corn > milo.

Information obtained can be used to synchronize the fermentation of protein and starch sources with similar rates of degradation to improve animal performance.

Introduction

Cereal grains are the most common sources of readily available energy for livestock and they can comprise up to 60 % of the total diet for high yielding dairy cows. Starch in grains is first subjected to microbial fermentation in the rumen with consequent production of microbial cells and volatile fatty acids and then to enzymatic digestion in the small intestine producing glucose.

Several in vitro (Trei et al., 1970; Herrera-Saldana et al., 1986), in situ (Frigoid et al., 1972; Erdman et al., 1987) and in vivo studies (MacRae and Armstrong, 1969b; Orskov et al., 1971 Spicer et al., 1986) have determined starch digestibility of different grains. However, most of these studies compared less than three grains using the same technique which may account in part for the wide variation in starch digestibility reported in the literature. Moreover, the wide number of techniques used to determine starch concentrations (Clegg, 1956; MacRae and Armstrong, 1968; Fleming and Reichert, 1980; Kartchner and Theurer, 1981; Salomonsson et al., 1984) add variation to reported data. Little information is available on rate of starch degradation in the rumen of the five common cereal grains used in livestock: corn, milo, barley, wheat and oats. Such information might allow combination of grains with protein sources of similar rumen degradation rates to stimulate more efficient utilization of energy and protein in the diet.

The objective of this study was to determine the rate of starch degradation of five cereal grains using a rapid in vitro technique developed in our laboratory and compare results obtained with those of an in situ trial in which the rates of dry matter and protein degradation were determined.

Materials and Methods

In vitro trial. Samples (5.0 kg each) of commercial grains (yellow corn, milo, barley, wheat and oats) of unknown variety and origin were obtained from a local milling company (Eagle Milling, Co., Tucson, Az). Grains were ground through a Wiley mill with a 1.0 mm mesh and analyzed for dry matter, organic matter (AOAC, 1975); crude protein using a nitrogen autoanalyzer (Technicon, Terrytown, NY); neutral detergent fiber, acid detergent fiber and acid detergent insoluble nitrogen by the Goering and Van Soest procedure (1971); and starch by the enzymatic method of Salomonsson et al. (1984) as modified in our laboratory and described as follows: Duplicate samples (50.0 mg) of ground grain (1.0 mm mesh, Wiley mill) were weighed into 35.0 ml Pyrex tubes fitted with teflon-lined screw caps, and 25.0 ml of acetate buffer (0.1 M, pH 5.0) were added to each tube along with 100.0 ul of alpha-thermoamylase enzyme (Taka-Therm L-170. Miles, Inc., Elkhart, IN). Tubes were placed in a boiling water bath (90-95 C) for 30 min. Caps were tightened after a few minutes and tubes were agitated three times during incubation. Tubes were then removed, cooled to approximately 60 C and 100.0 ul of glucoamylase (Diazyme L-200. Miles,

Inc., Elkhart, IN) were added. Tubes were recapped and incubated overnight in a waterbath at 60 C. Tubes were then centrifuged (2000 g, 10 min) and a 1.0 ml aliquot from the supernatant was diluted (1:10) with distilled water. From this dilution a 200 ul aliquot was mixed with 2.0 ml of a glucose oxidase (Glucose-Trinder, Sigma, Co.,St. Louis, MO) and left exactly 20 min at room temperature (20 - 25 C). Absorbance from samples was then read in a spectrophotometer at 505 nm. The starch content of the samples was calculated using a regression equation from a calibration curve obtained from varying dilutions of a standard glucose solution incubated with glucose oxidase. A pure starch sample also tested starch recoveries.

Starch degradation of grains was determined as follows: One g of ground grain (1.0 mm mesh, Wiley mill) with a known starch content was incubated overight at room temperature in a flask containing 100 ml acetate buffer (0.1 M, pH 5.0). Samples were then shaken in a oscillatory shaker (120 oscillations/min, 1.0 hour) and 1.0 ml of a glucoamylase enzyme (Diazyme L-200, Miles, Inc., Elkhart, IN) was added to each flask. Duplicate 1.0 ml aliquots were immediately removed after the enzyme addition at 0, 15, 30, 45 and 60 min. Aliquots were diluted to 10.0 ml with distilled water, centrifuged (2000 g, 10 min) to remove solids and 1.0 ml of supernatant was diluted again

(1:10) with distilled water. A 1.0 ml aliquot was then mixed in a separate tube with 2.0 ml of a glucose oxidase (Glucose Trinder, Sigma Co., St. Louis, MO) and left to react for 20.0 min at room temperature (20 - 25 C). The final concentration of grain in samples was 50 ug/ml. Sample solutions were then read in a spectrophotometer at 505 nm and starch content was calculated as described previously.

For estimation of the rate of starch degradation in vitro, zero time values from all grains were omitted since these values were inflated by soluble sugars and non-starch polysaccharides in original samples. Hence, starch concentrations observed between 15 to 60 min were transformed to their natural logarithms (Mertens and Loften, 1980) and regressed upon time to calculate the slopes of the lines, which were considered comparative indicators of the rate of degradation of starch. Values obtained from this trial were statistically analyzed by an univariate analysis of variance (BMDP Statistical Software, W.J. Dixon, 1985) and slopes from the regression lines compared by Duncan's Multiple range test (Steele and Torrie, 1960). The precision of the described method for starch analysis was determined by statistical analysis according to Steele and Torrie (1960).

In situ trial. Six rumen fistulated steers (650 kg average body weight) were used as replicates in two periods

to determine in situ dry matter (DM), crude protein (CP) and starch degradability of five cereal grains. Steers were housed in individual stalls and fed, for 15 days prior and during the study, a 60 % concentrate diet (table 4) at 1.5% bodyweight in two equal portions at 700 and 1400 h. Animals had free access to water and trace mineral salt.

Bags for the in situ trial were constructed of dacron cloth with an approximate pore size of 50 microns, as specified by the vendor (EWR Industries, Inc., Kansas City, MO., "Gloriosa" 60050 polyester lining). A single piece of cloth (19X20 cm) was cut with a hot soldering iron to prevent fraying at the edges. The cloth was then folded in half and glued with silicon rubber along the edge (glue line approximately 1.0 cm wide) rounding the corners to avoid pocket formation. Bags were then sewn with polyester thread along the midline of the glue to reinforce seams.

Grains used in this trial were obtained from the same batch (5.0 kg) as in the vitro trial. Grains were ground in a Wiley mill (1.0 mm mesh) and approximately 6.0 g were weighed into a previously dried (85 C) and tared bag. Bags were closed approximately 7.0 cm from the top using a polyester cord, tied to a weighted chain, and placed into the ventral sac of fistulated steers approximately two hours after the morning feeding. All grains were incubated

simultaneously in all six steers using one bag per grain for removal time. Bags for each grain were removed after 2,

Table 4. Ingredient and chemical composition of the diet consumed by rumen fistulated steers used for the in situ trial.

Ingredient	(%)
Roughage	
Chopped alfalafa hay	20.0
Chopped wheat straw	20.0
Concentrate	
Steam processed and flaked milo	50.7
Molasses	5.0
Tallow	2.0
Urea	1.0
Dicalcium phosphate	0.7
Limestone	0.3
Salt	0.5
Analysis	
Crude protein	12.2
NDF	28.1
Calcium	0.7
Phosphorus	0.3

4, 6, 8, 12, 24 and 48 hours of incubation. Immediately after removal from the rumen, bags were washed with tap water until rinse was clear. Bags were dried for 72 h at 60 C in a forced-air oven, after which residues were removed and analyzed for DM, CP and starch as described above.

Data obtained from this trial was analyzed using a 2-way factorial analysis of variance with a factor within (incubation times) and a factor between (grains) (BMDP Statistical Software. W.J. Dixon, 1985). Values for disappearance of DM (ISDMD), CP (ISCPD) and starch (ISSTD) for each incubation time were subjected to logarithmic transformation as described by Mertens and Loften (1980). Logarithmic values were regressed upon time and the slope from the equation was used as an estimator of the rate of rumen degradation. Observed values from each time and grain were used to construct graphs to aid in interpretation of the observed results. These observed values were fitted to a model similar to that described by Erdman and Vandersall (1983); but in this case, the slowly degraded fraction (P-R) was divided in two (from 2 to 12 h and from 12 to 48 h) to better relate to degradation kinetics in the rumen. The proposed model is as follows:

$$\text{DM, CP or starch disappearance} = R + (P - R)ct1 \quad (T - P)ct2$$

where

R = soluble and rapidly degradable fraction ($0 < R < 100$)

P = total potentially degradable fraction ($0 < P < 100$) at 12 h.

(P-R) = Insoluble degradable fraction degraded at

rate c during the first 12 h

(T-P) = Insoluble degradable fraction degraded at rate c during the period 12 to 48 h

T = Total degradable fraction at 48 h.

t = time

Results and Discussion

In vitro trial. The chemical composition of grains is in table 5. The starch content of the grains, as determined with our method, varied between 58.1 and 75.7 %,

Table 5. Chemical composition of cereal grains used for in vitro and in situ trials (DM basis).

Item	CORN	MILO	WHEAT	BARLEY	OATS
Organic matter, %	98.50	98.30	98.30	97.00	97.70
Crude protein, %	9.70	9.80	15.80	11.00	12.80
NDF, %	9.30	15.57	11.30	19.47	24.03
ADF, %	3.25	5.30	4.22	7.81	16.49
ADIN, %	1.08	3.64	1.13	0.55	0.32
Starch, %	75.72	71.34	70.25	64.34	58.11

from 23 replicated samples of each grain were used to determine the precision of the method (table 6). The standard deviation and the coefficient of variation tended to increase as the amount of fiber in a grain increased. Hence, oats, containing 16.5 % ADF, had the highest variation and corn, with 3.2 % ADF, had the lowest. Kartchner and Theurer (1981) also found greater differences in starch estimation, either by acid or enzyme hydrolysis, as amount of cellulosic material increased. This variation may be explained as contamination of fiber in the small sample of grain used for starch analysis. Mean and range values for starch , as determined with our method, agree quite well with results reported elsewhere (Waldo, 1973; Aman and Hesselman, 1984). Our method for total starch analysis was reproducible for grains, feeds and feces analyzed in this and other studies in our laboratory.

