RUMINAL PROTEIN DEGRADATION: EFFECTS ON LOW QUALITY FORAGE INTAKE AND UTILIZATION BY BEEF COWS

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CHAPTER I

INTRODUCTION

Fall calving beef cows have high energy and protein requirements due to lactation, postcalving tissue regeneration and environmental stress (NRC, 1984). The nutritive value of dormant winter range in Oklahoma is very low. Crude protein levels may fall below 3% of DM by November (Waller et al., 1972). The low protein content of dormant range usually is coupled with low dry matter digestibility. Consequently, dormant range will not meet the energy and protein requirements of lactating beef cows. Supplemental nutrients must be provided for the cowherd to perform satisfactorily (Rakestraw et al., 1986).

Cows meet a large proportion of their protein requirement from ruminal microbial synthesis (Orskov, 1982). Ruminal microbial growth is principally a function of diet fermentability. With low protein low digestibility forages, protein supplementation should increase fermentability, microbial growth and microbial protein flow to the duodenum (Stokes et al., 1988). The quantity of supplemental ruminally degraded protein (RDP) required to maximize microbial protein synthesis, forage utilization and intake of cows grazing dormant rangeland is unknown.

Commercial range supplements are formulated and sold on the basis of total protein. Most range cubes contain 12, 20, 32 or 40% protein. These supplements are formulated by mixing cottonseed meal or soybean meal with cereal grains or low-protein byproduct feedstuffs. Extent of ruminal protein degradation varies considerably among these feedstuffs (NRC, 1985). Consequently, protein degradation of range supplements can be markedly different, although total protein content may be identical. Ruminal protein degradation is an overlooked, yet vital characteristic that may deserve consideration in range supplementation programs. Because the

fermentability of dormant range is low, RDP is needed to stimulate fiber digestion. The quantity of RDP needed to maximize the utilization and intake of dormant range, however, is not known.

Microbial protein synthesis may be inadequate to meet the protein requirements of lactating cows when forage digestibility is low (Orskov, 1982; Owens and Bergen, 1983). In this case, cow performance might be enhanced by incorporation of ruminally undegraded protein (RUP) feedstuffs into range supplements (Lee et al., 1985; Hibberd et al., 1988; Miner and Petersen, 1989; Miner et al., 1990). The amino acid composition of feedstuffs is variable. Consequently, Klopfenstein et al. (1985) recommended that one feedstuff should not contribute more than 60% of the RUP fraction. Furthermore, the efficacy of incorporating high-protein byproduct feedstuffs into range supplements for lactating beef cows requires further evaluation. Optimum ratios of RUP have been evaluated in other classes of livestock. Matras et al. (1990) reported that the optimal proportion of RDP, independent of feed intake level, in lamb diets containing 10.5% protein. Recommendations for the proportion of RUP range from 20 to 55% for dairy cows consuming minimum levels of CP (NRC, 1985). These observations with lambs and dairy cows suggest that the requirement of supplemental protein for low quality forage diets be balanced for both RDP and RUP.

Because, the end products of ruminal fermentation are used to meet the energy and protein requirements of the ruminant, RDP requirements are critical. In addition, RUP might not be useful unless ruminal fermentation has been stimulated sufficiently by ruminal ammonia-N (Hibberd and Martin, 1990). Therefore, RDP would have a higher priority than RUP in supplements for low quality forage diets. Consequently, the effect of RUP cannot be evaluated until supplemental RDP requirements have been addressed. The objectives of this dissertation were to: a) determine the quantity of RUP required to maximize 1) the intake and utilization of hay and 2) microbial protein synthesis; b) evaluate the effects of incorporating RUP feeds into supplements at two levels of protein on hay intake and utilization, ruminal fermentation and composition of duodenal nitrogen flow; and c) justify results of objective b by feeding these same supplements to lactating beef cows grazing dormant native grass pastures.

CHAPTER II

LITERATURE REVIEW

Nutritional Status of Lactating Beef Cows

Grazing Dormant Pastures

Forage Quality and Nutrient Requirements

From December through March, the average TDN (36%; Lusby, 1985) and CP (2.5%; Waller et al., 1972) content of native range is extremely low. In addition, the quality of winter rangeland declines as the season progresses (Waller et al., 1972). This decline in forage quality primarily is due to a decline in the leaf to stem ratio (Minson, 1981; Poppi et al., 1981) caused by selective grazing and leaching of nutrients (Waller et al., 1972). Additionally, physical factors such as rainfall, snow cover, and trampling gradually render higher quality plant parts inaccessible. Therefore, the need for energy supplementation increases as the winter progresses.

The lactating beef cow (454 kg) with average milking ability (4.5 kg/d) requires 56.6% TDN and 9.6% CP in the diet (NRC, 1984). Absolute daily requirements for TDN and CP are 5.2 kg and 909 g, respectively. Consequently, this cow must consume forage at a daily rate of 3.2 and 8.0% of body weight to meet TDN and CP requirements, respectively. A lactating cow producing 4 kg milk/d requires substantially more TDN (1.3 kg) than a steer gaining 1.5 kg/d (Owens et al., 1991). This relationship illustrates the exacerbated energy demands due to lactation. In addition, cold stress increases maintenance energy requirements (NRC, 1984) and decreases energy available for milk production. Without sufficient energy intake, the postpartum interval will be lengthened and subsequent reproductive performance will be impaired (Wettemann et al., 1987;

Short et al., 1990). These relationships clearly illustrate the nutritional inadequacies of low quality forage relative to lactating cow requirements which in turn justifies supplementation.

Energy Status in Response to Supplementation

Energy intake has been increased by feeding supplemental protein due to increased ruminal ammonia-N, digestion, rate of passage and intake of low quality forage (McCollum and Galyean, 1985; Guthrie and Wagner, 1988; Stokes et al., 1988; DelCurto et al., 1990a). Although protein supplementation increases concentrations of total VFA (McCollum and Galyean, 1985; Stokes et al., 1988) and molar proportions of propionate (McCollum and Galyean, 1985; Judkins et al., 1987; Stokes et al., 1988; DelCurto et al., 1990a), energy intake still may limit the performance of lactating cows.

The energy status of cows can be improved by feeding moderate-protein (20% CP) energy supplements (Furr and Nelson, 1964; DelCurto et al., 1990b). These supplements must be fed at high rates (2 to 4 kg/d) to meet the energy requirements of lactating cows. Numerous energy supplements are formulated with cereal grains that supply large quantities of starch (Hibberd et al., 1982). Because a majority of microbes digest starch preferentially to fiber (Mertens and Loften, 1980), ruminal pH drops (< 6.2) rapidly and inhibits growth of cellulolytic bacteria (Stewart, 1977; Mackie et al., 1978; Orskov, 1982). In addition, the protein in cereal grains is degraded in the rumen only moderately; this may deprive the rumen of nitrogen (NRC, 1985). Consequently, large quantities of corn-based supplements have depressed forage utilization and intake to the extent that total energy intake was not improved (Chase and Hibberd, 1987; Pordomingo et al., 1991). In contrast, Lee et al. (1987) supplemented corn to steers fed low quality grass hay and reported that forage intake and subsequent energy intake increased.

Byproduct feedstuffs such as soybean hulls, wheat middlings (midds) and corn gluten feed contain little starch but are useful as energy supplements in forage diets (Fleck et al., 1987, 1988; Trautman, 1987; Martin and Hibberd, 1990; Ovenell et al., 1991; Sunvold et al., 1991; Chan, 1992). Byproduct feedstuffs offer digestible fiber that does not depress forage utilization (Highfill

et al., 1987, Martin and Hibberd, 1990). Therefore, digestible fiber feedstuffs offer a low-starch alternative to cereal grain supplements.

Maximizing Forage Utilization With Supplemental Protein

Protein supplementation increases forage utilization (Kartchner, 1981; McCollum and Galyean, 1985; Guthrie and Wagner, 1988). When excess ruminally degraded protein (RDP) is fed, microbes may digest more protein than they resynthesize (Broderick, 1990). Therefore, forage utilization would continue to increase to a point where excess supplemental protein would be preferred as an energy substrate by microbes. DelCurto et al. (1990a,b) suggested that excess supplemental protein reduced forage utilization. They reported that intake of hay and dry matter peaked ($P<.10$; quadratic) when moderate (1 kg of 28% CP) levels of supplemental protein were fed to 227 kg steers maintained on low quality (3.1% CP, OM basis) hay or native range (8.6% CP, OM basis). Supplements were formulated with increasing quantities of soybean meal whereas supplemental energy supply was equalized with dry-rolled grain sorghum. Comparisons were made with low protein-high starch supplements versus high protein-low starch supplements. Consequently, their interpretation is questionable because starch levels varied in supplemental treatments.

Hay intake increased curvilinearly (Guthrie and Wagner, 1988) or linearly (Stokes et al., 1988) in response to increasing quantities of supplemental soybean meal, however, hay intake did not peak. Those studies did not answer the question of how much supplemental RDP is required to stimulate the maximum utilization of low quality forage. Supplemental energy consumption increased concomitantly with supplemental protein; consequently, effects of supplemental protein could not be evaluated independently of supplemental energy because protein and energy were confounded. Graded levels of RDP supplementation could be evaluated more appropriately if supplemental energy consumption was equalized with a digestible fiber feedstuff that does not compete with cellulolytic bacteria. Soybean hulls appear to be a noncompetitive, high energy feedstuff (Hibberd and Martin, 1990) with protein characteristics that closely reflect those of

soybean meal. Changes in forage utilization and microbial protein synthesis then could be attributed to level of supplemental RDP.

Ruminal Nitrogen Status

Ruminal ammonia-N stimulates the utilization of ingested fiber by cellulolytic bacteria (Van Soest, 1982) and many cellulolytic species require ammonia-N as their sole source of nitrogen (Orskov, 1982). Ruminal ammonia-N concentrations are related to the CP content of forage (Hogan, 1981) and are regulated by the relative availability of protein and energy (Weston and Hogan, 1968). Concentrations of ammonia-N in ruminal fluid often are low (.7 to .9 mg/dl) in cattle consuming low quality forage diets (Guthrie and Wagner, 1988; Stokes et al., 1988). These concentrations are well below the quantity estimated to be required to maximize microbial protein synthesis (Satter and Slyter, 1974).

As plants mature, their protein content declines more rapidly than OM digestibility, and the ratio of digestible OM (DOM) to CP may rise (Hogan, 1981). Allden (1981) suggested that when the ratio between digestible protein and DOM falls below 2.5 g N/100 g DOM intake is reduced. Consequently, when this ratio is below 2.5, supplemental protein would be required. Guthrie and Wagner (1988) reported that low quality grass hay contained 5.57% CP (OM basis) and 41.1% DOM. This yields a nitrogen to digestible organic matter ratio (N/DOM = .891/.411) of 2.17. Protein supplementation (83 g of RDP) increased ruminal ammonia, hay utilization and digestible dry matter intake. DelCurto et al. (1990a) fed hay that contained 2.6% CP with a DDM content of 35.5%; this yields a N:DOM ratio of 1.17. Supplementation with a 25:75 blend of grain sorghum and soybean meal increased forage utilization. In contrast, supplements composed of a 90:10 ratio of sorghum grain to soybean meal depressed forage intake. These observations verify the positive effect of protein supplementation on the utilization of low quality forage diets.

In addition to providing ruminal ammonia-N (Guthrie and Wagner, 1988; Stokes et al., 1988), protein supplements provide branched-chain volatile fatty acids which are required at low concentrations by certain cellulolytic bacteria (Dehority et al., 1967). Supplementation with

branched-chain volatile fatty acids, however, has failed to substantially affect ruminal fermentation (McCollum et al., 1987; Gunter et al., 1990).

Estimates of ruminal ammonia-N requirements vary. Satter and Slyter (1974) utilized a mixed population of ruminal bacteria in continuous culture and reported that ruminal ammonia-N concentrations of 2 to 5 mg/dl maximized microbial protein synthesis. Hume et al. (1970) reported that ammonia-N concentrations of 13.3 mg/dl maximized nonammonia N (NAN) flow to the small intestine of sheep. Substantially higher values i.e., 19 mg/dl (Allen and Miller, 1976) and 23.5 mg/dl (Mehrez et al., 1977), increased rate of digestion and were suggested to increase duodenal NAN flow. These estimates appear to be very high for low quality forage; however, ruminal ammonia-N concentrations may need to be high to reach cellulolytic microbes located in isolated niches within the rumen (Owens and Bergen, 1983). Microbial protein synthesis has been maximized by intraruminal infusions of urea which yielded a ruminal ammonia-N concentration of 7 mg/dl (Nikolic et al., 1975). Variation in cell numbers and permeability of cells to ammonia make it improbable that one concentration of ammonia could maximize microbial growth under various dietary conditions (Smith, 1979).

