VARYING RUMEN AVAILABLE CARBOHYDRATE AND RUMEN AVAILABLE PROTEIN IN DIETS OF LACTATING CATTLE

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(ABSTRACT)

Two studies were conducted to evaluate the effects of varying dietary sources of rumen available carbohydrate (RAC) and rumen available protein (RAP) on milk yield and milk composition, nutrient flow to the duodenum, ruminal and total tract nutrient digestibilities, and ruminal pH, ammonia-N, and VFA concentrations in lactating cows. The first study was a response surface design utilizing nine dietary combinations of RAC and RAP. The response surfaces of all milk variables were saddle-shaped. Because of the saddle-shaped surfaces, an optimum combination of RAP and RAC for milk production variables was not obvious from the limited range of RAC and RAP used in this study. Ridge analysis of the saddle surfaces predicted maximum milk yield when dietary RAC was below 69 % of the DM and RAP below 60% of CP in alfalfa-corn silage based diets. In the second study, four cannulated (ruminal and duodenal) cows were utilized in a 4x4 Latin Square design. Four of the nine original diets were selected to provide the largest range of RAC and RAP. Nutrient flow, digestiblities and ruminal parameters were evaluated. Although the in situ incubations

indicated that rates of DM, CP, and NDF degradabilities differed among diets, no effects on overall ruminal pH and total VFA concentrations were detected. Additionally, DM, OM, NDF, ADF, and N flows to the duodenum were not affected by dietary treatment. Nonmicrobial N flow was greater for the barley-based diet, yet microbial flow was not different. The differences in rates of availability determined by in situ methods were not large enough to illicit a measurable difference in nutrient digestion and utilization. Additionally, the data implied that none of the diets were limiting in RAC and RAP for vigorous microbial activity. Fat-corrected (3.5%) milk production was greatest ($P<.05$) when alfalfa-corn silage based diets contained supplements providing intermediate (69 % RAC) carbohydrate availability (corn and barley) and low (60 % RAP) ruminal protein availability (BM and SBM). The increase in fat-corrected milk was consistent with the predicted milk production response in the previous study when RAP exceeded 62% of CP. However, the ruminal parameters, nutrient flow, and nutrient digestibility measurements did not adequately explain the increased milk production when diets contained increased concentrations of BM.

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INTRODUCTION

Microorganisms digest much of the feed ingested by ruminant animals, thus the interaction between carbohydrate and protein metabolism in the rumen is of particular importance. Protein deficiency in the rumen can decrease digestibility of carbohydrate. If carbohydrate is insufficient, nitrogen (N) can be inefficiently used as ruminal ammonia. The appropriate balance of the two for maximizing microbial protein synthesis and ultimately increasing milk production has yet to be identified for high producing dairy cattle. In order to meet this goal, nutritionists must become familiar with 1) factors affecting microbial protein synthesis, 2) quantitative and qualitative measurements of nutrients in feedstuffs, 3) potential for synchrony of carbohydrate and protein, and 4) animal effects that cause differences in production responses.

The objectives of these studies were: 1) to evaluate diets varying in carbohydrate and protein availabilities for their effect on intake, milk production and composition, and growth of primiparous cows, and 2) to determine differences in ruminal fermentation, nutrient flow, and nutrient digestibilities in dual-cannulated cows fed similar diets.

CHAPTER 1

LITERATURE REVIEW

Factors Affecting Rumen Microbial Yield

Rumen microbial nitrogen (MN) contributes 50 to 80 % of the nitrogen (N) reaching the small intestine of ruminant animals (29). Because of this significant contribution of N by the microorganisms, it is important to understand the numerous factors affecting microbial yield and flow of microbial matter to the small intestine. This discussion will be limited to dietary factors affecting microbial yield in cattle.

Microbial Yield

Microbial yield is usually defined as microbial flow to the small intestine in relation to organic matter apparently (OMAD) or truly digested (OMTD) in the rumen. Sniffen and Robinson (92) noted that problems exist with the interpretation of microbial yield data presented as OMAD, especially where undigested dietary organic matter (OM) residue is small and microbial OM flow is large. However, if N concentration of bacterial OM flow is not reported, estimation of bacterial N:OM can be misleading and inaccurate with unsupported assumptions. As a result, much

reported data define microbial yield as g MN/100 g OMAD for comparative purposes.

Positive relationships existed for OMTD (r^2 =.39) and OMAD (r^2 =.25) with passage of MN to the small intestine. These data suggested that OM fermented in the rumen is an important factor affecting the amount of MN that passes to the small intestine. Organic matter truly digested in the rumen appears to be a more accurate predictor of the quantity of MN leaving the rumen than OMAD, yet OM intake was more highly correlated with MN passage to the small intestine than either OMTD or OMAD. The authors suggested that factors other than the quantity of OM fermented in the rumen contribute to the quantity of MN passing to the small intestine.

Although OMAD and OMTD are most commonly reported to indicate measures of the efficiency of microbial yield, some reports indicate that microbial yields may be more closely related to carbohydrate rather than total organic matter intake (66). Net rumen microbial yield (efficiency) is a function of costs of maintenance and death. Sniffen and Robinson (92) described this relationship in the following equation: $1/Y = M/K + 1/Y_G$, where Y = yield, grams of bacteria per gram carbohydrate (CHO) fermented; M = grams of CHO per gram bacteria per hour; K = growth rate per hour; and Y_G = maximum growth yield, grams of bacteria per gram

glucose. Rohr (82) suggested that 90% of the variation in microbial protein yield could be explained by carbohydrate availability. However, a single measurement of carbohydrate potentially digested in the rumen has yet to be developed, thus more variation between laboratories would likely exist in comparison to organic matter measures.

Microbial growth is directly affected by various dietary factors and feeding management factors that influence the OMAD and the OMTD in the rumen. These factors include feed intake, ruminal dilution rate (15,95), forage to concentrate ratio, source and amount of carbohydrate and protein (10,92,95), feeding frequency, feed processing and storage (92), and nitrogen:sulfur ratio (95). This review will be limited to a discussion of the effects of feed intake, ruminal dilution rate, forage to concentrate ratio, source and amount of carbohydrate and protein on microbial yield and flow to the small intestine.

Feed Intake

Dry matter intake affects microbial yield because intake may vary up to fivefold or more during a lactation (92). Although it seems evident that increasing feed intake would result in greater flow of microbial N (MN) from the rumen, several studies have indicated no relationship between intake and microbial yield (18,101). However, these

studies reported intake of only 1.5 % to 2.5 % of body weight (BW), and only three levels of feed intake. Other studies have shown higher bacterial yield at higher intakes (19,56,74). Stern and Hoover (95) reported a lower mean yield of 27 g MN/100 g OMAD based on a summary of 64 observations from the literature. These data included beef cattle and sheep experiments in which intake was low.

In dairy cattle, higher feed intake resulted in consistently higher microbial yield and efficiency of microbial growth in comparison to data from sheep and beef (60). From calculations made by Oldham (69) on data from several studies reporting intake (greater than 12 kg DM/d) and microbial N/kg apparent digested OM (MN/OMAD), it appeared that cows at high intakes produced significantly higher levels of MN. Oldham suggested that dry matter intakes greater than 20 kg/d would likely result in an average of 35 g MN/100 g OMAD.

Dairy cattle data demonstrate the positive relationship between high levels of intake and MN synthesis. Clark et al. (10) summarized an extensive dairy cattle data set to evaluate the relationship of OM intake (OMI) to the passage of N fractions to the small intestine. In a summary of 36 experiments in which 145 different diets were fed, a positive relationship between OMI and nonammonia N (NAN) passage to the small intestine was detected (r^2 = .83). The

relationship was not as strong when the MN fraction was excluded (r^2 = .54). Additionally, the relationship between OMI and the passage of MN to the small intestine was positive (r^2 = .62) when OMI increased from about 3 to 23 kg/d. These positive relationships between increasing OMI and passage of both nonammonia nonmicrobial N (NANMN) and MN out of the rumen indicated that both of these fractions (NANMN and MN) contributed significantly to the total passage of NAN.

Greater feed intake also increases rumen turnover rates. As turnover rates increase, the likelihood of increased amounts of incompletely digested particles being emptied from the rumen would be increased. Coincidently, increased MN flow from the rumen would result (106). Thus, microbial efficiency can be directly affected by rumen dilution rate (or rumen turnover) as a response to increased feed intake.

Rumen Turnover

Increased feed intake often results in greater rumen particulate and liquid turnover rates (16,15). Increased passage of fluids and solids to the small intestine increases quantity of OMTD, amount of N (including MN), and amino acids (AA) passed to the small intestine. Increasing the OMTD provides additional nutrients for microbial growth.

An increased rate of growth coupled with faster passage of microbes to the small intestine may reduce recycling of energy and N within the rumen. Reduced recycling would occur because of decreased cell lysis, resulting in decreased maintenance requirements of the microbes, thus more nutrients available for microbial growth (95).

Rumen liquid and particulate turnover rates have a significant effect on microbial protein synthesis. Harrison and McAllan (25) illustrated from a theoretical calculation based on in vitro reports, that maximal yield of microbes would be approached by increasing the dilution rate. However, Sniffen and Robinson (92) cited four studies in which attempts to increase microbial yield in vivo by increasing liquid fractional turnover rate have been unsuccessful. Lack of consistency between in vitro and in vivo data probably reflects fundamental differences between the two. Oldham (69) noted some confusion in the literature in attempting to relate fractional rate of liquid outflow from the rumen to dilution rate of bacteria in continuous culture. The two were not synonymous because the microbial pool does not relate only to the fluid of the rumen.

It is likely that the relationship between increased microbial yield with greater liquid outflow reflects its close relationship to particulate turnover rate (16,15). Evan's reviews indicated a close relationship between liquid

and particulate turnover over a wide range of reported values from the literature from cattle and sheep.

Rode and coworkers (77) reported a 15% increase in efficiency of microbial protein synthesis when ground hay replaced long hay in cattle diets supplemented with corn and soybean meal. With ground forage in the diet, ruminal OMAD decreased, feed N flow increased, and particulate turnover rate increased. The increased efficiency of microbial protein synthesis was directly related to ruminal particulate turnover rate and inversely related to liquid dilution rate. Because of less residence time in the rumen, ground forage would be fermented to a lesser extent than long forage.

In a second study (77), MN synthesis was positively related to the turnover of the particulate digesta in the rumen, regardless of liquid dilution rate. Efficiency was greater with long hay compared with chopped, and with inclusion of 75 % forage compared with 25%. No effect due to barley or corn supplement was related to efficiency of bacterial growth.

On the contrary, efficiency of bacterial growth (g MN/100g OMTD or OMAD) was improved by increasing *liquid* dilution rate caused by increased feed intake (9.1 vs 6.1 kg/d) in beef cattle (19). Particulate dilution rate was unaffected by intake. The positive relationship between

liquid dilution rate and efficiency of bacterial synthesis could be attributed to a decreased proportion of the total energy expenditure used as maintenance energy with faster liquid flow.

An explanation for the differences in influence of liquid and solid turnover on efficiency of microbial growth in the rumen may be explained by work with dairy cattle by Rode and Satter (77). Efficiency of microbial protein synthesis increased by increasing solids turnover regardless of changes in liquid dilution rate. However, when little or no change occurred in particulate turnover, liquid turnover rate positively influenced the efficiency of microbial protein synthesis (74,77). Additionally, solids turnover was increased with higher intake in dairy cattle. The level of intake was much higher when compared with the beef cattle data (19). Although turnover rate of both the particulate and liquid fractions have been reported to modify microbial yield, these relationships were less strong than the ratio of microbial protein to dietary OM in the rumen of dairy cattle (10,92).

Forage to Concentrate Ratio

The ratio of forage to concentrate in the diet also affects amount of microbial protein reaching the small intestine (77). Clark et al. (10) noted that effects due to

changes in forage to concentrate ratio may be more appropriately attributed to the amount and rate of OM fermentation in the rumen rather than specific levels of forage and concentrate. If additional OM were fermented in the rumen, more available energy should be provided for increasing microbial protein synthesis. As cited by Sniffen and Robinson (92), Hagemeister et al. (24) suggested that the efficiency of energy utilization for synthesis of bacterial protein is greatest in diets containing between 30 and 70% concentrate.

Diets high in concentrate may decrease efficiency of microbial protein synthesis. Potentially, with fewer large particles available for attachment, flow of MN may be reduced concomitant with increased microbial recycling. High concentrate diets may also result in a rapid rate of starch degradation which would cause uncoupled fermentation, thus inefficient energy utilization. Inclusion of a more slowly degraded forage OM source would allow more energy to be trapped by the microorganisms. Adding forage to high concentrate diets increased total feed intake and increased liquid and particulate flow to the small intestine (23). This allowed for greater washout of microbial proteins attached to small feed particles.

With very high levels of forage (> 70%), microbial yield is depressed (51,78,77). The decreased quantity and

efficiency of microbial protein flow to the small intestine may be due to a deficiency of energy (from concentrate) or a diversion of energy toward microbial maintenance resulting in slower growth. When the concentrate portion consisted of high fiber by-product ingredients, instead of grain, Tamminga et al. (101) observed no relationship between forage:concentrate ratio and microbial yield. By-product ingredients are commonly fed as dairy feeds, yet, little research has been reported concerning ruminal utilization of these feeds in dairy cattle. Further research is needed in this area.

Few dairy cattle studies have been designed to specifically evaluate the effects of varying the forage to concentrate ratio on MN synthesis in the rumen (38,78,77). Although forage levels in these studies ranged from 24 to 81 %, an optimum ratio of forage to concentrate that would maximize ruminal production and passage of N to the small intestine was not determined. The *ingredient composition* of the forages and concentrates selected for dairy cattle feeding apparently have a greater influence on microbial protein synthesis and rumen fermentation than simply altering the forage to concentrate ratio.

The composition of individual feed ingredients has long been recognized as significant to efficiency and yield of microbial protein synthesis. Many articles and reviews have

been published concerning effects of carbohydrate fractions and protein fractions on microbial growth in vitro, and more recently in vivo. However, characterization of these fractions in feedstuffs and their availability in the rumen continue to be evaluated.

Carbohydrate Sources

Carbohydrates are the most important source of energy for rumen microbes. Carbohydrates are classified as structural carbohydrates (SC), such as those in NDF, and nonstructural carbohydrates (NSC), such as starches, soluble sugars, and other reserve carbohydrates (107).

Nonstructural carbohydrates include sugars, starches, and pectins. The soluble, non-starch polysaccharides, pectins, are precipitated by sodium laurel sulfate of neutral detergent solution and are 90-100% digested in the rumen (68). They are primarily found in citrus, beet pulp, and legumes but are low in grasses (105). Galactans are unique to legumes as the carbohydrate reserve, in place of starch (105). Fructosans are the storage carbohydrate in temperate grasses and β - glucans are unique to the cell wall of grasses and to the bran of oats, barley, rye, and triticale (105).

The largest portion of NSC is starch. Seventy to 80 % of most cereal grain carbohydrate is starch (66). Starch is

arranged in a highly organized fashion composed of two major molecules, amylose and amylopectin. Amylose is a linear polymer of alpha-1-4-D-glucose units. Amylopectin is a branched polymer with linear chains of alpha-1-4-D-glucose that have an alpha-1-6 branch point every 20 to 25 glucose residues (20). Starch granules are "pseudocrystals" that contain regions of organized "crystalline" form (primarily amylopectin) and nonorganized "amorphous" areas (20).

Structural carbohydrates are associated with the cell wall. The maturity and plant species greatly affect the ratio of cellulose, hemicellulose, and lignin. In turn, the insoluble digestible and indigestible fractions vary greatly between feeds.

Carbohydrate Measurements

The starch and sugars of feedstuffs can be measured quantitatively (105). A commonly reported enzymatic method of measuring total nonstructural (TNC) carbohydrate content of feedstuffs is that of Smith (86). This procedure utilizes Taka-diastase, an alpha-amylase derived from *Aspergilus oryzae* that represents more than 30 different enzymatic functions (67). Its action is not only amylolytic, but also proteolytic and lipolytic, thus in some feeds the TNC would be overestimated. A type A amylase of *Bacillus subtilus* has also been used, but it is specific for

alpha-1 to 4 glucosidic linkages of starch (67). Certain readily fermentable carbohydrates may be underestimated in the type A procedure. A single reliable technique that can quantitate several digestible carbohydrates (starch and sugars) across a range of feeds has yet to be developed.

The NSC fraction can also be calculated by difference: NSC = 100 - (NDF + CP + EE + ASH) (66) or NSC = 100 - [(NDF - NDF protein) + CP + EE + ASH] (105). Van Soest noted that the calculated NSC could be close to determined starch and sugar values for many feeds but was larger when the feed contained large quantities of nonstarch polysaccharides, i.e., pectin. The significance of this difference lies in the different fermentation pathways of starches, sugars and pectins. Starch and sugars in large amounts in the rumen can cause a switch to lactic acid fermentation, but pectins and *B* - glucans are not fermented to lactate (99).

A further evaluation of the quantitative measurements of feed carbohydrates was reported by Nocek and Tamminga (68). Fifty NSC determinations of common grain, by-products, and forage sources were extracted from the literature and compared. They identified values from the total nonstructural carbohydrate (TNC) procedure of Smith (86) with Taka-diastase, nonstructural carbohydrate (NSC) by difference calculation (100 - (NDF + CP + EE + ASH)), and enzymatic methods of starch using bacterial amylases

(STARCH). STARCH measurements were most commonly reported in the literature.

Determinations of TNC, NSC, and STARCH averaged 74.6, 73.7, and 76.1% of DM for corn; 69.4, 58.0, and 60.6% for barley; and 12.9, 25.2, and 1.3% for soybean meal, respectively. Forage sources were more variable than grain sources. Alfalfa silage was 5.8% TNC, 24.7% NSC, and 8.1% STARCH; whereas, corn silage averaged 32.0% TNC, 36.1% NSC, and 39.4% STARCH. The largest variations between measurements could possibly be due to the peculiarities of the carbohydrate composition of the specific feed sources (proportions of starch and types of sugars).

The structural carbohydrate (SC) fraction of feedstuffs is measured most conveniently as NDF. The NDF fraction includes cellulose, hemicellulose, and lignin as the major components. The NDF procedure was originally developed for forages, thus, modifications for concentrate sources were developed. Various modifications using amylases have been proposed (105).

Because NDF is easily measured and commonly reported in the literature, it has been used as a means of defining physical and chemical restrictions of feeding the dairy cow. Mertens (57) suggested values of 31% NDF for cows producing 29 to 36 kg milk; 28% NDF when milk production exceeds 36 kg. The NDF is highly related to dry matter intake (DMI)

and the depression in digestibility asociated with high intakes (57). However, NDF is a chemical entity that does not necessarily reflect rumen availability (66). A measurement of the availability is critical to providing the proper combination of rapidly available (NSC) and slowly available (SC) or degradable carbohydrate sources that should promote maximum microbial yield (36).

Nocek and Russell (66) suggested that in situ measurements of NDF availability could be used to estimate ruminal carbohydrate availability. The following equation was developed as a prediction of the rumen available carbohydrate as a percent of total carbohydrate: Rumen available carbohydrate (RAC) =

$$\frac{[.9\ (NDS - (protein + lipid)) + (NDF \times NDF\ availability)]}{[(NDS - (protein + lipid)) + NDF]}$$

where NDF = neutral detergent fiber and NDS = neutral detergent solubles (100 - NDF). The .9 value was included because unpublished work estimated that 90% of NSC was almost completely digested in the rumen by 24 h. The numerator represented the portion of the carbohydrate fraction that was considered potentially available in the rumen; whereas, the denominator represented a sum of the NSC and SC or total carbohydrate. The ease of using this equation is attractive since the NDF, CP, and EE values of most feedstuffs can be easily obtained. However, the data

base of NDF availability is very limited. Eighty-seven reported measurements of NDF rumen availability were obtained from the literature by Nocek and Russell (66), yet many of these were from the same lab and only a few of different grains, forages, and by-product feeds.

In general, the rank of ease of degradation of commonly fed feedstuffs is: wheat, barley, oats, corn, and sorghum greater than legumes (107). Potential rate of fermentation of all carbohydrates largely determines efficiency with which the microbial population can use them.

True characterization of degradability of carbohydrate fractions has not been adequately determined. However, several attempts have been made (6,27,53). Standardization of a technique describing qualitative characteristics of feed carbohydrate is needed, but several dynamic components are involved (67,68).

The largest data base of both quantitative and qualitative determinations is of the starch component of NSC. Ruminal starch degradabilities have been reported from in vitro (27), in vivo (68), and in situ (101) determinations. In vitro techniques use enzymes and short incubation times to estimate starch degradability rates. These values should be used for comparative purposes since they may not represent actual rates in vivo.

Nocek and Tamminga (68) compared the quantitative and qualitative measurements from 83 determinations in vivo or 47 in situ or in vitro measurements. The relationship between starch content and ruminal degradability of starch (in situ, in vivo) was significant, but the starch content (quantitative measure) accounted for little of the variability associated with rate of degradability (r^2 = .29). More of the variability associated with degradability was accounted for by the in situ measures. The relationship between in situ and in vivo starch degradability was in situ = -89.8 + 2.01 (in vivo), r^2 = .65, $P<.005$. These data suggested that the various methods were comparable, but the authors warned that the data set was small and more comparisons were needed from various laboratories since the in situ data was from only 5 different reports.

Barley and Corn as Carbohydrate Sources

Barley and corn grains have been reported most often as comparative carbohydrate sources varying in carbohydrate digestibility. Waldo (110) reported 23 observations in the literature of barley starch digestibilities at the abomasum or duodenum. The mean starch digestibility was 94 % +/- 2.4. Very little variation occurred in these data from different sources or loads, different ruminant species, different processing methods, or by changing percentage in

the ration. Thirty published observations of corn starch digestibility were compared by Waldo (110). The average corn starch digestibility was 78 % +/- 12.5. Much more corn than barley starch escaped ruminal degradation. Variations in the corn data were also much higher than found in the barley data. Variations could be attributed to different loads or lots, plant varieties, processing methods, and differences between species (beef and sheep).

Matras et al. (52) evaluated the effect of barley, steam-flaked sorghum, and dry-rolled sorghum in combination with urea, urea:bloodmeal:corn gluten meal (50:25:25), or bloodmeal:corn gluten meal (50:50) on N utilization in growing lambs. Greatest microbial N synthesis (allantoin N excretion) occurred when dry-rolled barley was fed. However, grain sources did not differ in N balance or the proportion of N retained. This implied that greater microbial N synthesis did not overcome the amount of NANMN provided to the small intestine by other grain sources.

Oldham et al. (70) reported greater bacterial yield when dairy cows were fed barley grain compared to corn grain in diets containing 10 or 40 % poor quality hay diets at moderate intakes. The more rapid degradation of barley starch than corn starch may have supported higher bacterial yield.

Higher microbial yield also occurred when pelletted ryegrass hay was supplemented with rolled barley compared with cracked corn (68). However, when chopped hay diets were fed, microbial yield was not different regardless of concentrate source. Pelleting may have increased hay degradation by posing more sites for bacterial attachment. The rapidly degraded barley could have provided a more readily available energy source to support growth when availability of hay nitrogen was increased. However, measurements of rates of degradability of the hay or concentrate sources were not reported.

Dairy cows consuming 14 to 15 kg/d of isonitrogenous corn- or barley- based diets (60 % concentrate, 40 % hay) did not produce differences in OMAD (70). However, cows fed the barley-based diets produced 64 g/d more MN flowing to the duodenum than those fed corn-based diets. The NAN was not different between corn- and barley-fed diets because the corn-based diet increased passage of NANMN by 50 g/d.

In dairy cattle (150 d post-partum) consuming dry-rolled barley and milo with cottonseed meal (CSM) or brewer's dried grain (BDG), MN/OMAD was significantly higher with the inclusion of barley in diets. The highest level of MN was detected in cows fed the barley and cottonseed meal combination (28). However, similar amounts of NAN passed to

the small intestine because barley diets decreased NANMN flow.

Contrary to the previously cited work, others have reported no differences in microbial yield with barley- or corn-based diets. In crossbred steers fed 20% forage diets, no influence of barley, corn, or sorghum feeding on g MN/OMAD was detected (93). Similarly, Theurer (102) adapted data from McMeniman et al. (54) and found no difference in MN synthesis between corn and barley diets (22 and 21 g MN/OMAD, respectively). However, the amount of MN that entered the abomasum in steers on the previously cited study was higher when barley was included in the concentrate compared with corn- or sorghum-based diets (112 vs 76 and 81 g/d, respectively), even though efficiency did not change.

Dairy cattle data have also indicated no influence of barley or corn on MN synthesis. Cows fed total mixed rations consisting of 26 % alfalfa-grass silage, 19 % corn silage, and 55 % concentrate that were corn- or barley-based produced no measurable differences in the efficiency of synthesis and amount of MN passage to the small intestine regardless of grain source in the concentrate (53). These cows were in early lactation and consumed 23.8 and 20.7 kg DM/d of the corn- and barley-based diets, respectively. Although MN yield was similar across diets, feeding barley increased the OM and starch apparently digested in the

rumen. However, AA flow to the duodenum in cows fed the corn-based diets was increased, probably due to the low ruminal protein degradability of corn and greater DMI of corn compared with barley.

Rode and Satter (77) fed ground corn- or ground barley-based concentrates to cows receiving either long or chopped full-bloom alfalfa hay. Forage to concentrate ratios were either 25:75 or 75:25. Cows were in midlactation, producing > 20 kg milk/d. No differences due to source of cereal grain were detected for OMI, NI, OMAD, or passage of MN, NANMN, or NAN to the duodenum, or MN/OMAD (efficiency of microbial synthesis). However, the trends were similar to McCarthy et al (53).

Nitrogen Sources

Nitrogen source and the degree of ruminal degradation play a major role in determining efficiency of microbial protein synthesis. Microbial protein synthesis and rumen OM digestibility will be depressed if the basal diet is deficient in N. Providing the proper amount and type of nitrogen to the rumen is primarily dependent on the source of feed protein selected.

Dietary protein can be partitioned into two pools that are soluble or insoluble in rumen fluid and into pools that

are degraded or undegraded by ruminal micro-organisms. Under practical feeding regimes, the extent of protein degraded is a function of the rate of proteolysis and retention time in the rumen.