Results from the in vitro starch degradation trial are in table 7. Values obtained at zero time, normally considered as starch, might also contain other soluble and readily degradable carbohydrates as mentioned in other studies (Aman and Hesselman, 1984; Van Soest, 1986). Aman and Hesselman (1984) found that the amount of soluble sugars (glucose, fructose, sucrose and fructans) and non-starch polysaccharides (arabinose, xylose, mannose, with corn highest and oats lowest ($P<.01$). Data obtained

Table 6. Statistical parameters to determine precision of the described method for total starch analysis[1].

Item	CORN	MILO	WHEAT	BARLEY	OATS
# of replications	23	23	23	23	23
Mean value (% of DM)	75.72	71.34	70.25	64.34	58.11
Range of values	72-78	68-78	67-77	60-74	52-69
Stand. Deviation	1.84	2.67	2.86	3.33	4.27
Coefficient of variation (%)	2.44	3.74	4.07	5.18	7.09

[1] Starch recovery range 94.5 to 99.9 %, in pure starch refence samples.

Table 7. Starch degradability and rate of degradation of five cereal grains during in vitro incubations[1].

Time (min)[2]	CORN	MILO	WHEAT	BARLEY	OATS	SE
			(% of total starch)			
0	7.72[b]	2.94[d]	1.72[e]	10.60[a]	3.89[c]	.08
15	8.47[c]	7.04[d]	6.82[d]	11.36[b]	16.50[a]	.05
30	9.44[d]	7.84[e]	12.73[c]	13.77[b]	19.86[a]	.06
45	11.49[d]	8.52[e]	19.34[b]	15.54[c]	23.09[a]	.07
60	13.10[d]	9.43[e]	24.21[b]	18.07[c]	27.99[a]	.05
Degradation rate from 15-60 min, (%)	11.0[c]	5.0[d]	39.0[a]	15.0[c]	25.0[b]	.58

[1]Initial starch concentration, (ug/ml), corn 37.75; milo 35.63; wheat 35.02; barley 32.08 and oats 28.98.
[2]Average of four samples for each grain at each incubation time.
[a,b,c]Values with different superscripts are different (P<.01).

galactose, glucose and uronic acids) were 1.8, 12.6; .09, 16; and 3.6, 7.7 % of the DM for barley, oats and wheat, respectively. However, not all these carbohydrates are soluble, Salomonsson et al. (1984) found that only 1.5 to 2.3 % of the DM in the same grains was water soluble. In a previous study (Herrera-Saldana et al., 1986), "starch" solubility in a bicarbonate-phosphate buffer was 3.7, 2.2, 5.3 and 5.3 % of the total starch in corn, milo, barley and oats, respectively. Although these values are lower than those in this trial, they follow a similar trend.

Significant differences ($P<.01$) were found in amount of starch degraded at all times in all grains. It was interesting to observe that oats had the highest values of starch degradability from 15 to 60 min. This undoubtedly reflects the physico-chemical characteristics of the starch and protein in oats. Delfino (1986) described the different structural components of grains and pointed out the importance in the grain type of starch (corneous vs. floury), type of protein, and the relationship between starch and protein matrix on starch degradability. Another factor might be the relationship between fiber and starch, which in the cases of oats, barley and milo may be of significance (Doyle, 1978). For wheat, degradation of starch was slow at first and after 15 min increased to almost 100 % . This may be due to the presence of thick,

rigid aleurone cells, (Delfino, 1986), that surround the endosperm and prevent the immediate access of starch degrading enzymes. Starch in corn and milo was degraded at an expectedly slow rate. Rooney and Pflugfelder (1986) explained the low digestibility of milo starch was due to the type of protein matrix associated with starch granules, the type and proportion of protein bodies found in the endosperm and the proportion of corneous endosperm. A similar explanation may also relate to corn.

Degradability rates observed for each grain were significantly different ($P<.01$) and allowed an easy ranking for the grains tested. The method proved to be rapid and simple for estimating starch degradability in grains and information obtained might be used as an index to match ingredients with similar rates of degradation in order to improve efficiency in protein and energy utilization in the rumen.

In situ trial. Values for ISDMD, ISCPD and ISSTD for the five grains are in tables 8, 9, and 10. Significant differences ($P<.01$) on DM disappearance were observed among grains. Wheat had highest values and milo lowest at all times. These differences were expected and explained previously. Oats also contained a highly degradable fraction which disappeared before 2 h, but thereafter DM was degraded more slowly, probably reflecting degradation of fiber in the

Table 8. In situ degradation of dry matter in five cereal grains.

Time (h)	CORN	MILO	WHEAT	BARLEY	OATS	SE
			% of total DM			
2	26.9 d	15.9 e	63.2 a	43.8 c	55.7 b	0.96
4	32.0 d	29.0 e	68.1 a	51.1 c	57.3 b	1.15
6	37.0 c	24.2 d	73.1 a	58.4 b	58.8 b	1.08
8	42.1 d	28.3 e	78.0 a	65.7 b	60.4 c	1.17
12	52.1 d	36.6 e	87.9 a	80.3 b	63.5 c	1.34
24	62.7 c	46.6 d	90.2 a	82.2 b	65.3 c	1.75
48	85.1 b	72.9 c	91.9 a	84.3 b	66.9 d	1.08

a,b,c,d,e
Values withdifferentsuperscript are different (P<.01).

Table 9. In situ crude protein degradability of five cereal grains.

TIME (h)	CORN	MILO	WHEAT	BARLEY	OATS	SE
			% of total crude protein			
2	39.2^{c}	15.9^{d}	51.4^{b}	50.4^{b}	77.9^{a}	0.79
4	42.8^{c}	19.6^{d}	60.7^{b}	58.1^{b}	80.2^{a}	1.04
6	46.4^{d}	23.3^{e}	70.0^{b}	65.8^{c}	82.5^{a}	1.31
8	49.9^{d}	27.1^{e}	79.3^{b}	73.6^{c}	84.9^{a}	0.89
12	57.1^{c}	34.5^{d}	97.9^{a}	89.1^{b}	89.6^{b}	0.96
24	66.3^{c}	45.6^{d}	98.2^{a}	90.0^{b}	92.5^{b}	1.11
48	84.0^{b}	71.7^{c}	99.1^{a}	94.9^{a}	95.2^{a}	1.38

^{a,b,c,d,e} Values with different superscripts are different ($P<.01$).

Table 10. In situ starch degradability of five cereal grains

TIME	CORN	MILO	WHEAT	BARLEY	OATS	SE
			% of total starch			
	c	c	b	b	a	
2	8.8	3.4	33.3	35.5	79.6	2.24
	c	c	b	b	a	
4	11.6	6.9	42.5	44.3	81.9	1.90
	c	c	b	b	a	
6	14.4	10.5	51.8	53.1	84.2	1.73
	c	c	b	b	a	
8	17.3	13.4	61.0	61.9	86.4	1.83
	c	c	b	b	a	
12	23.0	21.0	79.5	79.4	91.0	1.94
	c	c	b	b	a	
24	33.6	28.2	83.6	82.0	91.5	1.90
	c	d	a	b	a	
48	67.6	45.5	91.8	85.8	92.7	2.62

a,b,c,d,e
Values with diff. superscripts are different (P<.01).

grain (16.5 ADF). Varga (1986) reported similar results but observed a lag phase of almost 4 h, considerably longer than in our study. Dry matter in corn and barley was degraded at rates intermediate between wheat and milo. Barley had higher ($P<.01$) DM degradability up to 12 h, however, by 48 h both grains were degraded to a similar extent, which indicates that most of the degradable fraction in barley disappeared

Protein and starch degradability had a trend similar to that of DM. However, oats was an exception, since almost 80 % of the total protein and starch disappeared during the first 2 h of incubation. Possible explanations are: 1) Globulins, a highly degradable protein, constitute approximately 80 % of total protein in oats (Sniffen, 1980); 2) The proportion of soluble sugars and readily available carbohydrates is one of the highest among cereal grains (Aman and Hesselman, 1984); 3) The mixed (simple and compound) type of starch granules; and 4) The floury type of starch in oats (Betchel and Pomeraz, 1981). All these factors have been related to high degradability values for protein and starch. Erdman et al. (1987) found results similar to those reported here, when they compared in situ degradation of oats, barley, corn and several protein sources.

Figures 1 to 3, for DM, CP and starch degradability were constructed using observed values. Graphs clearly show

that more than one fraction of protein and starch are present in these grains. These fractions might be characterized as follows: 1) Soluble and/or rapidly degradable fraction (R); 2) Insoluble degradable fraction, which in this study was divided into (P-R) and (T-P); and 3) Rumen undegradable fraction (fraction remaining after 48 h).

It is interesting to observe the parallelism that exists between CP and starch curves for the five grains. In all cases, with the exception of oats, protein was degraded faster than starch which may indicate, as has been suggested by other studies (Hale, 1973; Lichtenwalner et al., 1978; Rooney et al., 1983) that the protein matrix and protein bodies must first be degraded to allow starch degradation.