Nitrogen Recycling

Recycled N functions as a mechanism to conserve N that might be lost via the urine (Schmidt-Nielsen, 1977). The supply of N available for recycling is greater early than late in pregnancy and during pregnancy than during lactation due to protein needs of the developing fetus or protein deposition in milk (Owens et al., 1991). During lactation, mobilization of body reserves to meet energy demands also may increase protein mobilization which could increase the availability of N for recycling (Owens et al., 1991). This relationship would be particularly true for fall calving cows grazing dormant pastures. Although low N diets result in low concentrations of plasma urea, the ratio of urea excreted to that which is filtered by the glomerulus was reduced in ruminants consuming low N diets (Scott and Mason, 1970; Phillips et al., 1975). Low N diets reduced the rate of glomerular filtration (Ericcson and Valtonen, 1982) and enhanced tubular

reabsorption of urea (Harmeyer and Martens, 1980). It is unclear, however, if low N diets induce a special mechanism by which ruminants conserve N. It seems plausible that reduced excretion of urinary urea for ruminants fed low N diets is the result of recycling to the alimentary tract (Moir and Harris, 1962). Regardless of cause and effect relationships, N appears to be conserved when low N diets are consumed by ruminants.

Ruminal microbes can utilize recycled urea as a source of N (Harris and Phillipson, 1962). Consequently, the supply of ammonia-N to ruminal microbes should be more constant than feeding patterns would suggest. Urea entry across the ruminal wall has been observed in Merino sheep fed a low N roughage diet. Recycling also can occur in the abomasum and intestines; however, the utilization of low quality forages would be more greatly impacted by entry of recycled N into the rumen. Fermentation characteristics effect the contribution of urea to the ruminal ammonia pool (Egan et al., 1986). High concentrations of carbon dioxide and butyrate have increased the permeability of urea across the ruminal wall; in contrast, high ruminal ammonia-N concentrations inhibit recycling (Englehardt et al., 1978; Kennedy and Milligan, 1980; Kennedy et al., 1981). The mechanism proposed for these relationships is related to ureolytic bacteria that line the ruminal wall (Wallace, 1979; Cheng and Costerton, 1980). They suggest that urea diffusion from blood through the ruminal wall is accelerated by these bacteria. Consequently, when ruminal ammonia-N concentrations are low, the concomitant urease activity is high; this could increase N recycling to the rumen (Orskov, 1982).

Ruminal Microbial Status

Under normal feeding conditions, 25 to 60% of microbial N has been derived from NAN sources (Mathison and Milligan, 1971; Oldham et al., 1981; Steinhour et al., 1982). Replacing urea N with isonitrogenous quantities of amino acid N increased both microbial protein production and microbial efficiency (Maeng and Baldwin, 1976; Cotta and Russell, 1982; Russell, 1983). Consequently, efficiency of microbial growth may be limited by amino acid supply. However, with low quality (.5% N) grass hay diets, microbial protein synthesis was not decreased when urea

replaced up to 75% of supplemental soybean meal at isonitrogenous and isocaloric levels; however, OM digestion was depressed (Kropp et al., 1977a). Microbial efficiency was actually greater for supplements containing greater proportions of urea. In that study, feed intake was restricted; thus energy supply or passage rate may have limited ruminal microbial synthesis. Therefore, ruminal OM fermentation of low quality forage diets might be limited by the supply of peptides and(or) preformed amino acids.

In addition to N, bacterial growth is dependent upon the supply of fermentable carbohydrate (Orskov, 1982). Digestible OM must be supplied in a synchronous rate with the synthetic ability of ruminal microbes for the efficient utilization of ruminally degraded nitrogen (Oldham et al., 1977). High lignin content of dormant range decreases its potential extent of digestion (Mertens, 1977). Therefore, reduced digestibility of low quality grass combined with a low level of nitrogen may limit bacterial growth due to the low rate and extent of carbohydrate and protein digestion (Stern and Hoover, 1979). In addition, energetic uncoupling (fermentation without ATP production) theoretically may occur when N levels are inadequate in forage diets (McMeniman and Armstrong, 1977).

Supplementation of low quality forage with protein increased rate and extent of digestion, intake and digesta passage (Barton and Hibberd, 1984; Guthrie and Wagner, 1988). Consequently, microbial protein flow to the small intestine should have increased (Bergen et al., 1982; Firkins et al., 1986) and bacterial maintenance requirements probably were reduced (Hespell, 1979). Hibberd and Martin (1990) substituted graded levels of ruminal undegraded protein (RUP) for RDP in low quality grass hay diets. Increasing the proportion of RUP to RDP deprived ruminal microbes of nitrogen; thus decreasing the rate and extent of fiber digestion and forage intake. Consequently, intake of low quality forage appears to be highly dependent upon the supply of ruminally degraded N.

Microbial efficiency, expressed as grams of microbial N produced per kg of OM truly fermented in the rumen, is associated with rate of flow from the rumen (Owens and Isaacson, 1977). Enhanced microbial efficiency due to increased ruminal dilution may be the result of: a)

reduced bacterial autolysis; b) reduced protozoal predation; c) shifts in microbial population or d) increased washout of microbes possessing slow generation intervals, i.e., a lower proportion of microbial energy is expended on maintenance functions (Kennedy et al., 1976). Hespell (1979) suggested that concentrations of inhibitory products and cell density also may be associated with improved microbial efficiency. Protein supplements might be expected to increase microbial efficiency; however, increasing the quantity of soybean meal did not improve the microbial efficiency of steers fed low quality grass hay (Stokes et al., 1989).

The technique of Zinn and Owens (1982) which precipitates free purines with silver nitrate was utilized to estimate microbial protein synthesis in this manuscript. It is commonly referred to as the RNA procedure (even though purines are present in RNA and DNA) because RNA is used as the reference standard and results often are cited as RNA equivalents. In that assay, components that could interfere with the estimation of purine content are centrifuged prior to the determination of purine concentrations by spectrophotometry. Other analytical procedures used to quantify DNA and RNA measure either intact polymers or the component sugars, but not purines. The validity of the purine procedure relies on the assumption that nearly all RNA from dietary sources is degraded ruminally (McAllan and Smith, 1973). Buttery and Cole (1977) expressed doubt as to whether all RNA from feed sources are digested ruminally. Consequently ruminal microbial protein synthesis could be overestimated by the purine procedure when large portions of dietary protein and nucleic acids have been exposed to heat or chemical treatment. Smith et al. (1978) also indicated that the RNA method slightly overestimated ruminal microbial protein synthesis. Another potential error is variability in the purine to total N ratio of attached verses free floating bacteria (Smith and McAllan, 1974). This error can be accounted for if isolated bacteria are used.

Small Intestinal Nitrogen Status

Ruminal microbes are a source of high quality protein for the host animal (Owens and Zinn, 1988). Owens and Bergen (1983) reported that 40 to 80% of the total protein reaching the

small intestine is of microbial origin. Consequently, ruminal microbial protein synthesis can greatly influence the supply of amino acids reaching the small intestine. The primary limiting amino acids in microbial protein alone relative to requirements for growing cattle appear to be those containing sulfur, plus lysine and threonine (Richardson and Hatfield, 1978). Furthermore, lysine and methionine appear to limit milk production on a wide variety of diets (Clark, 1975). Because microbial amino acid composition is relatively constant (Storm and Orskov, 1983), the only way to alter amino acid flow to the small intestine is by the addition of RUP feedstuffs that contain high concentrations of these limiting amino acids. Blends of slowly degraded protein sources with complementary amino acid profiles may supply a more balanced profile of amino acids to the small intestine than individual protein sources or diets supplemented with soybean meal (Cecava et al., 1990). Stock et al. (1981) fed a combination of corn gluten meal and blood meal to growing calves; this resulted in a 28% improvement in protein efficiency above either of the sources alone. Corn gluten meal is rich in ruminal escape sulfur amino acids, whereas blood meal is rich in ruminal escape lysine. The effective use of combinations of protein sources to alter the quantity and profile of amino acids supplied to the host animal depends on satisfaction of the following criteria: 1) microbial protein synthesis must be maintained by including an RDP source in the diet which provides ammonia-N and other products of ruminal proteolysis to microbes and 2) complementary proteins must be resistant to ruminal degradation yet remain digestible in the small intestine (Cecava et al., 1990).

Egan and Moir (1965) increased forage intake of sheep fed low quality forage by infusing casein or urea at the duodenum. Egan (1965) concluded that casein alleviated a protein deficiency and thereby stimulated the rate of removal of metabolites by tissues to promote an intake response. In contrast, urea may have acted primarily by increasing ruminal digestion and passage by recycling to the rumen. Intake of low quality forage by beef steers was increased less by postruminal infusions of casein than urea/glucose (Garza-F et al., 1991). Ruminal fill appeared to be different between treatments although fecal output was not altered. These data support the study by Egan (1965) in which glucogenic substances influenced intake. In a second study,

Garza-F et al. (1991) directly compared urea to urea/glucose and reported that added glucose did not increase intake of low quality forage.

In addition to supplying amino acids and(or) glucogenic precursors, postruminal casein infusion has altered the hormonal status of portal-drained viscera of steers by increasing the release of insulin, glucagon and somatostatin (Guerino et al., 1991). Because amino acid flux accounted for only 26 to 30% of the infused casein, amino acid catabolism or utilization would appear to be extensive during absorption (Owens et al., 1991). Consequently, Owens et al. (1991) suggested that increased forage intake and N retention afforded by postruminal casein infusion might be attributed partly to altered hormonal concentrations.

Ruminally Undegraded Protein Supplementation of Low Quality Forage Diets

Ruminal Protein Degradation

Ruminal protein degradation varies between feedstuffs due to different rates of proteolysis. Sources with a low extent of protein degradation e.g., blood meal and fish meal possess relatively low rates of proteolysis following an immediate burst. In contrast, oilseed meals from soybeans, sunflowers, and cottonseeds are degraded more continuously; therefore, their extent of degradation in the rumen is higher.

Access to protein by proteolytic enzymes is influenced by the three-dimensional structure of the protein molecule. Casein, which possesses an essentially linear structure, is more rapidly degraded than proteins containing more complex tertiary structures (Wallace, 1979). Proteins with extensive crosslinking, such as disulfide bonds, are less accessible to proteolytic enzymes and are relatively resistant to degradation (Nugent and Mangan, 1978). Proteins in hair and feathers are examples of highly crosslinked proteins. Treatment of protein with formaldehyde can create methylene crosslinks, which will reduce the rate of proteolyis by ruminal microbes (Ferguson et al., 1967). Disruption of disulfide bonds allows degradation rates to increase

(Mahadevan et al., 1980; Nugent et al., 1983). For example, ovalbumin, a soluble yet cyclic protein, has no terminal amino or carboxyl groups. The cyclic feature of its tertiary structure greatly reduces its rate of proteolysis (Mangan, 1972).

Solubility is the most widely used estimator of ruminal protein degradation. Soluble proteins differ, however, in rates of microbial hydrolysis (Nugent and Mangan, 1978). Depending on the specific protein, the soluble portion may require disruption of the secondary and tertiary structure for proteolysis to proceed rapidly. Stern and Satter (1982) proposed that the amino acid composition of the soluble fraction of a feedstuff usually differs from that of the more insoluble fraction. Because ruminal microbes have the ability to adapt to soluble organic compounds (Owens and Bergen, 1983), correlations between in vitro and in vivo solubilities are debatable. Solubility has been assumed to reflect extent of degradation; however, alone it is a poor indicator of the extent of ruminal degradation across a variety of diets and feeding conditions (Owens and Bergen, 1983).