Pichard and Van Soest (8) proposed that feed proteins are degraded at different rates. They classified proteins as soluble and insoluble proteins. The water-soluble nonprotein nitrogen fraction degraded rapidly and completely is referred to as fraction A. Ammonia, amines, free amino acids, and nitrates belong in this class. Fractions B_1, B_2, and C are considered insoluble. The rapidly degraded fraction (0-2h) is B_1, more slowly degraded fraction is B_2, and C is considered completely unavailable for digestion. The rate of rumen turnover will most greatly affect the rapidly degraded B_1 fraction. In situ measurements have become an accepted measure of determining the insoluble fractions. A discussion of factors affecting in situ measurements can be found in a later section of this review.

Several methods of determining fraction A have been employed: distilled and hot water, borate-phosphate buffer, 70% ethanol, sodium chloride, McDougal's artificial saliva, Burrough's mineral mixtures, and autoclaved rumen fluid (67). However, values for soluble N for the same feedstuff varies with solvent, thus selection of an appropriate solvent is difficult.

Primary sources of N for rumen bacteria are provided by fractions A and B_1. Almost all rumen bacteria utilize mostly ammonia; some, however, also utilize peptides (12) and amino acids (41). An estimated 50 - 80 % of MN is derived from the rumen ammonia pool (41). The remainder probably is obtained as peptides and amino acids from dietary or endogenous sources. Branched-chain C_4 and C_5 acids are required by some species for protein synthesis (60) but crossfeeding of microbial populations may meet this need.

The amount of ammonia required for microbial growth has been researched, modelled, and reviewed extensively (60). In vitro observations of Satter and Slyter (85) showed maximum microbial growth to occur when the ammonia concentration in rumen fluid reached 5 to 8 mg/100 ml. An in vivo observation consistent with Satter and Slyter indicated maximal protein synthesis was achieved when rumen ammonia reached 5 mg/100 ml. Other in vivo studies have indicated desired concentrations of 9 mg/100 ml (32) and 29 mg/100 ml (58). In a review by Hoover (31), a rumen-fluid ammonia concentration of 6.2 mg/100 ml appeared to be optimum when the dietary protein level exceeded 6% CP. These data consisted of a summary of 9 experiments with rumen ammonia ranges from 7 to 76 mg/100 ml rumen fluid. In general, the microbial requirement for ammonia is related to

substrate availability, fermentation rate, microbial mass, and yield (84).

Although rumen bacteria are considered efficient scavengers of ammonia and can grow at low concentrations, uncoupled fermentation could occur if the N level in the rumen becomes too low. This would result in fermentation without useful ATP production for microbial use (69). Rumen ammonia concentrations averaged less than 4 mg/100 ml (53) and about 2 mg/100 ml (38) in high producing dairy cows without any detrimental affect on MN synthesis. However, the amount of OMTD appeared to be more directly related to MN synthesis than concentration of ammonia in both studies.

If the N level is too high, energy may be the limiting factor for efficient utilization of N by the microbes (95). As increasing levels of corn gluten meal were fed to dairy cows consuming corn silage - alfalfa hay total mixed diets, rumen ammonia levels increased to 14.4 mg/100 ml. No difference was detected in OMTD in the rumen or MN flow, thus energy appeared to be limiting MN synthesis as levels of rumen undegradable protein increased.

In a survey of the dairy literature, Clark et al. (10) identified four studies designed to compare the effects of altering N intake on rumen ammonia concentrations and MN synthesis and flow to the duodenum. As expected, the relationship between CP content of the diet and the

concentration of ruminal ammonia was relatively high (r^2=.50) in the combined data sets. The relationship between the concentrations of rumen fluid ammonia and MN passage to the small intestine was very low (r^2=.08) when the concentrations varied from 2 to 30 mg/100 ml. This relationship implied that passage of MN to the duodenum is more highly correlated with OMTD than concentration of rumen fluid ammonia when the level exceeds 2 to 5 mg NH_3/100 ml.

A significant amount of nitrogen can be derived by rumen microbes from sources other than ammonia, such as amino acids and peptides. Energy usually limits microbial growth in the rumen; yet, underfeeding conditions utilizing large amounts of rumen undegradable protein, ammonia or other sources of N (amino acids, peptides) may become more limiting than energy.

Soybean meal and Blood meal as Protein Sources

Several studies have been reported that indicate lower MN synthesis with inclusion of the rumen undegradable protein supplement, bloodmeal. These studies compared bloodmeal-containing diets with those containing soybean meal as the primary protein supplement in vitro (2), cattle (37, 111) and sheep (7, 34). Feeding a more degradable source of protein (in this case, soybean meal) may provide more amino acids and peptides required for microbial growth

since microbial nitrogen flow was lower when bloodmeal was fed in comparison with soybean meal.

In vitro results reported by Bas et al. (2) indicated that diets containing blood meal had lower organic matter and fiber digestion, ammonia-N, VFA concentrations, and N degradation but higher NAN, dietary N, total AA, and essential AA flows than diets containing lignosulfate-treated soybean meal, feather meal, or soybean meal. All diets contained 1.4% of DM as urea. Combining lignosulfate-treated soybean meal and blood meal resulted in similar organic matter and fiber digestibilities compared with the soybean meal diets. Additionally, this combination was similar to the treated soybean meal in VFA concentration, NAN, and essential and total AA flows. These data indicated that blood meal limited MN synthesis although urea was included in the diet. Ammonia-N or other products of ruminal protein digestion may have been limiting MN synthesis when blood meal was included, yet the addition of the treated-soybean meal may have provided these needed products. A combination of blood meal and soybean meal also changed the AA composition by increasing histidine and leucine flows to small intestine.

Feeding supplemental protein with low ruminal degradability increased passage of NAN only when it composed 35% or more of the total dietary CP in dairy cattle diets

(10). The amount of NANMN flowing to the duodenum needs to exceed the amount of decrease in MN flow caused by feeding high levels of supplemental undegraded protein. When feeding protein of low ruminal degradability (fishmeal, bloodmeal, corn gluten meal, feathermeal, distiller's dried grains with solubles, or bloodmeal:fishmeal, bloodmeal:feathermeal, or dehydrated alfalfa and corn gluten meal) compared with feeding soybean meal higher in degradability, NAN flow was lower data from several dairy cattle experiments (10). This decrease was attributed to a decrease in the amount of MN provided to the small intestine. A lack of available N and energy synchrony probably decreased MN synthesis.

Synchrony of Ruminal Carbohydrate and Protein

Few studies have been designed specifically to evaluate varying carbohydrate and protein availabilities in the rumen for the appropriate synchrony to affect microbial growth. Differences in describing rumen available carbohydrate and protein fractions still exist between laboratories. Particularly, rates of degradability are poorly described. These differences in characterization probably are attributed to the variability in milk production and rumen fermentation responses to varying carbohydrate and protein sources.

In 1958, Lewis and McDonald (43) emphasized the importance of providing a carbohydrate that can be attacked by the microbes at a steady rate that is roughly comparable with that at which N becomes available in the rumen.

Researchers from West Virginia University consider the *ratio* of NSC to degradable intake protein (DIP) as a primary factor affecting maximum bacterial growth (30,97). Varga et al. (108) found that microbial growth decreased in vitro when the NSC:DIP exceeded 6.0. Bacterial efficiency (24 to 29 g MN/kg OMAD) increased nonsignificantly as the ratios narrowed to less than 3.4. The NSC level was 53% in these diets. A possible explanation was that an imbalance in NSC:DIP may have resulted in uncoupled fermentation, thus reduced microbial growth.

When diets were formulated with 3 levels of NSC (25, 37, 54% of DM) and varying levels of DIP in continuous culture (97), the NSC:DIP ratio where maximum bacterial efficiency occurred were those ratios less than 3.0. Across all levels of NSC, bacterial efficiency decreased as DIP decreased until NSC:DIP widened to greater than 8:1. Regardless of DIP level, bacterial efficiency (g MN/kg DM digested) was lower for diets containing 25% NSC. Apparently, available energy was limiting regardless of the availability of N sources when 25% NSC was used.

When the NSC:DIP concept was applied to lactating cattle diets, microbial efficiency was unaffected by diet (96) when NSC varied from 24 to 38% of the DM and DIP varied from 9.0 to 13.2% of the DM (49.9 to 73.3% of CP). The lowest flow of MN occurred when the diet containing low NSC (24%) and DIP (49.9%) was fed. Microbial N synthesis was maximized at the intermediate level, 31% NSC and 64.4% DIP. Enhanced MN flow was measured when NSC exceeded 24% and DIP was greater than 49.9% of the CP. However, total N flow was not different across diets. Milk production (>30.4 kg/d) and dry matter intake (>18.3 kg/d) did not differ regardless of the NSC:DIP. These data implied that synchrony of available carbohydrate and protein could maximize MN synthesis when the NSC:DIP ratio was less than 2.6 and at least 31% NSC and 49.9% DIP was provided. In turn, one would expect less supplemental rumen undegradable protein (RUP) would be needed for meeting the animal's amino acid requirements. The lack of a response in milk production may have indicated that some amino acids that were not provided by microbial or supplemental protein with these dietary combinations were limiting milk synthesis.

Likewise, when MacGregor et al. (45) fed diets differing in NSC (24.9 vs 32.9% of DM) and high in DIP (60 - 80% of CP), no differences in milk yield (>31 kg), fat-corrected milk, or solids-corrected milk were measured.

Milk production tended to be highest on the high NSC diet. Forage source was mixed timothy/legume silage and the concentrate contained primarily corn and corn byproducts (corn gluten feed, corn gluten meal, corn distiller'dried grains, hominy, corn solubles, corn grain, potato pulp, wheat bran, soybean meal, brewers dried grains, oats grain, and wheat midds).

Both the high (32.9%) and low (24.9%) levels of NSC and high level of DIP fed by MacGregor et al. (45) may have provided adequate carbohydrate and N for optimal MN synthesis. These levels were above the 24% NSC (% of DM) and 49.9% DIP (% of CP) suggested by Stokes et al. (97) for enhancing MN flow. Theoretically, these diets may have been low in dietary lysine because of the high proportion of corn products in the concentrate. However, MN typically contains a relatively high level of lysine, thus lysine flow to the duodenum may have increased (86). Yet, amino acid determinations were not conducted in this study.

A lack of a response in milk production and composition due to changes in dietary NSC and DIP had also been reported by others. Casper and Schingoethe (6) varied NSC by providing dried whey (high carbohydrate and protein degradability), rolled barley (high carbohydrate degradability and solubility), or ground shelled corn (low carbohydrate degradability and solubility) in combination

with rapidly available N (urea) or a less rapidly available N supplement (soybean meal). The synchrony of carbohydrate and protein availability did not influence milk production. In a second study (5) in which the same supplements were fed except the dried whey diets, again no differences in milk yield were detected as a result of carbohydrate-protein synchrony. In both experiments, DMI was decreased by inclusion of barley in the diets. In turn, milk production was higher in the corn-based diets.

Likewise, DMI was lower in barley-based as compared to corn-based diets, regardless of protein source (fishmeal or soybean meal) (53). Again, the higher DMI of corn diets compared with barley resulted in increased milk production with the corn-based diets. No protein x carbohydrate affect on milk yield was reported. Microbial nitrogen efficiency (g MN/kg OMTD) was also unaffected by carbohydrate or protein sources.

However, when the NSC sources fed were barley (high starch degradability, 90.5%) and milo (low starch degradability, 70.5%) in combination with either cottonseed meal (high N degradability, 56.6%) or brewers dried grains (low N degradability, 65.0%), the barley-cottonseed meal diets resulted in highest milk production (28). The barley-cottonseed meal diet (27) was also highest in efficiency of microbial protein synthesis (expressed as g MN/kg TFOM).

Barley diets caused higher flow of MN passing to the duodenum than milo diets, regardless of protein source. The increased milk production may have resulted from the stimulated MN yield caused by synchronization of the rumen degradable starch and protein from barley and cottonseed meal.

While actual differences in milk production due to changes in dietary NSC and DIP have been difficult to find, US patents have been issued for quantitating NSC and SC and further utilizing these in dairy cow ration formulations (64,65). Use of this patented procedure has resulted in significant increases in milk production (68), yet supportive research has not been reported. However, when milk production responses were pooled from 15 nutrition studies from cows producing > 30 kg milk, "optimal" concentrations were predicted: 78% carbohydrate (CHO = NSC + NDF), 53% RAC (as a percent of CHO intake), 15.6% CP, and 66% rumen available protein (RAP). The average 8.5 kg/d rumen available carbohydrate (RAC) intake was similar to the amount predicted by these author's theoretical rumen model for maximum yield of microbial protein (66). The use of RAC differs from NSC by including an availability value of the SC in addition to considering NSC.

Actual milk production response to rations balanced for RAC and RAP may vary due to the variations in chemical

analyses characterizing the feed nutrients, variations in in situ results from cows at high and low intakes, variations due to feed sources, and animal variations (parity, stage of lactation).

In Situ Determinations of Feedstuffs

The in situ bag technique has received extensive evaluation as a technique for estimating contribution of feed protein and carbohydrate to the rumen. This technique involves the suspension of feed materials in the rumen of a cannulated animal to allow contact of the test feed with the ruminal environment (proper pH, buffer, temperature, enzymes, microbes). Mastication, rumination, and feed passage are not simulated by this technique.

The key to the usefulness of this technique is the standardization of the procedure among laboratories. Some key factors that must be considered include bag porosity, particle size, sample size to bag surface area, dietary and animal effects, and microbial contamination. Nocek (67) presented an excellent review of how these variables affect the accuracy of the nylon bag technique. His recommended guidelines focused on the variables that could be standardized between laboratories (Table 1).

Another variable that could easily be standardized between laboratories not considered by Nocek (67) is that of

the position of the bag in the rumen. Stritzler and coworkers (98) compared dry matter disappearance of barley straw or rye grass hay in nylon bags attached to cords of 25, 40, 75, or 105 cm suspended from the rumen cannulae. Increasing cord length increased the dry matter disappearance from the bags as well as microbial contamination in the bags. As an example, barley straw incubated for 24 h increased dry matter disappearance from 12.1, 16.2, 20.3, to 31.9% as cord lengths increased from 25, 40, 75, to 105 cm. Shorter cord lengths decreased motility of the bag within the digesta. Additionally, colonization of microbes was decreased when the bags were limited to the dorsal sac. The authors recommended a need for standardization of cord length. Orskov (71) suggested that the cord length be at least 50 cm to permit free movements of the bag and to allow the bags to be squeezed by ruminal contractions.

Several dynamic mathematical models that incorporate several components of ruminal digestion have been developed (40,51,71,106,109). These models allow for kinetic interpretation of data in terms of nutrient availability in the rumen.

Table 1. Recommended guidelines for ruminal in situ digestion procedure (Adapted from Nocek (67)).

VARIABLE		RECOMMENDATION
A.	Bag porosity	40 to 60 um
B.	Particle size	Protein, energy suppl., 2mm Whole grains, 5mm Fibrous by-products, 5mm Hays (>80% DM), 5mm Silages (air or freeze-dry), 5mm
C.	Sample size to bag surface area	10 to 20 mg/cm^2
D.	Microbial contamination	Use correction, regardless of marker (DAPA, RNA, N^{15}, S^{35}, etc.)
E.	Diet	Meet nutrient requirements Document ingredient composition Include test ingredient in basal Feed a total mixed ration, ad-lib
F.	Animal/period	Use test animal that matches animal for which determinations will be applied Replicate at least twice if only one test animal used For each animal and period, insert bags at the same time in relation to feeding
G.	Preruminal incubation	Soak bags in water or buffer (39C) prior to ruminal incubation
H.	Bag insertion	Insert at specific time intervals and remove as a group
I.	Postruminal washing	Rinse in tap water until rinse water is clear
J.	Incubation times	0 to 6 h: 3 to 6 time points 6 to 24 h: 3 to 6 time points >25h: 6 to 12-h intervals
K.	Expression of results	Estimated ruminal availability as suggested by Orskov and McDonald (71) or Van Soest et al. (106)

Orskov and McDonald (71) calculated protein degradability (D) as a percent with the following equation:

$$D = A + (B \times k_d)/(k_dN + k_r)$$

when D = protein degradability (%);

A = readily degradable protein fraction (%);

B = protein fraction degraded at a measurable rate (%);

k_d = protein degradation constant of B fraction;

k_r = rumen turnover rate (.05/h).

Madsen and Hvelplund (49) found a close relationship between the in situ estimates determined by the nylon bag technique and in vivo degradation, especially when a rumen turnover rate of .08/h was used in the model. Others have also reported good agreement between the two measurements (51,114).

In vivo Digesta Markers

Measurements of postruminal nutrient flow and digestion requires the use of animals fitted with cannulae at specific sites in the tract (abomasum, proximal duodenum or terminal ileum). Also, a reliable method for calculation of flow rates at these sites is needed. Two cannula types are commonly used: the re-entrant cannulae and the simple T-type cannula. Use of each cannulae has been reviewed and advantages and disadvantages exist for each (48,103). The re-entrant allowed for total digesta collection, thus

eliminated the need for digesta phase markers (67). The T-type cannula require spot sampling and indigestible solid- (or particulate) and liquid-phase markers (71).

Although the in vivo determinations of nutrient flow and digestion are used as the standard of comparison, disadvantages to this system exist. First, digesta flow is contaminated with endogenous proteins that usually are not measured. Tamminga et al. (101) corrected the difference of total N and MN at the duodenum using an endogenous N estimate of 4 g N/kg of DM. Second, digesta flow is inherently intermittent thus representative sampling of the digesta is difficult. Extreme variation in digesta and microbial marker estimates have also been reported (56,67). The coefficient of variation for digesta flow measurements was 5 - 20%, and 50 - 90% of the variation was attributed to between animal differences as determined by MacRae (48). Third, limited numbers of animals are usually used and the between-animal variation is high (67,56). Sutton and Oldham (100) determined that to detect a 10% difference in treatment responses using in vivo procedures, two 6x6 Latin square design experiments would be needed.

Advantages and disadvantages also exist for the use of various digesta marker systems in ruminants. Markers have been used as experimental tools for many years and a large number of materials have been evaluated as markers for

studying digestion in animals (56). The "ideal" marker should: be inert without toxic effects; unabsorbable and nonmetabolizable in the tract; nonbulky; mix and distribute uniformly within the digesta; have no influence on gastro-intestinal function or secretions; have no influence on the microflora of the tract; and have physico-chemical properties that can be measured quantitatively (56). None of the materials currently utilized as digesta markers can be considered "ideal".

Ellis (14) suggested that dual-phase marker systems should be used since the digestive process in ruminants is considered to be a two compartment system. In two marker systems, one inert marker is selected to estimate liquid flow and another inert marker is selected to estimate solid or particulate flow.

Commonly used liquid flow markers include polyethylene glycol (PEG), cobalt ethylenediaminetetraacetate (CoEDTA), and chromium ethylenediaminetetraacetate (CrEDTA). CoEDTA and CrEDTA are easily measured using atomic absorption. However, quantitation of PEG is difficult with a high degree of error (112). Additionally, PEG may adsorb to certain dietary ingredients. CoEDTA and CrEDTA have largely replaced PEG as liquid phase markers of choice (56).

Particulate, or solid-phase markers used most widely include chromic oxide, rare-earth elements (La, Am, Ce, Yb,

and Dy), and chromium-mordanted fibers. Chromic oxide has been more widely used as a digestibility and digesta flow marker than any other (56).

Many studies have indicated almost complete chromic oxide recovery in feces of sheep (47, 73). However, disadvantages exist for the use of chromic oxide. Chromic oxide is a dense powder which travels in suspension in digesta at a rate sometimes independent of either the particulate or liquid phases (56). Because of its density, it may form a sediment in the bottom of the rumen resulting in sporadic flow to the lower gastrointestinal tract. Like many digesta markers, chromic oxide is subject to diurnal and daily variation. Frequent marker administration and digesta sampling is needed to account for these variations in excretion. The use of chromic oxide for measuring total tract digestibility has been well accepted (56). However, its use as a marker for measuring flow rates in animals spot-sampled from simple T-type cannulaes has been questioned because of the questionable use of any single marker (vs a dual marker system) with simple T-type cannulae. Faichney (17) suggested that samples from simple T-type cannulae may not be typical of normally distributed liquid- and solid-phase digesta. Use of dual phase markers and analyses of both phases would help correct for this nonhomogeneity (1). Others indicated that calculated

digesta flows obtained using chromic oxide as a marker in spot sampling were in close agreement with those obtained by total collection utilizing re-entrant cannulae (11,82).

Rare earths are excreted quantitatively and methods of detection are readily available. Some have suggested that rare earths may be inappropriate particulate markers because the degree of association with particulate digesta is variable and migration to unlabelled feeds has been detected (26).

The use of Cr-mordanted fiber is gaining wider acceptance as a solid-phase digesta marker. "Mordanting" forms a strong complex between chromium and plant cell walls (104). This complex has been shown to be stable in rumen fluid and acidic media and is essentially indigestible when Cr content of the complex is greater than 8% (14). A potential drawback to this type marker is the possibility that its density may not allow it to behave similar to the other feed particles in the diet (14).

Numerous markers for measurement of microbial yield and flow in vivo have also been suggested. The microbial markers include DNA, RNA, DAP, AEP, nucleic acids, and isotopes (49) that can incorporate into the microbes (^{35}S,^{15}N,^{32}P). Stern and Hoover (95) discussed the advantages and disadvantages of these markers in a review.

The use of purine and pyrimidine bases, rather than nucleic acids in the polynucleotide form, as microbial markers have also been reported and evaluated. The major obstacle to the use of nucleic acids as a microbial marker has been the lack of simple, laboratory procedures for isolating and quantifying the nucleic acid content of feeds and digesta.

Quantitative recovery of purine and pyrimidine bases was first reported by Marshak and Vogel in 1951 (50) with a perchloric acid hydrolysis procedure. However, the procedure was tedious and recovery was low. Jackson (39) modified the perchloric acid hydrolysis procedure to quantitate nucleic aid content of digesta by high pressure liquid cation exchange chromatography (HPLC). The modified procedure improved accuracy, precision, and rapidity of analysis. Jackson's procedure was further modified by Koenig (39) to improve the precision and ease of the analysis.

Koenig (39) indicated that cytosine, adenine, guanine, uracil, and xanthine all appear to be acceptable markers for indicators of microbial protein synthesis. Hypoxanthine and thymine were the least stable over time and the most difficult to analyze by separation. Shelling (89) noted that cytosine and adenine should be considered the bases with the greatest potential as indicators.

The use of a base as a valid marker must assume that dietary nucleic acids are completely digested in the rumen. This is unlikely, yet Koenig (39) suggested that any errors due to dietary cytosine would be small. In vivo work (39) indicated no statistically significant amounts of dietary cytosine were present in sheep abomasal digesta at 0, 6, 9, 12 or 15h post-feeding. At 3h, 14.1% cytosine of dietary origin was detected when finely ground, alfalfa hay was fed. Alfalfa hay was found to contain 7 - 10 times more nucleic acids per unit weight than corn in this study. Further analysis of feed cytosine content and degradation in the rumen are needed (89).

Schelling (89) suggested that because cytosine has been shown to degrade rapidly in the rumen, it would be a more desirable indicator compared to adenine. With low survival in the rumen, cytosine would be the least likely to cause erroneous estimations due to its presence in undigested feedstuffs. Koenig (39) developed a prediction equation from 168 observations for the prediction of microbial nitrogen from cytosine: ug MN = 1.838 (nM cytosine) - 3.374, r = .92.

Although the cytosine to MN ratio as a technique for estimating MN flow from the rumen is not ideal, it appears to be accurate with a minimal expenditure of effort on sample preparation and analysis (90). Its lack of greater

widespread use may be the lack of equipment for HPLC analyses in many laboratories (113) and the lack of access to the written procedure since it has not been published in a refereed journal.

The lack of an "ideal" digesta or bacterial marker combined with inherently high between-animal variation in in vivo studies consequently results in great potential for cumulative error. Without standardized procedures for these studies, it is difficult to compare nutrient flow data between laboratories, between animal species, and across feed types.

Production Responses of Primiparous Cattle

Primiparous cattle tend to respond differently from mature cows when varying planes of nutrition are compared. Oldham (69) attributed these differences to decreased secreting capacity and an increased metabolic drive to achieve mature size. This increased metabolic drive for growth is not present in mature cattle. Increased partitioning of nutrients toward growth (tissue gain) corresponds to the decreased milk production often reported when comparing primiparous with multiparous cattle.

Increasing levels of CP in diets resulted in higher dry matter intake in mature cows but not in heifers (13,79,81). A concomitant increase in milk yield was reported in cows,

not in heifers (13,79,81). In these studies, rations were not fed as complete diets. When CP level was decreased from 16 to 13 %, milk production declined in mature cows but remained steady for heifers (81).

In studies designed for primiparous cattle only, mixed responses were again reported due to varying nutrition parameters. In general, differences in intake dictated milk production responses. No differences in milk production were detected when heat treated soybeans replaced raw soybeans to decrease N solubility in rations (42). Fat-corrected milk was higher with higher CP (20.7 vs 15.4 %) regardless of solubility (22.8 and 53 % of CP). The greatest response in FCM occurred during wk 2 through 6 of lactation. When actual CP intake was compared to NRC recommendations (61), CP intake was above requirements during this time. During the next five weeks of the experiment when DMI was restricted to the level of those consuming low protein diets, differences in FCM were smaller. Intake as a percent of requirement was similar across diets during the latter period.

Likewise, when milk yield was adjusted for intake of diets formulated to contain 12, 14, or 18 % CP, no difference in milk yield was detected (46). However, a linear increase in dry matter intake, fat-corrected milk, and milk fat yield occurred in response to increasing

dietary protein (not adjusted for dry matter intake). When milk yield was adjusted for dry matter intake, milk yield increased with greater energy density as forage:concentrate changed from 75:24, 55:45, to 35:65. No interactions of energy x protein were reported for production measurements.