Degradation rates of DM, CP and starch were first calculated using the natural logarithm (ln) obtained from the observed potential degradable residues remaining at the end of each fermentation time. However, when rates were obtained using all the fermentation times, corn and milo had rates that were unrealistic, being not different from those in wheat and barley. This confounding effect, due to the low initial degradability and the extent of digestion of corn and milo, was avoided by regressing only the values from 2 to 12 h. At 12 h, an

inflection point was observed in the curves of DM, CP and starch of wheat, barley and oats, which may indicate the exhaustion of insoluble degradable (P-R) protein and starch, with plant structural compound as the remaining substrate. As expected after 12 h, degradation rates for wheat, barley and oats were slower than for corn and milo reflecting the rapid early disappearance of the former compared to the latter group of grains.

The insoluble fraction degraded by 48 h was then divided in two fractions; one corresponding to the 2 to 12 h period (P-R), and the second to the 12 to 48 h period (T-P). The (T-P) value was obtained by subtracting the value observed at 12 h, P, from the value observed at 48 h, T. Fractions R, P-R and T-P and the degradation rate (C) calculated at 12 h were statistically different ($P<.01$) among all grains (Tables 11, 12 and 13).

The amount of CP and starch degraded at 12 h in wheat, barley and oats ranged from 79 to 98 % of the total suggesting that most of the protein and starch were available for microbial utilization in the rumen. At 12 h, less than 60 % of CP and starch in corn and milo were degraded but by 48 h degradability of these components increased to 72 and 84 % and 45 and 68 %, respectively. This suggests that a fraction of CP and starch from these grains

Figure 5. in situ DM DISAPPEARANCE OF 5 GRAINS

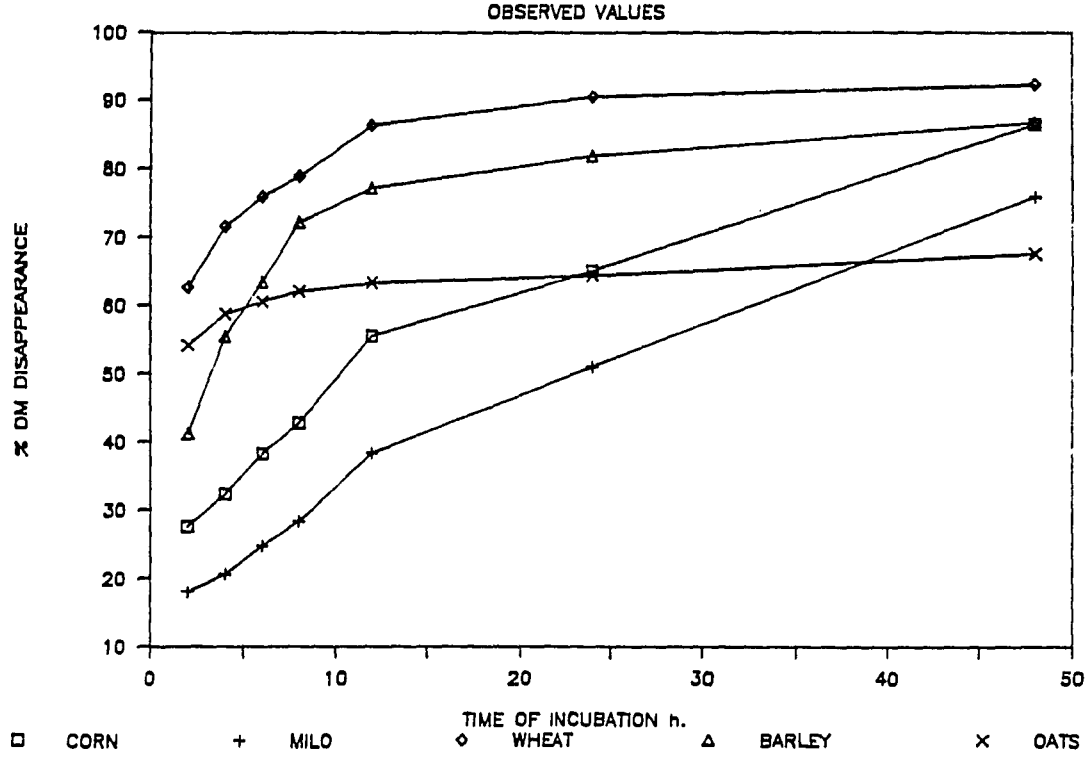

Figure 6. in situ CP DISAPPEARANCE OF 5 GRAINS

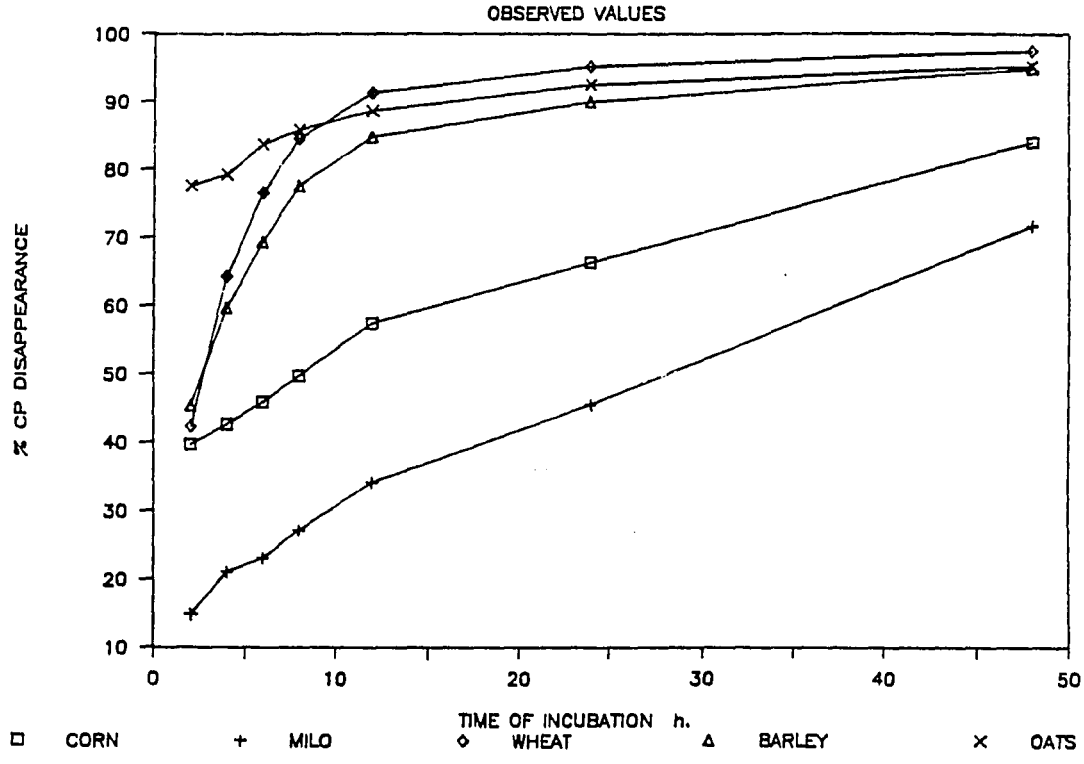

Figure 7. in situ STARCH DISAPPEARANCE OF 5 GRAINS. OBSERVED VALUES

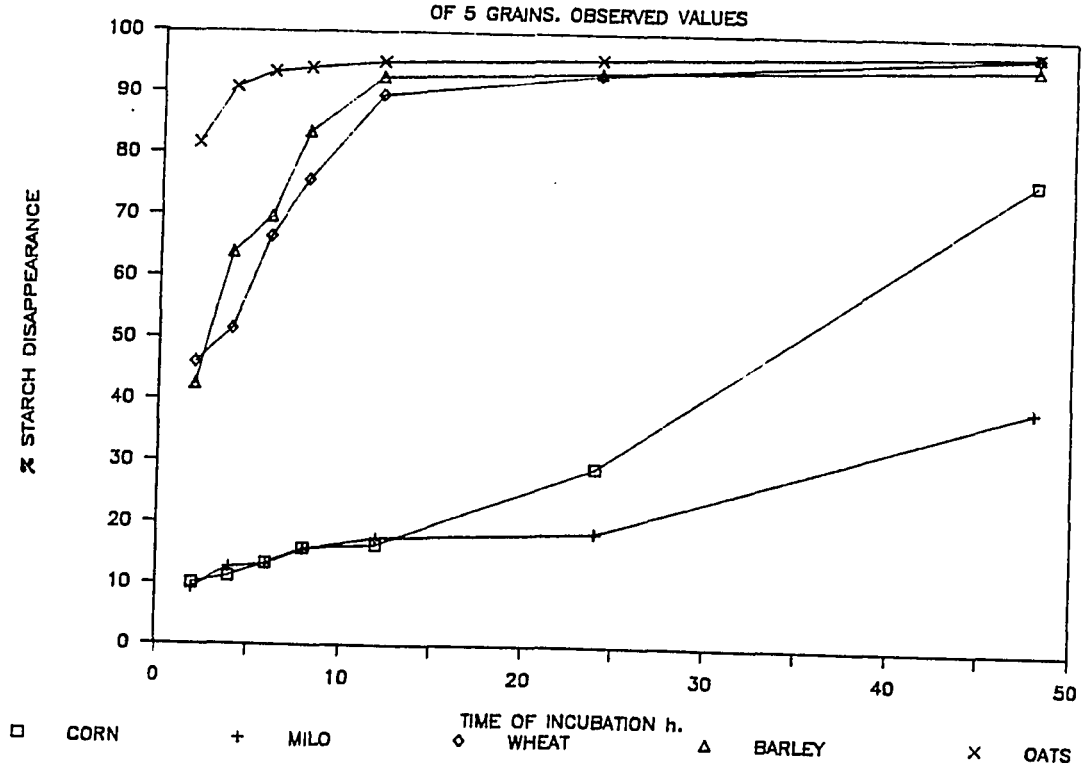

Table 11. Soluble and degradable fractions and degradation rate of dry matter in five ceral grains incubated in situ.