The extent of protein degradation is also influenced by the amount of time spent within the rumen. Faster rates of passage decrease ruminal residence time; subsequently, protein degradation is depressed due to a reduced exposure time of protein to microbial attack. Estimates of ruminal passage rate for many protein supplements and most feeds fall between 3 and 7% per hour (Ganev et al., 1979; Stern and Satter, 1982). Ruminal degradation has often been evaluated from the equation: $a + [(bc)/(c + k_d)]$ where a is the soluble component, b is the potentially degraded fraction, c is the rate of disappearance and k_d represents the passage rate constant (Orskov and McDonald, 1979). Interrelationships between individual fractions and their effect on extent of degradation as a whole are more readily understood if fractions a, b and c are set at .20, .80 and .10, respectively. In this case an increase in the passage constant from 3 to 7% per hour will decrease ruminal degradation by 18% (from .82 to .67, respectively). In contrast, when potential extent of degradation is lowered (b=.40) and fraction a remains at .2, an increase in passage from 3 to 7% per hour will decrease extent of ruminal degradation by 14% (from .51 to

.44, respectively). These calculations imply that passage rate depresses ruminal protein degradation to a greater degree when feedstuffs contain a more highly degraded protein fraction.

Level of feed intake may impact extent of ruminal protein degradation by influencing passage rate. Higher producing ruminants, consuming large quantities of feed are more likely to have a smaller fraction of feed protein digested within the rumen than counterparts consuming low to moderate amounts of feed. Increased feed intake in steers (Zinn and Owens, 1983) and dairy cattle (Tamminga et al., 1979) decreased the quantity of feed protein degraded within the rumen. The effect of feed intake on ruminal retention time, however, can be small (Varga and Prigge, 1982) and may not alter extent of ruminal degradation of protein (McAllan and Smith, 1983). Increased feed intake often is accompanied by a lower ruminal pH which may reduce bacterial and proteolytic activity (Satter, 1986). Normal ranges of ruminal pH are from 5.5 to 7.0; thus, proteins with an isoelectric point above this range would have increased solubility and increased protein degradation as pH increased (Satter, 1986).

Environmental temperatures influence residence time of feed within the rumen. Exposure to lower ambient temperature reduced total tract retention time in forage-fed sheep (Kennedy et al., 1976) and cattle (Warren et al., 1974). Studies with forage-fed sheep suggest that the reduction in total tract retention time is largely due to reductions in ruminal retention time, with little to no change in postruminal passage rate (Christopherson and Kennedy, 1983). Consequently, cold-exposure may result in greater ruminal escape of forage protein due to increased ruminal passage rate (Kennedy et al., 1986).

Physiological Effects of Ruminally Undegraded Protein

Ruminal nitrogen status should be considered when undegradable protein sources are supplemented. If ruminal ammonia-N is deficient, microbial growth will be depressed and animal performance may be reduced. When ruminal N requirements are satisfied, however, the addition of feed protein which escapes ruminal degradation may or may not be beneficial. Flow of ruminal microbial nitrogen can meet 50% or more of the amino acid requirements of ruminants under all

states of production (Orskov, 1982). Animals with high protein requirements, however, may benefit from dietary protein that escapes ruminal degradation (Orskov, 1982). Undegraded protein sources may only be useful for growing calves and lactating dairy cows.

Protein supplements have increased forage intake without concomitant increases in the rate and extent of digestion and(or) rate of passage (Egan and Doyle, 1985; Krysl et al., 1987; Hunt et al., 1989). These increased intakes have been attributed to increases in ruminal capacity (Egan and Doyle, 1985; Krysl et al., 1987). Greater ruminal capacities were suggested to be the result of a 35% increase in NAN flow (Egan and Doyle, 1985). Flow of duodenal NAN was increased when protein supplements were fed to cows consuming low quality grass hay (Stokes et al., 1989; Moberg et al., 1989). In addition, increasing duodenal NAN flow could improve the protein/energy (P/E; g digestible protein/ MJ digestible energy) ratio in digestion products (Egan, 1977). With roughage diets Egan (1977) concluded that when the P/E ratio in digestion products is less than 5.5, voluntary intake could be increased by additional flow of digestible protein to the small intestine. Because the P/E ratio required for various functions of production differs, one specific P/E ratio may not be a reliable index. If supplemental RUP increased duodenal NAN flow, then production responses to supplemental RUP (Hibberd et al., 1988; Miner et al., 1990) could be attributed to meeting specific amino acid deficiencies (NRC, 1984) or to improved duodenal P/E ratios.

Growing and lactating ruminants require high quantities of amino acids for protein deposition, whereas both amino acids and propionate can be utilized for glucose synthesis (Preston and Leng, 1984). MacRae and Lobley (1986) suggested that postruminal amino acids may increase the efficiency of maintenance energy utilization. Orskov and Macleod (1982) infused casein abomasally and reported that milk production was increased by a greater amount than the ME equivalent of the infused casein. Apparently cows in negative energy balance during early lactation are particularly sensitive to changes in amino acid supply at the tissue level. Thus, increasing amino acid supply to cows in negative energy balance could increase milk production at further expense of body tissue (Orskov et al., 1981).

Glucogenic amino acids, from supplemental RUP sources, could play an important role in energy metabolism. When propionate is limiting, carbon skeletons of nonessential glucogenic amino acids may supply additional oxaloacetate for the TCA cycle, thereby increasing the utilization of acetate. Therefore, the heat loss from futile cycles involving acetate would be reduced if glucogenic compounds were provided (Preston and Leng, 1984). Supporting this theory is work conducted by Tyrrell et al. (1979) in which infused acetate was utilized by nonlactating cows more efficiently when consuming a corn/alfalfa diet versus a 100% alfalfa diet. Reducing equivalents in the form of NADPH are needed in adequate amounts for body fat and milk fat synthesis from acetate and butyrate (Annison and Armstrong, 1970). In ruminant adipose tissue, TCA cycle intermediates provide the majority of NADPH used for fat synthesis via the isocitrate dehydrogenase shuttle (Leat, 1983). In ruminant mammary tissue, however, glucose oxidation via the pentose phosphate pathway provides two-thirds of the $NADPH_2$ required for milk fat synthesis (Black et al., 1967). Glucose is a major precursor of both lactose and glycerol phosphate, the latter of which is required for fatty acid esterification by ruminant mammary tissue (Baldwin and Smith, 1983). These interrelationships illustrate the metabolic role of NAN or, more specifically, glucogenic amino acids as a substitute for glucose in the metabolism of acetate.

Supplementation of RUP improves reproductive efficiency of beef cattle presumably via mediations of insulin in metabolic pathways (Wiley et al., 1991). Cows with depleted adipose reserves and(or) a restricted supply of nutrients prepartum and postpartum have reduced plasma insulin concentrations (McCann and Reimers, 1985). Low insulin concentrations in postpartum cows may extend the time required by ovaries to respond to LH or other ovarian functions vital to reproduction (Wiley et al., 1991). An additional supply of protein stimulates pancreatic secretions of insulin in cattle independent of body condition (Kaneko, 1989; Weekes, 1991). This increase in plasma insulin may partition more nutrients toward maternal tissue replacement and increase weight gain of cows postpartum (Hunter and Magner, 1988) at the expense of milk production. Consequently, ruminally undegraded protein may act as a catalyst of metabolic and hormonal

activity rather than merely as a nutrient to satisfy protein requirements for milk production and weight gain (Wiley et al., 1991).

In summary, supplemental RUP potentially could: 1) correct a specific amino acid deficiency, 2) supply nonessential amino acids to enhance utilization of metabolizable energy and(or) milk fat synthesis, 3) improve the P/E ratio of digestion products or 4) modulate hormonal activity. In this manner, depending on diet and physiological status, RUP substitution for RDP in range supplements may spare glucose.

Production Responses to Ruminally Undegraded Protein

Milk production of lactating dairy cows has been increased with RUP additions to the diet (Orskov, 1982). If ruminal microbial synthesis is optimized with supplemental RDP, then lactating range cows may benefit from RUP supplementation. Feedstuffs containing RUP are protein-rich so that relatively small additions considerably alter the protein characteristics of a supplement. The value and optimal proportion of high quality RUP for ruminants with high protein requirements have not been defined conclusively (Kirkpatrick and Kennelly, 1987).

Petersen et al. (1985) illustrated the concept of replacing RDP with RUP in soybean meal (SBM) or blood meal/urea supplements for low quality (5.1% CP) forage diets. Additions of supplemental protein to low quality forage did not alter bacterial nitrogen flow to the abomasum, forage utilization or passage rate; however, forage intake (2.2% BW) in their study was held constant. Performance of gestating cows supplemented with RUP was not different from those with SBM supplements (Petersen et al., 1985).

Hibberd et al. (1988) supplemented lactating cows grazing dormant range with soybean hulls and soybean meal at two energy levels with and without added blood meal. Ruminally undegraded protein increased ($P<.05$) milk production ($P<.05$) and calf weight gain. Cows lost less ($P<.05$) body weight with RUP supplementation. Similarly, Lee et al. (1985) reported that a cottonseed meal/fish meal/meat meal blend supplemented in incremental quantities to cows consuming low quality (2.7% CP) hay increased hay intake and milk production.

Miner and Petersen (1989) fed SBM, SBM/blood meal or SBM/urea/corn gluten meal supplements to gestating cows grazing winter pastures. All supplements provided 200 g/d of RDP. Soybean meal/RUP supplements increased the rate of ruminal NDF digestion. Supplements containing RUP were degraded more slowly and were suggested to supply amino acids or certain carbon skeletons over a longer period of time, thus enhancing fiber fermentation. In a companion study, fecal output was greatest ($P<.05$) for SBM/blood meal supplements. Therefore, Miner and Petersen (1989) suggested that intake was increased by SBM/blood meal supplements. The increase in intake was attributed to an enhanced duodenal protein status (Egan and Moir, 1965).

Miner et al. (1990) supplemented gestating cows for two consecutive years with SBM, SBM/blood meal or SBM/corn gluten meal. All supplements provided 200 g/d of RDP. Cows receiving RUP supplements lost less body condition in both trials and less body weight in one trial. Serum urea-N concentration in cows was greater for those fed the RUP supplements than those fed the control. Miner et al. (1990) suggested that lower bilirubin concentration for SBM/blood meal supplemented cows could indicate that protein status was improved (Bull et al., 1974). Production from first calf beef heifers fed 250 g/d of RDP or 250 g/d of RUP was evaluated by Wiley et al. (1991). Supplements containing additional RUP increased body weight gain and improved reproductive performance of heifers.

CHAPTER III

RUMINALLY DEGRADED PROTEIN FOR BEEF COWS FED LOW QUALITY GRASS HAY

Abstract

Five mature beef cows fitted with ruminal, duodenal and ileal cannulae were used in a 5 x 5 Latin square design to determine the effect of incremental levels of supplemental ruminally degraded protein (RDP) on the utilization of low-quality hay. Cows were fed coarsely chopped native grass hay (4% CP) free choice and blends of soybean hulls and soybean meal to supply similar amounts of energy but graded levels of RDP (175, 294, 428 and 544 g RDP/day). Supplementation increased the majority of parameters above the control. Higher amounts of RDP increased hay OM intake (P=.10), hay OM digestibility (P=.36) and digestible OM intake (P=.05) quadratically; these peaked at 428 g RDP. Digestible OM intake was maximized with 88.6 g supplemental RDP per kg digestible OM intake. True ruminal OM disappearance also increased quadratically (P=.06), however, microbial N flow was not altered (P=.26) by RDP level. Duodenal N flow tended to increase (P=.12) quadratically due to greater feed N flow (quadratic, P=.07). Increased intake can be partially attributed to linear increases in total tract passage rate (P=.01) and rate of in situ hay OM digestion (P=.0001). Ruminal ammonia-N concentrations increased with added supplemental RDP and were correlated (r=.82) with rate of in situ hay degradation. In summary, RDP stimulated rate of OM disappearance via enhanced ruminal ammonia-N. Consequently, digestible OM intake increased due to enhanced hay digestibility, not due to changes in ruminal volume. With an estimate of energy intake, the value of 88.6 g supplemental RDP per kg digestible OM intake can be used to predict supplemental RDP requirements of low quality forage diets.