Protein degradability may have influenced intake and intake may have influenced protein degradability in these studies. A good explanation of this concept was described by Newbold and coworkers (63). If a deficit of rumen degradable protein occurred, increased dietary degradable protein concentration should lead to increased intake. With increased intake, the degradability of each additional input of degradable protein declines, so that the increments of rumen degradable protein, and its positive effects on intake, are progressively reduced until an equilibrium is achieved. However, as energy becomes limiting in the rumen, the positive effect on intake of added rumen degradable protein will cease. These combined factors affect the amount of undegraded protein needed to complement the decreased microbial protein flow when intake is lowered.

In addition to differences in milk production responses between primiparous and multiparous cows, changes in body weight also differ significantly. In both primiparous studies previously cited (46,42), body weight increased over the experimental period. Similarly, higher protein rations

resulted in less body weight loss in early lactation in heifers but not in cows, with a subsequent recovery of body weight in both (80). Heifers receiving high protein, low degradability diets lost less weight in early lactation; whereas, all heifers gained weight from wk 7 through 11 of lactation. These responses were similar to those observed by Roffler and Thacker (80). Cressman et al. (13) reported an overall gain of 26.2 kg in primiparous cattle and a loss of 6.8 kg in mature cattle during the first 13 wk of lactation. Ultrasound measurements of loineye fat cover also differed as heifers decreased .11 cm compared with an average loss of .41 cm in adult cattle. These differences indicated a larger partitioning of nutrients toward muscle and fat deposition in heifers, rather than increased milk output found in the mature cows.

Overall, data from studies evaluating first-lactation performance are limited. The recommendations of NRC (62) include an increase in maintenance requirements by 20 percent for all nutrients except vitamins A and D. However, studies were not cited to verify these recommendations. Further research is needed to identify nutrient requirements of growing animals during first lactation.

Response Surface Methodology

Response surface methodology (RSM) is a statistical means of finding the combination of levels of two or more factors that will simultaneously produce an optimum response (maximum or minimum) (21,22,55,59). It may be used for specification of a region of treatment combinations in which certain practical requirements desired by the experimenter are met (22). It evaluates multi-factor dependence by determining how factors jointly influence the response (55). RSM can indicate if variables, when changed together, will cause a response even though no change may be measured when only one of the variables are considered.

Box and Wilson (3) are credited with the initial development and description of RSM in 1951. However, many of the fundamental ideas were reported as early as 1929 as noted in a review by Mead and Pike (55). Box and Wilson designed experiments to find the point on a response surface at which the maximum output, or yield, could be achieved with the smallest possible number of observations. They were the first to introduce the "central composite" design.

A "central composite" design can be used when adequate knowledge is available to identify a specific area within the composite that merits study. After identification of this point, the design would be symmetrically placed with respect to that region (3).

Response surface designs are usually expansions of factorial designs or may be simply geometrical patterns of design points that allow for the combining of levels of specific treatment factors of interest (22). The advantage of RSM over other factorials is that combinations of factors are evaluated simultaneously. By allowing for measurements of responses to synergistic effects, the RSM could save an experimenter considerable money and experimental effort compared with varying only one factor at a time (21).

In RSM, the dependent variable is considered to be a *response* to a treatment (a combination of variables), whereas, the *surface* is a graphical depiction of the response represented by a height above the plane. Gardiner, et al. (21) explained the *surface* with an example using an experiment in which the treatments were a combination of two factors, X_1 and X_2. They described X_1 and X_2 as being within the same plane, but X_1 was measured on an axis perpendicular to X_2. Any point in this plane would represent a combination of X_1 and X_2, or treatment combination. By erecting a scale perpendicular to this plane of X_1 and X_2, a response designated as Y could be measured. Thus, the response to any treatment was represented by a height above the plane. The *surface* would be described when one considered all of the points in that plane. Thus, response surface experiments are "three-dimensional" factorials in

which two or more factors could be considered simultaneously.

Briefly, the mechanics of RSM involves selection of a mathematical model, estimation of regression coefficients by least squares procedures, followed by the analysis of the fitted surfaces (3). Location of the stationary points (SP) where the derivatives are simultaneously equal to zero are determined (59). To find the nature of the SP, the regression equations are transformed to canonical form to reduce the number of parameters by including only quadratic terms (59).

Interpretation of the RS curve is determined by observing the sign and magnitude of the latent roots of the canonical matrix. If both latent roots are negative, responses at the SP are assumed maximum ("peak"). If both latent roots are positive, responses at SP are assumed minimum ("sink-hole"). If both positive and negative occur, then a saddlepoint or minimax is identified (3,88,59). If the sample responses miss one maximum or minimum because of poor choice of levels for one or more factors, the resulting RS may be a "rising ridge" or "sinking valley" (22). In this case, one of the latent roots would be zero or near zero and the change in response would be small for changes in the level of the corresponding factor (59). Ranking of the absolute value of the latent roots corresponds to the

rank of the variable with most influence on the response (88). In SAS, the RSREG procedure (94) produces output with the term "eigenvalue" instead of "latent root", yet these terms are synonymous.

A specific limitation of the RSM as indicated by the data reported by Sharma et al. (87), is that RSM may not always provide the values that lie in the experimental region or within biological function. In this study (87), Holstein and Jersey records (n=11,092 and 11,120) from the University of Florida Dairy were pooled and adjusted for genetic and environmental effects to study the variation accounted for by stage of lactation and stage of pregnancy in 17 milk yield and milk constituent traits. Response surface methodology was used to characterize the combinations of effects of stages of lactation and pregnancy for optimal milk yield and milk constituent production. Analyses of milk yield data showed the SP for Holsteins at maximums of 49.9 and 14.6 mo for stages of lactation and pregnancy. These values fall beyond normal lengths of lactation and pregnancy for cattle. Overall, the RSM was a simple and effective procedure to measure the detectable interactions between effects of stage of lactation and stage of pregnancy in this study, yet it demonstrated that RSM does not guarantee that SP values will fall within the experimental region. Users of RSM must be aware of the

unreliability of extrapolation outside the range of independent values that may lead to impossible predicted values.

In a review by Mead and Pike (55), the importance of the presentation of response surface data were stressed. They reported several citings in which data was presented as means with Duncan's Multiple Range Test utilized for means separation. They felt these cited workers had "obscured the pattern of response remarkably successfully". These reviewers noted that one of the benefits of using RSM (when looking for a pattern of yield response to varying levels of a quantitative factor) is the expression of that pattern through a graph or fitted response surface instead of identifying only tests of significance. These authors concluded that RSM can be used successfully in helping to unravel complex biological responses, even though the RSM was originally developed for use in the chemical industry.

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Chapter 2

INFLUENCE OF RUMEN AVAILABLE CARBOHYDRATE AND RUMEN UNDEGRADABLE PROTEIN ON GROWTH AND MILK PRODUCTION OF PRIMIPAROUS HOLSTEIN CATTLE

(ABSTRACT)

A central composite, response surface experimental design was utilized to evaluate the effects of varying dietary rumen available carbohydrate (RAC) and rumen available protein (RAP) on milk production and growth in 54 primiparous Holstein cattle from wk 4 through 15 of lactation. Nine isonitrogenous diets contained (% of DM) 31 % alfalfa silage, 17 % corn silage, and 52 % concentrate. Rumen available carbohydrate varied from 67 to 73 % of DM by replacing cracked corn with ground barley. Rumen available protein varied from 73 to 59 % of CP as blood meal replaced soybean meal in the total mixed diets. The response surface was saddle-shaped for all milk production variables, thus maximum responses were predicted from ridge analysis. Decreases in RAP caused the greatest influence on each response. Predictions for maximum milk and fat-corrected milk yield indicated that levels of RAP below 59 % of CP (blood meal) and RAC below 69 % of DM (ground barley) were needed in diets of an alfalfa-corn silage base. Milk composition, component yields, and growth were less responsive to changes in RAC and RAP within the narrow range of RAC and RAP intake provided by our design.

Introduction

Traditional methods for determining carbohydrate or protein requirements have included factorial dietary arrangements, rather than an integrated scheme of dietary factors. Because the rumen is the primary site of carbohydrate and protein digestion in dairy cattle, the action and interaction of these two nutrients in the rumen need to be evaluated simultaneously.

Early work has focused on these components as individual nutrients, yet little research has attempted to describe their interaction. If there is a deficiency of available carbohydrate to match available protein, excess N will be absorbed across the rumen wall, assimilated into urea, and excreted in the urine. If there is a deficiency of available protein to match available carbohydrate, the digestibility of carbohydrate may decrease. In order to study a range of protein and carbohydrate combinations on ruminal function and animal productivity, a response surface, central composite design is useful. This design would allow for response measurements resulting from changes in several factors simultaneously. Resulting maxima and minima can be calculated.

Characterization of both rumen available carbohydrate and protein is difficult. Recently, attempts at defining

the carbohydrate fraction have been made (4,15,27) particularly the nonstructural and available carbohydrate. This entity has been described most commonly as NSC (nonstructural carbohydrate, determined enzymatically or calculated by difference), TNC (total nonstructural carbohydrate, enzymatic procedure), or NFC (non-fiber carbohydrate, calculated by difference).

One of the limitations of these fractions is that they do not consider the degradability of the structural carbohydrates. A proposed system to include both structural and nonstructural carbohydrate availability was suggested by Nocek and Russell (27). In their system, structural carbohydrates are defined as NDF; nonstructural carbohydrates as the components that are not measured as CP, NDF, and EE; and availability of the NDF fraction determined in situ.

Ruminal availability of protein (RAP), whether soluble N or degradable N, has been more extensively reviewed than carbohydrate availability (34,38). The degradability of protein is commonly determined from ruminal in situ incubations. However, differences in methodology for determinations of protein degradabilities still exist (25, 26). In vitro determinations have also been developed (12). The undegradable protein fraction is calculated as 100-RAP. It has been described as UIP (undegraded intake

protein), RUP (rumen undegraded protein), BYPASS (protein that bypasses the rumen undegraded), and ESCAPE (feed protein that escapes ruminal degradation).

The objective of this study was to find the optimum combination of both ruminal available carbohydrate (RAC) and protein (RAP) in diets of primiparous heifers for maximum milk production and growth. A response surface design was used to evaluate the responses to simultaneous changes of RAC and RAP.

Materials and Methods

Experimental Plan

Fifty-four, first-lactation Holstein cows were assigned to nine diets (6 cows/diet) in a response surface, central composite design (Figure 1). Diets (Table 1) varied in rumen available carbohydrate (RAC) as a percent of carbohydrate from 67.7 to 72.3 percent, and rumen available protein (RAP) as a percent of crude protein from 58.6 to 73.0. Rumen available carbohydrate was calculated according to Nocek and Russell (27) as follows:

$$RAC = \frac{[.9((NDS - (CP + EE)) + (NDF \times NDF\ availability)]}{[NDS - (CP + EE)) + NDF]}$$

where NDS = (100 - NDF). Neutral detergent fiber availability was determined by in situ ruminal incubations. Residues were corrected for microbial nitrogen contamination before calculation of crude protein availability.

Variations in RAC (Table 2) were made by substituting cracked corn (CC) with ground barley (GB) at the following increments: 100% CC; 75% CC, 25% GB; 50% CC, 50% GB; 25% CC, 75% GB; 100% GB. Dietary rumen available protein was decreased by replacing soybean meal (SBM) (fast protein availability) with blood meal (BM) (slow protein availability) so that dietary CP was approximately 18%.

Table 1. Chemical composition of diets varying in RAP and RAC.

	Diets									
Component	ADJ[1]	1	2	3	4	5	6	7	8	9
DM, %	60.1	62.9	62.2	62.7	62.4	62.8	62.6	62.6	62.9	61.7
CP, % DM	18.9	18.4	18.1	18.3	18.0	18.3	18.1	18.0	18.3	18.3
RAP, % CP[2]	66.4	65.4	62.6	67.2	58.6	65.2	73.0	62.0	67.5	64.0
CHO, % DM[3]	73.9	73.2	72.6	73.1	73.8	72.9	74.9	72.7	73.1	73.1
RAC, % CHO[4]	70.8	72.3	71.7	71.7	70.1	70.2	70.7	69.2	69.5	67.7
NSC, % DM[5]	39.9	47.3	45.9	45.7	46.2	44.6	46.2	43.5	43.1	41.5
NDF, % DM	34.0	25.9	26.7	27.4	27.6	28.3	28.7	29.2	30.0	31.6
ADF, % DM	18.0	18.5	19.5	19.4	19.7	19.9	19.9	20.6	20.6	21.7
NE_l, Mcal/kg	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.6	1.7
Ca, % DM	.79	.78	.79	.78	.76	.76	.78	.79	.75	.76
P, % DM	.44	.44	.42	.44	.40	.43	.43	.41	.43	.42

[1] ADJ = adjustment diet fed during pre-experimental period

[2] RAP, % CP = rumen available protein as a % of crude protein

[3] CHO, % DM = NSC + NDF

[4] RAC, % CHO = rumen available carbohydrate as a % of total carbohydrate

[5] NSC, % DM = 100 - (CP + NDF + EE + ASH)

Table 2. Ingredient composition of experimental diets.

Ingredient	ADJ[1]	1	2	3	4	5	6	7	8	9
					% of DM					
Alfalfa silage	31.1	30.3	31.6	30.9	31.0	31.0	31.0	31.0	30.5	31.7
Corn silage	17.6	17.0	17.4	17.4	17.6	17.5	17.1	17.6	17.0	17.7
Soybean meal	17.4	13.0	8.5	13.2	4.0	11.4	7.6	5.5	12.5	7.6
Blood meal	.0	.8	2.1	.4	3.9	1.6	.0	2.9	1.1	2.5
Urea	.0	.0	.0	.0	.0	.0	.8	.0	.0	.0
Cracked corn	32.5	37.5	28.5	27.2	21.0	19.0	21.0	9.5	8.5	.0
Ground barley	.0	.0	10.5	9.5	21.0	19.0	21.0	32.0	30.0	39.0
Vit-Min	1.4	1.4	1.4	1.4	1.5	1.5	1.5	1.5	1.4	1.5

[1] ADJ = adjustment diet fed during pre-experimental period

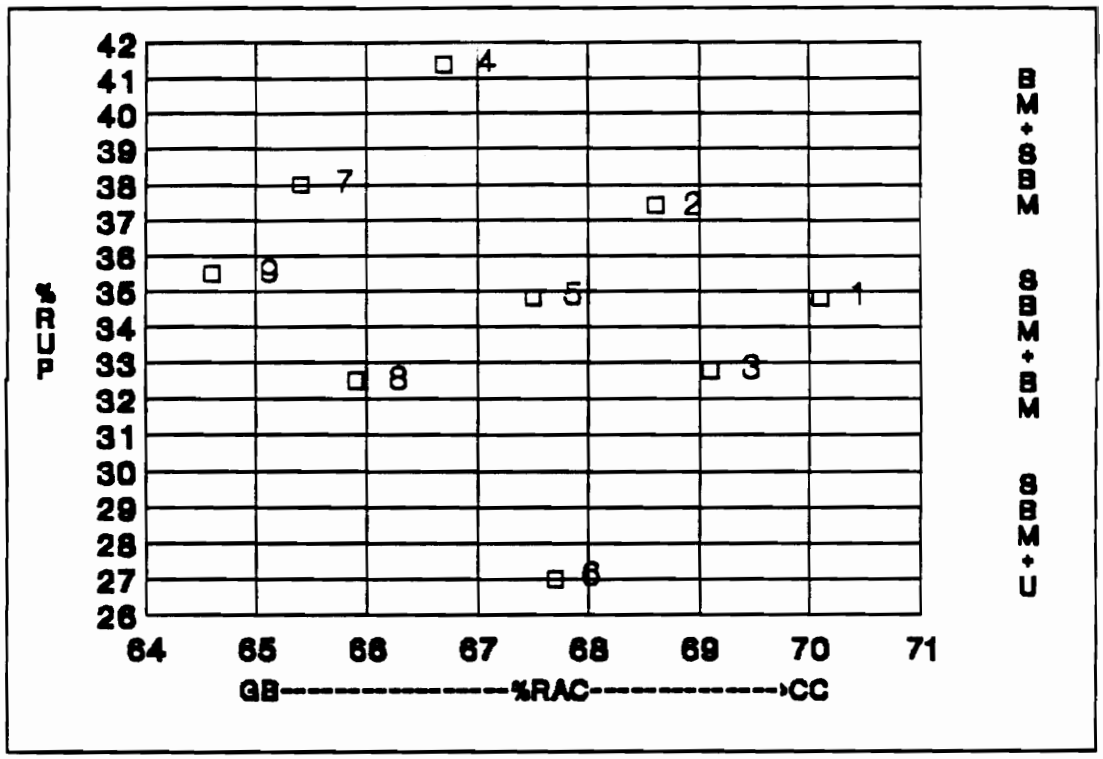

Figure 1. Arrangement of experimental diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC) in a response surface design. B=ground barley, C=cracked corn, BM=blood meal, SBM = soybean meal, NPN = urea

In the diet formulated for the highest RAP, supplemental protein included NPN (urea) and SBM. The carbohydrate and protein sources were chosen initially based on in situ incubations showing large differences in rates of DM and CP disappearance.

The forage to concentrate ratio was 47:53 with approximately 65% of the forage as alfalfa silage (AS) and 35% as corn silage (CS) on a dry matter basis. Only the concentrate sources varied between diets. Diets were formulated to meet or exceed NRC recommendations (24) for first-lactation animals averaging 500 kg BW and producing 32 kg milk containing 3.6% fat.

Animal Management

Animals were fed an adjustment diet similar to diet 1 (Table 1) from calving through d 21 of lactation. The study began September 25 and ended April 28, yet more than seventy percent of animals received experimental diets from October through January. Because of limited facilities, animals were housed in two locations at the Virginia Tech Dairy Center. Animals were blocked across treatments within locations. The first eighteen animals to calve (two per diet) were housed in a free stall area and were individually fed via Calan doors (American Calan, Inc., Northwood, NH). As they calved, the next group of eighteen animals (two per

diet) were housed in a tie stall barn with individual mangers. The last group of eighteen animals (two per diet) were housed in the free stall area with access to Calan doors.

Animals were selected from the three genetic populations of the Virginia Tech herd (Control, Selection, College). Two animals from each herd were assigned to each of the nine experimental diets. The Control genetic herd was established in 1968 and has been maintained as a herd with zero genetic improvement. The sires for the Selection and College herds are selected from the top ten percent of U.S.D.A. proven sires for production and type improvement. Since the animals on this study had no previous lactation, assignment to treatment was random with the restriction that equal numbers from each herd and feeding location be represented in each treatment. In the original plan, milk production during wk 2 and 3 of lactation was to be used as a covariate. Training to the Calan doors and to the tie stalls during this period resulted in wide variations in intake which adversely affected milk production in many animals. Therefore, a reliable covariate estimate for milk production was not attained. However, genetic herd was used as a covariate because production was consistently lower ($P<.01$) in the Control herd.

Sample Collection and Analyses

Animals began dietary treatments on d 22 of lactation and remained through 105 d in milk (DIM). Animals were fed a total mixed diet at 0600 and 1500 h daily. Refusals were weighed and recorded at 1000 h Tuesday through Friday. Total mixed diets and individual ingredients were sampled three times per week and were stored at 4° C until composited by month. Individual ingredients were analyzed for DM, ASH, CP, EE (1), NDF (41), and ADF (13) in the Virginia Tech Forage Testing laboratory.

Animals were milked twice daily at 0100 and 1300 h. Duplicate milk samples for component analysis were taken at consecutive PM/AM milkings at 28 ± 4 d intervals. Milk fat, protein, lactose, and solids-not-fat content were determined by a four-channel spectrophotometer (Multispec Mark I, Foss Food Technology, Eden Plains, MN) in the Virginia Tech DHI laboratory. Lactose and solids-not-fat are not reported because of problems with sample storage and analysis. Milk yield was recorded daily and weekly averages calculated.

Body condition scores were assigned at 28 d intervals (21, 49, 77, 105 DIM) by a team of three trained scorers. The Virginia Tech Body Condition Score scale, modified to include tenths, resulted in possible scores from 1.0 - 5.0 as determined by visual observation. A score of 1.0

indicated very thin body condition, whereas, a score of 5.0 indicated very fat body condition. An average score was calculated per cow per scoring session. On the same day, at 1800 h, samples of jugular vein blood were drawn into heparinized tubes for analysis of plasma urea nitrogen. Samples were centrifuged for 15 min at 5000 x g. The plasma was frozen at -20° C until analyzed by the procedure of Coulombe and Favreau (9) for plasma urea nitrogen.

Body weights were recorded weekly immediately following milking at 1300 h. At 28 ± 4d intervals (21, 49, 77, 105 DIM), wither and hip heights were measured while animals were standing on the scale.

Ruminal Availability Determinations

Carbohydrate sources, including cracked corn, ground corn, high moisture shelled corn, cracked barley, or ground barley, were evaluated in situ as potential sources of high and low ruminally available carbohydrate prior to the initiation of the lactation study. A second set of in situ incubations included blood meal, soybean meal, dried brewer's grains, and corn gluten meal as potential rumen available protein sources. In situ incubations were performed in three mature, rumen-cannulated cows (two lactating, one non-lactating). Each set of incubations was

replicated 10 days following the first series using the same test animals.

The two lactating animals averaged 19.1 kg/d DM intake, 590 kg BW, 22 kg milk yield, and 225 DIM. Animals consumed a total mixed diet containing 24% alfalfa silage, 54% ammoniated corn silage, 15% high-moisture shelled corn, 6% soybean meal, and 1% herd mineral at 0700 and 1500 h. Chemical analysis indicated diets contained 55.7% DM, 16.5% CP, 19.6% ADF, and 1.5 Mcal NE_l/kg DM.

The in situ procedure followed the guidelines of Nocek (25, 26). Bags (10 x 20 cm) were spun of dacron polyester (59μm) and were filled with approximately 5 g DM for a sample size to bag surface area of 14.9 mg/cm^2. Prior to incubation, dry barley and corn grains were processed in a hammermill fitted with a 3.2 mm screen ("ground") or a 9.2 mm screen ("cracked"). Dried-brewer's grains were ground through a 3 mm screen in a Wiley-Thomas mill. Blood meal, soybean meal, corn gluten meal, and high-moisture corn (hammermilled) were used in the original form. Urea was not incubated, but was considered completely soluble in the rumen. All bags were soaked 15 min in 39° C water prior to insertion into the rumen.

Bags were suspended from the rumen cap on 60 cm nylon cords weighted with three-link segments of 3.2 mm chain.

Duplicate bags containing individual ingredients were placed in the rumen in reverse order for the following incubation times: 0, 1, 2, 3, 4, 6, 8, 12, 16, 24, 48, and 72 h beginning at 1700 h on d 1. Bags were forced to the bottom of the ventral sac of the rumen upon insertion. All bags were removed on d 4 at 1700 h, washed in a Maytag washing machine on delicate cycle, and dried in a forced-air oven at 50 C. Dried residues were weighed and analyzed for DM, CP, NDF, and cytosine content (bacterial marker). Due to limited quantities, residues were composited from the two lactating cows before NDF analysis. The data from the nonlactating cow were not used in calculation of degradabilities due to inconsistencies in DM disappearance between duplicate bags and replications of sets at various incubation times in comparison with the lactating animals.

Component degradability (D) in percent was calculated per the equation of Orskov et al. (30):

$$D = A + (B \times k_d B)/(k_d B + k_r), \text{ where}$$

D = DM, N, or NDF degradability, (%);
A = fraction readily degraded, (%);
B = fraction degraded at a measurable rate, (%);
k_d= component degradation constant of B fraction;
k_r= rumen turnover rate (.08/h).

Estimations of fractions A, B, and C followed Zerbini (43). Fraction C was calculated from the residue present

after 72 h incubation, as a percent of the original. Nitrogen content of the residue at each incubation time was corrected for microbial nitrogen contamination, using cytosine as a microbial marker, as described by Kwak (18).

Hydrolytic procedures and quantification of samples for cytosine content was similar to Koenig (17) with modifications. Approximately 250 mg of in situ residues from incubation times of 0, 6, 12, 24, and 72 h were weighed into 15 ml, screw-cap glass tubes. To each tube, 2.5 ml 70% perchloric acid was added, gently vortexed, and allowed to set until the acid was soaked through the sample (overnight). Samples were hydrolyzed at 90° C for 1 h in a Fisher Dry Bath (Fisher Scientific, Pittsburgh, PA) with mixing at 10-min intervals. Following hydrolysis and cooling, samples were diluted with approximately 10 ml .2 M $NH_4H_2PO_4$ buffer.

Samples were quantitatively transferred to a 100 ml volumetric flask. Concentrated NH_4OH was added to adjust pH to 5.0 before bringing to volume with .2 M $NH_4H_2PO_4$ buffer. A magnetic stirrer was used to thoroughly mix the diluted sample before filtering approximately 20 ml through a .45 μ millipore filter into glass, screw-cap tubes for storage until analysis.

Cytosine was determined by high performance liquid chromatography. Separation of cytosine was by a 25 cm Partisil-10 SCX L column at room temperature. The mobile phase was .15 M $NH_4H_2PO_4$ with pH adjusted to 3.5 with concentrated HCl.

A Varian 2510 pump was used to pump eluent at a rate of .6 ml/min. All bases were monitored at 254 nm as they passed through a 25 μl loop. A 10 μl injection loop was selected for cytosine detection of the rumen microbes. Cytosine was detected by a 200 UV/VIS detector in a linear mode. Normal elution was approximately 11.5 min.

Cytosine standards (Sigma Chemical Co., St. Louis, MO) were prepared in 1.75% $HClO_4$ in .2 M $NH_4H_2PO_4$ to provide concentrations of 10, 20, 30, 40, and 50 μM. Concentrated NH_4OH was used to adjust pH to 3.5. Standards were injected prior to analysis of each sample type and intermittently within sample type for detection of possible analytical variations.

The cytosine to microbial nitrogen (MN) ratio was used to calculate the MN contamination of in situ residues. The N content of rumen bacteria was determined from bacterial pellets obtained from a subsequent study in which four rumen-cannulated animals were consuming diets similar to diet 4.

The MN as a percent of the total N for each sample type was determined by the following equations:

$$\% MN_s = (MN/MCYT \times SCYT/SN) * 100$$

where $\%MN_s$ = sample MN, %,
MN = microbial nitrogen, g/g DM,
MCYT = microbial cytosine, umoles/g DM,
SCYT = sample cytosine, umoles/g DM,
SN = sample nitrogen, g/g DM.