Item	CORN	MILO	WHEAT	BARLEY	OATS	SE
			% of Dry matter			
Degraded at 12 h, P	d 52.1	c 36.6	a 87.9	b 80.3	c 63.5	1.34
Sol. and readily degradable, R	d 26.9	e 15.9	a 63.2	c 43.8	b 55.7	0.96
Insoluble and degradable, P-R	b 25.2	b 20.7	b 24.7	a 36.5	c 7.8	1.32
Degradation rate, C, % /h	b 4.5	b 3.0	a 10.3	a 9.7	c 1.8	0.79
Degraded from 12-48 h, T-P	a 33.0	a 36.3	b 4.1	b 4.0	b 3.4	1.23
Total DM degraded at 48 h, T	b 85.1	c 72.9	a 91.9	b 84.3	d 66.9	1.08

a,b,c,d,e
Values with different superscript are different (P<.05)

Table 12. Soluble and degradable fractions and degradation rate of crude protein in five cereal grains incubated in situ.

Item	CORN	MILO	WHEAT	BARLEY	OATS	SE
			% of Crude protein			
Degraded at 12 h, P	c 57.1	d 34.5	a 97.9	b 89.1	b 89.6	0.96
Sol. and readily degradable, R	c 39.2	d 15.9	b 51.4	b 50.4	a 77.9	0.79
Insoluble and degradable, P-R	c 17.8	c 18.6	a 45.5	b 39.5	d 11.7	1.26
Degradation rate C, % / h	d 3.6	d 2.5	a 20.2	b 13.3	c 7.6	0.87
Degraded from 12-48 h, T-P	b 26.9	a 37.1	d 1.2	c 5.8	c 5.6	1.16
Total CP degraded at 48 h, T	b 84.0	c 71.7	a 99.2	a 94.9	a 95.2	1.38

a,b,c,d
Values with different superscripts are different (P<.05)

Table 13. Soluble and degradable fractions and degradation rate of starch in five cereal grains incubated in situ.

Item	CORN	MILO	WHEAT	BARLEY	OATS	SE
			% of Starch			
Degraded at 12 h, P	23.0 c	21.0 c	79.5 b	79.4 b	91.0 a	1.94
Sol. and readily degradable, R	8.8 c	3.4 c	33.3 b	35.5 b	79.6 a	2.24
Insoluble and degradable, P-R	14.21 b	17.6 b	46.2 a	43.8 a	11.4 b	2.09
Degradation rate C, % / h	1.8 c	2.0 c	11.8 a	11.7 a	7.8 b	0.89
Degraded 12-48 h, T-P	44.6 a	24.5 b	12.3 c	6.4 d	1.7 d	1.37
Total degraded at 48 h, T	67.6 b	45.5 c	91.8 a	85.8 a	92.7 a	2.62

a,b,c,d,e
Values with different superscript are different (P<.05).

may escape degradation in the rumen and be available for intestinal digestion.

Oats had the smallest (P-R)1 for CP and starch and milo had the highest, with corn, barley and wheat intermediate. In one of the few reported studies which included oats, Erdman et al., 1987 agreed with these results.

Values for total DM degradability at 48 h in all grains agree well with values obtained with in vivo studies (Parrot et al., 1969; Waldo, 1973; Galyean et al., 1979; Orskov, 1986). However, CP degradabilities for all grains were overestimated compared to in vivo studies (Satter and Whitlow, 1977; Spicer et al., 1986), and starch degradabilities were slightly underestimated (Orskov et al., 1969; Kartchner, 1972; Galyean, 1979; Spicer et al., 1986; Theurer, 1986).

Degradabilities obtained in this study are not absolute values and would have been different if factors such as sample size and grind (Galyean et al., 1981; Weakley et al., 1983), pore size of the bag material (Orskov, 1982; Nocek, 1985) and diet (Loerch et al., 1983) had been different.

Based on the CP and starch degradability rates (C) obtained for the P-R fraction (from 2 to 12 h) in the grains studied, they might be ranked as follows: Wheat >

barley > oats > corn > milo. However, if ranked according to their R value for CP and starch, oats would be highest, followed by wheat, barley, corn and milo in that order.

The information obtained in both trials help to better understand the kinetics of protein and starch degradability in the rumen of the five grains. Knowledge concerning rates of CP and starch degradation will allow combination of grains with protein sources of similar rates of degradation in order to control efficiency of protein and energy utilization in the rumen. Such an approach might result in improved animal performance.

CHAPTER 5

INFLUENCE OF SYNCHRONIZATION OF PROTEIN AND STARCH DEGRADABILITY ON PERFORMANCE OF HIGH YIELDING DAIRY COWS

Summary

We demonstrated that starch degradability in barley (HS) was higher than in milo (LS), and that crude protein in cottonseed meal (HP) higher than in brewer dried grains (LP). Thus, a milk production trial with 32 cows (8 cows/treatment) was conducted for 75 days in a randomized block design with a 2 x 2 factorial arrangement (2 protein and 2 starch sources). Four diets were compared, HSHP, HSLP, LSHP and LSLP in cows fed 65 % concentrate and 35 %forage. Apparent digestibility of diets was estimated by using chromium bread. Results obtained indicated increased ($P<.05$) organic matter digestibility in diets containing CSM. Starch digestion coefficients were higher ($P<.05$) in diets containing barley. Although HSHP diet had the highest crude protein digestibility, high variability prevented significance. Rumen fluid and blood samples were collected at about 21, 42 and 63 days of treatment, at approximately 0, 2, 4 and 6 hours after the morning feeding. Rumen ammonia-N, acetic acid and total volatile fatty acids were higher ($P<.05$) in diets containing CSM, indicating the influence of this protein source in the increased digestion

of organic matter in the rumen. Blood urea nitrogen, however, was higher for diets containing barley grain. Milk production was highest for the HSHP diet with mean adjusted values of (kg/day), 37.4, 34.9, 34.6 and 34.2, for the respective treatments. Milk fat was lower ($P<.05$) for barley diets thus, lowering the relative advantage of the HSHP diet in production of 3.5 % FCM. From results obtained in this study, the following was concluded: 1) The response from varying protein degradabilities can be altered by rate of starch degradation in the rumen; 2) Protein of higher rumen degradability does not always adversely affect milk production provided there is a synchronous release of energy; 3) Protein and starch that escape rumen degradation may compensate for their low rumen digestibilities by rendering more dietary nutrients available to the small intestine.

Introduction

Milk production and composition are influenced by the fermentation and absorption of feeds in the rumen and small intestine. Fermentation in the rumen depends, among other factors, upon the intrinsic physico-chemical characteristics of nutrients in feeds, the ability of rumen microbes to degrade these nutrients and retention times in the rumen. Feeds which are easily degraded tend to ferment rapidly rendering available nutrients for bacterial

use. This condition stimulates microbial cell synthesis, but also reduce the amount of nutrients reaching the small intestine, which might be a disadvantage for high producing dairy cows. It is now a common practice to feed low degradable proteins to increase supply of amino acids to the intestine for improved milk production. Flow of amino acids is determined by ruminal degradation of the dietary protein and the synthesis of microbial protein (Chalupa, 1982). The protein contribution from rumen microbes has been estimated to supply, under normal physiological conditions, from 60 to 70 % of the protein required by high producing dairy cows (Kaufmann and Lupping, 1982). However, these percentages are not constant and depend on from an optimum balance between microbial requirements for growth and substrate availability. Russell and Hespell (1981) observed that optimum synthesis is dictated, in part, by the utilization of degraded protein and carbohydrates from ingredients used in diets. Protein degradation in the rumen, in some cases, exceeds carbohydrate availability and protein (NH3) wastage ocurrs. In other cases, protein degradation is too slow to support optimum digestion of carbohydrates in the rumen. Both situations depress microbial protein synthesis. It is, therefore, important to provide sources of energy and protein with similar rates of degradation that will allow

microbes to obtain, simultaneously, ATP and NH3 and/or amino acids for microbial cell synthesis. This condition should ultimately result in a better utilization of nutrients in the rumen and an increase of microbial protein supply to the small intestine.

In a previous study (R. Herrera-Saldana et al., 1986) we found large differences in the in vitro rates of protein and starch solubility and degradability from a group of protein sources and several cereal grains. Protein degradability was higher in cottonseed meal and barley than in brewers dried grains and milo; moreover, starch degradability of barley was higher than milo. Hence, a study was planned to test the hypothesis that the synchronization of protein and starch degradation in the rumen will increase microbial mass, nutrient utilization and milk production in high yielding dairy cows.

Materials and Methods

The study was for 75 days using a randomized block design in a 2x2 factorial arrangement. Cottonseed meal (CSM), a common protein supplement in the southwest US, and brewers dried grains (BDG) were the two protein supplements; while dry rolled barley (B) and dry rolled milo (M) were the two grains and served as the main sources of starch. Protein degradability, as determined by the

ficin procedure (Poos et al., 1985), was higher for CSM and B (56.6 and 65.0) than for BDG and M (38.8 and 45.0 %). Starch degradability was measured with the maltose-glucosidase procedure developed in our laboratory (R. Herrera-Saldana et al, 1986) and was higher in B than in M, with values of 90.5 and 70.5 %, respectively. Four complete diets (table 14) were formulated with a ratio of 65:35 concentrate to forage. The forage portion was alfalfa hay and cottonseed hulls. Barley was mixed with CSM for a diet synchronized for rapid fermentation (HSHP) and M was mixed with BDG for a slow fermentation diet (LSLP). Two "uncoupled" diets were also formulated mixing B with BDG (HSLP) and M with CSM (LSHP). All diets were formulated to contain 17 % CP and 1.5 Mcal NEL/kg of dry matter.