Key Words: Beef Cattle, Grass Hay, Protein Degradation, Protein Supplementation

Introduction

From December through March, the average concentrations of CP (2.5%; Waller et al., 1972) and TDN (36%; Lusby, 1985) in dormant native pastures are extremely low. Fall-calving beef cows grazing dormant native grass can satisfy a large proportion of their energy and protein requirements from the fermentation of consumed forage, but pastures still are deficient in protein and energy relative to the lactating beef cow's requirement. Therefore supplementation with protein and energy is vital for adequate cowherd performance (Rakestraw et al., 1986).

Concentrations of ruminal ammonia-N have ranged from .7 to .9 mg/dL in cattle consuming low quality hay (Guthrie and Wagner, 1988; Stokes et al., 1988); these concentrations are below recommendations for maximal microbial growth (Satter and Slyter, 1974). Supplementation of low quality hay with soybean meal has increased hay intake via an increase in ruminal ammonia-N and microbial protein synthesis (Stokes et al., 1988). Consequently, the utilization of low quality forage probably is dependent upon ruminal ammonia-N, which can be provided by supplemental protein degraded within the rumen. The extent of ruminal degradation of protein in feedstuffs varies (NRC, 1985). Feedstuffs with low ruminal protein degradation could deprive ruminal microbes of ammonia-N (Martin and Hibberd, 1990) and decrease microbial growth and forage utilization. Thus, ruminal protein degradation appears to be a vital characteristic of protein feedstuffs that often is overlooked when formulating range supplements.

Supplementing low quality hay with soybean meal has increased forage digestion and intake (Guthrie and Wagner, 1988; Stokes et al. 1988); however no peak in hay intake was achieved. In those studies, supplemental energy was increased concomitantly with supplemental protein; consequently supplemental protein and energy were confounded. The quantity of ruminally degraded protein (RDP) required to maximize forage utilization could be determined if the supplemental energy supply were equalized among the several levels of supplemental protein. In addition, the degree to which altered ruminal fermentation influences microbial protein

synthesis and duodenal N flow in low quality forage diets justifies further evaluation. The objective of this experiment was to determine the relationship between incremental levels of supplemental RDP and forage intake and digestion, ruminal fermentation and composition of duodenal N flow in beef cows fed low quality native grass hay.

Materials and Methods

Preliminary trial. Soybean meal was labeled with ytterbium (Teeter et al., 1984) to evaluate the flow of supplemental soybean meal from the rumen. Six mature cows (523 kg) consuming low quality grass hay free choice were fed 544 g of Yb-labeled (1,075 mg Yb) and 364 g of unlabeled soybean meal (as fed basis). Duodenal samples (550 ml) collected at 0, .5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 29, 36 and 48 h postsupplementation were dried (forced air oven, 55^{o} C) and ground (1 mm screen). Ytterbium was extracted from duodenal samples with EDTA and concentrations were determined by atomic absorption spectrophotometry using a nitrous oxide flame (Karimi et al., 1987). Zero-h duodenal samples and unlabeled soybean meal were extracted with EDTA for preparation of standards.

Ruminally degraded protein experiment. Five mature, nonpregnant Limousin x Angus/Hereford beef cows (538 kg) fitted with a permanent ruminal cannula, and double-L type cannulas in the proximal duodenum and distal ileum (Streeter et al., 1991) were allocated randomly to five treatments in 5 x 5 Latin square. Cows were housed in individual pens (4.7 x 2.3 m; concrete-slatted floors) inside an environmentally controlled barn. Coarsely chopped (5-cm screen) native grass hay and fresh water were available free choice. The native grass hay, harvested in July, contained 4.0% CP (Table 1). The control supplement, consisting of 108 g mineral plus 58 g dried molasses (as-is), was used to assess the digestibility of unsupplemented hay. The remaining four treatments supplied various quantities of ruminally degraded protein (RDP, Table 2). Supplements, composed of soybean hulls and soybean meal, provided 175, 294, 418 and 540 g RDP/d. Calcium, phosphorus, trace mineralized salt and vitamin A were added to meet the requirements of a 550 kg lactating beef cow (NRC, 1984). In addition, sodium sulfate

TABLE 1. CHEMICAL COMPOSITION OF NATIVE GRASS HAY[a]

Item	% (DM basis)
OM	93.1
CP	4.0
NDF	79.9
Lignin	7.3
Acid insoluble ash	4.7

[a]Major species include Andropogon gerardi, Schizachyrium scoparium, Panicum vergatum and Sorghastrum nutans.

TABLE 2. COMPOSITION, NUTRIENT SUPPLY AND FEEDING RATE OF SUPPLEMENTS PROVIDING INCREMENTAL LEVELS OF RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d			
Item	Control	175	294	428	544
Feed composition, % (DM basis)					
Soybean hulls		91.00	62.71	32.49	
Soybean meal			28.04	57.95	90.11
Molasses	33.78	3.00	3.00	3.00	3.00
Dicalcium phosphate	38.05	3.24	3.34	3.46	3.58
TM salt[a]	27.62	2.35	2.43	2.51	2.60
Sodium sulfate		.36	.43	.55	.66
Vitamin A (30,000 IU/g)	.54	.05	.05	.05	.05
Nutrient, % of DM					
Crude protein[b]	3.0	12.9	22.1	33.1	43.4
TDN[d]	22.2	71.0	73.4	75.8	78.4
Intake, g/d					
DM	160	1,890	1,850	1,790	1,740
CP					
Total[b]	7	245	408	594	755
Ruminally degraded[c]	3	175	294	428	544
TDN[d]	38	1,342	1,358	1,357	1,364

[a]Trace mineralized salt contained 92% NaCl, .25% Mn, .20% Fe, .033% Cu, .007% I, .005% Zn and .0025% Co.

[b]Actual analysis.

[c]Estimated from NRC (1985), ruminal degradation of soybean hulls was assumed to equal that of soybean meal.

[d]Estimated from NRC (1984).

was included to obtain a nitrogen:sulfur ratio of 12:1 in the supplement. Ruminal degradation of soybean meal protein was estimated at 72% (NRC, 1985). Ruminal degradation of soybean hull protein also was assumed to be 72%. Supplemental energy supply (1,360 g TDN/d) was equalized with soybean hulls (TDN estimated at 75%) to prevent confounding effects between supplemental protein and energy. The highest level of RDP was formulated to provide 140% of a gestating or 120% of a lactating beef cow's CP requirement, respectively (NRC, 1984).

The experiment consisted of a 5 by 5 Lation square. The 21-d experimental periods included 15 d of adaptation to supplements followed by 6 days of intensive sampling. Cows were weighed on d 4 and d 11 of each adaptation period. Full cow body weight reduced by 6% was assumed to equal shrunk body weight basis. Supplements were fed between 0700 and 0800 each morning. Fresh hay at a level of 2.3 kg plus the previous day's consumption was offered immediately after supplements were consumed. On day 1 through 12 of each period, refused hay was refed. On day 13 through 21, fresh hay was fed daily and orts were weighed and discarded. Hay offered and refused was recorded throughout the experiment. From d 16 through 19, hay and supplements were sampled daily and samples were composited across days. Hay refusals were subsampled (10% of weighback) daily from d 16 through 19, composited by cow and subsampled. Hay, hay refusals and supplements were ground (1-mm screen) and stored at 4^{o} C.

Duodenal (500 ml), ileal (250 ml) and fecal (450 g as-is) samples were collected eight times during d 16 through 19 to represent each three h interval of a 24-h day. Digesta samples, composited by animal within each period, were subsampled and stored (-15^{o} C) until being lyophilized and ground (1-mm screen).

Hay, hay refusals, supplements and digesta were analyzed for dry matter, ash, crude protein (Kjeldahl-N x 6.25; AOAC, 1975), neutral detergent fiber (Goering and Van Soest, 1970) and acid-insoluble ash (AIA; Thonney et al. 1985). Acid-insoluble ash was used as an indigestible marker to determine nutrient flow and digestibility (Chase and Hibberd, 1985). Cows were in slatted floor pens and had no access to soil that can increase fecal AIA output. Hay OM digestibility was estimated by subtracting an estimate of indigestible supplement OM flow from

fecal OM flow. Estimates of TDN (employed as OM digestibility herein) were 82% for soybean meal (NRC, 1984) and 75% for soybean hulls (Streeter and Horn, 1983; Hsu et al., 1987).

To determine particle passage rate, coarsely ground (5-cm screen) hay was labeled with ytterbium by immersion (Teeter et al., 1984). Ytterbium-labeled hay (250 g) was fed with the supplements at 0700 on d 16. Fecal grab samples (250 g as-is) were obtained at 0, 24, 36, 48, 60, 72 and 96 h postdosing, dried (55^{o} C, forced-air oven) and ground (1-mm screen). Ytterbium was extracted with EDTA and concentrations were determined by atomic absorption spectrophotometry using a nitrous oxide flame (Karimi et al., 1987). Zero-h fecal samples, composited and extracted with EDTA, were used for preparation of standards. Particle passage rate was estimated from the slope of the regression of the natural logarithm of Yb concentration over time.

To evaluate ruminal kinetics, Co·EDTA (1 g Co in 500 ml water, prepared according to Uden et al., 1980) was dosed intraruminally at five locations between 0650 and 0710 on d 19. Ruminal contents (500 ml) were sampled at 0, 2, 4, 6, 9, 12, 18 and 24 h postdosing. Ruminal pH of whole contents was measured immediately using a combination electrode. Ruminal samples were strained (four layers of cheesecloth) to obtain ruminal fluid (100 ml), which was acidified (2 ml 20% H_2SO_4) to halt fermentation and then frozen (-15^{o} C). Prior to laboratory analyses, acidified ruminal fluid was centrifuged at 10,000 X g for 20 min to remove suspended particles. Ammonia content of the supernatant fluid was analyzed by the phenol-hypochlorite assay (Broderick and Kang, 1980). Cobalt concentrations were analyzed by atomic absorption spectrophotometry using an oxygen-acetylene flame (Chase and Hibberd, 1987). Ruminal fluid dilution rate (%/h) was estimated from the regression of the natural logarithm of Co concentration against time. Liquid retention time was calculated as the inverse of fluid dilution rate. Ruminal fluid volume (L) was calculated by dividing the Co dose by the extrapolated Co concentration at time 0. Volatile fatty acid concentrations were determined on ruminal fluid samples collected 4 h postsupplementation. Subsamples (2 ml) were combined with .333 ml of 25% metaphosphoric acid containing 2-ethylbutyric acid (internal standard) and centrifuged (20,000 X g, 20 min). The

supernatant fluid (1 μl) was injected into a Perkin Elmer AutoSystem1 gas chromatograph equipped with a spiral J & W fused silica Megabore column[2] (30 X .533 mm; acidified (TPA) polyethylene glycol liquid phase; 1.0 μm film thickness). Helium served as the mobile phase at a flow rate of 40 ml/min. Column temperature was programmed to increase from 110° to 235° in three stepwise increments. Inlet port and detector temperatures were both 250° C.

To estimate microbial composition, additional ruminal fluid (800 ml, 200 ml per collection) was obtained at 0, 6, 12, and 18 h postsupplementation on d 20. A solution of 37% formaldehyde/.9% NaCl was added (25 ml/100 ml ruminal fluid) to stop microbial growth and maintain cell tonicity. Isolated bacteria were recovered by centrifugation (Merchen et al., 1986), frozen (-20° C), lyophilized and ground with a mortar and pestle. Analyses of isolated bacteria included DM, ash, N (KjelTec 1030 Auto Analyzer[3]) and purine concentration (Zinn and Owens, 1980). Microbial N in duodenal digesta was estimated from the bacterial N:purine N ratio. The SE of the estimate of duodenal microbial N flow was decreased from 5.55 to 4.08 by using the treatment average of the ratio of microbial nucleic acid-N to microbial-N.

To determine rate of OM and NDF degradation, duplicate dacron bags[4] (10 X 20 cm; 53±10μm pore size) containing 4.8 to 5.0 g undried ground (5-mm) native grass hay were suspended in the rumen of each cow beginning on d 14 at 1900. Bags were tied to a weighted 65-cm tygon tubing line (4 cm between bags). Lines were placed in the ventral sac of the rumen at times representing 6, 12, 18, 24, 48 and 96 h of incubation. All bags were removed at 1900 on d 18 and immediately washed individually with lukewarm tap water until effluent was clear. Bags were dried (50° C) in a forced air oven for 48 h. Residue was subsampled (.5 g as-is) and analyzed for DM and ash. Bags then were grouped by period for simultaneous NDF analyses (Moore et al., 1987). Rate of digestion was estimated from the slope of the regression of the natural logarithm of residual OM and NDF over time.