Statistical Analysis

Analyses of the response surface models for each dependent variable were performed using the response surface least squares regression procedure (33) (RSREG). Ridge analysis (22) was performed when a unique optimum of the response surface could not be determined. The models included:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_1^2 + b_4x_2^2 + b_5x_1x_2 + b_6z + e,$$

where Y = measured response,
x_1 = variable for RAP,
x_2 = variable for RAC,
z_1 = variable for genetic herd (covariate),
b_0 = intercept,
b_1-b_2 = linear effect of RAC and RAP, respectively,
b_3-b_4 = quadratic effect of RAC and RAP, respectively,
b_5 = crossproduct of RAC and RAP,
b_6 = regressor used as covariate, and
e = residual error.

All data were also subjected to least squares analysis of variance by the general linear model procedure (33) for determination of a possible covariate. The model was:

$$Y_{ijkl} = u + G_i + L_j + D_k + (GD)_{ik} + (LD)_{jk} + C_l\,(GLD)_{ijk} + e_{ijkl}$$

where Y_{ijkl} = dependent variable of cow l of genetic herd in location j on diet k,
u = population mean,
G_i = genetic herd i, i = 1, control; i = 2, selection,
L_j = location j, j = 1, freestall, j = 2, tie stall,
D_k = dietary treatment k, k = 1 - 9,
$(GD)_{ik}$ = interaction between genetic herd i and diet k,
$(LD)_{jk}$ = interaction between location j and diet k,
$C_l(GLD)_{ijk}$ = interaction of genetic herd i, location j, and diet k within cow l, and
e_{ijkl} = residual.

RESULTS AND DISCUSSION

Evaluation of Ingredients

Chemical composition of individual dietary ingredients is presented in Table 3 and ruminal degradabilities and estimated rates of degradability are in Table 4. Only the concentrate ingredients were ruminally incubated prior to the lactation experiment. Selection of feed ingredients was based on the kd (rate, %/h) determined from the DM degradability of in situ incubations of several carbohydrate (cracked corn, ground corn, high-moisture shelled corn, cracked barley, ground barley) and protein sources (soybean meal, blood meal, dried brewers grains, corn gluten meal). Rates of DM degradability of cracked corn (4.8 %/h) and ground barley (7.2 %/h) differed most among the carbohydrate sources. The selected protein sources included blood meal and soybean meal. Because the NDF degradabilities had not been determined when the lactation study began, the original formulations of RAC were calculated from NDF degradabilities reported by Nocek and Russell (27) for rolled barley and cracked corn. The decision to use these values for preliminary estimates was made because the DM degradabilities reported by Nocek and Russell (27), and the corresponding DM degradabilities determined in this study for corn and barley, were similar.

Table 3. Formulation values and actual chemical compositions of ingredients used in total mixed diets varying in RAP and RAC.[1]

	DM, %	CP, % of DM	NEL, Mcal/kg	ADF, % of DM	NDF, % of DM	EE, % of DM	ASH, % of DM	RAP, % of CP	NDF-D, % of DM
Formulation Values									
Ground Barley	88.0	13.5	1.9	14.0	25.0	2.1	2.6	73.0	28.0
Cracked Corn	89.0	10.0	1.8	5.0	11.0	4.3	1.6	48.0	73.0
Soybean Meal	89.0	44.0	1.9	7.0	7.0	1.5	7.3	65.0	65.0
Blood Meal	90.0	96.0	1.8	1.2	5.0	1.4	5.8	15.0	20.0
Alfalfa Silage	58.0	20.5	1.4	32.0	46.0	2.6	9.1	77.0	36.9
Corn Silage	39.0	9.2	1.5	21.2	51.0	3.1	4.5	69.0	30.3
Actual Values									
Ground Barley	89.6	11.3	1.8	12.2	21.8	1.0	3.1	68.3	53.3
Cracked Corn	88.6	9.9	1.9	5.4	9.0	2.2	1.6	49.0	65.6
Soybean Meal	90.1	48.0	1.8	11.2	12.2	1.0	7.3	67.8	71.2
Blood Meal	91.6	96.5	1.8	3.8	3.5	.4	5.8	17.5	.0
Alfalfa Silage	51.9	20.7	1.3	36.2	44.3	9.1	1.8	77.0	36.9
Corn Silage	40.9	8.3	1.5	23.8	43.9	2.5	4.5	69.0	30.3

[1] RAP = rumen available protein; RAC = rumen available carbohydrate

[2] NDF-D = neutral detergent fiber degradability as determined by in situ

[3] Standard error of means in appendix

Table 4. In situ fractions and degradabilities of DM, CP, and NDF of cracked corn, ground barley, soybean meal, and blood meal.

Fraction	CC[1]	GB	SBM	BM
	DM Degradability			
A[2]	.8	14.3	27.4	1.3
B	95.4	69.2	70.2	17.5
C	3.7	16.5	2.4	83.8
kd[3]	4.8	7.2	8.9	.5
D[4]	46.7	55.1	72.3	3.9
	CP Degradability [5]			
A	10.6	23.0	20.4	10.0
B	59.5	61.6	65.3	11.1
C	29.9	15.3	14.2	78.9
kd	9.1	13.8	13.1	.1
D	49.0	68.3	67.8	17.5
	NDF Degradability			
A	48.1	37.2	24.0	NM[6]
B	27.7	28.1	69.1	NM
C	24.2	34.7	6.9	NM
kd	8.6	9.3	12.8	NM
D	65.6	53.3	71.2	NM

[1] CC = cracked corn, GB = ground barley, SBM = soybean meal, BM = blood meal.

[2] Estimated fractions are A = rapidly soluble, B = degraded at a measurable rate, C = undegraded residue after 72 h in the rumen.

[3] Kd = degradability rate of fraction B (%/h).

[4] D = estimated percent ruminal degradability of ingredient at rumen turnover of .08/h.

[5] Corrected for microbial nitrogen contamination.

[6] NM = nonmeasurable.

The CP degradabilities of BM and SBM for use in the original formulations were also from (27).

In the original RAC formulations, calculated RAC was lowest for cracked corn-based diets and highest for ground barley-based diets. The final analysis indicated that the diets containing cracked corn had the highest calculated RAC and those containing ground barley the lowest RAC, although the rates (kd) of NDF degradabilities were numerially higher for barley (9.3) than corn (8.6). The numerical order of the rate of NDF degradability followed the original assumption as ground barley degradability was faster than cracked corn. In vitro and in situ determinations of ground barley and cracked corn degradability rates reflected the same relationship (4,14,15,27,28). Although rates of degradability of soluble starch determined in vitro and in vivo reflected barley greater than corn, the percentage starch in corn was greater than barley (4,15,28).

The calculated RAC does not reflect differences in specific rates of availability. Instead, it reflects the availability of the total carbohydrate based on two assumptions: 1) nonstructural carbohydrates are 90%

available in the rumen, and 2) the availability of the structural carbohydrates can be accurately estimated by in situ determinations of NDF degradabilities.

The microbial-corrected degradability of the CP of ground barley (68.3%) was higher than cracked corn (49.0%), but similar to other reported values (27). As a result of this difference in CP degradability, slightly more BM was included in the ground barley diets compared with the cracked corn diets in order to achieve specified lower levels of RAP (Table 2).

Chemical composition, CP degradability and NDF degradability of SBM was similar to other reports (24,27,39). Source of BM (porcine) used in this study was consistently high in CP and provided the least variation in DM and CP degradabilities. Because NDF content of BM was very low (Table 4), an accurate measurement of NDF degradability could not be determined.

Chemical composition of the forage sources was similar to values used in the original formulation. Ruminal degradability of the forages was not determined in situ. Instead, in situ values from (27) were used to estimate these degradabilities for determination of the RAP and RAC of the complete diet.

As expected, NDF varied the most among diets. Although the actual CP and calculated CHO percentages (NSC + NDF) were similar among diets, the availabilities of CP and CHO differed among diets (RAP ($P<.05$), RAC ($P<.12$)). However, the variation in RAC was not as large as expected, due primarily to the high NDF content.

Dry Matter Intake

No differences in DMI, or DMI expressed as a percentage of BW, were detected among dietary treatments from wk 4 through 15 of lactation (Table 5). High intakes were maintained throughout the study with a linear increase through wk 15 ($P<.01$). On the contrary, Casper et al. (4), Casper and Shingoethe (3), and McCarthy et al. (21) reported lower DMI of barley-based than corn-based total mixed diets in mature lactating cows. Others have shown no depression of intake when cows were fed barley-based diets (11,14,15,37,42).

Plasma Urea Nitrogen

Although diets varied widely in ruminal availabilities of CP, no differences in plasma urea nitrogen (PUN) were found (Table 6). All diets exceeded the recommended level of dietary CP for first-lactation Holstein heifers (24), thus the relatively high levels of PUN on all dietary treatments reflect the high dietary CP content.

The PUN levels exceeded the recommended minimum of 8-10 mg N/dl required to maximize organic matter digestion in the rumen during lactation (24). The values are similar to other studies in which SBM-based, high protein diets were fed (6,31).

The PUN samples were determined from blood samples taken 3 h post-feeding. It is possible that PUN may have been higher prior to this time with diets containing higher levels of ground barley as a result of the more rapidly degraded CP of ground barley. When blood samples were drawn at 0, 2, 4, and 6 h post-feeding from lactating cows fed barley and milo-based diets, blood urea N was higher for barley than milo diets (15).

Growth measurements

Since these animals were in the first lactation, they would be expected to partition nutrients toward growth (29) in addition to that required for lactation. All animals increased in BW over the experimental period, and no differences were observed in response to diets. However, large variations existed when comparing the change in BW from the beginning to the end of the study by dietary group

Table 5. Dry matter intake (DMI), BW, and height measurements of primiparous cows fed diets varying in rumen available carbohydrate (RAC) and rumen available protein (RAP).

	Diet[1]									
Item[2]	1	2	3	4	5	6	7	8	9	SE
DMI, kg/d[3]	20.5	19.0	19.6	19.7	21.0	19.2	19.5	19.3	20.3	.51
DMI, % BW	4.1	3.9	3.8	4.1	4.2	4.0	3.9	3.9	4.1	4.1
BW, kg[3]	502	489	515	490	495	478	507	498	498	1.02
BW, gain[5]	39.3	65.1	42.5	39.3	42.7	35.5	36.4	53.6	46.3	1.13
WH, cm[4]	134	132	134	132	135	133	134	135	135	1.47
WH, change[5]	.6	1.1	2.6	1.0	2.6	1.3	2.0	1.4	.5	.69
HH, cm[4]	140	136	140	139	138	138	139	140	140	1.37
HH, change[5]	0.0	1.6	.8	.9	1.1	0.0	.4	.3	1.6	.94
BCS[4, 6]	2.7	2.8	2.7	2.7	2.6	2.7	2.7	2.7	2.7	.10
BCS, change[5]	.3	.5	.4	.2	.4	.4	.5	.2	.3	.08

[1] See text for dietary descriptions

[2] WH=wither height, HH=hip height, BCS=body condition score

[3] LSMeans of weekly measurements recorded wk 4 through 15 of lactation

[4] LSMeans of measurements recorded wk 7, 11, and 15 of lactation

[5] Change in variable from wk -1 through 12 of the experiment

[6] Body condition scores, scale (1.0-5.0): 1.0=very thin, 5.0=very fat

Table 6. Plasma urea nitrogen (PUN) concentration 3 to 4 h post-feeding of primiparous cows fed diets varying in RAP and RAC.

	PUN[1]	
Diet	mg/100 ml	SE
1	17.7	1.4
2	18.4	1.3
3	16.2	1.4
4	14.6	1.4
5	17.4	1.4
6	16.5	1.3
7	17.4	1.4
8	17.9	1.3
9	16.2	1.4

[1] Least squares means of 6 cows per diet.

(Table 5). All animals increased in BW, yet the six animals receiving diet 2 had a greater recovery of weight loss (+ 29.6 kg) than the slower gaining animals on diet 6. Others (40, 36) have reported BW gains in lactating primiparous heifers. Cressman et al. (10) showed an overall gain of 26.2 kg in primiparous heifers during wk 1 through 12 of lactation. During the same period, the mature cows lost 6.8 kg. Gains in our study were considerably higher than (10), yet the initial BW measurement was wk 3 of lactation compared with wk 1 in (10). A decrease in BW is expected during wk 0-3 in high producing cows. The initial BW was probably the lowest of the lactation. Roffler and Thacker (32) reported lower BW loss in early lactation in lactating heifers fed high protein diets vs low protein diets, with a subsequent recovery of BW with high protein supplementation.

Height measurements (WH and HH) taken at 4-wk intervals indicated small increases in stature during the 12-wk study. Change in body condition score (BCS) was also slight over the experimental period, yet animals in early lactation would not be expected to increase greatly in body condition. The average BCS 1 wk prior to the initiation of the study was 2.3, thus most animals began the experimental diets in thin condition. The animals used in this study had been maintained previously on pasture.

A quadratic response ($P<.01$) of 3.5 % fat corrected milk (FCM) yield (kg) was determined across weeks of lactation from the repeated measures analysis of variance. Peak milk yield occurred between wk 7 and 11 of lactation. Baseline production curves of heifers in winter, established by (2), showed peak milk production between wk 7 and 9 of lactation. Production was persistent through wk 11. Heifers in summer peaked approximately 50 d later than those in winter (2). In a comparison of various forage to concentrate ratios (F:C) fed to primiparous heifers, Macleod et al. (19) reported maximum production at wk 6 in high forage diets (80:20 and 65:35), wk 7 in 50:50 diets, and wk 8 in high concentrate diets (35:65).

The least squares means (6 animals per diet) of the daily milk production and milk composition are included in Table 7. Production at 4 wk intervals are found in Table 8. However, due to the low numbers of animal per treatment, an evaluation based on the central composite analysis is a better test of animal response to dietary treatment.

Response Surfaces

Prior to response surface analysis, analysis of variance procedures indicated no significant differences in milk production or milk components due to diet (six animals/diet). However, the test for differences in

Table 7. Daily milk production and milk composition from primiparous cows fed diets varying in rumen available carbohydrate (RAC) and rumen available protein (RAP).

	Diet									
Item	1	2	3	4	5	6	7	8	9	SE
RAC, %CHO	70.1	68.6	69.1	66.7	67.5	67.7	65.4	65.9	64.6	
RAP, %CP	65.4	62.6	67.2	58.6	65.2	73.0	62.0	67.5	64.0	
Milk, kg	24.7	22.6	26.7	27.2	26.1	22.8	25.1	25.6	26.5	1.4
3.5%FCM, kg	25.7	23.7	26.2	27.9	26.8	23.4	25.5	25.7	26.3	1.5
Fat, %	3.8	3.8	3.5	3.7	3.7	3.8	3.6	3.6	3.5	.16
Fat, kg	.93	.86	.90	.99	.96	.84	.91	.91	.92	.06
Protein, %	3.3	3.3	3.2	3.3	3.4	3.3	3.3	3.4	3.3	.15
Protein, kg	.83	.73	.85	.88	.86	.75	.81	.86	.86	.05

Table 8. Least squares means of milk yield and component responses by experimental period of primiparous cows fed diets varying in RAP and RAC.[1]

		Diet[2]									
Item	Per[3]	1	2	3	4	5	6	7	8	9	SE
Milk, kg[4]	1	25.2	22.8	26.4	28.6	27.0	23.4	26.7	26.1	26.8	1.5
	2	25.0	22.6	27.2	27.4	26.4	23.3	23.9	26.1	27.2	1.4
	3	23.9	22.5	26.5	25.5	24.8	21.8	24.6	24.5	25.4	1.4
FCM, kg[5]	1	26.3	23.6	25.7	28.6	26.5	23.6	26.9	26.3	25.7	1.5
	2	25.7	24.5	26.9	28.7	26.8	24.2	24.3	26.2	27.0	1.5
	3	25.2	23.1	25.9	26.3	27.2	22.3	25.3	24.7	26.3	1.5
Fat, kg[5]	1	.95	.85	.88	1.00	.92	.83	.95	.93	.87	.06
	2	.92	.91	.94	1.04	.95	.87	.86	.93	.94	.06
	3	.92	.83	.89	.94	1.02	.83	.91	.87	.95	.06
Protein,	1	.82	.72	.80	.89	.87	.74	.83	.85	.85	.05
kg,[6]	2	.83	.73	.89	.91	.88	.76	.78	.91	.90	.04
	3	.83	.74	.86	.85	.83	.73	.82	.82	.84	.05
Fat, %[4]	1	3.8	3.7	3.5	3.5	3.4	3.6	3.5	3.6	3.3	.18
	2	3.7	4.0	3.5	3.8	3.7	3.8	3.6	3.6	3.5	.15
	3	3.9	3.7	3.4	3.7	4.1	3.9	3.7	3.5	3.7	.19
Protein,	1	3.3	3.2	3.0	3.1	3.3	3.2	3.1	3.3	3.2	.15
kg,[4]	2	3.3	3.3	3.3	3.3	3.4	3.3	3.3	3.5	3.3	.16
	3	3.5	3.3	3.2	3.4	3.4	3.3	3.3	3.3	3.3	.15

[1] RAP = rumen available protein, % of CP
RAC = rumen available carbohydrate, % of CHO

[2] See text for dietary descriptions

[3] Period 1 = wk 4 through 7 of lactation.
Period 2 = wk 8 through 11 of lactation.
Period 3 = wk 12 through 15 of lactation.

[4] Effect of period, P<.10.

[5] Quadratic effect of experimental period, P<.05.

[6] Quadratic effect of experimental period, P<.08.

production due to genetic group was significant. Therefore, genetic herd was used as a covariate in the response surface analyses approximated by a second order model.

Response surface analysis prediction equations were developed for milk production variables (Table 9). These equations were used to produce three-dimensional, response-surface graphs for the milk production parameters. Discussion of the response-surface results will follow presentation of the surface data.

Response Surface: Milk

The response surface of milk yield with independent variables RAP and RAC is shaped like a saddle (Figure 2). The eigenvalue of 1.27 shows that the valley orientation of the saddle is less curved than the hill orientation, with eigenvalue of -4.29 (Table 10). The coefficient of the associated eigenvectors show that the valley is more aligned with RAC and the hill with RAP. The canonical variables are linear functions of the original variables, thus a decrease in RAP (X_1) would increase milk yield and an increase in RAC (X_2) would decrease milk yield. Up to a maximum of 71.2 % RAC, a decrease in RAP from 67.1 % would cause an increase in milk yield.

Table 9. Regression coefficients of rumen available protein (RAP) and rumen available carbohydrate (RAC) on milk production responses.

Response	Intercept	X_1	X_2	$X_1 * X_1$	$X_1 * X_2$	$X_2 * X_2$	COV[2]
Milk, kg	302.88	-19.1168	10.4742	-.0346	.3339	-.2311	-6.2677
FCM, kg	44.23	-12.8258	11.9214	-.0215	.2196	-.1876	-6.0258
Fat, kg	-5.30	-.2839	.4573	-.0004	.0047	-.0054	-.2053
Protein,kg	-3.30	-.3387	.4530	-.0011	.0069	-.0065	-.1532
Fat %	-33.44	1.2716	-.1618	.0029	-.0236	.0123	.1099
Protein, %	-49.44	1.1410	-.4460	.0000	-.0161	.0042	.2275

[1] Model: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_1 + b_4X_1X_2 + b_5X_2X_2 + b_6Z$, where Y = response, X_1 = RAP, % CP, X_2 = RAC, % CHO, and Z = genetic group.

[2] COV = covariate Z, genetic group.

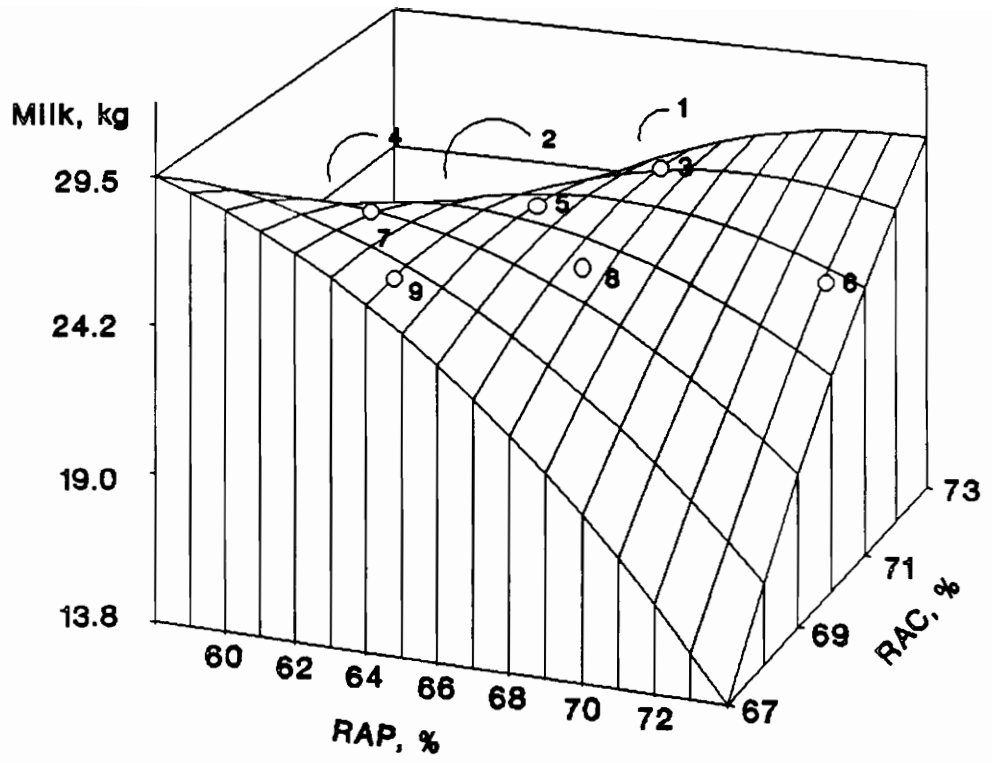

Figure 2. Response surface of milk yield from diets varying in RAP and RAC in 54 primiparous cows (R-Square=.46; C.V.=13.78; Lack of fit, P<.20).

Table 10. Solutions for response surface analysis of milk and milk components.

Solutions	Milk	3.5% FCM	Fat	Prot	Fat	Prot
	kg	kg	kg	kg	%	%
Stationary points (SP)[1]						
RAP (X_1)	67.1	68.3	72.1	72.5	67.0	64.4
RAC (X_2)	71.2	71.8	73.1	73.3	70.5	70.5
Estimated mean at SP	25.6	25.7	.90	.82	3.6	3.3
Eigenvalues[2]						
X_1	1.27	.767	.014	.011	.308	.144
X_2	-4.29	-2.87	-.064	-.105	-.092	-.124

[1] RAP = rumen available protein, % of CP
RAC = rumen available carbohydrate, % of CHO

[2] Based on coded data

The model used for predicting milk yield had the lowest proportion of variation attributed to factors other than the model (R^2=.46) when compared to the R-Square values of the models for the other dependent variables (Table 11). Both RAP (P<.02) and RAC (P<.03) influenced the predicted response of milk yield (Table 11).

Since a unique optimum could not be obtained from these data, a ridge analysis was performed to predict an optimum response. The ridge could be interpreted as the area of the surface of the predicted response on which the response rapidly 'climbs' or 'falls'. The starting point of the ridge analysis has each coordinate equal to the midpoint between the highest and lowest factors in the design. The average value of the covariate is used in the ridge equation, thus the predicted responses would be expected to fall between the maximum production of the control and selection genetic herds. The ridge analysis is based on coded data.

The ridge analysis for milk yield (Table 12) suggested that a decrease in RAC and RAP would increase milk yield. The suggested combination of RAC and RAP, within the constraints of this experiment, would be 60.8 % RAP and 68.7 % RAC. A diet similar to diet 4 would provide this combination.

Table 11. Tests of significance of RAP and RAC for the model of each response surface.

Variable	Prob > F [2]		R-Square[3]	C.V.[4]
	X_1 [1]	X_2		
Milk yield	.02	.03	.46	13.78
3.5% FCM yield	.03	.26	.44	13.48
Fat yield	.09	.75	.35	15.34
Protein yield	.04	.08	.34	13.58
Fat, %	.39	.13	.06	11.46
Protein, %	.29	.36	.22	6.63

[1] X_1 = Rumen available protein, % of CP.
X_2 = Rumen available carbohydrate, % of CHO

[2] H0 for X_1 = X_1, X_1*X_1, $X_1*X_2=0$.
H0 for X_2 = X_2, X_2*X_2, $X_2*X_1=0$.

[3] Proportion of variation in the response attributed to the model, corrected for the effect of the covariate.

[4] Coefficient of variation = 100 * rootmse/mean

Table 12. Estimated ridge of maximum response for 3.5% FCM and milk yields based on experimental diets varying in RAP and RAC. [1]

Dependent Variable	Independent Variables		Estimated Response	
Variable$_i$	RAP(X_1)	RAC(X_2)	Y_i	SE
FCM, kg	65.8	70.0	25.9	.53
	65.1	70.0	26.2	.52
	64.4	69.9	26.4	.51
	63.2	69.8	26.5	.50
	62.6	69.6	26.7	.50
	62.0	69.5	26.9	.51
	61.5	69.3	27.1	.55
	61.0	69.2	27.4	.63
	60.4	69.0	27.6	.75
	59.9	68.8	27.9	.90
Milk, kg	65.8	70.0	25.7	.53
	65.1	69.9	25.9	.52
	64.4	69.8	26.1	.51
	63.9	69.7	26.3	.50
	63.3	69.5	26.5	.49
	62.8	69.4	26.8	.51
	62.3	69.2	27.1	.55
	61.8	69.0	27.3	.63
	61.3	68.9	27.7	.75
	60.8	68.7	28.0	.91

[1] Determined by ridge analysis of saddle-surface response with independent variables limited to the range of the experimental region.

When the independent variables were allowed to exceed the experimental range (Table 13), the ridge analysis indicated that decreasing RAP below 60 % and RAC below 68 % would increase milk yield more than 2.7 kg above the estimated mean at the SP of the surface. However, interpretation of these data must be done with caution. The standard errors of the means increase greatly when the responses are predicted from points outside of the experimental region. These predicted responses reiterate the questionable reliability of extrapolation outside the range of independent variables in response surface methodology.