Twenty-eight multiparous (2 to 4 lactations) and four primiparous cows were placed for two weeks on a pre-treatment diet consisting of the normal herd ration (corn, barley, alfalfa hay, whole cottonseed) at two to four weeks postpartum. Cows were then allotted to one of the treatment groups on the basis of the 10 d for pretreatment milk production. Groups were placed at random in open, shaded pens of 12 cows each, equipped with calan gates (American Calan, Co. Northwood, NH), for measuring individual feed intake. Even though most animals had eaten

Table 14. Ingredient composition of diets used in the study.

Ingredients	HSHP	HSLP	LSHP	LSLP
	----------(% of DM)----------			
Barley	43.5	33.0	....	....
Milo	....	....	41.8	30.1
CSM	12.7	....	14.6	....
BDG	....	23.6	....	25.5
Molasses	6.8	6.4	6.7	6.6
Fat	1.4	1.2	1.3	1.2
Min. & Vit.[1]	1.6	1.6	1.6	1.6
Alfalfa hay	21.3	22.2	21.7	22.8
CS hulls	12.7	12.0	12.3	12.2

[1] Ca_3CO .33; Biophos .74; Trace Min. salt .33; Vit A & D .20.

from the calan gates during previous lactations, they were allowed a 7-day adjustment period to gates and diets. Concentrate was divided into two equal portions and fed twice daily at 500 and 1500 h, hay (long form) was offered once a daily at 1500 h. Feed refusals were recorded before each afternoon feeding.

Cows were milked twice daily at 500 and 1700 h, and milk production recorded for each milking. A daily composite milk sample was obtained weekly from each cow and analyzed by the Arizona DHIA (Phoenix, AZ) for butterfat, protein, lactose, total solids and somatic cells.

At about 21, 42 and 63 days of treatment, samples of blood (coccygeal vein) and rumen fluid (via stomach tube) were taken from all cows at approximately 0, 2, 4 and 6 hours after morning feeding. Blood was allowed to stand at room temperature for two hours and serum was separated by centrifugation for 20 min. at 2000 g and was analyzed for urea-N. Rumen fluid samples were strained through 4 layers of cheesecloth and frozen immediately after pH determination. Rumen samples were then transfered to the laboratory, thawed and centrifuged for 15 min at 3000 g. Fifteen ml of the supernatant were saved and stored at -4.0 C until analyzed for NH3-N (10 ml) using the hipochlorite-phenol procedure (Beecher and Whitton, 1970), and for volatile fatty acids (5 ml acidified with 2 ml of

25% metaphosphoric acid) by gas liquid chromatography with a 80/120 carbopack B-DA/4% carbowax 20M column.

Digestion coefficients were determined by using chromium oxide (Cr_2O_3) as feed marker. Chromium oxide was offered as top dressing to the diet in the form of chromium bread (4:1, whole wheat flour: Cr_2O_3) at 4.4 g/kg of concentrate during the last 10 days of the study (6 days adjustment, 4 days collection). During the collection period, 3 grab samples of feces were obtained daily (at 500, 1300 and 1700 h) from each cow and frozen immediately. Fecal and feed samples (collected bi-weekly) were dried at 55 C in a forced-air oven for 72 h, ground in a Wiley mill (2 mm mesh) and analyzed for dry matter (DM) and organic matter (OM) using standard procedures (AOAC, 1970); crude protein (CP) using a nitrogen autoanalyzer (Technicon, Terrytown, NY); neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent insoluble nitrogen (ADIN) by the Goering and Van Soest (1971) procedure. Starch was determined by a method described by Herrera-Saldana and Huber (unpublished data). This procedure is a modification of that of Solomonsson et al. (1984) and was described in Chapter 4. Results of the study were analyzed statistically by analysis of variance using a block, factorial design.

Results and Discussion

Chemical composition of treatment diets is shown in table 15. Crude protein was similar for all diets, but was 1.0% higher than originally estimated. However, all diets contained sufficient protein to meet NRC (1978) requirements. Diets containing BDG had higher NDF and ADF and lower starch and rumen degradable protein (RDP). These differences were expected since BDG contains more fiber than CSM and was included in concentrate at almost double the amount of CSM. The M diets contained less rumen degradable starch (RDS) than those with B (74.7, 69.9 vs. 62.3, 48.3 %, for diets HSHP, HSLP, LSHP, LSLP, respectively).This difference was expected considering previous results obtained in our laboratory and elsewhere (Kay et al, 1972; Spicer et al., 1986).

Total DM and CP intakes were not different ($P>.05$) among diets (table 16), but intakes of total starch, RDP and RDS were different ($P<.05$). Animals eating diets with CSM consumed around 35 % more RDP than those on BDG. Consumption of total starch and RDS was highest for HSHP (8.3, 6.2 kg/d) and lowest for LSLP (5.8, 2.8 kg/d), with the other diets intermediate.

Digestion coefficients for OM, and starch were significantly different between diets ($P<.05$), as shown in table 17. Diets containing CSM had higher ($P<.05$) OM

Table 15. Chemical composition of the diets used in the study.

Item	Diets[1]			
	HSHP	HSLP	LSHP	LSLP
	(% of DM)			
Organic matter	93.2	93.9	93.2	94.5
Crude protein	17.3	17.6	17.2	17.6
Starch	32.8	30.1	30.1	24.4
Neutral detergent fiber	33.5	37.4	33.2	35.3
Acid detergent fiber	17.5	18.0	18.6	18.7
RDP, % of total N[1]	59.5	43.7	55.9	35.4
RDS, % of total starch[2]	74.7	69.9	62.3	48.3

[1]Values obtained using the Ficin method.

[2]Values obtained using the Maltose-glucosidase method.

Table 16. Influence of protein and starch degradability on dry matter and nutrient intakes in lactating cows (kg)

Item	Diets				SE
	HSHP	HSLP	LSHP	LSLP	
Total DM	25.3	23.7	24.3	23.8	2.50
Nutrients					
Protein	4.3 a	4.1 b	4.1 b	4.1 c	.11
Starch	8.3 a	7.1 b	7.4 a	5.8 b	.16
Rumen deg. protein[1]	2.7 a	1.8 b	2.5 b	1.5 c	.05
Rumen deg. starch[2]	6.2	4.9	4.6	2.8	.11

[1]Assuming 55 % protein degradability in alfalfa hay.

[2]Calculated only in the concentrate portion.

[a,b,c]Values with different superscripts are different ($P<.05$).

Table 17. Influence of protein and starch degradability on apparent digestibility of nutrients in lactating cows (%)

Item	Diets				
	HSHP	HSLP	LSHP	LSLP	SE
Organic matter	65.6[a]	60.1[b]	64.8[a]	62.7[b]	0.37
Crude protein	68.8	61.5	62.7	62.6	2.85
Starch	92.2[a]	87.8[a]	80.5[b]	80.5[b]	1.05
Neutral det. fiber	48.3	46.4	44.1	52.6	3.96
Acid det. fiber	26.7	27.3	28.4	28.3	2.11

[a,b,c]Values with different superscripts are different ($P<.05$).

digestibility than those containing BDG. This might reflect higher RDP and starch as well as synchronization of rumen degradation. The HSHP diet may have released amino acids, peptides and/or ammonia for rumen bacterial growth at a rate favoring greater overall breakdown of starch from barley. The low value for CP digestibility on the LSHP diet may have been due to slow degradation in protein from M which is less digestible than protein in B or other cereal grains (Hale, 1973; Thorne, 1983; Spicer et al, 1986). A less active rumen fermentation may have also retarded degradability of starch in the rumen, which is a primary site for its digestion (Theurer, 1986; Orskov, 1986). Hibberd et al. (1982) found that the differences in alpha-amylase digestion between sorghum and corn starches were small, and observed rumen microbial digestion was equally rapid for isolated corn and sorghum starches, but concluded that factors such as type of protein and tannins limit availability of sorghum starch in the digestive tract of animals.

For the LSLP diet (synchronized for a slow degradation), availability of RDP was limited because of the natural resistance of BDG proteins to microbial attack (Satter and Whitlow, 1977; Johnson, 1985). Armentano et al, (1986) estimated that in situ degradation rate for fraction "B" in BDG was less than a third of that in soybean

meal (.042 vs. .177 h-1). Slow release of nitrogen in BDG coupled with the slow degradability of protein in milo may have been responsible for the numerically lower CP and starch digestibility of LSLP compared to HSHP.

The reason for lowest CP digestibility on HSLP is not clear. Although the supply of CP from B (around 20% of dietary N) had the highest rumen degradability, it may not have been sufficient to sustain microbial breakdown of the protein contained in other ingredients.

Starch digestibility for all diets was lower than that observed in many other studies (Waldo, 1973; Hale, 1973; Spicer et al, 1986; Theurer, 1986). Probable reasons are: 1) The high intake of total DM and starch (23.7 to 25.3, and 4.3 to 4.5 kg/d, respectively), and 2) Processing of grains by the dry rolled method. Several researchers (Kartchner, 1972; Theurer, 1984; Owens et al, 1986), have suggested that both factors limit starch digestibility. The lower starch digestibility on LSHP and LSLP was probably due to less NH3-N availabe in the rumen for support of bacterial growth.

No differences in NDF and ADF ($P>.05$) digestibilities were observed among diets, even though less synchronized diets were numerically lower for NDF. Less timely availability of N and starch to the rumen flora might have depressed hemicellulose breakdown.