1Perkin Elmer, Norwalk, CT.
2J & W Scientific, Folsum, CA.
3Tecator Company; Tecator AB, Box 70, S-263 01 Hoganas, Sweden.
4Ankom, Fairport, NY.

To evaluate ruminal degradation of supplemental feeds, 1.2 g ground as-is (2-mm screen) soybean hulls or soybean meal were placed in duplicate dacron bags[4] (5 X 10 cm; 53±10μm pore size). Bags containing soybean hulls were incubated in cows receiving the low RDP (175 g RDP-100% soybean hulls) supplements whereas bags containing soybean meal were incubated in the cows receiving high RDP (544 g RDP-100% soybean meal) supplements. Bags incubated for 48 h were placed in the rumen on d 16 at 0700. The remaining bags were placed in the rumen on d 17 at 0700. Bags representing 6, 12, and 18 h of incubation were removed sequentially and immediately frozen. The 48 h and 24 h bags were removed simultaneously on d 18 at 0700 and immediately frozen. All bags were simultaneously thawed, washed until effluent was clear and then dried (80^{O} C for 24 h). The entire bag plus its contents was placed in Kjeldahl flasks for analysis of Kjeldahl-N. Empty bags were incubated in the rumen for 24 h to correct for N content of bags and microbial contamination. Rates of DM and N digestion were estimated from the slope of the regression of the natural logarithm of residual DM and N over time.

On d 21, ruminal contents were evacuated between 1300 and 1600 to evaluate ruminal fill. Liquid was separated from solid by squeezing whole contents by hand and then straining the liquid through a 2-mm screen. The liquid and solid portions were weighed and recorded, and duplicate subsamples (250 g) were frozen (-15^{O} C) and lyophilized. Ruminal liquid volume (L) and DM fill (kg) were estimated by multiplying the quantity of evacuated contents by the DM of ruminal liquid and solid subsamples.

Statistical analyses. Intake, flow and digestibility data were subjected to least squares analyses of variance with a model that included period, animal and treatment. Orthogonal contrasts were used to compare the control vs all supplements plus the linear, quadratic, and cubic (control omitted) responses to increasing level of supplemental RDP. Ruminal data were analyzed as a split plot over time with effects of period, cow, and treatment tested with period X treatment X cow; hour and treatment X hour were tested with the residual. A repeated measures analysis was conducted to determine an adjusted probability value for treatment X hour (Huynh and Feldt, 1976). Treatment effects were tested with the previously listed contrasts. The partial

correlation between in situ degradation of hay and ruminal ammonia-N concentration of supplemental RDP treatments was estimated with period and animal included in the model.

Results and Discussion

Preliminary trial. Ytterbium from labelled soybean meal first appeared in the duodenal samples at 1 h postdosing and increased slowly to 6 h (Figure 1). After 6 h, duodenal Yb concentrations increased at a substantially faster rate and peaked at 29 h postdosing. With steers fed weathered prairie hay, Johnson et al. (1981) reported that substantial quantities of a corn/soybean meal/dehydrated alfalfa supplement passed directly into the abomasum within seconds after feeding. They suggested that ruminal compaction of forage inhibited the mixing of the supplement with ruminal contents. Although abomasal and omasal retention time would delay the appearance of Yb in the duodenum, our data indicate that very little soybean meal directly bypassed the rumen due to lack of space afforded by ruminal compaction by fiber.

Supplement characteristics. Supplements provided 243, 408, 580 and 755 g/d of total CP and were very close to formulated values (250, 400, 550 and 700 g CP/d; Table 2). In situ rates of DM and N degradation were markedly faster ($P<.0001$) for soybean meal than for soybean hulls (Table 3). Supplements in this study were formulated under the assumption that ruminal N degradation was relatively similar for soybean hulls and soybean meal.

OM intake and digestion. Compared to the control, supplementation increased ($P<.0001$) hay OM intake by a mean of 63% (Figure 2). Greater hay OM intake with supplementation was partially attributed to an 8% increase ($P=.05$) in hay OM digestibility (Figure 3). Control hay OM digestibility (46.3%) was 6.7 percentage units lower than the TDN value for mature, fresh bluestem forage (NRC, 1984). Although TDN is assumed equivalent to digestible OM (NRC, 1985), our lower estimate probably is the result of a combination of differences due to plant species composition, season and harvesting method. Supplementation increased ($P<.0001$) digestible OM intake because supplements increased hay OM digestibility and provided substantial quantities of digestible OM (Figure 4). Disappearance of OM at all sites

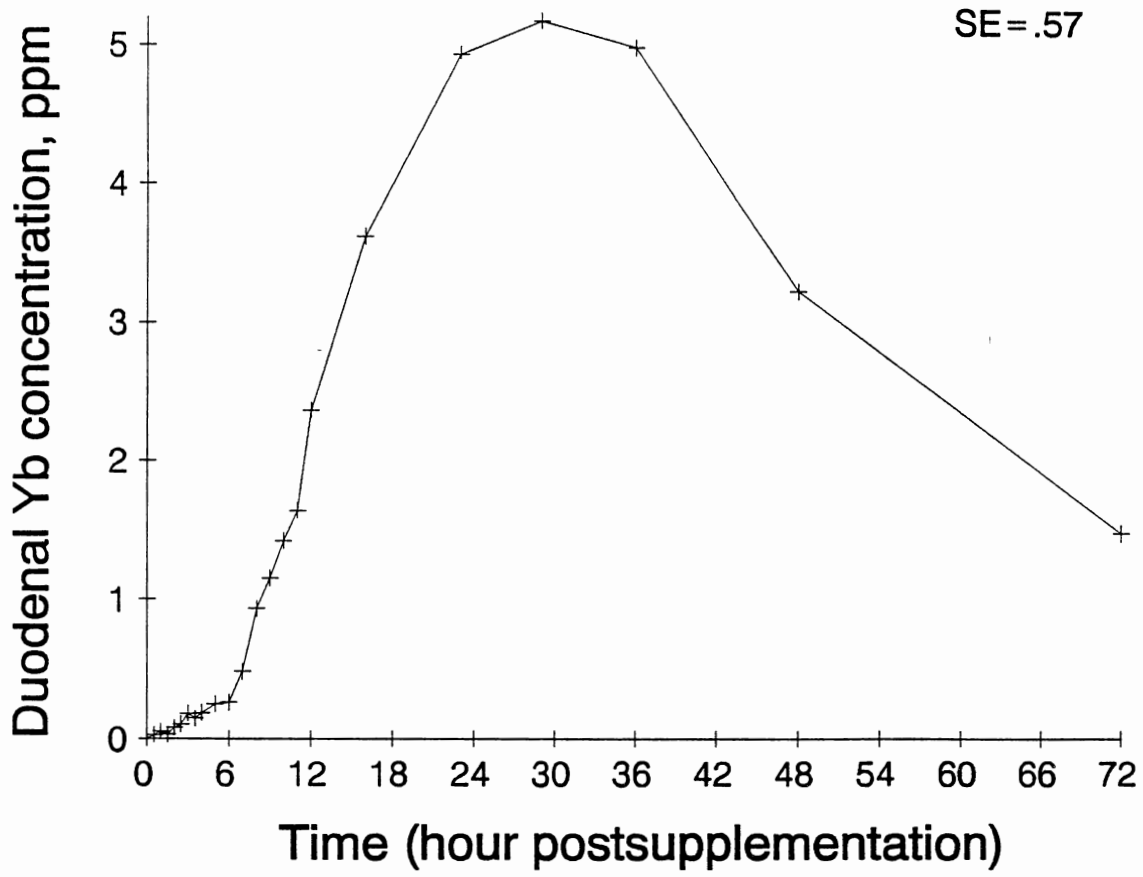

Figure 1. Appearance of ytterbium, from labeled soybean meal, in the duodenum of beef cows fed low quality native grass hay.

TABLE 3. IN SITU DISAPPEARANCE OF DM AND N FROM SOYBEAN HULLS AND SOYBEAN MEAL

Item	Soybean hulls	Soybean meal	SE	Probability
Nitrogen, %	2.31	7.78		
DM disappearance, %				
Hours of incubation				
6	33.4	52.6	.86	.0001
12	50.0	81.4	.65	.0001
18	57.0	93.0	.29	.0001
24	59.3	96.8	.46	.0001
48	82.5	98.6	.80	.0001
Rate of DM digestion, %/h	3.2	15.2	.13	.0001
N disappearance, %				
Hours of incubation				
6	49.6	46.0	.95	.02
12	72.4	81.9	1.00	.0001
18	77.9	95.4	.38	.0001
24	77.2	98.3	.35	.0001
48	86.6	99.5	.23	.0001
Rate of N digestion, %/h	4.9	20.0	.22	.0001

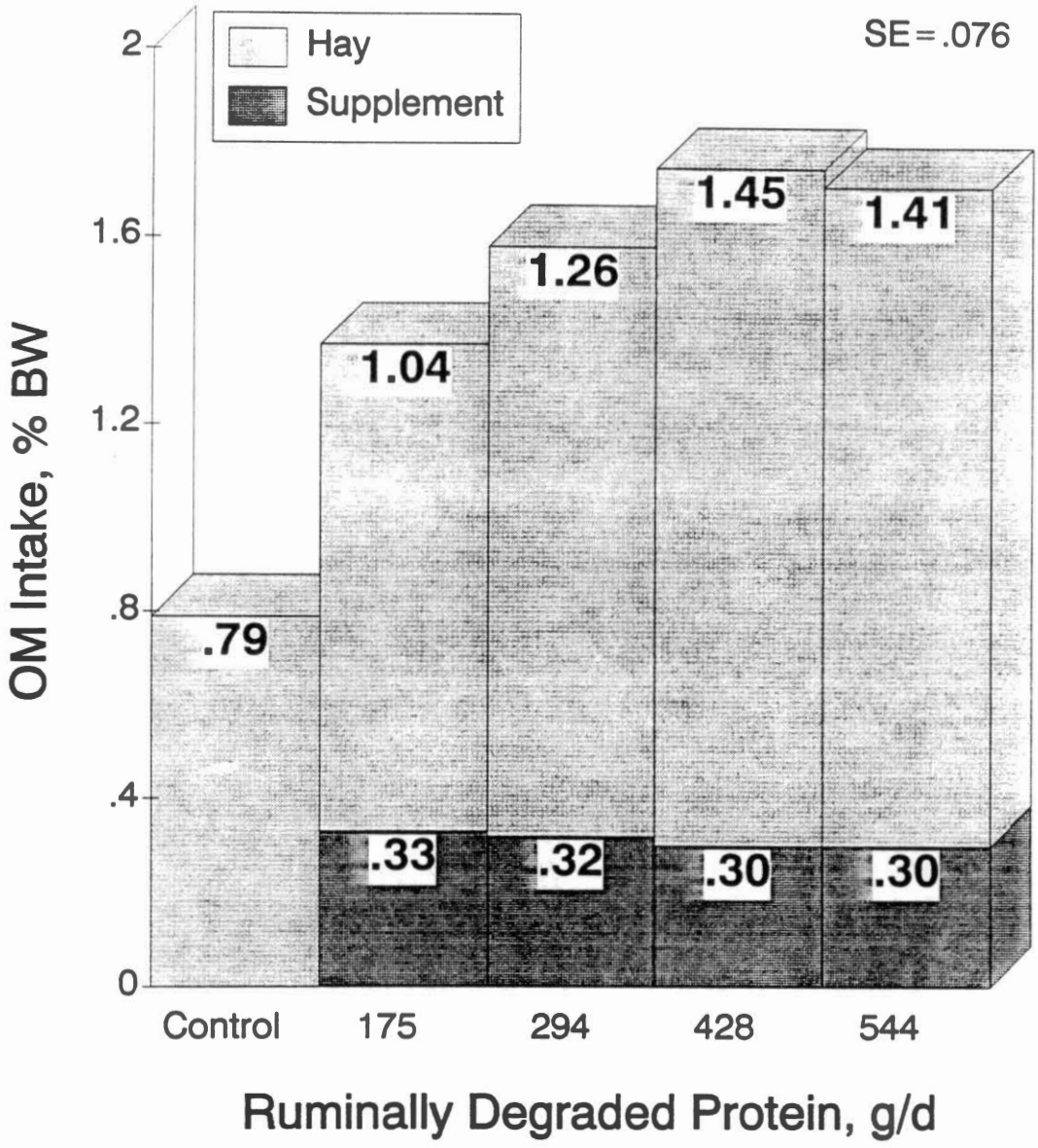

Figure 2. Organic matter intake (% BW) of beef cows fed low quality native grass hay as affected by incremental levels of supplemental RDP. Quadratic effect (P = .10) of level of supplemental RDP (control omitted).