Differences in milk yield by experimental period were also examined. Experimental periods were wk 4-7 (period 1), wk 8-11 (period 2), and wk 12-15 of lactation (period 3). Milk yield differed ($P<.07$) by experimental period.

Response surface of milk yield by experimental period resulted in similar trends as previously discussed for milk yield from wk 4 through 12 of lactation. Evaluations of the eigenvectors and corresponding eigenvalues indicated that decreases in RAP during periods 1 and 2 would increase milk yield, whereas, increases in RAC would decrease milk yield.

Table 13. Estimated ridge of maximum response for milk and 3.5% FCM yields. [1]

Dependent Variable	Independent Variables		Estimated Response	
Variable$_i$	RAP(X_1)	RAC(X_2)	Y_i	SE
FCM, kg	59.9	68.7	28.1	1.1
	58.9	68.3	28.7	1.5
	57.8	68.0	29.4	2.1
	56.8	67.7	30.1	2.8
	55.8	67.4	30.8	3.5
	54.8	67.0	31.7	4.4
	53.8	66.7	32.5	5.3
	52.8	66.4	33.5	6.4
	51.8	66.0	34.5	7.5
	50.7	65.7	35.6	8.7
Milk, kg	60.3	68.5	28.4	1.1
	59.3	68.2	29.2	1.6
	58.3	67.8	30.1	2.1
	57.3	67.5	31.1	2.8
	56.4	67.2	32.2	3.6
	55.4	66.8	33.5	4.4
	54.4	66.5	34.8	5.4
	53.5	66.1	36.2	6.4
	52.5	65.8	37.7	7.6
	51.5	65.5	39.4	8.8

[1] Determined by ridge analysis of saddle-surface response based on estimated combinations of RAP and RAC outside of the experimental region.

In period 3, changes in either independent variable had less effect on milk yield. In general, a decrease in RAC and RAP would tend to maximize milk production during each period.

The predicted milk production from the ridge analysis ranged from 26.4 kg (RAP=65.8, RAC=70.0) to 29.6 kg (RAP=60.5, RAC=68.6) during period 1 when the factor values were limited to the experimental region. In period 2, predicted milk yield ranged from 25.7 to 27.7 kg. Milk yield range was 24.9 to 27.0 kg in period 3. The independent variables used in these predictions are the same as found in Table 12. These data indicate that the level of RAP and RAC have a lesser influence on milk yield in primiparous animals past peak production compared to the period before and during peak.

3.5 % Fat-Corrected Milk

As was predicted for milk yield, the SP for 3.5 % FCM was for a minimum X_1 (RAP) and maximum X_2 (RAC) (Table 10). The estimated mean at the SP was 25.7 kg where a combination of 68.3% RAP and 71.8 % RAC were fed. The associated eigenvectors showed that the valley was more associated with the RAC variable and the hill with RAP, implying changes in RAP result in greater changes in FCM than RAC. The coefficient of determination (R^2 = .44) was similar to that of the model for milk yield.

A maximum response of FCM could not be determined from these surfaces because of the saddle-like shape (Figure 3). The estimated maximum responses determined by ridge analysis are shown in Tables 12 and 13. The responses were similar to the predicted milk yield responses, yet the overall range was smaller. No differences in FCM production by period were detected.

Response Surface: Milk Fat

The response surfaces of milk fat percentage and milk fat yield were saddle-shaped as determined by the corresponding positive and negative coefficients of the eigenvalues (Table 10). The surfaces are graphed in Figures 4 and 5, respectively. With RAC at a maximum 70.5 %, a decrease in RAP from 67.0 % would result in an increase in milk fat percentage from the predicted 3.6 % at the SP (Figure 4). Since the canonical variables are linear functions of the original variables, the analysis indicated that RAP would have a much greater influence on milk fat percentage than RAC within the range of these diets. Analysis of the corresponding eigenvectors also indicated that the hill of the saddle is more closely aligned with RAP, thus, greater changes in response would occur with changes in RAP. Also, the canonical variable, RAC, is very small and implies a relatively flat response to changes in RAC within the experimental region.

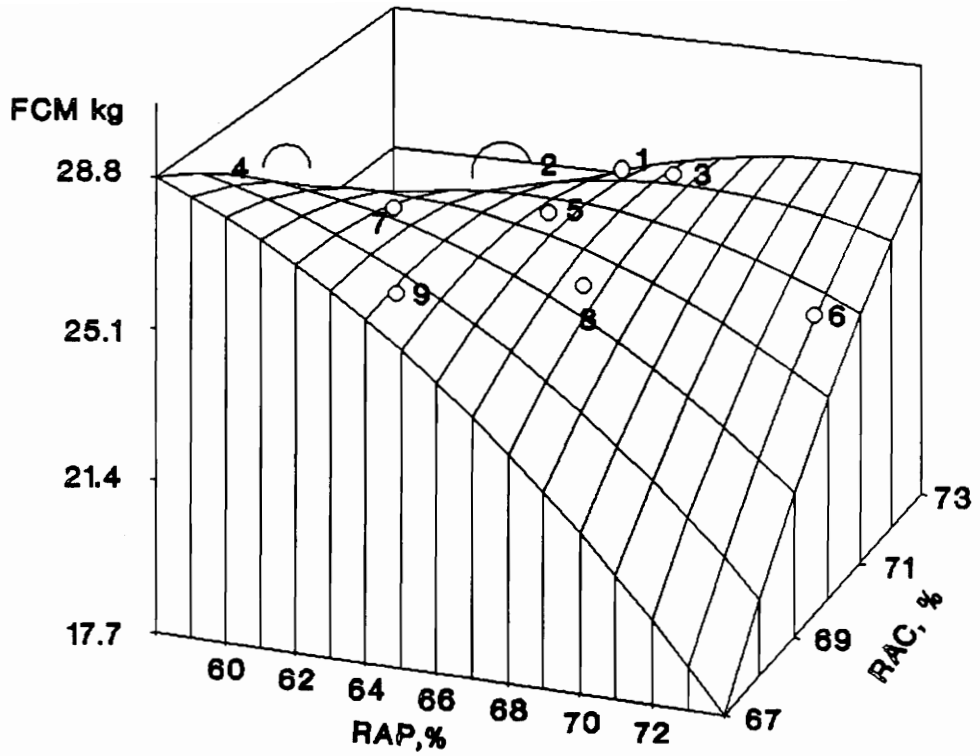

Figure 3. Surface of 3.5% fat corrected milk yield response to diets varying in RAP and RAC in 54 primiparous cows (R-Square=.44; C.V.=13.48; Lack of fit, P<.48).

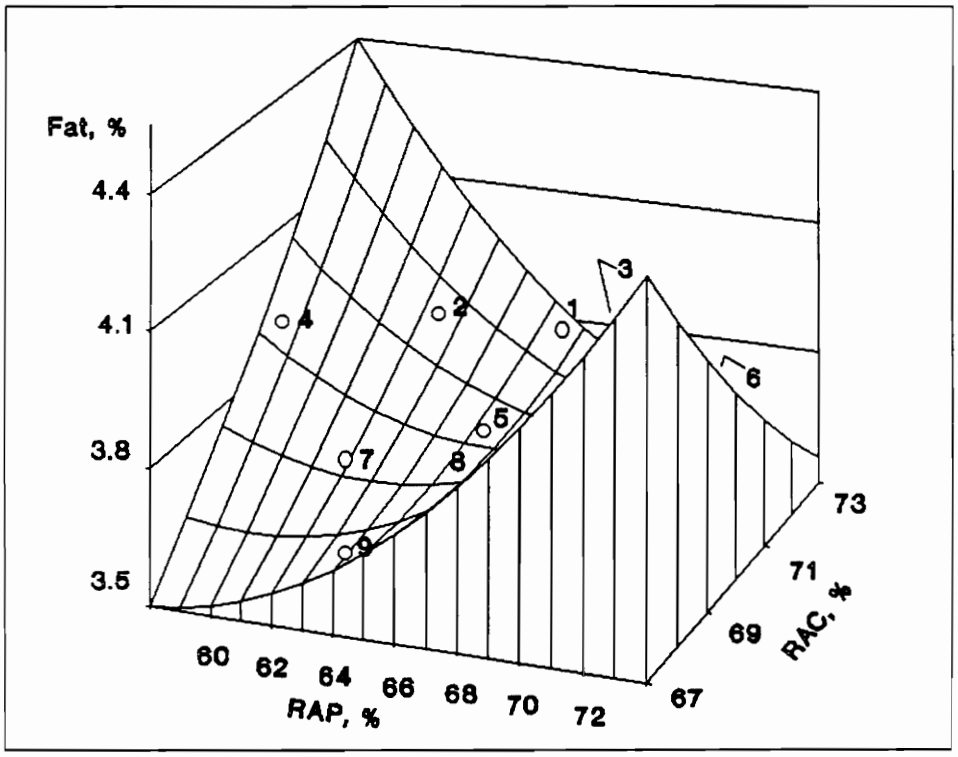

Figure 4. Surface of milk fat percentage response to diets varying in RAP and RAC in 54 primiparous cows (R-Square=.06; C.V.=11.46; Lack of fit,P<.03).

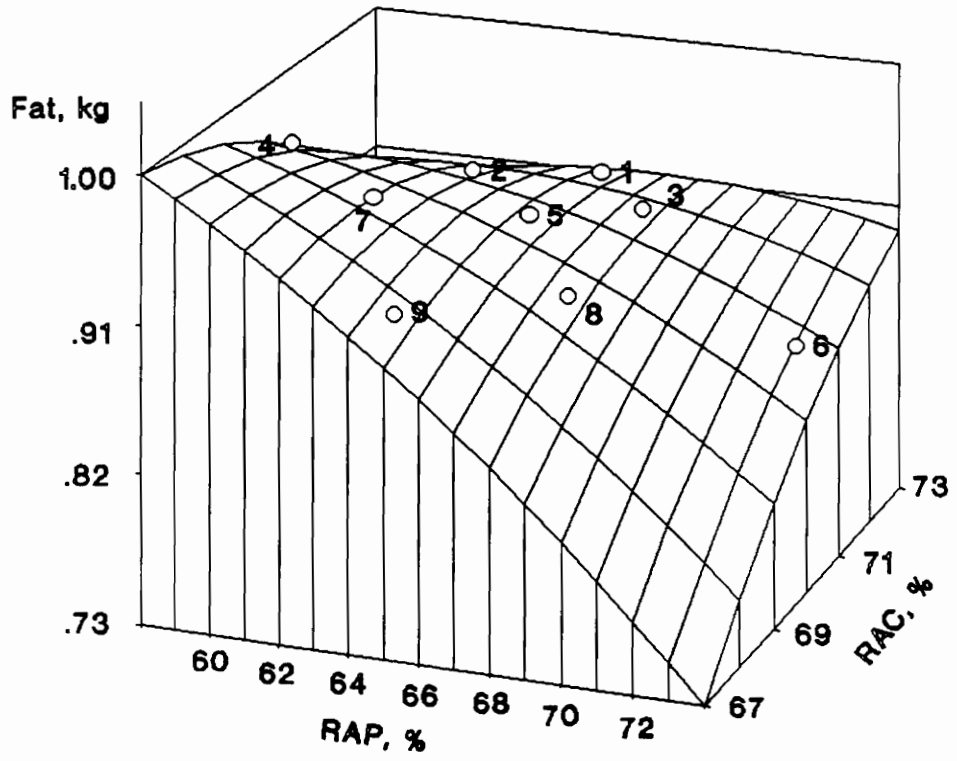

Figure 5. Surface of milk fat yield response to diets varying in RAP and RAC in 54 primiparous cows (R-Square=.35; C.V. 15.34; Lack of Fit, P<.58).

However, it should be noted that the model for milk fat percentage indicated significant lack of fit ($P<.03$). The variation in milk fat percentage was apparently due to something not accounted for in the model. When analyzed by period, lack of fit was no longer significant yet the coefficents of determination were less than .10 for each period. This indicated that some of the variation causing a poor fit of the data to the surface was due to normal biological differences in milk fat production associated with stage of lactation (before, during, and after peak).

The predicted SP for milk fat yield was for maximum X_1 and maximum X_2 (Table 10). However, the eigenvectors of both variables are small, indicating a surface of relative flatness. The eigenvectors are very similar numerically, indicating that changes in minimum RAP and maximum RAC will cause similar small changes in milk fat yield. The plotted surface in Figure 5 demonstrates the relatively flat response of milk fat yield to changes in RAP and RAC.

Response Surface: Milk Protein

The predicted SP of .82 kg protein yield was at the maximum X_1 and maximum X_2. From the absolute value of the

corresponding eigenvectors, RAP had greater influence on milk protein yield than did RAC. The model was significant for lack of fit ($P<.05$). Therefore, more data would be needed before a firm conclusion could be drawn from these combinations of RAP and RAC in primiparous cattle.

As with milk fat percentage, the predicted SP of 3.3 % milk protein was also at the minimum X_1 and maximum X_2 (Table 10). The corresponding eigenvectors indicated the greatest influence on milk protein percentage would result from changing RAC rather than RAP (Figure 6). At a maximum 64.4 % RAP, decreases in RAC from the SP would increase milk protein percentage.

Discussion of Response Surfaces

The ridge analyses of all the milk parameters indicated that the synchrony of rates of available carbohydrate and available protein in alfalfa-corn silage based diets should be diets lower than 68.8% RAC and 59.9% RAP.

These data appear opposite of common proposals when considering optimizing rumen fermentation, and ultimately maximizing rumen microbial protein synthesis (7,15,27). However, it is important to remember that these diets contained adequate soluble protein (estimated range: 29.7 to

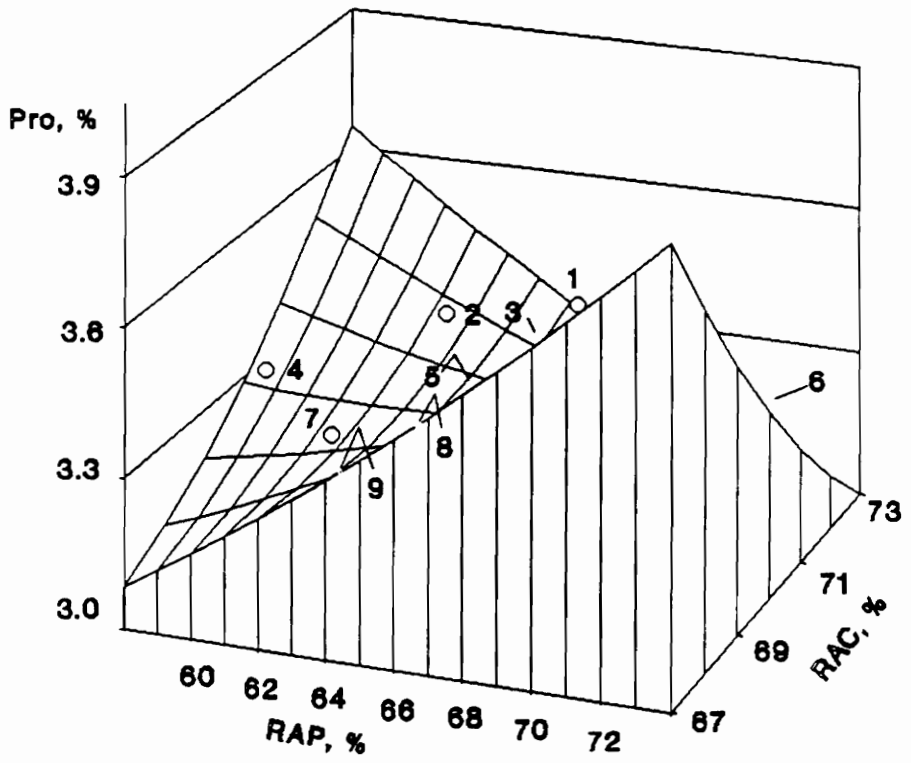

Figure 6. Surface of milk protein percentage response to diets varying in RAP and RAC in 54 primiparous cows (R-Square=.22; C.V.=6.63; Lack of fit, P<.04).

39.4 %) for potentially optimizing microbial protein synthesis (23), and adequate to excess nonstructural carbohydrate as a percent of total dietary dry matter (37).

The soluble protein, or urea equivalent, provided by alfalfa and corn silage, is required for those microbes considered structural carbohydrate fermenters (12). Additionally, peptides and amino acids are required by NSC fermenters (12), which were provided by the SBM. Clark (8) proposed that soy protein is considered a good source of amino acid nitrogen and/or energy needed by some microbes for optimum bacterial growth and efficiency when fed in diets containing other sources of high ruminal undegradability.

For this reason, the indication from the ridge analysis that sources high in RAC are needed in combination with low levels of RAP (high levels of rumen undegradable protein) may be misleading. Based on the rates of DM availability determined from in situ (kd) and reported by others (4,14,15,28), ground barley was more rapidly available in the rumen compared with cracked corn. The rapidly available carbohydrate source may have been synchronized with the soluble N from the alfalfa and corn silages, and the peptide release from SBM, for optimizing microbial growth. In theory, if this were true, the amino acids provided to the small intestine by the microbial protein could have been

prioritized for milk synthesis. The additional amino acids provided by the BM may have been used for both growth and lactation.

On the other hand, since no overall differences in milk production were detected due to dietary treatments (six animals/diet) in the analysis prior to response surface analyses (54 animals), it may be possible that heifers receiving BM diets may have been deficient in microbial N synthesis because of limited ammonia N. However, the complementary flow of BM-amino acids potentially flowing to the small intestine may have accounted for the potential deficiency, resulting in no differences in milk production.

Several studies have reported no response in milk production to varying dietary NSC and degradable protein (11,14,37,42). However, it was unclear in these studies whether the lack of response was due to feed sources used, the calculated characterization of the carbohydrate and protein fractions, or low cow numbers. DePeters et al. (11) compared two dietary treatments: ground corn and ground barley in SBM, chopped alfalfa hay-based diets. Beet pulp and molasses were included in the corn diet to widen the difference in NSC between the two diets. According to the in situ and in vitro data summarized by Nocek and Tamminga (28), the beet pulp and molasses, and the use of ground corn instead of cracked corn would have theoretically resulted in

both diets containing an equally large proportion of soluble and available carbohydrate. No supplemental rumen undegradable protein source was included in these diets.

Similarly, Grings et al. (14) fed beet pulp in the alfalfa-silage diets containing corn as a comparison to three rolled-barley diets that varied by barley variety and density. No supplemental rumen undegradable protein source was fed. Again, the beet pulp in the corn diets may have resulted in increased rate of carbohydrate availability as a result of increasing the soluble carbohydrate (primarily in the form of pectin) in the diet. In turn, no differences in milk production were attained.

In the other two studies, low cow numbers may have limited interpretation of the data. Weiss (42) fed 12 midlactation cows alfalfa silage diets with either barley or corn and SBM supplementation. No differences in milk yield or component yield were detected. Stokes et al. (37) fed three cows diets varying in NSC:DIP to three cannulated, lactating cows. Forage sources varied widely between diets in order to attain the range of NSC. Milk yield did not differ, yet MN synthesis was highest when diets were formulated to contain 31 or 39 % NSC and 11.8 and 13.7 % DIP as a percentage of the DM. All nine diets used in our study exceeded the NSC recommendation, but were within range for DIP.

Reports from three locations have been reported in which cows fed corn-based diets yielded more milk than those fed barley-based diets (3,4,21). In all three studies, DMI was lower in cows fed barley-based diets, thus results were confounded with intake.

In Jersey heifer studies conducted at Virginia Tech (39), similar positive responses to ground barley-BM supplemented diets varying in level of NSC were found. Supplemental CP sources included SBM and BM, whereas, supplemental NSC sources included ground barley and cracked corn. Nonstructural carbohydrates were determined by the difference method: NSC = 100 - (CP+NDF+EE+ASH), and were not based on rates of ruminal availability. Rumen undegradable protein levels were determined in situ following the growth trial. Diets were balanced for varying NSC (13.9 to 25.5 % of DM) and RUP (27.5 to 60.6 % of CP). Alfalfa haylage, chopped orchardgrass hay, and barley straw were included in varying amounts to help achieve the large range in NSC and RUP. The diets containing large amounts of BM (40 and 50 % RUP) resulted in the greatest efficiency of growth, measured as Mcal DE/kg BW gain. Additionally, diets containing the highest level of ground barley (high NSC) and BM (high RUP) produced the highest BW gain (.694 kg/d). Diets containing greater than 19 % of the DM as ground barley, and 6 to 47 % of the DM as alfalfa haylage,

resulted in gains above 600 g. An exception was found in the diet that replaced alfalfa haylage with chopped orchardgrass hay. The author contributed poor gains on this diet to either poor health or seasonal effects.

Within levels of NSC, as diets increased in RUP (BM) the trend was toward greater structural growth. As diets increased in RUP (BM) and NSC (ground barley), BW gain increased. Overall, increasing dietary RUP had a greater influence on heifer growth and apparent feed efficiency than dietary NSC.

Likewise, in our study, when the carbohydrate fraction was defined in terms of RAC, the response surfaces indicated that RUP (100-RAP) influenced responses more than RAC. Additionally, the ridge analysis projected maximum milk yields from diets containing BM in combination with barley or barley plus corn.

Although the partitioning of nutrients in primiparous animals remains unclear, a comparison of the growing heifer data and the primiparous data lends credence to the benefit of supplemental BM. It appears to provide nutrients for both growth and lactation when diets contain ground barley (source of soluble carbohydrate) and adequate soluble N.

Another benefit of BM may lie in its amino acid profile. The proposed first-limiting amino acid(s) for milk production is considered to be lysine or methionine (20,36).

Diets containing high levels of corn-based products would be considered marginal to low in dietary lysine (7,36). Clark (7) suggested that the greater ruminal undegradability of corn allows for higher passage of lysine to the small intestine in comparison with alfalfa hay, barley grain, and corn silage. This was true only when efficiency of microbial N (MN) yield was equal across diets (35 g MN/kg organic matter apparently digested in the rumen). If the MN yield was less, then the contribution of corn lysine would be less than the other sources mentioned.

Although amino acids were not measured in this study, diets 1, 2, and 3 may have been limiting in lysine compared with the ground barley diets (21). Additionally, diets 7, 8, and 9 were supplemented with higher levels of BM that is considered to provide greater amounts of lysine to the small intestine than grain-type sources and SBM (7,16,20). Methionine, however, is lower in BM than SBM (20), and lower in corn than barley (21). However, Clark (7) recommended supplemental BM, fish meal, or brewers dried grains in barley diets to account for the low methionine flow in cows fed barley-based diets.

The concept of balancing diets for RAC and RAP was established in order to provide a balance of available carbohydrate and available nitrogen in the rumen for use by the microbial population (27). The appropriate balance

should allow for maximum microbial protein synthesis (8,27). Microbial protein contains an amino acid profile that most closely matches the amino acid profile found in milk, in comparison to common feedstuffs (20). If microbial protein synthesis is maximized, two primary benefits should result. First, rumen fermentation is optimized when microbial protein synthesis is maximized. In turn, high dry matter intake results. Second, by providing a greater amount of protein containing the desired amino acid profile, less supplemental protein may be needed.

Nocek and Russell (27) proposed the original, patented system of dietary RAC based on data shown in Figure 7. These data included estimated RAC from 24 published studies using multiparous cows consuming an average 20.2 kg DMI and producing above 30 kg milk. From these data, optimum RAC and RAP were estimated. The proposed 'optimums' were: 15.6 % CP, 66 % RAP (% of CP), 78 % CHO, and 53 % RAC. Calculated as a percent of CHO, RAC would be equivalent to 67.9 %.

Figure 7 also includes the actual, unadjusted data from the primiparous cows used in our study. The milk yields were then adjusted for age and season based upon USDA adjustment factors. Our data indicated that the level of RAC intake was similar to the average of the 24 studies cited by (27). Although the calculated percentages of RAC

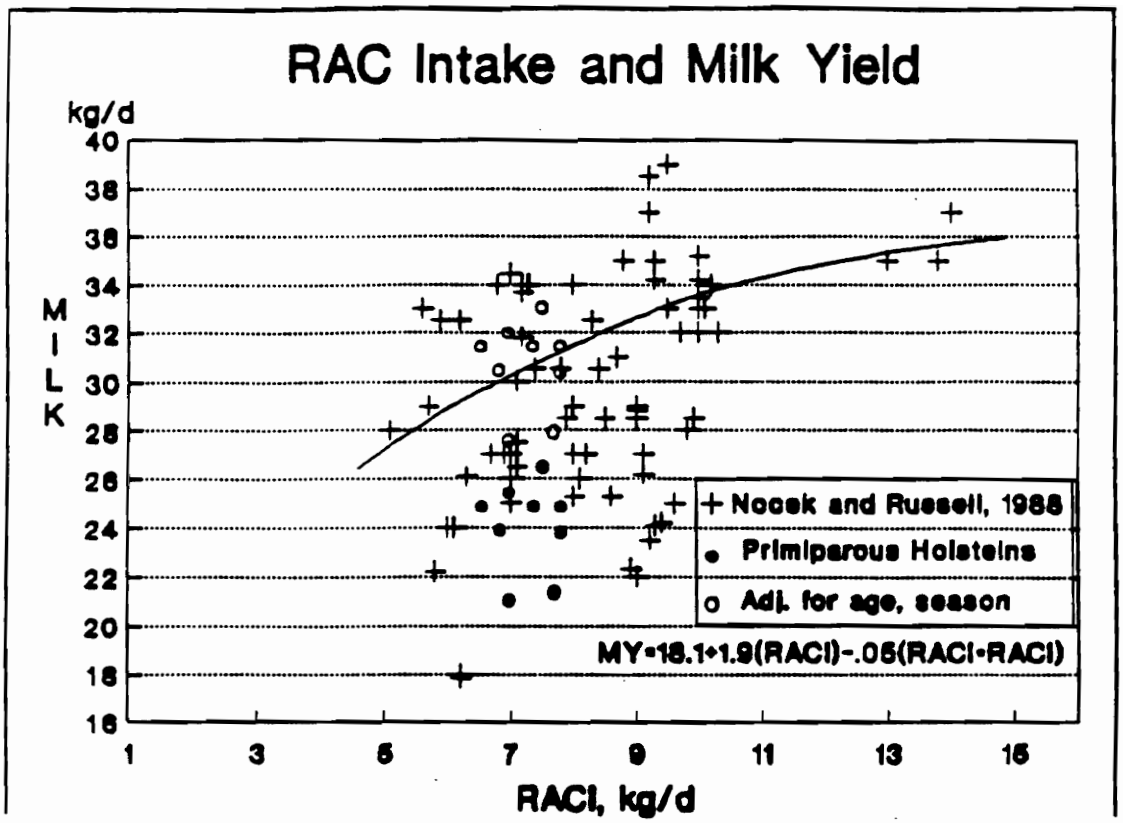

Figure 7. Comparison of milk yield response to estimated RAC intake (RACI) from 24 lactating cow experiments (27), and actual RACI of primiparous cows during wk 4 through 15 of lactation.

designed for this study appeared different, the actual amount of RAC intake (RACI) was not different ($P<.30$).