Rumen concentration of NH3-N and VFA's are in

table 18. As expected, diets containing CSM had higher ($P<.05$) rumen NH3 than those with BDG. Similar results have been shown by Satter and Whitlow (1977), when SBM or BDG were fed to sheep; and by Seymour and Polan (1986), who compared SBM and BDG in lactating dairy cows. The lower NH3 levels on BDG diets reflect less degradability of protein from both BDG and M. The slightly higher NH3 value in HSLP compared to LSLP was due to more rapid degradation of protein in B than M. Even though NH3 concentrations on HSLP were sufficient for bacterial requirements according to Satter and Slyter, (1974), digestibility of OM and CP were lowest in this diet. Mehrez et al. (1977) suggested that NH3-N concentrations for maximal microbial yield are probably lower than for optimal OM digestion. Blood urea-N levels were higher ($P<.05$) for diets containing B reflecting more rapidly degraded protein on B than S.

Concentrations of VFA's were influenced more by source of protein than starch with HSHP and LSHP showing higher acetic, propionic, butyric and total VFA's than diets with BDG. This might be associated with the higher OM digestibilities for these diets. Diets containing B had lowest ratios of acetic to propionic acids, with values lower than those considered adequate to maintain a normal milk fat percent.

Table 18. Influenceof protein and starch degradability on rumen fluid and blood components in lactating cows[1]

Item	Diets				
	HSHP	HSLP	LSHP	LSLP	SE
Ammonia-N (mg/dl)	16.4[a]	11.4[b]	12.5[ab]	10.2[b]	1.58
VFA's (mMole/l)					
Acetic	14.4[a]	11.0[ab]	13.1[ab]	10.4[b]	0.38
Propionic	7.3	6.0	6.4	4.7	0.35
Butyric	2.4	1.9	2.4	1.9	0.09
Ace./Pro.	2.0	1.9	2.2	2.3	0.35
TOTAL	25.5[a]	20.1[b]	23.1[a]	18.1[b]	1.07
Blood urea-N (mg/dl)	14.8[a]	15.0[a]	13.6[b]	13.7[b]	0.34

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

[1]Significant for protein source (P<.03).

Based on previous research with high producing dairy cows, it might be expected that diets containing BDG would result in higher milk production because of less degradable protein than CSM diets (Forster et al, 1983; Kung et al, 1983; Kung and Huber, 1986; Satter, 1986). However, milk yields were highest on HSHP (Table 19) although this was not significant ($P<.05$). Possible explanations for this are: 1) Higher DM intake; 2) The greater supply of digested nutriets (table 4); and 3) An increased rumen microbial mass which provided additional protein and energy to the animal. Milk fat percentages were lower ($P<.05$) for diets containing B (3.0%), than M (3.5%), as were acetic to propionic ratios. No difference ($P>.05$) was observed for concentrations of milk protein or lactose. When milk production was corrected for 3.5 % fat, the diet containing B and BDG was lower ($P<.05$) than all others. No difference ($P>.05$) in the utilization of protein and starch was observed among diets. An explanation might be that more protein and starch from BDG diets escaped rumen degradation and were digested in the small intestine resulting in a net increase in efficiency in these diets, despite a slightly lower digestibility in the total tract. Moreover, the lower efficiency in transformation of dietary starch to milk in diets containing CSM might be partially attributed to

Table 19. Influence of protein and starch degradability on milk production and composition.

Item	Diets				
	HSHP	HSLP	LSHP	LSLP	SE
Milk prod., (kg)[1]	37.4[a]	34.9[b]	34.2[b]	34.6[b]	
3.5 % FCM, (kg)[1]	34.3[a]	31.4[b]	33.6[a]	34.8[a]	
Butterfat, (%)	3.1[b]	2.9[b]	3.4[a]	3.6[a]	0.11
Milk protein, (%)	2.9	3.0	3.0	2.8	0.12
kg Milk/kg CP intake	8.70	8.51	8.34	8.43	0.74
kg Milk/kg ST intake	4.50	4.91	4.62	5.96	0.56
kg Milk/kg DMI	1.50	1.50	1.41	1.45	0.37

[a,b,c]Values with different superscripts are different ($P<.05$)

[1]Compared by orthogonal contrasts.

more NH3 escape from the rumen requiring additional energy to synthesize and excrete urea.

From results obtained in this study, we might conclude the following: 1) Productivity of dairy rations containing similar protein and available energy can be altered by rumen degradability of the starch and protein; 2) Protein of higher rumen degradability does not always adversely affect milk production, provided there is synchronous release of energy; 3) Slowly degradable protein and starch that escape rumen degradation may compensate for their low rumen digestibilities by rendering more dietary nutrients available to the small intestine.

CHAPTER 6

THE EFFECT OF SYNCHRONIZATION OF PROTEIN AND STARCH DEGRADABILITY IN THE RUMEN ON NUTRIENT UTILIZATION AND MICROBIAL PROTEIN SYNTHESIS

Summary

A study was designed to determine the effect of Cr_2O_3 synchronization of protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. A 4 x 4 Latin square design in a 2 x 2 factorial arrangement was used, with four multiparous cows in mid lactation (approximately 150 days in average), two fitted with duodenal and two with duodenal and ruminal cannulae. Diets used were those described in the lactation trial (HSHP, HSLP, LSHP and LSLP). Experimental periods were 12 days, 8-d adjustment and 4-d for collection. Chromium oxide was used as a marker to determine digestibility and rumen nutrient flow. Microbial protein synthesis was estimated from nucleic acid content in duodenal samples.

Results showed that degradable starch diets (containing barley) significantly increased ($P<.05$) apparent and corrected rumen digestibility of DM, OM, CP and starch, and no effect due to protein degradability was detected. The average amount of starch digested in the small intestine was estimated at approximately .17 and 1.05 kg per day for

barley and milo diets, respectively, which confirm the ability of the amylolytic enzymes in the small intestine to degrade escaped starch. No difference (P>.05) among diets was found in DM, OM and CP flow to the small intestine; however, microbial CP synthesis was higher (P,.05) for diets containing barley than milo. When microbial protein synthesis was expressed as g of N/kg of truly fermented organic matter (FOM), values obtained were very high and they approach the theoretical maximum of 50 g/kg of FOM.

Introduction

The use of a highly degradable starch source with a rapid or slowly degradable protein source stimulated a better nutrient utilization and microbial protein synthesis than a slowly degraded starch source. The better nutrient utilization observed in diets synchronized for a fast degradation of starch and protein can explain the increased milk production found in the lactation trial.

Rumen microorganisms provide to the host animal with microbial protein (amino acids) and available sources of energy in the form of volatile fatty acids. The magnitude of this contribution largely depends on the quantitative and qualitative characteristics of the substrates to be fermented. However, it has been demostrated that ruminal microorganisms cannot meet, the protein and energy

requirements of high yielding animals (Tamminga, 1982; Kaufmann and Lupping, 1982; Kung and Huber, 1983). Protein and energy (starch) sources that can escape ruminal degradation are required to allow for maximum production.

In a previous study (Herrera-Saldana and Huber, 1987), it was found that cows that consumed a rapidly fermentable diet (barley plus cottonseed meal) produced more milk than cows on a slowly fermentable diet (milo plus brewers dried grains), 37.4 vs. 34.6 kg/day. In this study, it was attempted to synchronize protein and starch degradability in the rumen in order to attain a higher efficiency in nutrient utilization.

Oldham and Smith (1982) reviewed results from different levels and sources of protein and energy (starch) fed to dairy cows. They concluded that the form of dietary energy has an important effect on protein utilization, in that starch has a protein-sparing effect. The probable explanation for this consists in the importance of matching energy and protein sources so that the rate of carbohydrate fermentation be similar to the rate of ammonia release from the dietary protein source.

The objective of this study was to determine the effect of synchronization of protein and starch degradation in the rumen on nutrient utilization, microbial protein synthesis and milk production and composition in dairy cows.

and milk production and composition in dairy cows.

Materials and Methods

A 4 x 4 Latin square design in a 2 x 2 factorial arrangement was used. Cottonseed meal (CSM), a common protein supplement in the southwest US, and brewers dried grains (BDG) were the protein supplements; while dry rolled barley and dry rolled milo were the grains and served as main sources of starch. Protein degradability, as determined by the ficin procedure (Poos et al., 1985), was higher for CSM and barley (56.6 and 65.0) than for BDG and milo (38.0 and 45.0%). Starch degradability was measured with the maltose-glucosidase procedure developed in our laboratory (Herrera-Saldana et al., 1986) and was higher in barley than in milo, with values of 90.5 and 70.5%, respectively. Four completely mixed diets (table 20) were formulated with a ratio of 65:35 concentrate to forage. The forage portion was alfalfa hay and cottonseed hulls. Barley was mixed with CSM for a diet synchronized for a rapid fermentation (HSHP) and milo was mixed with BDG for a slow fermentation diet (LSLP). Two "uncoupled" diets were also formulated mixing barley with BDG (HSLP) and milo with CSM (LSHP). All diets were to contain 17.0 % CP and 1.6 Mcal NEl/kg of dry matter.

Four multiparous Holstein cows in mid lactation (approximately 150 days in average), two fitted with

used to test the diets. Cows were housed in individual, shaded pens with dirt floors and had free access to water and to extra trace mineral salt. Feeds were divided into two equal portions and fed twice daily at 500 and 1500 h. Feed refusals were recorded before each afternoon feeding. Feeds

TABLE 20. Ingredientcomposition in diets used in the study (%).

Item	HSHP	HSLP	LSHP	LSLP
Barley	42.8	32.6	----	----
Milo	----	----	41.1	30.1
CSM	12.5	----	14.4	----
BDG	----	23.3	----	25.5
Molasses	6.7	6.3	6.6	6.6
Fat	1.4	1.2	1.3	1.2
Minerals and Vit.[1]	1.6	1.6	1.6	1.6
Alfalfa hay	25.0	25.0	25.0	27.0
Cottonseed hulls	10.0	10.0	10.0	8.0

[1] CaCO3 .33; Biophos .74; Trace mineral salt .33; Vit. A & D .20.

and weigbacks were sampled daily and composited prior to dry matter determination in a forced-air oven at 60 C for 72 h.