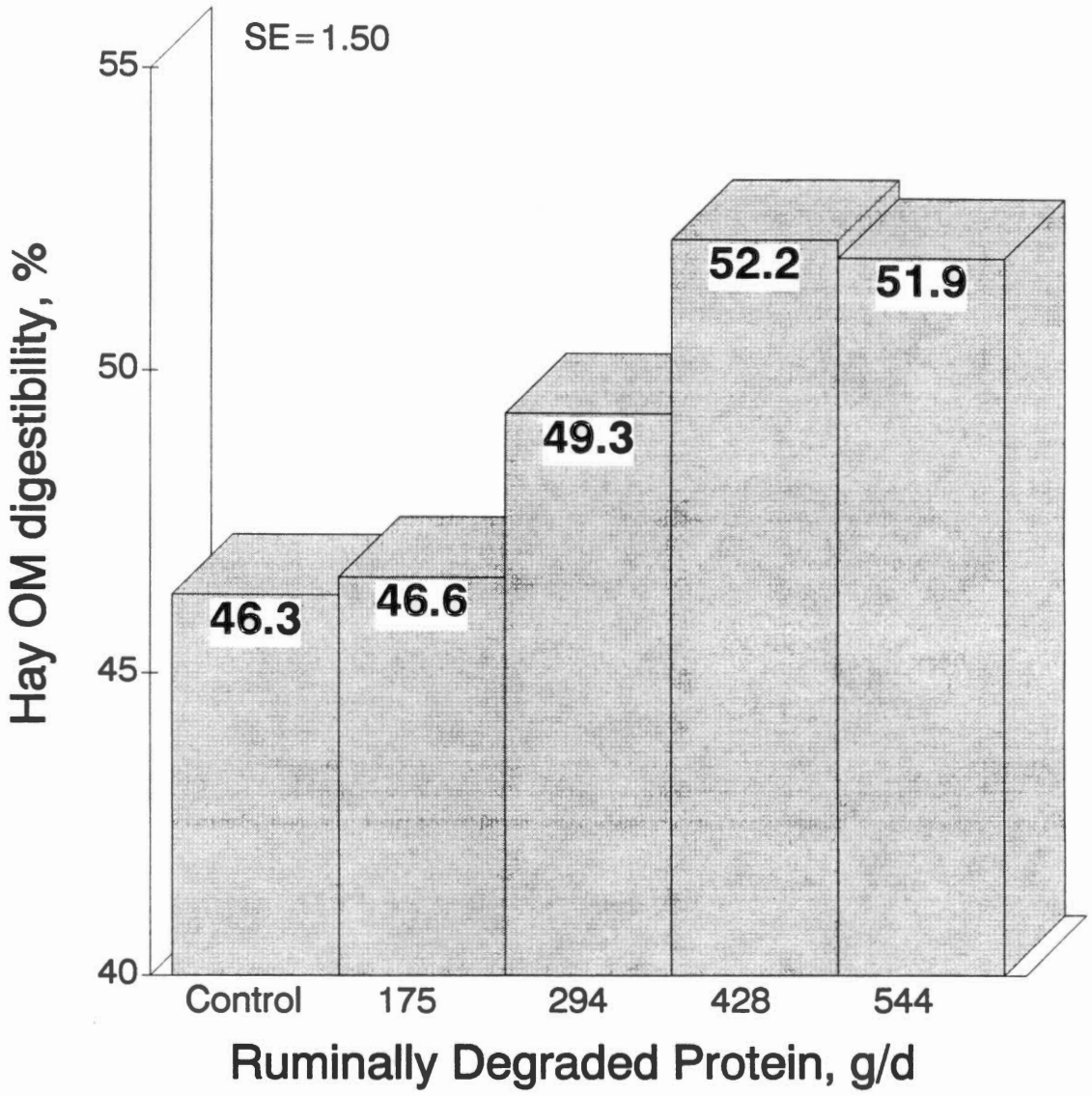

Figure 3. Organic matter digestibility (%) of low quality native grass hay as affected by incremental levels of supplemental RDP. Linear effect (P = .02) of level of supplemental RDP (control omitted).

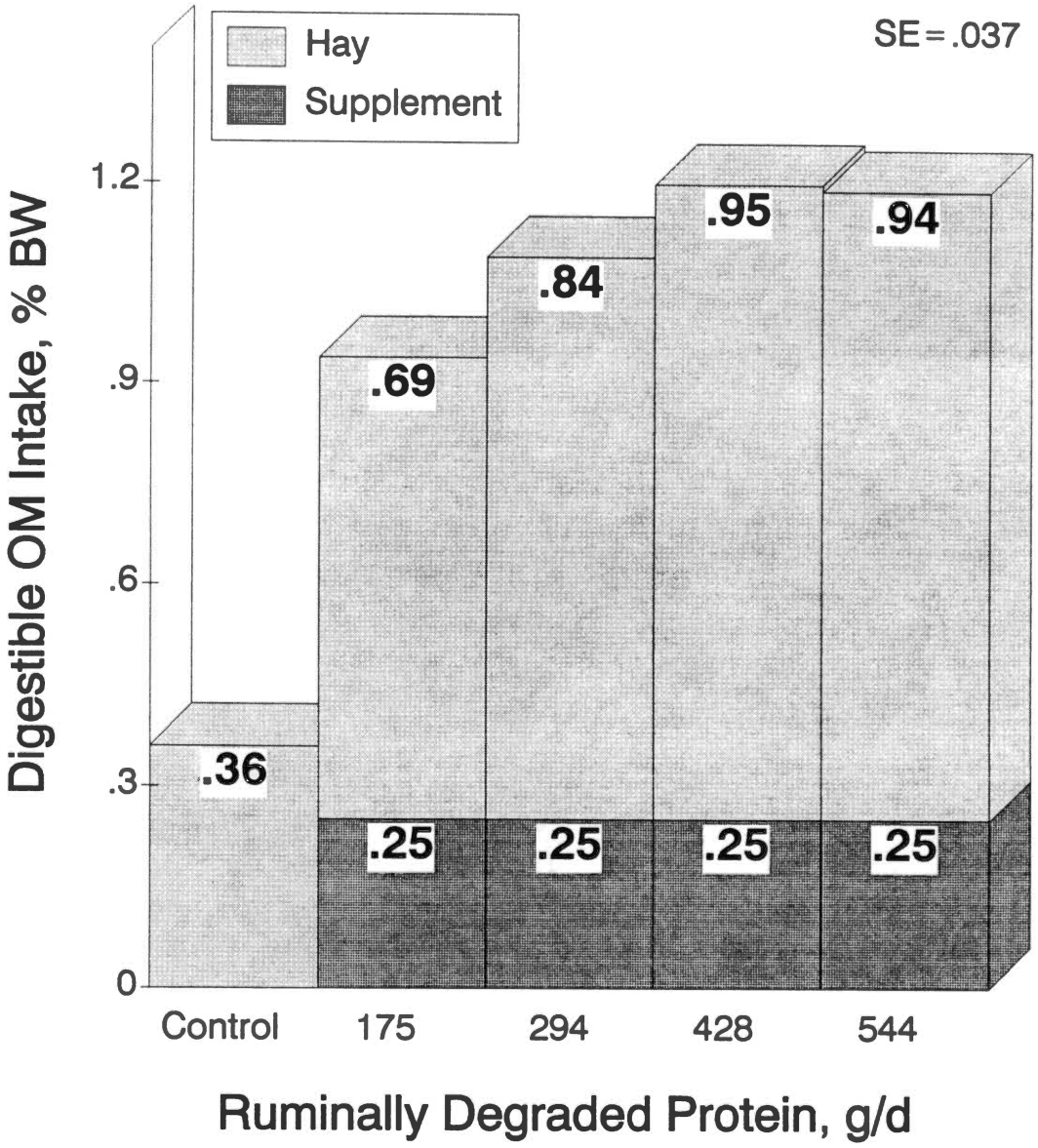

Figure 4. Digestible OM intake (% BW) of beef cows fed low quality native grass hay as affected by incremental levels of supplemental RDP. Quadratic effect (P = .06) of level of supplemental RDP (control omitted).

increased approximately two-fold with the first increment of RDP, suggesting that energy intake was effectively doubled by supplementation (Table 4). Supplementation increased ($P<.0001$) OM disappearance in and flow of OM from the rumen. The smaller postruminal OM disappearance (small intestinal plus hindgut) relative to ruminal OM disappearance emphasizes the importance of ruminal digestion on the utilization of low quality forage. The increased intake and utilization of hay is confounded with digestible fiber intake. Martin and Hibberd (1990), however, observed no decrease in hay intake when soybean hulls were fed at a rate of 1 or 2 kg/d. Consequently, we conclude that changes in the intake and utilization of hay observed in this study are attributed primarily to supplemental RDP.

Within RDP supplements, total OM intake (% BW) increased ($P=.10$) quadratically and peaked with 428 g RDP (Figure 2). This response is attributable solely to a quadratic increase ($P=.10$) in hay OM intake. Increased hay OM intake was largely a result of increased hay OM digestibility (linear, $P=.02$). Although supplemental OM intake was equalized, incremental levels of supplemental RDP up to 428 g RDP continued to increase hay intake. Unlike previous studies (Stokes et al., 1988; Guthrie and Wagner, 1988), supplemental RDP in this study was increased to a much higher level so that the maximum hay intake could be detected.

Digestible OM intake (% BW) increased (quadratic, $P=.06$) even though the RDP supplements supplied an equal quantity (.3% BW) of digestible OM (Figure 4). Because total tract OM disappearance is indicative of TDN intake (NRC, 1985), cows supplemented with 428 g RDP should have had the maximum daily energy consumption. Greater energy intake was the result of a quadratic increase ($P=.05$) in total tract OM digestibility (Table 4) and increased hay OM intake. Predicted total tract OM digestibility can be calculated with forage OM digestibility obtained from the control diet and the estimated TDN contribution of each supplement (Guthrie and Wagner, 1988). Comparison of predicted and observed digestibilities illustrates the positive (observed > predicted) associative effect that supplemental RDP exerted on total tract OM digestion (Figure 5). The advantage in digestibility (observed-predicted) increased up to 428 g RDP. Expected values calculated by Guthrie and Wagner (1988) increased from 38.7% to 42.0% but in that case,

TABLE 4. SITE AND EXTENT OF OM DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
Intake, g/d	3,862	6,954	7,997	9,091	8,676	381.1	.0001	.003	.08	.38
Flow, g/d										
Duodenal	2,176.9	3,538.5	4076.3	4,540.8	4,456.8	250.54	.0001	.01	.24	.68
Feed	1,759.6	2,642.9	3,182.0	3,709.8	3,658.9	206.45	.0001	.002	.18	.55
Microbial	417.4	895.6	894.2	831.1	798.0	55.91	.0001	.18	.78	.72
Ileal	2,071.1	3,414.0	3,610.8	4,120.7	3,736.7	232.00	.0001	.18	.23	.27
Fecal	2,043.0	3,156.8	3,487.2	3,838.4	3,610.4	203.81	.0001	.08	.20	.52
Disappearance, g/d										
Apparent ruminal	1,684.8	3,416.0	3,921.0	4,550.0	4,219.6	194.77	.0001	.004	.05	.24
True ruminal	2,102.2	4,311.5	4,815.3	5,381.1	5,017.6	212.61	.0001	.02	.06	.32
Postruminal	134.0	381.7	589.1	702.4	846.4	91.22	.0004	.003	.73	.76
Small intestinal	105.8	124.5	465.5	420.2	720.2	87.57	.006	.0008	.82	.09
Hindgut	28.1	257.2	123.6	282.3	126.2	73.3	.06	.49	.88	.09
Total tract	1,818.2	3,797.6	4,510.1	5,252.4	5,066.0	210.45	.0001	.0004	.05	.33
Digestibility, % of intake										
Apparent ruminal	43.3	49.4	49.0	50.5	48.8	1.3	.001	.95	.60	.39
True ruminal	54.4	62.2	60.1	59.5	57.8	1.18	.001	.02	.86	.66
Postruminal	3.3	5.5	7.4	7.6	9.9	1.02	.003	.01	.86	.39
Small intestinal	2.9	1.4	5.8	4.5	8.4	1.05	.09	.001	.82	.04
Hindgut	.4	4.0	1.6	3.0	1.5	1.12	.11	.25	.71	.20
Total tract	46.6	54.9	56.5	58.1	58.6	1.33	.0001	.05	.71	.86
Digestibility, % entering segment										
Small intestinal	5.6	2.6	11.5	8.3	16.4	2.26	.13	.003	.86	.04
Hindgut	.4	7.8	3.4	6.6	3.6	2.16	.06	.35	.76	.19

[a]Control vs average of supplemental treatments.
[b]Linear response to increased level of ruminally degraded protein (control omitted).
[c]Quadratic response to increased level of ruminally degraded protein (control omitted).
[d]Cubic response to increased level of ruminally degraded protein (control omitted).