The RAP intake was different among diets ($P<.05$). Figure 8 compares the RAP intake of our study with that of the same high producing herds used in determining optimum RAC. Protein intake appears high, yet the level of CP in all diets was 3 percentage units higher than the average of the reported studies. Additionally, DMI was high in our experiment compared with other first-lactation reports (10,32). These RAC and RAP intakes reiterate the importance of calculating nutrient intakes instead of relying on nutrient percentages of the diet.

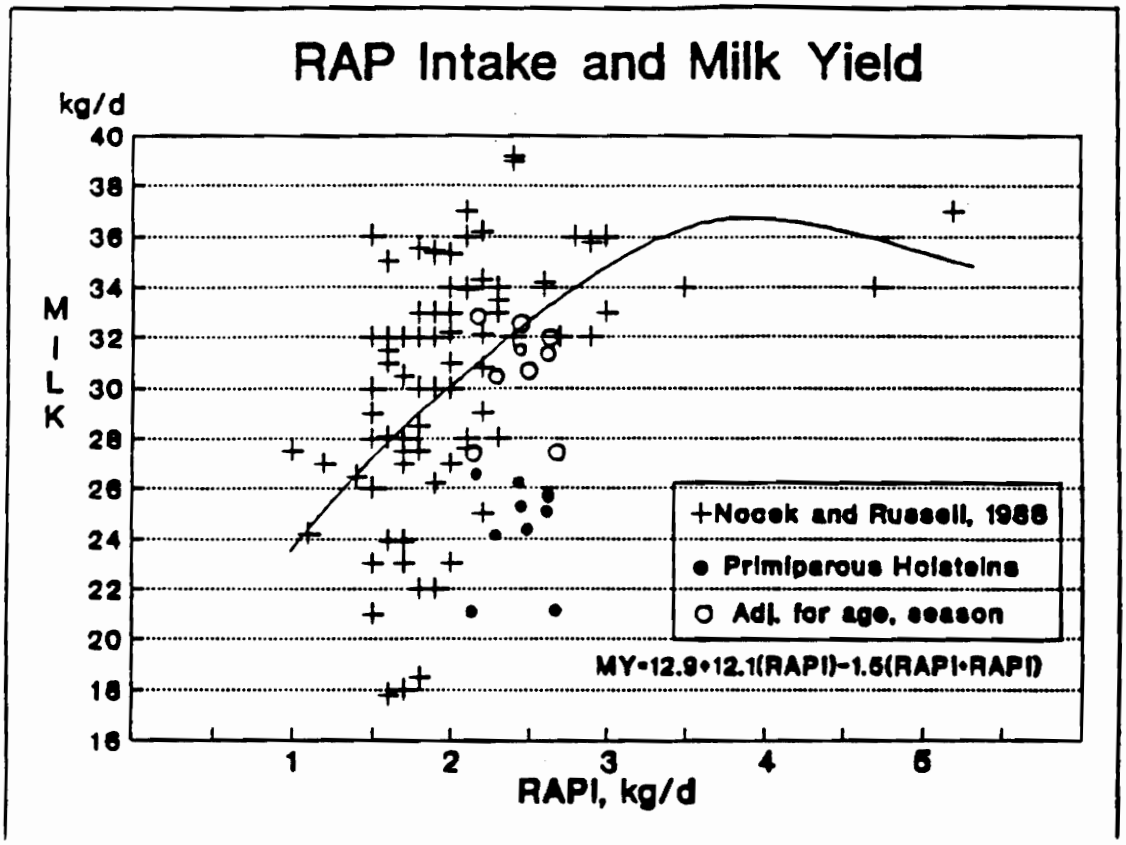

Figure 8. Comparison of milk yield response to estimated RAP intake (RAPI) from 24 lactating cow experiments (27), and actual RAPI of primiparous cows during wk 4 through 15 of lactation.

Conclusions

Primiparous and growing heifers respond positively to BM supplementation in diets containing adequate soluble N (provided primarily from alfalfa silage) and adequate nonstructural carbohydrate. Whether the BM response is due to the low ruminal degradability, amino acid profile, or the complementary nutrient profile to the other dietary ingredients cannot be determined from these data. While calculated NSC was similar across diets, and adequate to excessive for optimum rumen fermentation, the variation in RAC (rate of carbohydrate degradation) was not large enough to illicit a milk production or growth response.

The use of the response surface design was a useful tool for determining the optimum combinations of RAC and RAP. The ridge analysis provided information useful for future experiments utilizing RAC and RAP dietary parameters. If the range of RAC had been as large as RAP in the original design, data may have been more conclusive. The ridge analysis suggests that if further experimentation is undertaken, it might be best to fix RAP at a moderate to high level and to concentrate on lower levels of RAC in diets with grain to forage ratios commonly fed to lactating cattle. Additionally, the chemical analysis and in situ

determinations of the feed ingredients used in this study indicates that carbohydrate and nitrogen solubility should also be evaluated.

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CHAPTER 3

RUMINAL FERMENTATION, DIGESTION, AND FLOW OF DIETS VARYING IN RUMEN AVAILABLE CARBOHYDRATE AND RUMEN AVAILABLE PROTEIN

ABSTRACT

Four total mixed diets varying in rumen available carbohydrate (RAC) and rumen available protein (RAP) were utilized in a 4 x 4 Latin Square design to compare differences in ruminal activity, nutrient digestion, and nutrient flow. Variations in RAC resulted from substituting cracked corn (slowly available) for ground barley (rapidly available) in alfalfa and corn silage-based diets. RAP was increased by replacing soybean meal with blood meal. Calculated RAC ranged from 65 to 72 % of the DM, and RAP increased from 58 to 72 % of the CP. RAC and RAP, within the range of this experiment, did not cause differences in ruminal digestion of DM, OM, NDF, ADF, or N components, although in situ results indicated differences in rates of ruminal CP and NDF availabilities among individual ingredients. No differences in microbial N flow were measured, yet nonmicrobial N flow (g/d) was greater in barley than corn-based diets. Ruminal VFA concentrations were similar across diets and indicated high levels of fermentative activity. Ruminal ammonia-N was above the minimum considered adequate for vigorous microbial growth, regardless of the supplemental protein sources.

INTRODUCTION

Carbohydrates and proteins are the primary nutrients promoting growth of the microbial population in the rumen. Nocek and Russell (19) and Oldham (21) reviewed the significance of the synergy of these two nutrients during the dynamic process of ruminal digestion, such that microbial yield, fermentation endproducts, and nutrient outflow of the rumen will be maximized. The quantity and composition of carbohydrate and protein vary greatly by diet, and the characterization of these fractions are under investigation (5,8,20,29,33).

For increased supply of microbial N to the small intestine, Oldham (21) suggested that provision of available carbohydrate and protein at coordinated rates should allow microbes to simultaneously use the ATP and N (ammonia or amino acids) needed for maximum growth. As milk production continues to increase, additional amino acid flow to the duodenum may be needed to meet the greater nutrient demands. Feeding protein sources of low ruminal degradability could provide the additional amino acids, above that provided by microbial N, to meet the greater demands of increased growth and milk production (17). However, Clark et al. (8) suggested that feeding protein sources of low ruminal

degradability tended to decrease microbial N passage to the small intestine, compared with feeding soybean meal.

The objectives of this study were to determine if diets varying in rates and quantities of rumen available carbohydrate (RAC) and rumen available protein (RAP) were different in their capacity to affect ruminal fermentation endproducts, nutrient outflow from the rumen, and apparent ruminal and total tract nutrient digestibilities. Milk production and composition changes were also evaluated.

MATERIALS AND METHODS

Experimental Animals

Four lactating Holstein cows, fitted with ruminal and duodenal cannulae were used in a 4x4 Latin Square design. One of the three cows had been fitted with both cannulae two lactations previous to this study. The other cows were cannulated prior to the beginning of the experiment. Surgery was performed approximately 2 wk post-calving at the Virginia-Maryland College of Veterinary Medicine. Paravertebral (T13,L1,L2) nerve blocks with 2% lidocaine provided local anesthesia. The duodenum was exposed to the exterior of the body for emplacement of the flexible duodenal cannula approximately 10 cm posterior to the pyloric sphincter. The cannula and intestine were returned to the body cavity, positioned low on the body wall, and forced through the wall to the exterior of the body through a small incision. Duodenal cannulae were made of polyvinylchloride resin with a barrel length of 13.5 cm and inner diameter of 2.4 cm. A plexiglass, threaded plug (2.3 cm x 3.8 cm) fitted within a stainless steel sleeve (3.8 cm in length) fit inside the barrel. The length of the cannula in the intestinal lumen was 11.8 cm with an inner diameter of 3.0 cm.

After a 2-wk recovery, cows underwent a 2-stage rumen cannulation. Lidocaine (2%) provided local anesthesia by an inverted L-block in the lumbar fossa. In stage 1, a circular excision of skin was removed and an incision was made for entry into the abdominal cavity. The rumen wall was exposed and sutured to the muscle of the abdominal wall before both were sutured to the skin to seal the abdominal cavity. A small flexible rumen cannula (5 cm ID, 17 cm OD) was placed temporarily in the surgical opening and remained approximately 7 d. In stage 2, the permanent rumen cannula (ANKOM^{R}, Spencerport, NY) with 11 cm ID, 25 cm OD, and 7.5 cm animal wall thickness was inserted. Post-operative care included daily monitoring of sutures, cleansing, antibiotic (i.m.), and analgesic as directed by the attending veterinarian.

The four multiparous Holsteins weighed an average of 553 kg at the onset and averaged 65 d postpartum (41 to 77 d). Three animals were in the second lactation, one in the fifth lactation. Cows were milked daily at 0500 and 1600 h and were housed in individual boxstalls with free access to water and feed.

Diets and Feeding

All cows were fed a control diet of 59% concentrate and 41% forage on a DM basis for approximately 5 wk prior to the initiation of the study. Dietary ingredients included (DM

basis) 28.6% alfalfa silage, 7.2% corn silage, 5.2% long orchardgrass hay, 46.4% cracked corn, 5.3% soybean meal, 5.4% dried brewers grains, and 1.9% VPI herd mineral. Chemical analyses (DM, CP, NDF, ADF) of weekly composites of ingredients were performed in the Virginia Tech Forage Testing Laboratory, Blacksburg.

Four of the nine experimental diets that were evaluated in a previous lactation study (12) were selected for this study. Ingredient and chemical composition are shown in Tables 1 and 2. Diets varied in calculated rumen available carbohydrate (RAC) and rumen available protein (RAP) in an attempt to vary the rates of degradation of carbohydrate and protein in the rumen. Dietary combinations varying in ruminal availabilities of carbohydrate and protein are identified as follows: corn-soybean meal (C-SBM); corn,barley-blood meal (C,B-BM); corn,barley-urea (C,B-NPN); and barley-soybean meal (B-SBM). Rumen available carbohydrate was varied by replacement of cracked corn (C) with ground barley (B), whereas, RAP was varied by substitution of blood meal (BM) for soybean meal (SBM). Urea (NPN) was added to the diet containing the highest RAP (fast protein).

Table 1. Experimental formulations of diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

Ingredient	Diet[1]			
	C-SBM	C,B-BM	C,B-NPN	B-SBM
	----------% of DM----------			
Corn Silage	14.4	14.1	14.1	14.1
Alfalfa Silage	35.3	35.2	34.8	35.3
Cracked Corn	34.8	20.0	19.5	0.0
Ground Barley	0.0	20.0	19.5	38.2
Soybean Meal	14.7	6.8	11.3	9.7
Bloodmeal	0.6	3.7	0.0	2.5
Urea	0.0	0.0	0.6	0.0
Mineral premix	0.2	0.2	0.2	0.2

[1] C-SBM = corn, soybean meal; 65% RAP, 72% RAC
C,B-BM = corn & barley, blood meal; 59% RAP, 66% RAC
C,B-NPN = corn & barley, urea; 73% RAP, 68% RAC
B-SBM = barley, soybean meal; 65% RAP, 65% RAC

Table 2. Chemical composition of diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

	Diet[1]			
Component	C-SBM	C,B-BM	C,B-NPN	B-SBM
DM, %	63.0	63.1	63.3	63.1
CP, %DM	18.4	18.5	18.5	18.5
RAP, %CP[2]	65.3	59.3	72.2	65.2
CHO, %DM[3]	78.5	78.7	78.7	79.2
RAC, %DM[4]	70.8	66.0	68.8	64.9
NDF, %DM	22.1	24.3	22.5	23.1
ADF, %DM	20.1	21.3	21.3	22.6
NE_l, Mcal/kg	1.67	1.66	1.66	1.67
EE, %DM	3.1	2.8	2.8	2.3
Ca, %DM	.37	.36	.36	.38
P, %DM	.38	.35	.37	.37

[1] Dietary descriptions in Table 1.

[2] RAP = rumen available protein determined from in situ, as a percent of CP

[3] CHO = [(100-(NDF+CP+EE))+NDF]

[4] RAC = $\frac{[.9(100-(NDF+CP+EE)) + (NDF \times NDF\ availability)]}{[(100 - (NDF+CP+EE)) + NDF]}$

RAC was calculated according to the equation of Nocek and Russell (19): RAC = [.9(NDS - (CP + EE)) + (NDF x NDF availability)] / [(NDS - (CP + EE)) + NDF] where NDS = (100 - NDF). Crude protein (RAP) and NDF availabilities were determined by in situ.

All diets were formulated to meet or exceed NRC (17) recommendations. The total mixed diets were fed ad libitum at 0600 h and 1700 h with at least 10% refusal. Refusals were recorded daily at 1645 h, sampled and stored at -5° C until composited by period.

Experimental Periods

Each experimental period was 21 d with the first 10 d for dietary adjustment. Digesta markers were administered from d 10 through d 20 and in situ dacron bags were suspended in the rumen from d 19 through d 21. Duodenal digesta and fecal samples were taken d 17 through 21. Feed intake and milk production were also recorded during this period. Duplicate milk samples were taken from two consecutive milking on d 20 and d 21 of each period for component analysis. Milk was analyzed by the Virginia Tech Dairy Herd Improvement Association with a 4-channel spectrophotometer (Multispec Mark I, Foss Food Technology, Eden Plains, MN) for milk fat, protein, and somatic cell count. Cows were weighed on d 16 of each period.

Digesta Flow, Ruminal Fermentation Measurements and Chemical Analysis

Cows were dosed twice daily via rumen cannula with 15 g cobalt ethylenediaminetetraacetic acid (CoEDTA) and 30 g chromic oxide powder immediately prior to each feeding (0545 h and 1645 h). The CoEDTA was selected as a liquid-phase digesta marker and chromic oxide powder served as the particulate-phase marker. CoEDTA was prepared according to Uden et al. (26, 32) and administered as a liquid (240 ml/d) through the rumen cannula. Chromic oxide was pre-weighed in brown paper bags (7.5 cm x 15 cm), delivered through the rumen cannula, turned upside down in the dorso-cardia region, and left inside the rumen for complete dispersement of the chromic oxide. Prior to placement in the rumen, bags were cut into two pieces (6 cm from bottom) to improve the ease of chromic oxide delivery.

Digesta marker concentrations were measured for use in determination of nutrient flow to the duodenum, and the apparent nutrient digestibilities of the rumen and the total gastrointestinal tract. Freeze-dried digesta and fecal samples (.5 g) were digested with 4 ml HNO_3 and 2 ml $HClO_4$ in 50-ml digestion tubes held in a 60-tube block digestor for approximately 8 h (125 to 150° C). Digested samples were diluted to 50 ml and filtered (Whatman #1). The filtrate was collected for analyses of cobalt and chromium.

CoEDTA crystals, chromic oxide powder, and prepared cobalt and chromium standards were digested simultaneously with the biological samples. Concentrations of cobalt and chromium were determined by atomic absorption spectrophotometry (Varian AA-475, Varian Techtron Pty. Ltd., Springvale, Australia). Conditions for analysis followed Varian atomic absorption specifications.

Duodenal digesta and fecal samples were collected on d 17 (1000 h), d 18 (0200 h and 1800 h), d 19 (0600 h and 2200 h), and d 20 (1400 h) so that samples represented 4-h intervals of a 24-h period. At each sampling, 1 L of collected digesta was mixed vigorously to suspend particulate materials and two 240 ml subsamples were collected, sealed, and frozen at -20° C. On d 21 samples were brought to room temperature for compositing. After combining the samples from the six collection times per cow, equal volumes from each cow (500 ml) were centrifuged at 3000 x g for 15 min for separation of liquid and particulate fractions. A separate 150 ml subsample was taken for analysis of whole duodenal contents (not centrifuged). Whole digesta and separated phases were stored in previously weighed plastic cups, freeze-dried, ground (1 mm) in a cyclone mill, and sealed until chemical analysis. Liquid phase samples were ground by hand with a mortar and pestle to avoid excess loss in the cyclone mill. Fecal samples

were thawed on d 21, composited on an equal volume basis using wet feces, freeze-dried, ground (1 mm) in a cyclone mill, and stored in sealed plastic cups. Feed, fecal, and duodenal samples were analyzed for DM, ASH, OM, EE, CP (1), NDF (32), and ADF (13).

Rumen fluid was collected on d 21 of each period via the rumen cannula. Samples (approximately 250 ml) were taken at 30, 60, 120, 180, 240, 360, 480, and 660 min after the morning feeding. This sampling routine allowed for minimal interference with the normal routine of feeding and milking. The morning milking occurred approximately one-h prior to the initial sample (30 min), and the afternoon milking occurred approximately .5 h prior to the final sample (660 min). The maximum time allowed without feed was 1 h. A plastic PVC pipe (1 m in length) containing 4 mm holes along the sides to provide inflow of rumen liquid, was used to facilitate collection of fluid from the top to the bottom of the rumen. Suction through a 5 mm plastic tube fitted with a stainless steel screen completed the collection. Rumen fluid pH was recorded immediately by glass electrode (Orion Ionalyzer, Model 407A). One 5 ml aliquot was placed in a plastic tube containing 1 ml 25% phosphoric acid and 30.2 μmol internal standard (isocaproic acid) for determination of VFA concentrations. Ruminal VFA were measured by a procedure similar to that of Erwin (11)

using a Varian 6000 gas chromatograph and 4270 Varian Integrator (Palo Alto, CA). Prior to VFA analysis, samples were thawed and centrifuged (1000 x g for 10 min). Supernatant was filtered through a .45 μ millipore filter before injection. VFA were separated on a glass column packed with 10% SP-1200/1% H_3PO_4 liquid phase on 80/100 Chromasorb WAW packing (Supelco Inc.).

A second 5 ml aliquot of rumen fluid was collected in a plastic tube containing .5 ml 70% sulfuric acid for NH_3 analysis (6). Both aliquots were frozen at -20° C until analyzed.

On d 21 at 1000 h, a 1 L sample of rumen fluid was collected for isolation of bacteria for determination of bacterial DM, OM, and N. Formaldehyde (25 ml) was immediately added to each sample to stop fermentation. Rumen fluid was then centrifuged (IEC B-22 centrifuge) at 200 x g for 10 min for separation of feed particles and protozoa. The supernatant was aspirated, collected, and centrifuged at 35,000 x g for 16 min to form a bacterial pellet. The soft pellet was resuspended and respun three times to remove contaminating materials. Double-distilled, deionized water was used for each resuspension. The resuspended pellet was freeze-dried and stored in sealed plastic containers. The bacterial samples were analyzed for DM, OM, and CP by the methods previously described.

Rumen bacteria, duodenal digesta and in situ residues were analyzed for cytosine content, a bacterial marker, in order to correct for bacterial protein contamination. Hydrolytic procedures and quantification of samples for cytosine content was similar to Koenig (14) with modifications. Approximately 175, 175, 250, and 250 mg of rumen bacteria, duodenal liquid-phase, duodenal particulate phase, and in situ residues were placed into 15 ml screw-cap glass tubes. To each tube, 2.5 ml 70% perchloric acid was added, gently vortexed, and allowed to set overnight. Samples were hydrolyzed at 90° C for 1 h in a Fisher Dry Bath (Fisher Scientific, Pittsburgh, PA) with mixing at 10-min intervals. Following hydrolysis and cooling, samples were diluted with approximately 10 ml .2 M $NH_4H_2PO_4$ buffer.

Samples were quantitatively transferred to a 100 ml volumetric flask. Concentrated NH_4OH was added to adjust pH to 5.0 before bringing to volume with .2 M $NH_4H_2PO_4$ buffer. A magnetic stirrer was used to thoroughly mix the diluted sample before filtering approximately 20 ml through a .45 μ millipore filter into glass, screw-cap tubes for storage until analysis.

Cytosine was determined by high performance liquid chromatography. Separation of cytosine was by a 25 cm Partisil-10 SCX L column at room temperature. The mobile

phase was .15 M $NH_4H_2PO_4$ with pH adjusted to 3.5 with concentrated HCl.

A Varian 2510 pump was used to force eluent at a rate of .6 ml/min. All bases were monitored at 254 nm as they passed through a 25 μl loop. A 10 μl injection loop was selected for the rumen microbes. Cytosine was detected by a 200 UV/VIS detector in a linear mode. Normal elution was approximately 11.0 min.

Cytosine standards (10, 20, 30, 40, and 50 uM) were prepared in 1.75% $HClO_4$ in .2 M $NH_4H_2PO_4$. Concentrated NH_4OH was used to adjust pH to 3.5. Standards were injected prior to analysis of each sample type and intermittently within sample type for detection of possible analytical variations.

The cytosine to microbial nitrogen (MN) ratio of the rumen bacteria was used to calculate the duodenal liquid and particulate phase MN and the MN contamination of in situ residues. The MN as a percent of the total N for each sample type was determined by the following equations:

$$\% MN_s = (MN/MCYT \times SCYT/SN) * 100$$

where $\%MN_s$ = sample MN, %,
MN = microbial nitrogen, g/g DM,
MCYT = microbial cytosine, umoles/g DM,
SCYT = sample cytosine, umoles/g DM,
SN = sample nitrogen, g/g DM.

Dacron Bag Techniques

Dacron bags were incubated each period in the cow consuming the diet containing corn, barley, bloodmeal, and soybean meal in the concentrate, such that bags were incubated in all cows but during different periods. The in situ procedure followed the guidelines established by Nocek (18). Bags (10 x 20 cm) were spun of polyester dacron (59 um pore-size) and were filled with approximately 5 g DM for a sample size to bag surface area of 14.9 mg/cm^2. Prior to incubation, forages were frozen at -20° C then ground with dry ice in a Thomas-Wiley Laboratory Mill through a 6 mm screen. Dry barley and corn grain were ground in a hammer mill fitted with a 3.2 mm and 9.2 mm screen, respectively. Blood meal and soybean meal were used in the original form. Urea was not incubated, but was considered completely soluble in the rumen.

Bags were suspended from the rumen cap on nylon cords (60 cm) weighted with 3-link segments of 3.2 mm chain. Duplicate bags containing individual ingredients of the total mixed diet were placed in the rumen on d 19, 20, and 21 in reverse order for the following incubation times: 0, 2, 4, 6, 12, 24, 48 h. Forage sources were incubated for 0, 2, 4, 6, 12, 24, 48, and 72 h. Bags were forced to the bottom of the ventral sac of the rumen upon insertion. All bags were removed on d 21, washed in a Maytag washing

machine on delicate cycle, and dried in a forced-air oven at 60° C. Dried residues were weighed and analyzed for DM, CP, NDF, and cytosine content (bacterial marker).

Component degradability (D) in percent was calculated per the equation of Orskov et al. (22):

$D = A + (B \times k_d B)/(k_d B + k_r)$, where

D = DM, N, or NDF degradability, (%);
A = fraction readily degraded, (%);
B = fraction degraded at a measurable rate, (%);
k_d= component degradation constant of B fraction;
k_r= rumen turnover rate (.08/h).

Estimations of fractions A, B, and C were made as outlined by Zerbini (36). Fraction C was calculated as a percent of the original from the residual present after 48 and 72 h incubation for the concentrate and forage sources, respectively. Nitrogen content at each incubation time was corrected for microbial contamination as described by Kwak (15).

Statistical Analysis

All data were analyzed using General Linear Models of SAS (26). The model included:

$$Y_{ijk} = u + C_i + P_j + D_k + E_{(ijk)}$$

where Y_{ijk} = dependent variable,
u = overall population mean,
C_i = effect of cow_i,
P_j = effect of experimental $period_j$,
D_k = effect of dietary $treatment_k$,
E_{ijk} = residual error.

The model used for the ruminal ammonia, pH, and VFA data also included sample time * diet interaction. However, no significant interactions were found. Sums of squares for diet were partitioned into single degree of freedom orthogonal contrasts as follows: 1) carbohydrate source: corn vs barley, 2) protein source: blood meal vs urea, and 3) carbohydrate x protein interaction.

RESULTS AND DISCUSSION

Chemical Composition of Ingredients

The chemical composition of dietary ingredients were similar to that determined in a previous study in which the same ingredients were selected (12). Analysis of ground barley revealed greater NDF than found in cracked corn (19.5 and 9.5 %, respectively), and the percentage NDF in barley was higher than most reports (4, 17, 31). In situ degradabilities (Appendix 1) were also similar to the previously reported DM and CP degradabilities (12). Rates (Kd) of DM and CP were greater for corn than barley, yet NDF rates of degradability were lower for corn than barley. The percentage NDF degradability was 28 and 73 for ground barley and cracked corn, whereas, measurements taken in the previous study revealed degradabilities of 55.5 and 65.6 %, respectively. The barley used in this study was locally purchased, whereas, the ground barley fed in the previous study was grown at the Virginia Tech Dairy Center. The differences in degradability may be due to variety and growing season (4), yet the varieties of purchased corn and barley were unknown.