Cows were milked twice daily at 430 and 1630 h, and milk production was recorded for each milking. A daily composite milk sample was obtained weekly from each cow and analyzed by the Arizona DHIA (Phoenix, AZ) for butterfat, protein, lactose, total solids and somatic cells.

Experimental periods were 12 days. Day 1 to 8 for ration adjustment and day 9 to 12 for collection of samples. Chromium oxide (Cr_2O_3) in powder form was used as indigestible marker to determine digestibility and rumen output of nutrients. Two gelatin capsules (12 g) of Cr_2O_3 were given daily after each milking during the entire study period (48 d). During the last four days of each treatment period (12 d), rumen and duodenal samples were collected at intervals of 4 h and feces at 8 h. intervals, during the last four days of each period. Approximately 300 ml of duodenal contents, 100 ml of strained (through 4 layers of cheescloth) rumen fluid and 500 g of feces were collected at each sampling. Duodenal and fecal samples were frozen inmediately after collection; pH of rumen samples was determined inmediately after collection and samples were frozen until analysis for ammonia nitrogen (NH3-N) and volatile fatty acids (VFA's). Digesta samples from each cow and each period were thawed and homogenized prior to

compositing (on a volume basis) for subsequent analysis. Digesta and fecal samples were dried individually in a forced-air oven at 60 C for 72 h. Dried feed, fecal and digesta samples were analyzed for dry matter (DM), and organic matter (OM) using standard procedures (AOAC, 1975); crude protein (CP) using a nitrogen autoanalyzer (Technicon, Terrytown, NY); neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent insoluble nitrogen (ADIN) by the Goering and Van Soest (1970) procedure. Starch was determined by the method of Salomonsson et al. (1984) as modified by Herrera-Saldana and Huber (unpublished data). Rumen NH3-N was determined by the hyplchlorite-phenol procedure (Beecher and Whitton, 1970) on the supernatant of the centrifuged (3000 g, 15 min) rumen fluid samples. Volatile fatty acids were determined (using 5 ml of supernantant acidified with 2 ml of 25 % metaphosphoric acid) by gas liquid chromatography with a 80/120 carbopack B-DA/4 % carbowax 20M column. Microbial crude protein was estimated from the nucleic acid content in duodenal samples by the procedure of Zinn and Owens (1986) and was calculated as 6.25 x nucleic acid-N /.20; microbial protein was assumed to make up 80 % of the microbial N CP (McAllan and Smith, 1969). Organic matter truly fermented in the rumen was calculated by the equation :

OMI - [duodenal OM (kg) - microbial OM (kg)],

assuming 17 % ash in microbial cells (Van Soest, 1983). Nutrient output to the small intestine and rumen and total tract digestibilities were calculated using the Cr2O3 ratio technique.

Data was analyzed factorially to determine differences between starch and protein sources and interaction of starch x protein by standard procedures of analysis of variance. Variation from animal and period were removed from treatments by analysis of variance (Steele and Torrie, 1960).

Results and Discussion

Chemical composition of treatment diets is in table 21. Crude protein was highest and starch lowest for the LSLP as compared to other diets. However, all diets contained sufficient protein to meet NRC (1978) requirements. Diets containing BDG had higher NDF and lower rumen degradable protein (RDP). These differences were expected since BDG contains more fiber than CSM and was included at almost double the amount of CSM in the concentrate. Diets containing B had less ADIN and more rumen degradable starch (RDS) than those containing M. These differences were also expected and agree with results reported elsewhere (Kay et al., 1972; Spicer et al., 1986).

Total DM, OM and CP intakes (table 22) were lower for HSHP and LSLP diets, but no significant differences were

Table 21. Chemicalcomposition of diets used in the study (%).

Item	HSHP	HSLP	LSHP	LSLP
Organic matter	92.0	92.5	92.5	92.2
Crude protein	17.2	17.6	17.1	17.8
NDF	40.6	44.9	38.9	44.6
ADF	22.7	24.8	26.7	25.5
ADIN	0.92	1.14	1.20	1.30
Starch	31.7	30.5	31.1	24.4
RDP, % of total CP[1]	59.5	43.7	55.9	35.4
RDS, % of total St.[2]	74.7	69.9	62.3	48.3

[1] Values were obtained using the Ficin method.

[2] Values obtained using the Maltose-Glucosidase method.

Table 22. Theeffect of protein and starch degradation on feed and nutrient intake (kg/day, dry matter basis).

Item	HSHP	HSLP	LSHP	LSLP	SE
Dry matter	18.34	19.55	20.25	18.30	0.74
Organic matter	16.83	18.08	18.72	16.88	0.68
Crude protein	3.15 [a]	3.44 [a]	3.46 [a]	3.26 [b]	0.13
Starch	5.80 [a]	6.00 [b]	6.30 [a]	4.50 [b]	0.22
RDP	2.07 [a]	1.59 [a]	2.16 [a]	1.23 [b]	0.06
RDS	4.33	4.17	3.92	2.16	0.17

[a,b] Values with different superscript are different ($P<.05$).

detected. However, LSLP diet had lowered (P<.05) total starch and RDS intakes than the other diets. Animals receiving diets with BDG consumed around 33 % less RDP than those consuming CSM diets. Apparent and corrected rumen digestion coefficients are shown in table 23. Starch source had a significant effect on rumen digestibility of DM, OM, CP and starch; however, no effect due to protein source could be observed. This may be related to the protein-sparing effect suggested by Oldham and Smith (1982). Diets containing barley had higher (P<.05) digestibility values for corrected and apparent DM, OM, CP and starch than milo diets. No difference (P>.05) was found in digestibility of fiber fractions (NDF and ADF), although barley diets tended to have higher values.

Orskov et al. (1974) reported a somewhat greater value (74 %) for corrected OM (OMc) in barley diets fed to sheep, whereas Spicer et al. (1986), using steers, found a OMc digestibility value of 61.7 % similar to that observed in this study (63.1 and 63.3, for HSHP and HSLP, respectively). Diets containing milo had higher digestibility values for OMc than those reported by Spicer et al. (1986) and Rahnema et al. (1987), with values of 56.6 vs 42.6 and 35.8 %, respectively.

Corrected CP digestibility was about 15 % higher in barley than milo diets. Percent ruminal digestibility of

Table 23. The effect of protein and starch degradation on rumen digestion coefficients (%).

Item	HSHP	HSLP	LSHP	LSLP	SE
Apparent DM	34.48[a]	35.94[a]	30.10[b]	27.10[b]	1.80
Corrected DM[1]	58.92[a]	59.02[a]	49.32[b]	47.06[b]	1.33
Apparent OM	40.14[a]	43.99[a]	37.92[b]	36.65[b]	1.76
Corrected OM[1]	63.12[a]	63.22[a]	56.97[b]	56.06[b]	0.07
Apparent CP	-14.71[a]	- 7.76[a]	- 6.33[b]	-12.33[b]	4.38
Corrected CP[1]	51.57[a]	54.12[a]	43.01[b]	38.41[b]	2.94
Starch	79.89[a]	80.00[a]	49.80[b]	48.75[b]	4.50

[1] Values corrected for microbial DM, OM and CP.

[a, b] Values with different superscript are different ($P<.05$)

starch in milo diets was almost 40 % less than for barley diets. This low digestibility in milo starch has been reported by other researchers (Kartchner, 1972; Spicer et al., 1986; Theurer, 1986). Values for starch digestibility in barley diets were similar to those reported by Mathers and Miller (1981) and McCarthy et al. (1987), but lower to those reported by Orskov et al. (1969) and Theurer (1986).

The absence of effect due to protein source on ruminal digestibility can not be explained at the present, but it may also be related to the protein-sparing effect of starch and the effect of the protein in grain sources.

Total tract digestibility values (table 24) were only different for CP and starch. Again, diets containing barley had higher ($P<.05$) digestibility values for CP and starch than milo diets. Values for CP digestibility in barley diets were similar to those in the literature. Parrot et al. (1969) found that CP digestibility in cattle varied from 65.8 to 72.1 % when barley was dry rolled or steam processed and flaked. Hale (1973) reported a higher value (77 %) for dry rolled barley fed to feedlot steers. Other results (Spicer et al., 1983; Theurer, 1986) have a range between 68 and 72 %, for minimally processed barley. Values for CP digestibility in milo diets (61.9 and 59.8%, for LSHP and LSLP) were also similar to those reported elsewhere (Hale, 1967; Rahnema, 1972; Theurer, 1986; Rahnema et al., 1987).

Table 24. The effect of protein and starch degradation on total tract digestion coefficients (%).

Item	HSHP	HSLP	LSHP	LSLP	SE
Dry matter	67.11	66.62	66.51	62.49	1.34
Organic matter	72.90	72.64	71.62	68.25	1.25
Crude protein	67.32[a]	68.62[a]	61.96[a]	59.83[b]	1.70
NDF	62.32	62.27	61.84	62.60	1.52
ADF	30.51	27.07	33.61	29.62	2.31
Starch	94.23[a]	93.69[a]	89.76[b]	85.78[b]	0.65

[a,b] Values with different superscript are different ($P<.05$).

Total starch digestibility values were lower for both barley and milo diets, as compared to those observed by other researchers (Waldo, 1973; Mcneill et al., 1971; Owens et al., 1986). However, most of these studies were conducted using sheep or beef animals and the consumption of starch was much more smaller than that in this study. It is interesting to observe that the amount of starch flowing to the small intestine was from 2 to 2.5 times bigger in diets containing milo. The average amount of starch digested in the small intestine was estimated to be approximately .17 and 1.05 kg per day for barley and milo diets. This confirm the capacity of the amylolytic enzymes in the small intestine to digest escaped starch (Owens et al., 1986) and contradict the conclusions made by others (Orskov, 1986).