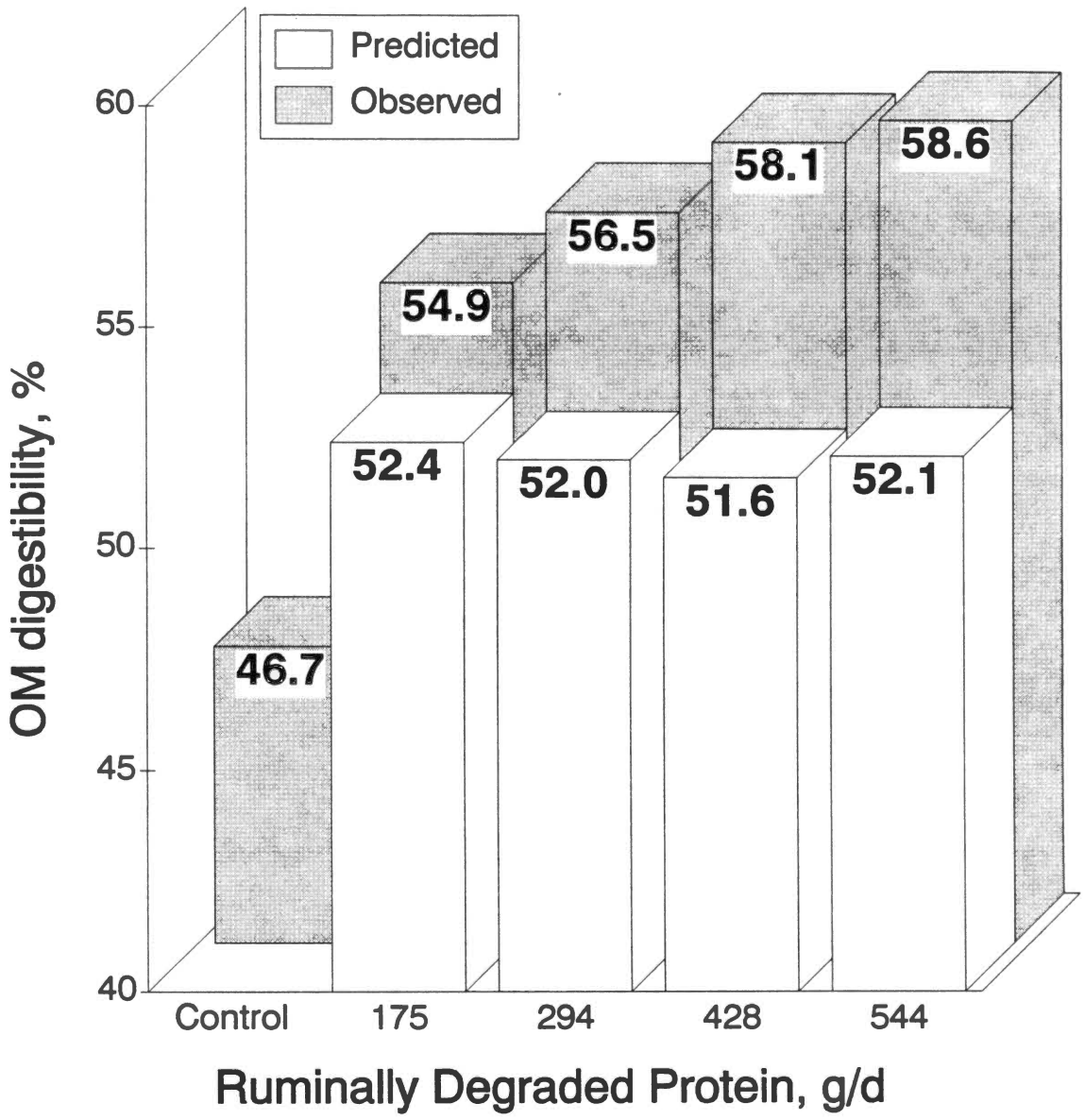

Figure 5. Comparison of observed versus predicted total tract OM digestibility in beef cows fed low quality native grass hay as affected by incremental levels of supplemental RDP.

supplemental OM intake increased concomitantly with supplemental protein. In contrast, the digestible OM contribution of supplements in our study was equalized. Consequently, expected values for total tract OM digestibility declined slightly with increasing supplemental RDP due to dilution of supplemental OM with hay OM.

The relationship between supplemental RDP and energy intake was evaluated by plotting digestible OM intake against the ratio of supplemental RDP to digestible OM intake (Figure 6). This relationship suggests that 88.6 g of supplemental RDP per kg digestible OM intake was required to maximize energy intake. This implies that an appropriate balance between RDP and digestible OM intake must be achieved to maximize energy intake. This estimate is a theoretical optimum generated from one data set. Whether or not this RDP requirement is ideal for all low quality forage diets is unknown. Nonetheless, these data offer a reasonable basis for predicting supplemental RDP needs once energy requirements have been determined.

Incremental quantities of supplemental RDP increased duodenal feed OM flow linearly, ($P=.002$) but microbial OM flow tended ($P=.18$) to decline linearly (Table 4). Stokes et al. (1988) fed two levels (.12% and .24% BW) of soybean meal and reported that microbial OM flow was increased. In that study, microbial OM flow may have increased because the .24% BW supplement supplied twice as much energy. In our study, supplemental energy supply was equalized; therefore, changes in microbial OM yield can be attributed directly to incremental levels of supplemental RDP. The explanation for the decline in microbial OM flow with higher amounts of supplemental RDP, however, is unclear.

Apparent ($P=.05$) and true ruminal ($P=.06$) OM disappearance (g/d) increased quadratically and peaked with 428 g RDP (Table 4). Maximal ruminal OM disappearance matched the peak in hay OM intake. True ruminal OM disappearance, however, was greater than total tract OM disappearance for all treatments except 544 g RDP. This discrepancy probably is the result of cumulative errors in marker flow and the contribution of microbial OM in the feces. Although true ruminal OM disappearance (corrected for bacterial OM) on a percentage basis increased due to

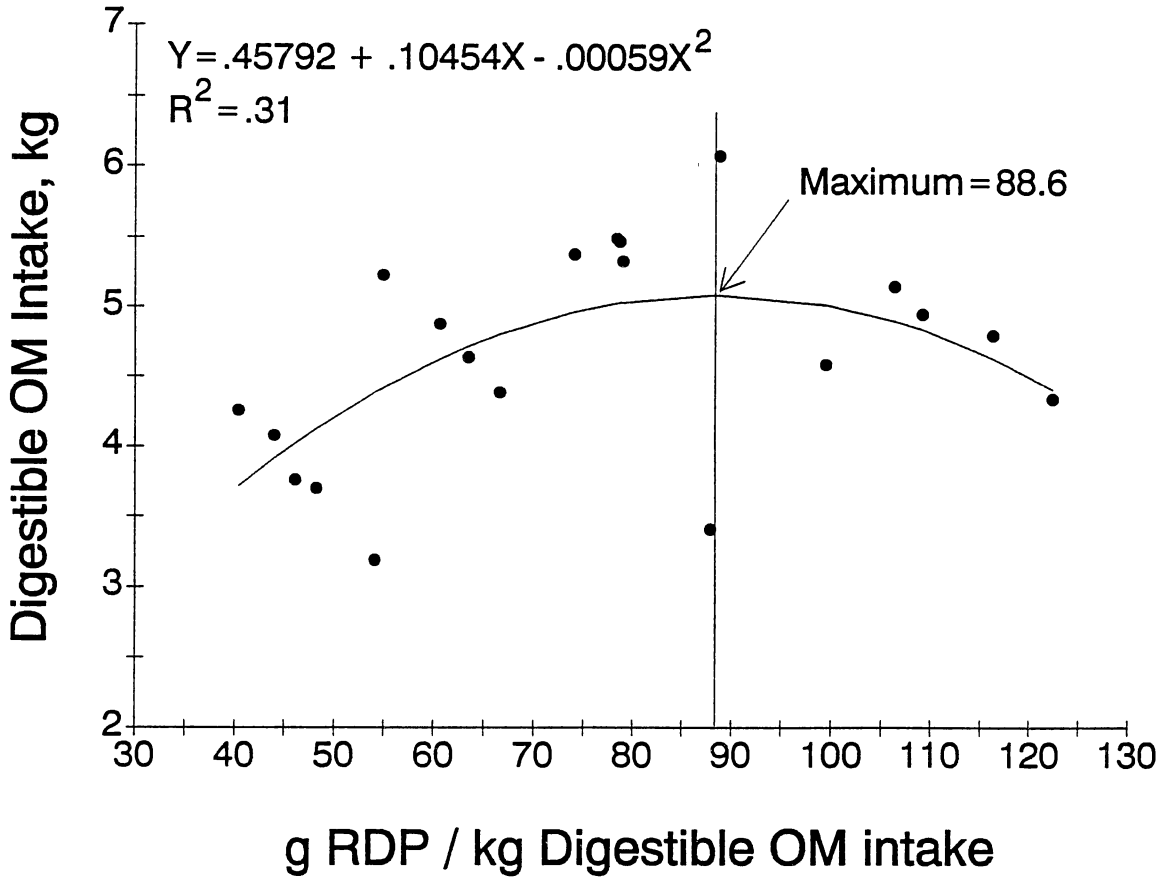

Figure 6. Relationship of digestible OM intake to supplemental RDP/digestible OM intake ratios.

incremental levels of supplemental RDP, true ruminal OM digestibility declined (P=.02) when more supplemental RDP was fed.

Postruminal OM disappearance increased (P=.003) linearly as a result of greater (linear, P=.0008) disappearance of OM in the small intestine; hindgut disappearance was not increased significantly (Table 4). Small intestinal OM flow is composed primarily of microbes and feed. Because microbial OM flow was not increased above 294 g RDP, greater disappearance of OM in the small intestine probably was the result of greater passage of undigested feed particles from the rumen. Similar values for postruminal OM digestion by cattle consuming forage have been observed (Funk et al., 1987; Stokes et al., 1988). Because the native grass hay utilized in this study was composed primarily of cell wall (79.9% NDF), ruminal fermentation was essential for utilization of the diet.

Fiber intake and digestion. Intake of NDF increased (P=.07) quadratically with incremental levels of supplemental RDP, although there was only a tendency (linear, P=.35) for increased total tract NDF digestion (Table 5). Because the percentage of NDF disappearing ruminally did not change (P>.46), increased ruminal NDF disappearance (quadratic, P=.03) was a function of greater NDF intake. Similar to total tract NDF digestibility, ruminal NDF digestibility (% of intake) was not affected (P=.69) by incremental levels of RDP. Postruminal utilization of NDF was negligible and similar to prior studies (Funk et al., 1987; Stokes et al., 1988). Negative digestion in the small intestine probably resulted from cumulative errors in marker flow and fiber analysis (Funk et al., 1987).