Intakes of DM, OM, and Fiber

Total DM, CP, and CHO intakes were not different (Table 3). Rumen available protein intake was lowest for C,B-BM and

Table 3. Nutrient intakes (I) of cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

	Diets [1]				
Item	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
DMI, kg/d	23.65	23.43	23.78	23.63	.78
DMI, % of BW	4.10	4.15	4.20	4.13	.16
CPI, kg/d	4.44	4.46	4.59	4.66	.15
RUPI, kg/d[2]	1.54	1.82	1.28	1.62	.15
CHOI, kg/d	18.57	18.44	18.71	18.71	.18
RACI, kg/d[3]	13.20	12.00	12.72	11.79	.16
NDFI, kg/d	5.57	6.86	6.89	8.03	.23

[1] Dietary descriptions in Table 1.

[2] Orthogonal contrast: BM vs NPN, P<.05.

[3] Orthogonal contrast: C vs B, P<.09.

highest for C,B-NPN as designed. Calculated rumen available carbohydrate intake was higher when cracked corn (C-SBM) replaced ground barley (B-SBM). Much of this difference in available carbohydrate intake can be explained by the lower NDF and ADF intake of cows fed C-SBM (corn).

Nutrient Flow and Digestibility

Although two digesta markers were administered prior to sampling, the data will be presented from analyses of the whole digesta only. Chromic oxide was used as the digesta marker for predicting nutrient flow and digestibility from the whole, uncentrifuged samples. The data from the whole digesta was used because the analyses of the data from the particulate and liquid phase samples resulted in biologically unfeasible digestibilities and nutrient flows. However, the results from calculations based on the whole digesta were similar to other reports using chromic oxide as a single marker (16, 35). The cause of the erroneous data is unclear, however, several possibilities exist. These include the possibility of errors occurring during cow-side sampling, compositing and subsequent subsampling, inappropriate separation of phases during centrifugation, cross-contamination of markers between digesta phases, and analytical error during final digestion and dilution of samples. Error in digestion of the particulate phase samples is likely. Subsequent to analyses of the samples in

this study, others in our laboratory have found similarly large variations in marker concentrations (chromic oxide and cobalt) of primarily the particulate digesta phase. Most recently, variations between duplicates were lowered by decreasing the amount of nitric acid by 1 ml and replacing it with 1 ml 72 % sulfuric acid. It appeared that in previous analyses, the nitric and perchloric acids were evaporating rapidly, prior to complete digestion of the particulate phase samples. Additionally, a means of regulating the temperature of the heating block at 125 to 130° C has been implemented. These changes in the procedure have improved duplication, yet further modifications of the procedure are under investigation. The whole digesta samples in this study were digested at 125 to 135° C with nitric and perchloric acid only.

DM, OM, and Fiber Digestibility and Flow

Differences in ruminal carbohydrate and protein availabilities did not influence DM flow to the duodenum (Table 4). Apparent digestibility of DM in the rumen was not different, even after correcting for microbial DM contribution. Total tract apparent digestibility of DM was numerically different by protein source, as digestibility was greater for diets containing urea (C,B-NPN) than blood meal (C,B-BM). No differences in total tract digestibility were attributed to the rate of carbohydrate availability.

Table 4. DM intake, flow, and digestibility in the digestive tract of cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

	Diets[1]				
	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
DM Intake, kg/d	23.65	23.43	23.78	23.63	.78
Flow to duodenum, kg/d	14.37	14.69	15.23	14.80	1.23
Nonmicrobial flow to duodenum, kg/d[2]	11.99	11.83	12.49	12.03	.93
Ruminal digestibility, %					
Apparent, %	39.05	36.77	35.53	37.07	4.33
Corrected, %[3]	49.14	49.12	47.09	48.77	3.04
Flow to feces, kg/d[4]	9.09	8.57	7.92	9.22	2.35
Total tract digestibility, %[5]	61.65	63.63	66.72	61.07	1.49

[1] Dietary descriptions in Table 1

[2] Nonmicrobial DM = Duodenal DM flow - microbial DM flow

[3] 100-[(Duodenal DM flow - microbial DM flow)/DM intake * 100]

[4] Orthogonal contrast: BM vs NPN, P<.05

[5] Apparent digestibility

The numerical differences in apparent total tract digestiblity of DM were due primarily to the lower (P<.05) flow of DM to the feces in C,B-NPN. Apparently, more post-ruminal digestion of DM occurred when diets contained both corn and barley in combination with SBM and urea, rather than diets containing either corn or barley when supplemented with more undegradable protein sources than SBM. Organic matter intake, flow and digestibility (Table 5) followed the same pattern as DM.

Apparent ruminal digestibility of NDF of C-SBM was less than B-SBM (P<.05)(Table 6). McCarthy et al. (16) reported greater ruminal NDF degradation of corn-based diets than barley-based diets. However, these authors reported lower intake of barley-based diets compared with corn, and overall lower fiber digestibility than found in our study.

Table 5. Intake, flow and digestibility of OM in the digestive tract of cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

	Diets [1]				
	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
OM Intake, kg/d	22.31	22.17	22.43	22.23	.78
Nonmicrobial flow to duodenum, kg	11.11	11.27	11.83	11.24	1.23
Ruminal digestibility, corrected, %[2]	49.93	48.99	46.86	49.19	.95
Flow to feces, kg	7.67	7.17	6.60	7.65	4.33
Apparent total tract digestibility, %	65.61	67.60	70.58	65.58	3.00

[1] Dietary descriptions in Table 1.

[2] 100 - [(duodenal OM - microbial OM)]/OM intake * 100.

Table 6. Intake, flow and digestibility of NDF in the digestive tract of cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

	Diets [1]				
	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
NDF Intake, kg/d[2]	5.56	6.86	6.89	8.03	.23
Flow to duodenum, kg	2.99	3.63	3.51	3.78	.21
Apparent ruminal digestibility, %	45.70	46.65	48.25	52.60	3.68
Flow to feces, kg[2,3]	2.89	2.93	2.74	3.53	.11
Apparent total tract digestibility, %[2,3]	47.93	57.30	60.40	56.15	2.02

[1] Dietary descriptions in Table 1.

[2] Orthogonal contrast: C vs B, P<.01.

[3] Orthogonal contrast: carbohydrate x protein, P<.05.

Others have reported higher total tract digestibilities of NDF with corn-based diets than barley (10, 16), yet barley intake was significantly lower than corn in those studies, unlike our diets.

Intake of ADF was greatest in barley diets; whereas, apparent ruminal digestibility and apparent total tract digestibility was lowest (Table 7). The most total tract apparent digestion occurred with C,B-NPN, yet no differences in ruminal apparent digestion were detected. These data imply greater post-ruminal apparent digestibility in diets containing cracked corn, regardless of the degradability of protein source. Weiss et al. (35) reported similar apparent, total tract digestibility of ADF in diets containing alfalfa silage-barley (42.6%) and alfalfa silage-corn (54.5%).

Nitrogen Digestibility and Metabolism

Although not significant, greater N intake and N flow to the duodenum (Table 8) occurred with B-SBM. In turn, nonmicrobial N flow to the duodenum was higher ($P<.10$) in B-SBM than C-SBM. The ratio of microbial N to cytosine used in this study was 2.3. The ratios varied from 1.9 to 2.4 with the largest variations due to differences between cows. No differences in microbial N to cytosine ratio due to carbohydrate or protein source were detected. Microbial

Table 7. Intake, flow and digestibility of ADF in the digestive tract of cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

	Diets [1]				
	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
ADF intake, kg/d[2]	4.27	4.25	4.43	4.62	.11
Flow to duodenum, kg[2]	2.12	2.52	2.53	2.60	.06
Apparent ruminal digestibility, %	41.85	38.93	39.41	39.69	1.62
Flow to feces, kg[2,4]	1.97	2.07	1.92	2.48	.05
Apparent total tract digestibility, %[3]	53.95	51.30	56.60	46.45	1.43

[1] Dietary descriptions in Table 1.

[2] Orthogonal contrast: C vs B, P<.05.

[3] Orthogonal contrast: C vs B, P<.07.

[4] Orthogonal contrast: carbohydrate x protein, P<.05.

Table 8. Nitrogen intake, flow, and digestibility in the gastrointestinal tract of cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

	Diets[1]				
	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
N Intake, g/d	710.3	717.6	734.0	744.9	23.2
Flow to duodenum, g/d					
Total, g/d	515.3	583.5	577.2	591.3	39.9
Microbial, g/d	240.5	275.9	281.3	260.7	33.3
Nonmicrobial, g/d[2]	274.8	307.6	295.9	330.6	21.0
Ruminal digestibility, %					
Apparent, %[3]	61.1	56.5	59.5	55.4	3.2
Corrected, %[4]	27.1	17.5	20.9	20.5	5.4
Recovery, % N intake	72.9	82.5	79.1	79.5	5.4
Flow to feces, g/d[5]	249.1	247.6	226.9	259.2	9.4
Total tract digestibility, %[6]	65.0	65.6	69.1	65.1	1.8

[1] Dietary descriptions in Table 1

[2] Orthogonal contrast: C vs B, P<.10.

[3] (N intake - N flow)/N intake.

[4] (N intake - nonmicrobial N flow)/N intake.

[5] Orthogonal contrast: carbohydrate x protein, P<.05.

[6] Apparent digestibility, not corrected for endogenous or protozoal N.

N flow was unaffected by source of protein or carbohydrate, unlike those reporting greater microbial N flow with supplemental barley (16, 23, 25). The numerically highest microbial N flow was found in cows fed C,B-NPN, yet differences were not significant. The average ruminal ammonia N and ruminal pH were also highest for C,B-NPN, indicating greater available N for microbial protein synthesis. When microbial N was determined from NRC (17), based on dietary NE_l of 1.66 Mcal/kg, the estimated microbial N was 385 g. The prediction equation was: microbial N (g/d) = 6.25 (-30.93 + 11.45 NE_l). The mean microbial N flow measured in this study was 68.7% of predicted. The discrepency between predicted and measured may be partly attributed to our method of harvesting the microbial pellet. Only the liquid associated bacteria are measured in our procedure, since no attempt is made to harvest bacteria from the particulate fibers of the rumen. Also, the predicted quantity is based on energy density of the diet, estimated as NE_l, which does not take into consideration the proportion of structural and nonstructural carbohydrates provided by the diet. Large variations in these carbohydrate fractions could directly increase or decrease microbial yield (20).

No differences in total N flow to the duodenum was attributed to carbohydrate or protein sources, which is

similar to the data of (16, 25). The measured N flow to the duodenum was similar to predicted (17), except when C-SBM was fed. Nitrogen flow was lowest with C-SBM, and can probably be attributed to the lower N intake. As calculated from NRC (17), the predicted N at the duodenum was 588 g. Actual N flow exceeded that predicted by the equations of Journet (475 g) and Verite (479 g) as cited in (17).

Ruminal and total tract N digestibilities were also unaffected by carbohydrate and protein source. Similar to our data, McCarthy et al. (16) found numerically higher total tract apparent digestibility of N when barley replaced corn in total mixed diets.

Ruminal pH, Ammonia, VFA

Overall, average ruminal fluid pH was not different (Table 9). However, at 3 and 4 h post-feeding, ruminal fluid pH was affected by rate of protein availability (Figure 1). Diets containing low RAP (C,B-BM) produced lower pH than diets containing high RAP (C,B-NPN). At 11 h post-feeding, a carbohydrate x protein interaction occurred. Diets containing blood meal (BM) resulted in the lowest ruminal pH (C,B-BM) and diets containing cracked corn produced the highest pH (C-SBM).

Ruminal pH was lower than expected at all sampling times for suggested optimum fermentation. Optimum pH for high cellulolytic activity has been proposed to be 6.8 (30).

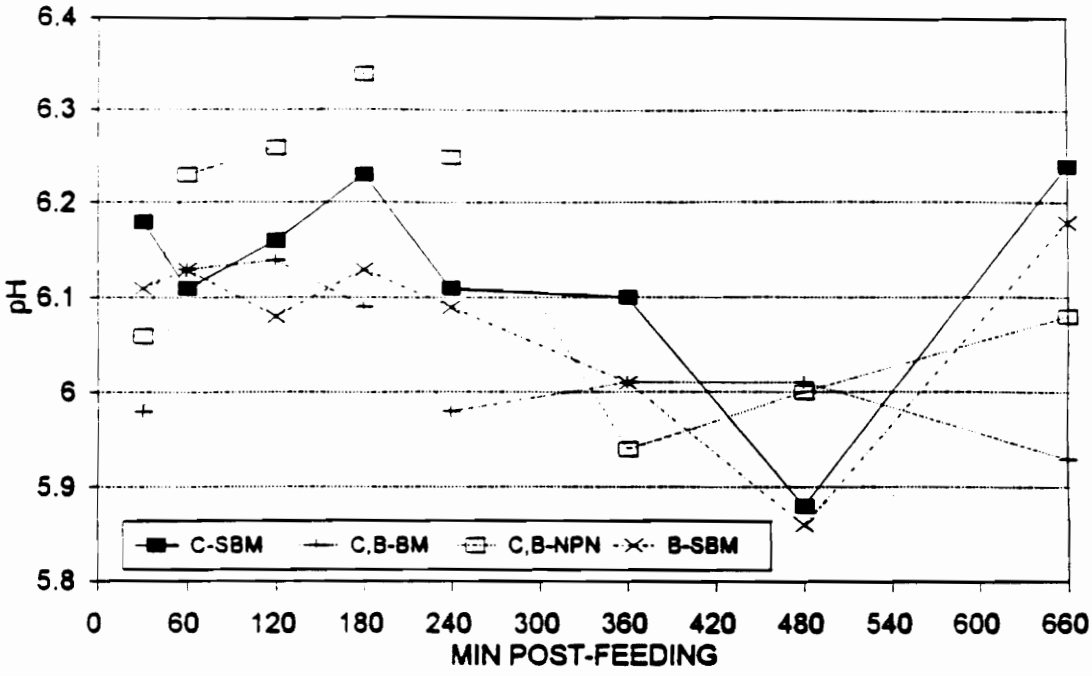

Figure 1. Ruminal fluid pH in cows fed diets varying in ruminal available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.

Table 9. Changes in rumen fluid pH in cows fed diets varying in rumen undegradable protein (RUP) and rumen available carbohydrate (RAC).

Post-Feeding	Diets[1]				
Min	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
30	6.18	5.98	6.06	6.11	
60	6.11	6.13	6.23	6.13	
120	6.16	6.14	6.26	6.08	
180[2]	6.23	6.09	6.34	6.13	
240[2]	6.11	5.98	6.25	6.09	
360	6.10	6.01	5.94	6.01	
480	5.88	6.01	6.00	5.86	
660[3]	6.24	5.93	6.03	6.18	
Overall mean	6.13	6.03	6.14	6.07	

[1] Dietary descriptions in Table 1.

[2] Orthogonal contrast: BM vs NPN, P<.05.

[3] Orthogonal contrast: carbohydrate x protein, P<.05.

Ruminal fiber digestibility declines as ruminal fluid pH declines, especially at pH less than 6.0 (27). The overall low ruminal pH in all diets may have resulted in decreased cellulolytic activity, thus potentially lower fiber digestibility.

Ruminal ammonia N concentrations reflected the excess dietary N in these diets (Table 10). The optimum ruminal ammonia required to maximize growth of ruminal bacteria has received much debate, yet Satter and Slyter (24) suggested a minimum of 5 mg/dl of ammonia N. The overall average concentration of ammonia N in this study exceeded the suggested minimum indicating potential for optimizing bacterial growth. The lowest concentration of ruminal ammonia occurred 8 h post-feeding when high levels of BM were fed (C,B-BM). Ruminal ammonia was significantly higher ($P<.05$) in C,B-NPN than C,B-BM at 1, 2, 3, and 8 h post-feeding (Figure 2). The greater ruminal ammonia N concentrations in C,B-NPN correspond to the higher ruminal pH values at the same sampling times, post-feeding. Additionally, the lower average ruminal ammonia concentration from 3 h through 11 h post-feeding in B-SBM compared with C-SBM reflects the slower degradability and ruminal deamination of the N in corn than barley.

The adequate ruminal ammonia N levels apparently promoted equivalent fermentation across diets, since no

Table 10. Post-feeding ruminal ammonia concentrations in cows fed diets varying in rumen undegradable protein (RUP) and rumen available carbohydrate (RAC).

Post-Feeding	Diets [1]				
Min	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
	----------mg/dl----------				
30	15.4	14.3	17.5	13.2	1.4
60[2,3]	18.3	17.2	24.3	16.3	1.4
120[2]	17.6	17.6	23.6	18.6	1.6
180[2,3]	15.0	14.2	23.8	12.3	1.8
240	15.8	16.8	21.7	16.8	2.0
360	14.4	15.3	19.0	12.8	2.7
480[2]	14.5	10.9	20.5	12.9	2.7
660	14.0	14.9	19.4	12.4	1.9
Overall mean[2]	15.6	15.2	21.2	14.4	.7

[1] Dietary descriptions in Table 1.

[2] Orthogonal contrast: BM vs NPN, P<.05.

[3] Orthogonal contrast: carbohydrate x protein, P<.05.

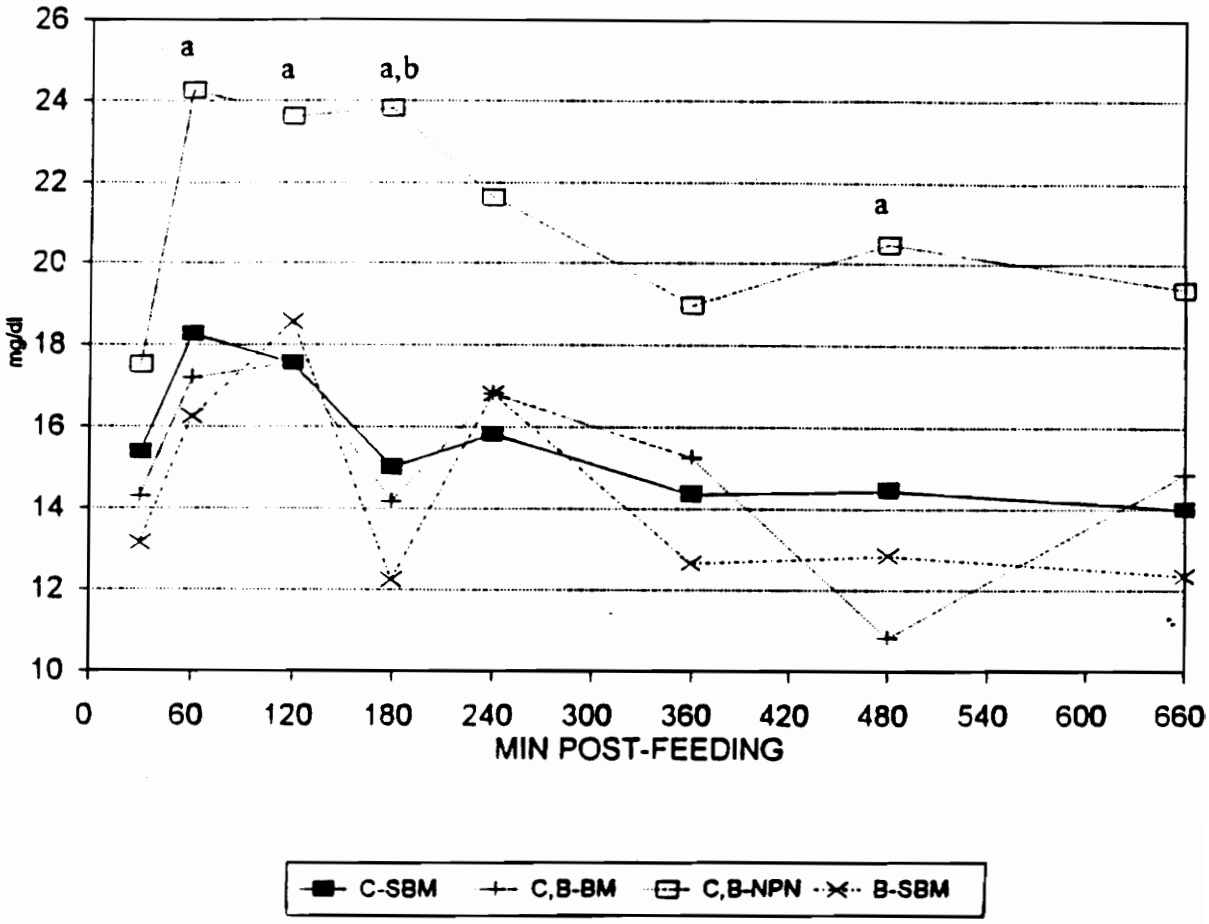

Figure 2. Ruminal ammonia concentrations in cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal. [a] Blood meal vs soybean meal, $P<.05$. [b] carbohydrate x protein, $P<.05$.

differences were found in apparent ruminal DM (Table 4), OM (Table 5), NDF (Table 6), and ADF (Table 7) digestibilities attributed to protein source. These data infer that ruminal ammonia was not limiting for optimum cellulolytic microbial activity, as has been suggested when blood meal was the primary protein source in vitro (2). In a study using cannulated lactating cows (34), rumen ammonia concentrations were 16.1 mg/dl when blood meal was the only supplemental protein source. When SBM replaced blood meal on an equal protein basis, ammonia N averaged 32.2 mg/dl (34). Contrary to our results, low levels of ruminal ammonia were reported by McCarthy et al. (16) in cows fed barley or corn combined with either supplemental SBM or fish meal in alfalfa silage, corn silage-based total mixed diets. However, McCarthy et al. (16) detected no differences in apparent ruminal degradation of OM, NDF, starch, or ADF, as well as OM truly digested in the rumen, as a result of ruminal ammonia N averaging less than 4 mg/dl.

The high concentrations of VFA in ruminal fluid (Table 11) reflected rapid and extensive carbohydrate fermentation in the rumen. The high VFA concentrations also depressed ruminal pH to an average of 6.1 (Table 9). At 2 h post-feeding, total VFA was higher ($P<.05$) in B-SBM than C-SBM diets. The highest concentration of total VFA (Figure 3) occurred in all diets 8 h post-feeding and is reflected in

Table 11. Total VFA concentrations in rumen fluid of cows fed diets varying in rumen undegradable protein (RUP) and rumen available carbohydrate (RAC).

		min post-feeding							
VFA	Diet[1]	30	60	120	180	240	360	480	660
		umol/ml rumen fluid							
Total VFA,	C-SBM	143.40	163.21	128.74[2]	142.76	144.26	161.12	179.71	133.95
umol/ml	C,B-BM	146.07	144.64	157.87	147.04	150.40	158.89	165.56	160.46
	C,B-NPN	158.40	168.48	140.40	135.88	140.91	173.29	175.33	155.10
	B-SBM	152.98	149.57	157.65	141.67	147.27	170.44	178.73	148.09

[1] Dietary descriptions in Table 1.

[2] Orthogonal contrast: C vs B, P<.05

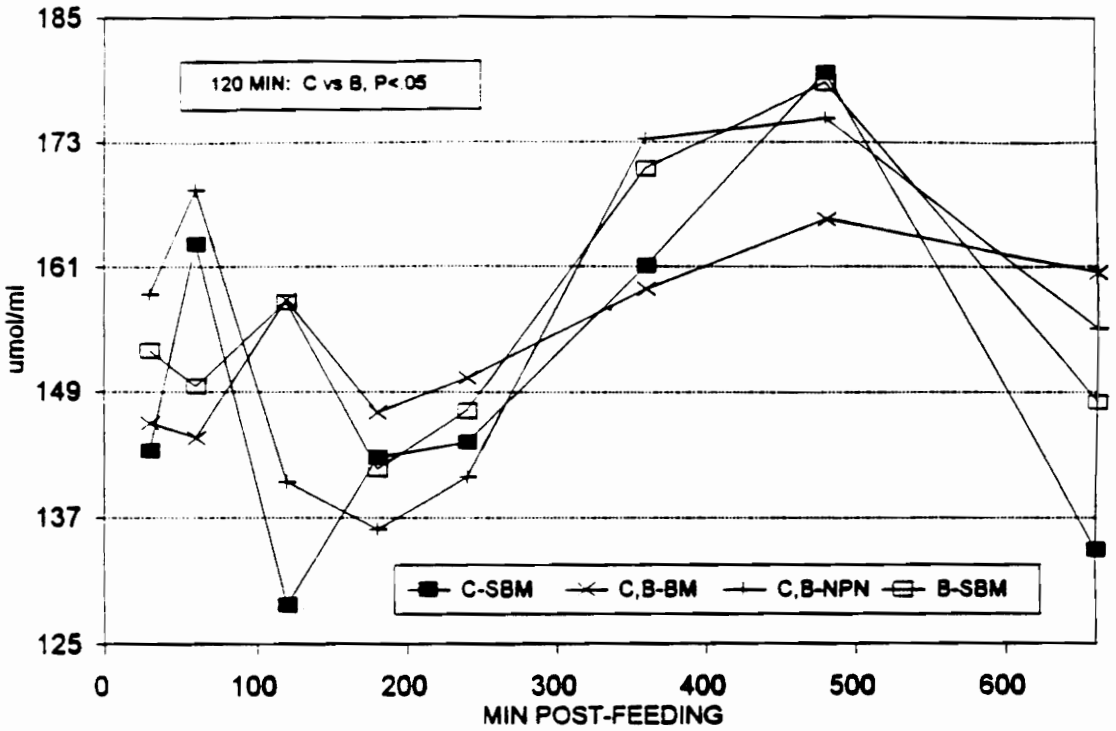

Figure 3. Total VFA concentrations of ruminal fluid from cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.

the lowest average ruminal pH at 8 h post-feeding. Similar to our results, DePeters and Taylor (10) found low ruminal pH (mean = 6.1) in cannulated heifers fed corn or barley based total mixed diets of a chopped alfalfa hay base, with total VFA concentrations unaffected by carbohydrate source.