Rumen concentration of NH3-N and VFA's are in table 25. Due to the fact that only two of the cows had rumen cannulae, the number of observations obtained was not sufficient to make valid statistical comparisions. However, the data obtained showed a trend for lower pH, NH3-N production and acetic/propionic ratio in diets containing barley. The significance of this values may account for some of the differences observed in rumen nutrient utilization.

With regard to output of nutrients to the small intestine and microbial protein synthesis (table26), only the amount of microbial CP synthesis was different ($P<.05$).

Table 25. The effect of protein and starch degradation on rumen pH, ammonia-N and volatile fatty acids.

Item	HSHP	HSLP	LSHP	LSLP
pH	5.6	5.7	5.9	6.0
NH3-N, (mg/dl)	13.18	13.59	15.27	14.23
VFA'S, (mM/l)				
Acetic	23.72	22.21	26.65	24.35
Propionic	12.13	11.56	8.63	7.69
Butyric	4.72	4.82	4.85	5.15
Ace./prop.	1.95	1.92	3.09	3.17
Total	42.11	40.28	41.82	38.77

Table 26. The effect of protein and starch degradation on nutrient output to the small intestine and microbial protein synthesis (kg /d).

Item	HSHP	HSLP	LSHP	LSLP	SE
Rumen Output					
Dry matter	12.04	12.68	14.15	13.10	0.82
Organic matter	10.12	10.59	11.62	10.59	0.68
Crude protein	4.31	4.41	4.54	4.24	0.35
CP /CP intake	1.36	1.28	1.31	1.30	0.27
Truly FOM	10.59	11.37	10.67	9.57	0.33
Microbial DM	4.59	4.01	4.17	3.83	0.26
	a	a	b	b	
Microbial CP	3.00	2.64	2.36	2.14	0.19
	a	a	b	b	
g micr. CP/kg FOM	283.2	231.9	220.8	223.3	0.78
	a	a	b	b	
g micr. N /kg FOM	45.32	37.11	35.33	35.73	0.28

a,b,c
Values with different superscript are different (P<.05).

The LSLP diet had the lower (9.57 kg) and HSLP the highest (11.37 kg) value for FOM, the other diets were intermediate. It was expected that the HSHP diet would have the highest FOM value, However, OM intake was the lowest on this diet (18.3 kg per day). The amount of nutriet output to the small intestine were higher than those in literature (Oldham, 1984; Spicer et al., 1986; Wanderly et al., 1987), but values were from steers and non-lactating dairy cows with smaller feed intakes.

With regard to microbial crude protein synthesis, the HSHP diet was higher ($P<.05$) than the other diets. When microbial protein synthesis was expressed as g of N / kg of FOM, values obtained were higher than those observed by Oldham and Smith (1982) with a range of 22.2 to 28.2 g microbial N / kg FOM) using non-lactating cows consuming a 50 % concentrate diet. Zinn et al. (1981) also found lower values (18 to 30 g microbial N /kg FOM) when using steer calves fed protein sources with different rumen degradabilities. In that study, they did not find any difference on microbial protein synthesis due to different sources of protein. Stern and Satter (1982) found values that are similar to those in this study with a range between 39 to 44 g microbial N / kg of FOM. The values obtained by Stern and Satter (1982) and those from this study are considered extremly high (Oldham, 1984) and close to the

theoretical maximum (around 50 g) calculated by Leng and Nolan (1984).

No differences (P>.05) were observed in milk production (table27), although milk composition was different (P<.05). However, the relevance of this data is limited because of the high variability in state of lactation, the short duration of the treatment periods, and the small number of cows used in this trial.

The conclusion from the results obtained in this study are: 1) The source of starch have a definitive and important effect on the utilization of nutrients in the rumen; 2) The effect of starch appeared to be higher than the effect of protein on nutrient utilization; 3) Synchronization of a high degradable starch source with a high degradable protein source stimulated a higher microbial protein synthesis; 4) Rumen escaped starch and protein were well degraded in the small intestine, showing that the limit for starch digestion in the intestine was not reached with the amounts of escaped starch observed in this study; 5) The better nutrient utilization observed in diets synchronized for a fast degradation of starch and protein can explain the increased milk production observed in our previuos study (Herrera-Saldana and Huber, 1987).

Table 27. The effect of protein and starch degradation on milk production and composition.

Item	HSHP	HSLP	LSHP	LSLP	SE
Milk Prod., (kg)	18.99	20.40	20.17	20.21	0.70
Fat, (%)	3.33[a]	3.13[b]	3.71[a]	2.90[b]	0.14
Protein, (%)	3.08[b]	3.21[a]	3.22[a]	3.08[b]	0.02
3.5% FCM (kg)	18.46	18.82	20.67	18.22	0.57
kg milk/kg DMI	1.05	1.05	1.00	1.10	0.03
kg milk/kg CPI	6.02	5.93	5.82	6.19	0.16
kg milk/kg starch I.	3.30	3.45	3.22	4.76	0.18

[a,b] Values with different superscript are different ($P<.05$).

CHAPTER 7

GENERAL SUMMARY AND CONCLUSION

Results obtained from the in vitro and in situ degradability studies (chapters 3 and 4), indicated that significant differences occurred in extent and rates protein and starch degradabilities These differences permitted the classification of grains and protein supplements according to their rate of starch and protein degradation as follows:

Grains; wheat > barley > oats > corn > milo.

Protein supplements; SBM > CSM > CGM > BDG > BM.

This information allowed the selection of two protein and two starch sources varying in rates of degradation, in order to match them and determine the effect of synchronization of protein and starch degradation on milk production (chapter 5).

Cottonssed meal, (highly degradable protein) was combined with dried rolled barley (highly degradable starch) for a rapidly degraded synchronized diet (HSHP) and brewer dried grains (low degradable protein) was combined with dry rolled milo (low degradable starch) for a slowly degraded synchronized diet (LSLP). Two uncoupled diets were also formulated HSLP and LSHP. Diets were fed to 32 dairy cows (8 cows/treatment) during a 60-day period in a randomized block design with a 2 x 2 factorial arragement (2 protein

and 2 starch sources). Apparent digestibility of diets was estimated by using chromium oxide; results obtained indicated increased ($P<.05$) organic matter digestibility in diets containing CSM and starch digestion coefficients were higher ($P<.05$) in diets containing barley. Although HSHP diet had the highest crude protein digestibility high variability prevented significance. Rumen ammonia-N, acetic acid and total volatile fatty acids were higher ($P<.05$) in diets containing CSM, indicating the influence of this protein source in the increased digestion of organic matter in the rumen. Blood urea nitrogen, however, was higher for diets containing barley grain.

Previous work (Kung and Huber, 1986; Satter, 1986) with high producing dairy cows fed diets containing slowly degraded protein supplements have resulted in higher milk production than diets with rapidly degraded supplements. In contrast to this studies and other, milk production was highest for the HSHP diet, which contained the most rumen degradable protein. This might be associated with the rapidly degradable starch also present. Milk fat was lower ($P<.05$) for barley diets thus, lowering the relative advantage of the HSHP diet in production of 3.5 % FCM. From results obtained in these studies, the following was concluded: 1) Productivity of dairy rations contaning similar protein and available energy (by NRC standards) can

be altered by rumen degradability of the starch and protein. This is supported by the higher milk yields on the HSHP than other diet; 2) Protein of higher rumen degradability does not always adversely affect milk production provided there is a synchronous release of energy; 3) Protein and starch that escape rumen degradation may compensate for their low rumen digestibilities by rendering more dietary nutrients available to the small intestine; such was suggested by equal FCM yields on the milo diets as on the HSHP diet.

A fourth study was then conducted (chapter 6) in order to determine the effect of synchronization of protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. A 4 x 4 Latin square design in a 2 x 2 factorial arrangement was used. Diets were the same as those in the lactation study (chapter 4). Four multiparous cows in mid lactation (approximately 150 days in average), two fitted with duodenal and two with duodenal and ruminal cannulae were used to test diets. Experimental periods were 12 days, 8-d adjustment and 4-d for collection. Chromium oxide was used as a marker to determine digestibility and rumen nutrient flow. Microbial protein synthesis was estimated from nucleic acid content in duodenal samples.

Results showed that degradable starch diets (containing barley) significantly increased ($P<.05$)

apparent and corrected rumen digestibility of DM, OM, CP and starch, and no effect due to protein degradability was shown, perhaps due to a protein-sparing effect suggested by Oldham and Smith (1982). Total tract digestibility was different ($P<.05$) only for CP and starch with barley diets higher ($P<.05$) than milo.

The average amount of starch digested in the small intestine was estimated at approximately .17 and 1.05 kg per day for barley and milo diets, respectively, which confirms the ability of the amylolytic enzymes in the small intestine to digest escaped starch. No difference ($P>.05$) among diets was found in DM, OM and CP flow to the small intestine; however, microbial CP synthesis was higher ($P<.05$) for diets containing barley tan milo. When microbial protein synthesis was expressed as g of N /kg of truly fermented organic matter (FOM), values obtained were very high (Oldham, 1984) but were similar to those reported by Stern and Satter (1982), they approach the theoretical maximum (around 50 g/kg) calculated by Leng and Nolan (1984).

General conclusions of this dissertation can be summarized as follows:

1) The rate of starch degradation is different among cereal grains and the source of starch has a definitive and important effect on protein degradability in the rumen.

2) It is feasible to synchronize the degradation of

protein and starch sources in the rumen. The use of a highly degradable starch source with a rapid or a slowly degradable protein source stimulated a better nutrient utilization and microbial protein synthesis than a slowly degraded starch source.

3) The better nutrient utilization observed in diets synchronized for a fast degradation of starch and protein in the rumen, might stimulate an increase in milk production in high yielding dairy cows.

CHAPTER 8

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