Total VFA concentrations were high in all diets and reflected rapid and extensive degradation of carbohydrate in the rumen, regardless of carbohydrate source. At 2 h post-feeding, total VFA concentrations were lower ($P<.05$) in corn based diets (C-SBM) than barley (B-SBM), probably as a result of the more rapid rate of barley degradation than corn (20). From 3 through 11 h post-feeding, total VFA were similar in C-SBM and B-SBM, while both increased 6 and 8 h post-feeding.

No differences in molar proportions of acetate (Figure 4) or propionate (Figure 5), or the ratio of acetate to propionate (Figure 6) were detected. However, the greatest difference in acetate occurred 6 h post-feeding. The diets containing either corn (C-SBM) or barley (B-SBM) peaked, whereas, those containing both barley and corn with differing protein degradabilities were lowest (C,B-BM and C,B-NPN). Conversely, molar proportions of propionate was lowest in C-SBM and B-SBM, whereas, C,B-BM and C,B-NPN were intermediate 6 h post-feeding. The overall molar

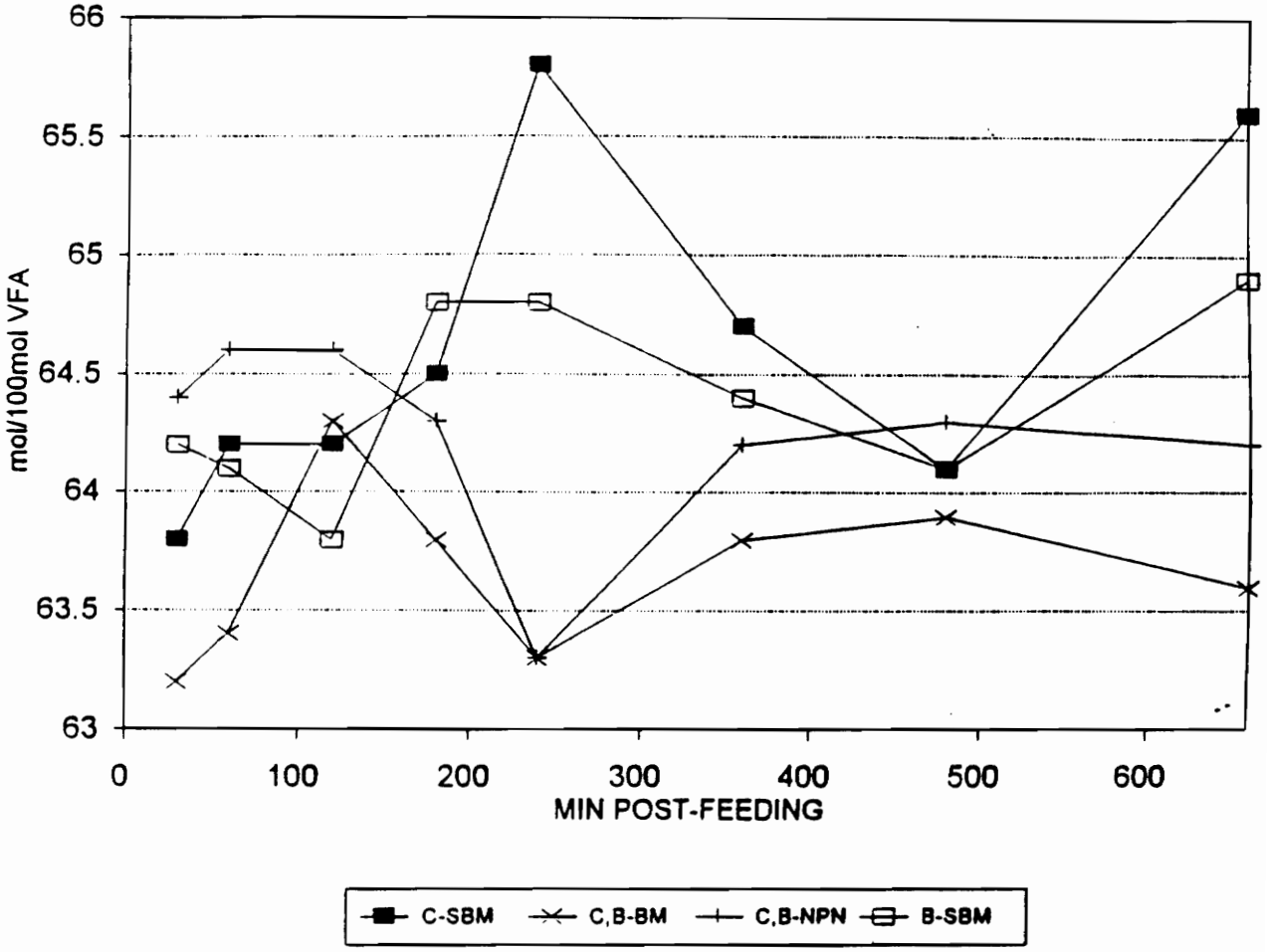

Figure 4. Molar proportions of ruminal acetate in cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.

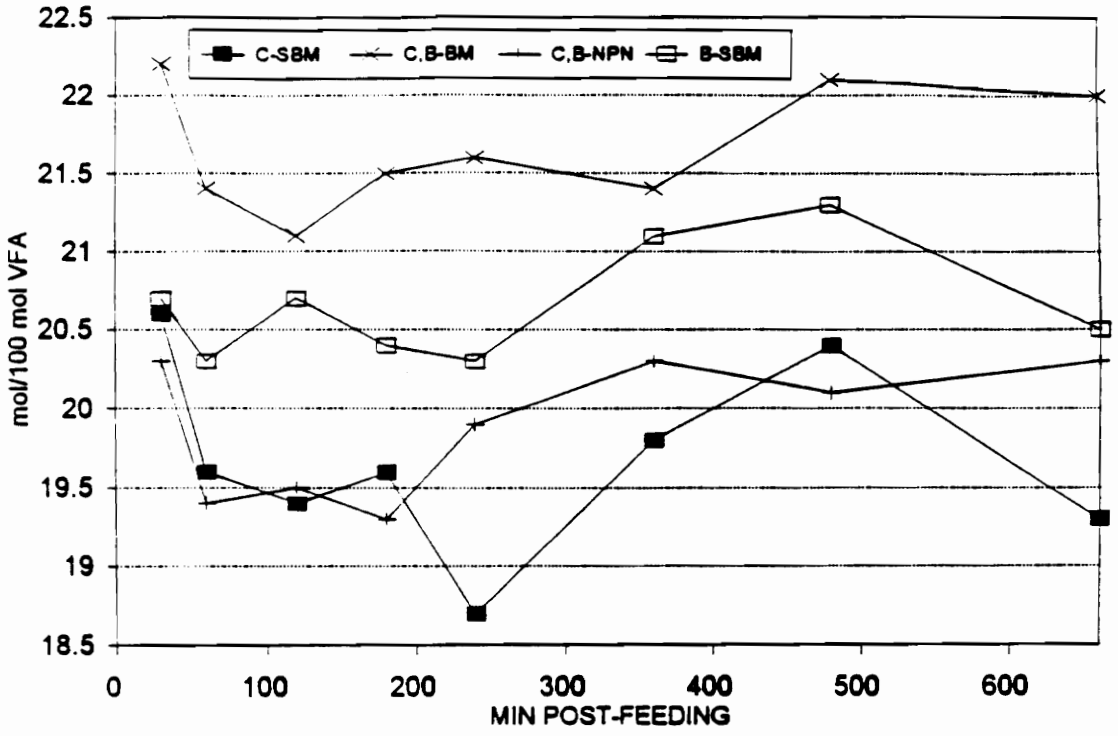

Figure 5. Molar proportions of ruminal propionate in cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.

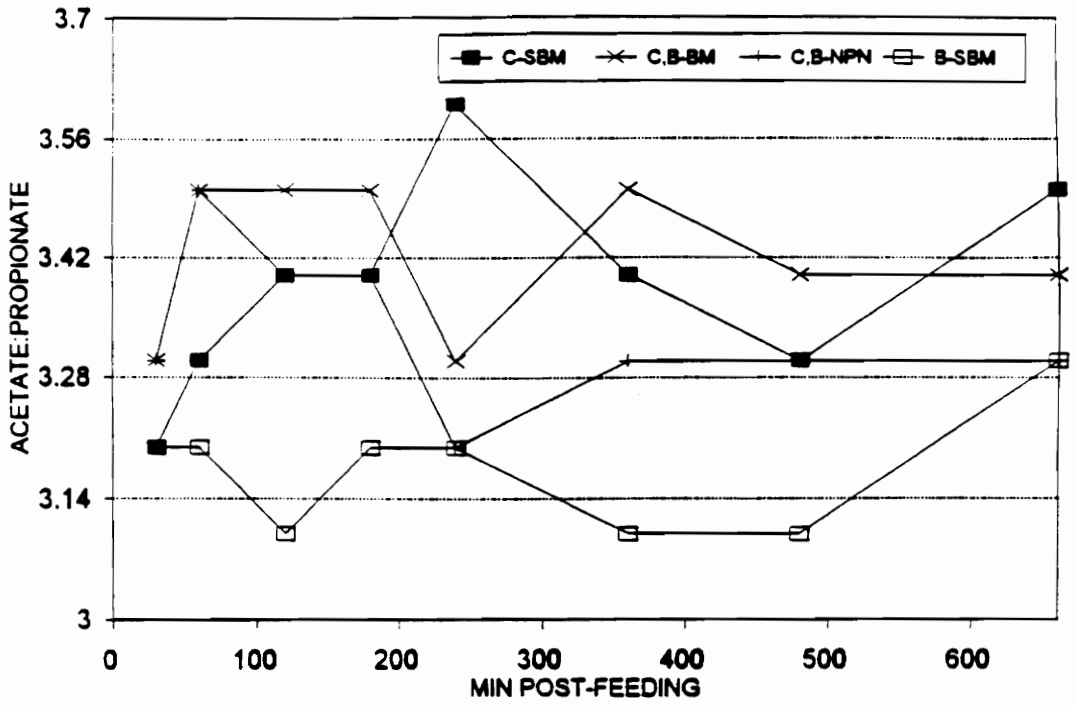

Figure 6. Ratio of ruminal acetate to propionate in cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.

proportions of propionate was numerically higher in C,B-BM than the others. This response was unexpected, since it has been suggested that increases in propionate occur when proteins are readily fermented by bacteria (34), implying that diets with less degradable protein would result in less propionate production.

Isobutyrate (Figure 7) and butyrate (Figure 8) ruminal molar proportions were not affected by source of protein or carbohydrate. However, the average molar proportions of both isobutyrate and butyrate were lowest with C,B-BM. In continuous culture, Bas et al. (2) found lowest isobutyrate when BM was fed, compared with feather meal, soybean meal, and calcium-lignosulfonate treated SBM. Stock et al. (28) also reported lower isobutyrate and isovalerate when BM was fed, compared with SBM. These data imply that the branched chain amino acids required by some ruminal bacteria (3) could be limiting when high levels of BM are fed. However, differences were not significant at the level of BM fed in our study.

The molar proportions of isovalerate (Figure 9) were highest in corn diets (C-SBM) 1 to 4 h post-feeding, then declined. Although concentrations were less than C-SBM, the B-SBM also peaked early and declined at an even faster rate than C-SBM. No differences due to protein source were detected. The numeric differences in

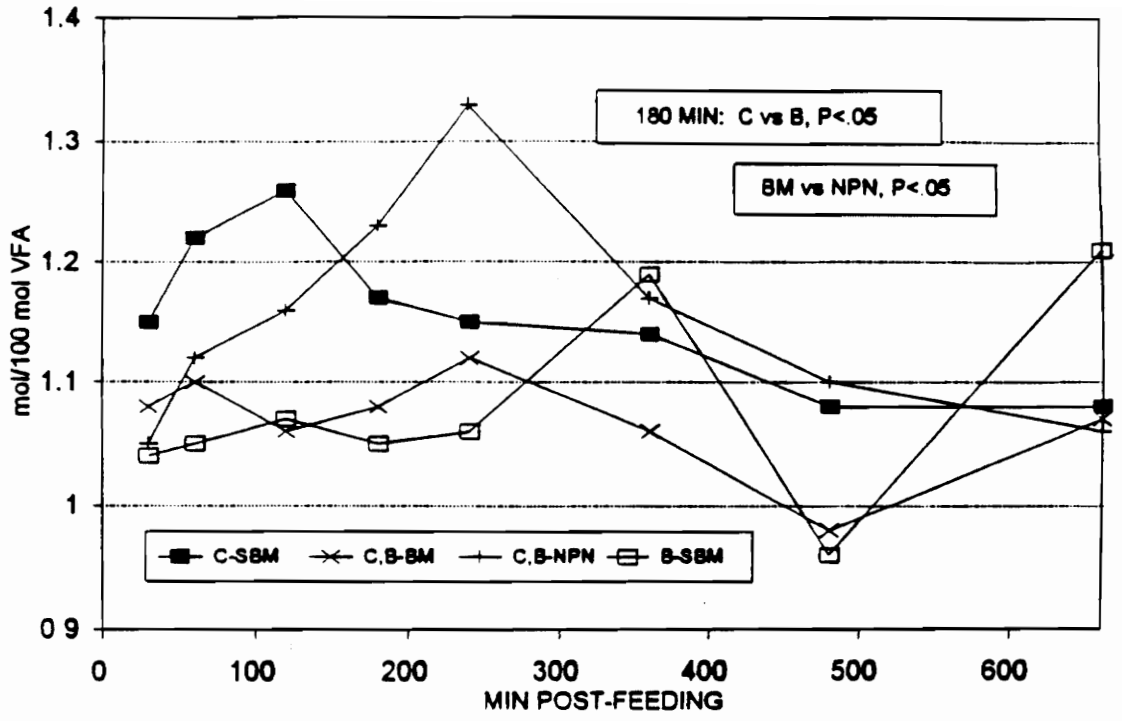

Figure 7. Molar proportions of ruminal isobutyrate in cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.

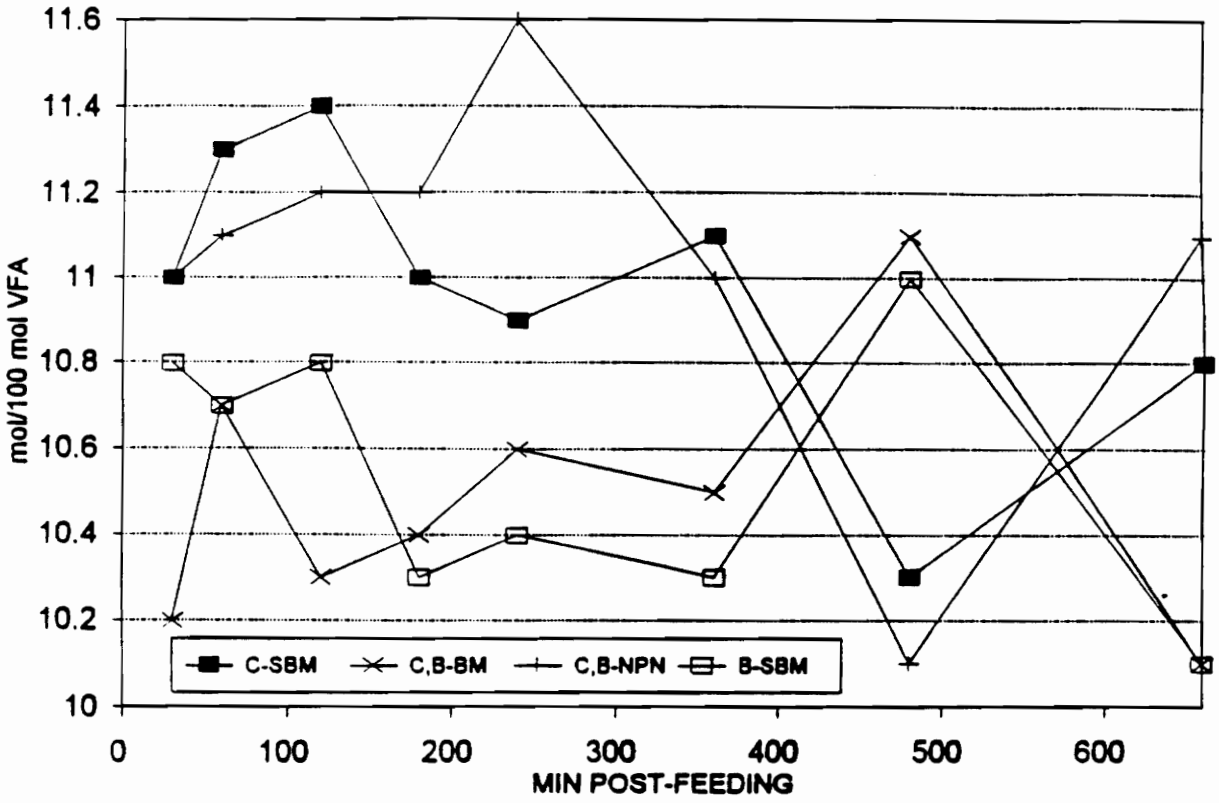

Figure 8. Molar proportions of ruminal butyrate in cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.

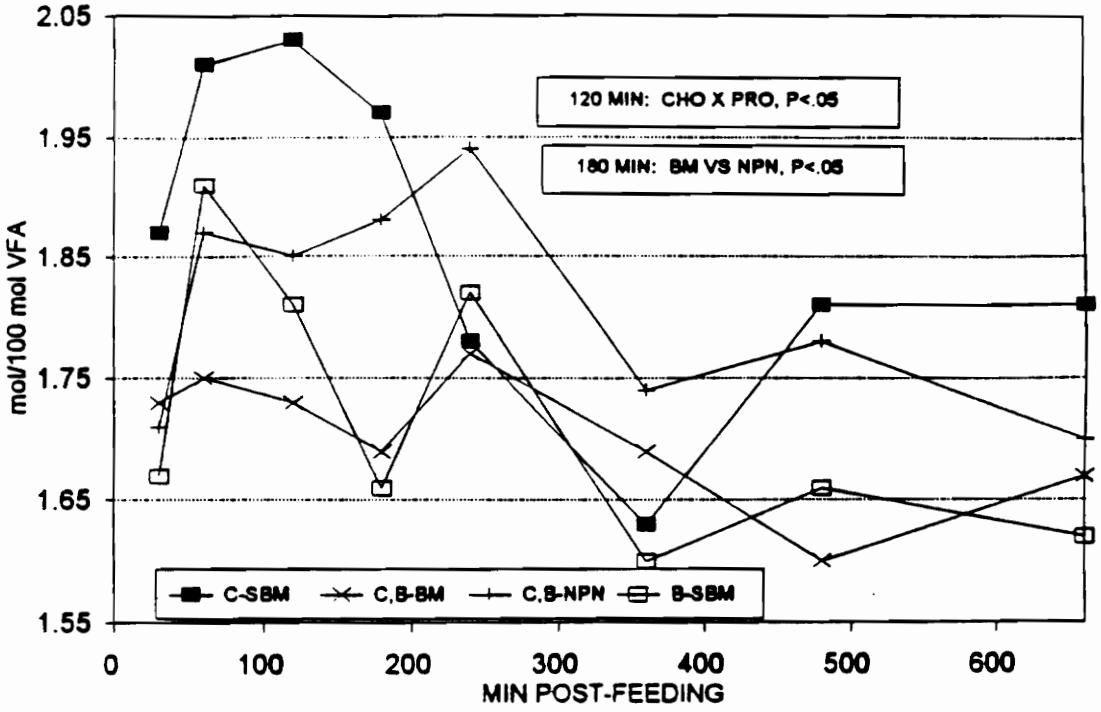

Figure 9. Molar proportions of ruminal isovalerate in cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.

isovalerate may be attributed to the high level of leucine commonly reported for corn (7). Leucine is a precursor of the branched chain VFA, isovalerate (2). Casper et al. (4) also found higher ($P<.05$) molar proportions of isovalerate in corn than barley-based diets when cows were sampled three h post-feeding. Valerate molar proportions (Figure 10), were greater for C,B-NPN than C,B-BM ($P<.05$), but were not different due to source of available carbohydrate.

Milk Production

Milk yield was not affected by carbohydrate and protein availabilities, yet 3.5% fat-corrected milk was higher ($P<.05$) when greater ruminal undegradable protein (BM) was fed (Table 12). Fat yield was also improved by feeding BM (C,B-BM) compared with urea (C,B-NPN) when both barley and corn were included as carbohydrate sources. The greater fat yield can be attributed to higher milk yield, and numerically higher milk fat percentage. Milk fat percentage was higher than reported by others (4,16) feeding diets varying in carbohydrate availability. This may be attributed to the acetate:propionate being greater than 2.25 in all diets. Davis (9) suggested that acetate:propionate molar ratios less than 2.25 were associated with depressed milk fat percentages and yields. Additionally, ruminal NDF

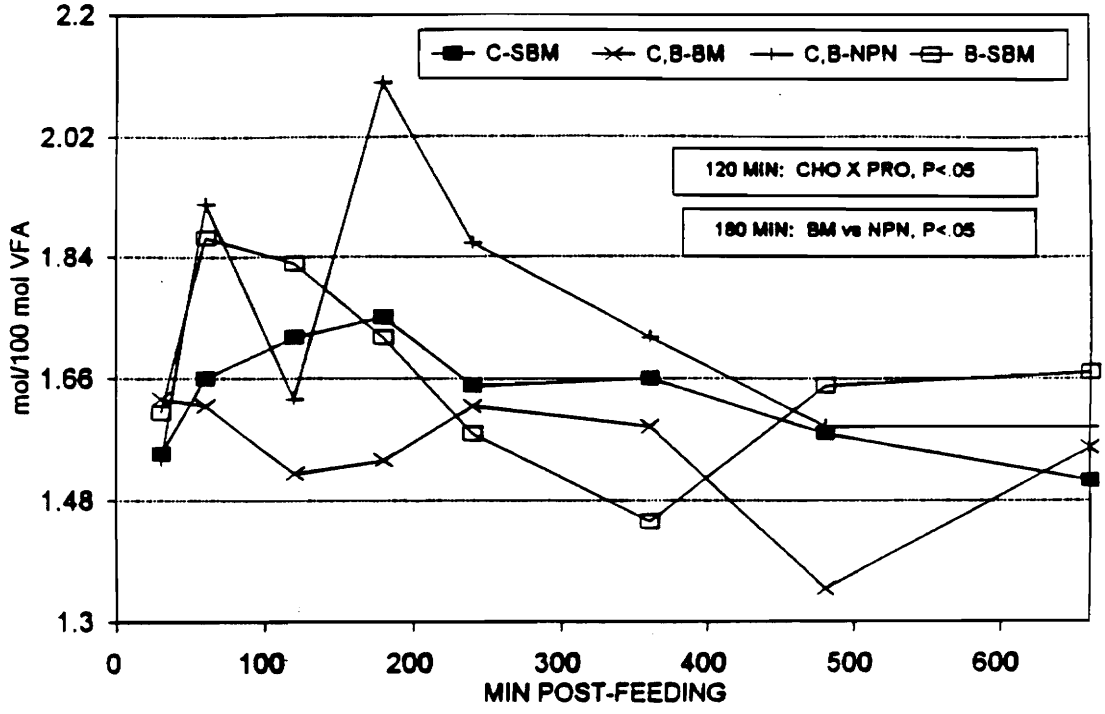

Figure 10. **Molar proportions of ruminal valerate in cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC). C-SBM = corn, soybean meal; C,B-BM = corn & barley, blood meal; C,B-NPN = corn & barley, urea; B-SBM = barley, soybean meal.**

Table 12. Milk yield and composition of Holstein cows fed diets varying in rumen available protein (RAP) and rumen available carbohydrate (RAC).

Component	Diets [1]				
	C-SBM	C,B-BM	C,B-NPN	B-SBM	SE
Milk, kg/d	30.20	30.13	28.78	30.65	.72
3.5% FCM, kg/d[2]	31.11	33.07	30.01	32.12	.69
Fat, %	3.70	4.12	3.78	3.82	.12
Fat yield, kg/d[2]	1.12	1.24	1.09	1.17	.03
Protein, %[2]	3.15	3.06	3.21	3.22	.03
Protein yield, kg	.96	.92	.92	.99	.03

[1] Dietary descriptions in Table 1.

[2] Contrast: BM vs NPN, P<.05

and ADF digestion promoted high acetate production in all dietary treatments. Milk protein percentage was greater ($P<.05$) for B-SBM than C-SBM. McCarthy et al. (16) found no differences in milk fat percentage, milk fat yield, 4% FCM production, or protein percentage due to source of energy (barley, corn) or supplemental protein (fish meal, SBM). The reported increase in milk production could be attributed to the greater DM intake of corn diets compared to barley in their study.

Conclusions

The differences in rumen available carbohydrate and rumen available protein as characterized in this study, were not great enough to illicit a change in ruminal fermentation endproducts, as reflected by ruminal pH and VFA measurements. As a result, no differences in microbial N flow to the duodenum were detected. Total tract apparent fiber digestibility (NDF and ADF) was greater in diets containing corn than barley. Ruminal NDF digestibilities were also greater in corn than barley.

The varying rates of carbohydrate and protein availability, as determined by in situ incubations, did not affect ruminal pH or total VFA concentrations. However the greater ruminal ammonia-N concentrations found when C,B-NPN was fed indicated a lack of synchrony of the amounts of readily available carbohydrate and N in the rumen. The

other dietary combinations appeared to provide an appropriate amount of carbohydrate and N for efficient utilization of the available N by the rumen microbes.

The use of RAC as an estimate of available carbohydrate should be reconsidered for feedstuffs such as the source of corn and barley used in this study. Difficulties and inconsistencies in analysis of feed grains for NDF content can result in misleading RAC estimations. Other factors, such as starch availability and nonstarch polysaccharide availability, measured quantitatively, may be a viable alternative to the NDF availability currently used in the RAC equation. However, the use of NDF was convenient, since it is commonly used by nutritionists and dairymen. The determinations of individual carbohydrate fractions is less convenient and is not accepted as routine analysis.

Additional information on effects of varying amounts, sources, and rates of rumen available carbohydrate and protein on nutrient utilization is needed. Although all combinations of RAP and RAC used in this study were adequate for effective nutrient utilization and adequate milk production, more studies are needed to determine the effects of varying RAP and RAC (over a wider range of RAC than used in this study) in diets utilizing various carbohydrate and protein feed ingredients. A comparison of differing forage sources in total mixed diets is also needed.

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