Nitrogen intake, flow and digestion. Supplementation dramatically altered N intake and digestion parameters (Table 6). The lowest level of RDP increased hay N intake by 40% and duodenal N flow by 95%. Nitrogen efficiency, duodenal N flow expressed as % of N intake, was 212% for the control diet (.70% N) which was substantially higher than values reported by Stokes et al. (1988) with steers fed unsupplemented prairie hay (.77% N, nitrogen efficiency=133%) and by Funk et al. (1987) with steers grazing dormant blue grama rangeland (1.13% N, nitrogen efficiency=129%). Our higher estimate of nitrogen efficiency may be partially attributed to

TABLE 5. SITE AND EXTENT OF NDF DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
Intake, g/d	3,315	5,683	6,375	7,139	6,537	325.1	.0001	.01	.07	.34
Supplemental	20	1,130	909	684	400	6.7	.0001	.0001	.0005	.08
Hay	779	1,112	1,356	1,604	1,541	89.0	.0001	.002	.10	.48
Disappearance, g/d										
Ruminal	1,817.5	3,270.8	3,672.0	4,169.3	3,671.8	178.78	.0001	.05	.03	.20
Postruminal	-103.2	-62.7	-12.6	3.7	114.9	63.45	.13	.08	.64	.66
Small intestinal	-125.8	-245.6	-81.7	-180.5	77.2	60.72	.79	.008	.45	.04
Hindgut	22.6	183.0	69.1	184.3	37.7	67.19	.24	.31	.81	.13
Total tract	1,714.3	3,208.1	3,659.4	4,173.1	3,786.8	181.65	.0001	.02	.04	.26
Digestibility, % of intake										
Ruminal	54.8	57.6	57.2	58.6	56.1	1.61	.17	.69	.51	.46
Postruminal	-3.8	-.9	.0	.0	2.1	1.32	.02	.15	.67	.61
Small intestinal	-3.9	-4.5	-1.2	-2.6	1.4	1.03	.09	.004	.78	.05
Hindgut	0	3.6	1.2	2.6	.7	1.38	.22	.26	.84	.27
Total tract	50.9	56.6	57.2	58.6	58.2	1.44	.001	.35	.73	.70
Digestibility, % entering segment										
Small intestinal	-8.6	-10.7	-2.7	-7.2	3.1	2.66	.18	.009	.68	.04
Hindgut	-.2	.3	.1	.2	.1	.16	.10	.52	.81	.50

[a]Control vs average of supplemental treatments.
[b]Linear response to increased level of ruminally degraded protein (control omitted).
[c]Quadratic response to increased level of ruminally degraded protein (control omitted).
[d]Cubic response to increased level of ruminally degraded protein (control omitted).

TABLE 6. SITE AND EXTENT OF N DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
N intake, g/d	25.6	74.0	107.4	146.0	169.0	2.85	.0001	.0001	.09	.13
Supplemental	.7	38.9	65.3	95.0	120.8	1.18	.0001	.0001	.83	.20
Hay	24.9	35.0	42.1	51.0	48.2	2.68	.0001	.002	.09	.28
Flow, g/d										
Duodenal-N	53.4	104.2	134.3	162.6	170.1	6.70	.0001	.0001	.12	.54
Feed-N	23.0	36.6	58.2	84.4	90.9	3.78	.0001	.0001	.07	.18
Microbial-N	28.6	63.9	71.6	71.4	71.1	4.08	.0001	.26	.35	.68
Ammonia-N	1.9	3.6	4.5	6.8	8.1	.38	.0001	.0001	.67	21
Ileal NAN	26.1	50.3	55.3	67.0	64.2	2.93	.0001	.002	.21	.12
Fecal NAN	26.0	47.5	55.2	60.2	60.9	2.15	.0001	.0005	.13	.87
NAN disapearance, g/d										
Apparent ruminal	-27.8	-30.2	-26.9	-16.6	-1.1	5.40	.16	.002	.28	.94
True ruminal	2.6	37.3	49.2	61.7	78.1	3.11	.0001	.0001	.48	81
Postruminal	25.8	53.7	75.3	96.3	101.8	5.14	.0001	.0001	.14	52
Small intestinal	25.6	50.7	75.0	89.3	98.4	4.64	.0001	.0001	.13	.82
Hindgut	.3	3.0	.2	7.0	3.4	1.73	.13	.31	.80	.02
Total tract	-.1	27.0	52.9	86.6	108.8	2.21	.0001	.0001	.43	.08
NAN digestibility, % of N intake										
Apparent ruminal	-111.6	-39.7	-24.6	-10.5	.2	7.42	.0001	.002	.78	.94
True ruminal	8.8	51.0	46.0	42.5	46.7	2.96	.0001	.24	.14	.66
Postruminal	104.5	71.6	70.0	65.4	59.7	7.01	.0004	.22	.78	.96
Small intestinal	103.8	67.6	69.6	60.7	57.7	7.36	.0004	.26	.74	.62
Hindgut	.8	4.1	.3	4.7	2.1	1.68	.30	.83	.74	.06
Total tract	.3	36.7	49.5	59.5	64.7	1.90	.0001	.0001	.07	.82
NAN digestion, % entering segment										
Small intestinal	49.8	49.2	57.6	56.9	60.6	2.27	.03	.006	.32	.21
Hindgut	.6	6.0	.9	10.5	5.6	2.61	.10	.49	.97	.03
Microbial efficiency[e]	14.14	14.82	14.95	13.33	14.09	.642	.83	.21	.63	.17
Nitrogen efficiency[f]	212	140	125	110	100	7.4	.0001	.002	.78	.94

[a]Control vs average of supplemental treatments.

[b]Linear response to increased level of ruminally degraded protein (control omitted).

[c]Quadratic response to increased level of ruminally degraded protein (control omitted).

[d]Cubic response to increased level of ruminally degraded protein (control omitted).

[e]grams microbial N flow/true ruminal OM disappearance.

[f](duodenal N flow/N intake) X 100.

extremely low true digestibility of hay N (5.8%, SE=13.0, n=5) estimated from control diets. Supplementation greatly stimulated (P<.0001) microbial N synthesis thus estimates of nitrogen efficiency were reduced (P=.0001) below the control. As little as 175 g of supplemental RDP increased microbial protein synthesis by 123%. These data verify the severe N deficiency afforded by low quality forage diets which necessitates RDP supplementation. Despite the dramatic improvement in ruminal N status, microbial efficiency (grams microbial N flow/kilogram true ruminal OM disappearance) was not increased (P=.83).

Supplemental N increased linearly (P<.0001) with RDP and contributed from 52 to 71% of total N intake (Table 6). Incremental levels of supplemental RDP increased (P=.09) total N intake quadratically due to a quadratic increase (P=.09) in hay N intake. Duodenal N flow was greater than N intake; this is typical for low N forages (Egan et al., 1975). Decreased nitrogen efficiency (linear, P=.002) with incremental levels of RDP may suggest that microbial capture of supplemental N was facilitated by a faster onset, rate and extent of ruminal hay fermentation (Stokes et al., 1988).

Greater N intake, with added supplemental RDP, increased (P=.12) duodenal N flow quadratically (Table 6). Above 175 g RDP, greater duodenal N flow was the result of more (quadratic, P=.07) feed N flow. True ruminal N disappearance, corrected for microbes, increased (P<.0001) linearly as supplemental RDP increased. When expressed as % of N intake, however, true ruminal N digestibility tended (P=.14) to decline quadratically. Because incremental levels of RDP increased ruminal OM fermentation linearly, microbial protein synthesis also should increase (Zinn et al., 1981). Microbial N flow, however, was not increased above 294 g RDP. Microbial efficiency tended (P=.21) to decline with incremental levels of supplemental RDP. Zinn et al. (1981) illustrated that lower microbial efficiencies were associated with greater true ruminal OM digestion. Because the potential quantity of microbial cell growth is related to an upper limit of ruminal fermentation (Bergen et al., 1982) our data suggest that low levels of readily fermentable carbohydrate, such as in prairie hay, may have limited microbial efficiency.

Ileal NAN flow (linear, P=.002) and fecal NAN flow (linear, P=.0005; quadratic, P=.13) increased as supplementation of RDP increased (Table 6). Postruminal NAN disappearance tended to increase (quadratic, P=.14) primarily due to greater NAN disappearance from the small intestine. Small intestinal NAN disappearance (% of entry) also increased (P=.006) linearly. In contrast, postruminal (P=.22) and small intestinal (P=.26) NAN digestion (% of N intake) tended to decline linearly. This inverse relationship between incremental levels of RDP and postruminal NAN digestion probably is the result of more highly digestible microbial N being diluted by larger quantities of poorly digestible hay N.

Passage rate and fill. Greater intake due to supplementation can be attributed partially to faster (P<.0001) particle passage (Table 7). Supplementation increased Yb passage rate by an average of 172%. Incremental levels of supplemental RDP increased (linear, P=.01) particle passage rate by 50%. Total tract OM digestibility, however, was not depressed (linear increase, P=.05). Faster particle passage, due to incremental levels of RDP, appeared to depress total tract NDF digestion to a greater degree than OM digestion. Guthrie and Wagner (1988) also reported a linear increase in OM digestibility even when passage rates increased by as much as 50%. This relationship could be attributed partially to the increased quantity of highly digestible soybean meal fed by Guthrie and Wagner (1988). In our study, however, supplemental OM was fed at similar rates. Nonetheless, the positive associative effect of supplementation appeared to outweigh the negative impact of increased passage on digestion.

Ruminal fluid dilution rate (%/h) based on cobalt as a marker was nearly doubled (P<.0001) by supplements (Table 7). In other studies, protein supplements have markedly increased liquid dilution or passage rate (McCollum and Galyean, 1985; Stokes et al., 1988; DelCurto et al., 1990a). Increased roughage intake and microbial protein synthesis have been related to increased fluid dilution rate (Bergen et al., 1982). There was no effect, however, of incremental quantities of RDP supplementation on ruminal dilution rate or retention time. Ruminal dilution rate significantly impacts microbial flow (NRC, 1985). Thus, unchanged microbial N flow in this study might be expected because liquid dilution rate was not altered by RDP supplements.

TABLE 7. PASSAGE RATES AND RUMINAL FILL IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

	Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
Total tract										
Particle passage rate, %/h	.80	1.64	2.36	2.19	2.53	.214	.0001	.01	.32	.10
Ruminal										
DM fill, kg[e]	8.25	10.23	10.76	11.01	10.66	.405	.0002	.41	.30	.86
DM fill, % BW[e]	1.70	2.00	2.12	2.12	2.10	.074	.0006	.35	.34	.73
Ruminal fluid										
Dilution rate, %/h	4.68	7.36	8.15	7.80	8.17	.468	.0001	.34	.66	.39
Retention time, h	24.04	13.98	12.50	13.22	13.18	1.865	.0002	.84	.70	.73
Volume, L[f]	69.38	86.34	83.57	94.94	90.06	4.371	.002	.27	.81	.15
Volume, L[e]	51.30	55.79	59.13	58.70	55.39	1.216	.0009	.77	.02	.87
Volume, % BW[e]	10.63	10.92	11.72	11.31	10.95	.231	.04	.77	.03	.24

[a]Control vs average of supplemental treatments.
[b]Linear response to increased level of ruminally degraded protein (control omitted).
[c]Quadratic response to increased level of ruminally degraded protein (control omitted).
[d]Cubic response to increased level of ruminally degraded protein (control omitted).
[e]Estimated from ruminal evacuation.
[f]Estimated with cobalt.

Ruminal volume, estimated from cobalt dosed 0 h postsupplementation, overestimated the volume of ruminal liquid compared to total evacuation 13 to 16 h postsupplementation (Table 7). Volume of ruminal liquid, estimated from ruminal evacuation, was increased (P=.0009) by supplementation. Increasing the level of supplemental RDP resulted in a quadratic (P=.02) response for ruminal liquid volume; however, causes for this change are unclear.

Ruminal DM fill (kg, estimated from ruminal evacuation) tended (quadratic, P=.30) to parallel hay OM intake (Table 7). Consumption of DM to a constant fill (% BW) suggests that hay intake in this study was limited by ruminal distension (Balch and Campling, 1962). Rumens of the control cows were not completely full (visual observation). If the rumen is not full on a forage diet, then some factor other than bulkiness of the undigested forage must limit intake (Owens et al., 1992). Bulk fill probably influences the maximum intake rather than the minimum intake of low quality forage. These observations suggest that N deficiency reduces ruminal fill. Intake responses due to supplementation probably are modulated by a combination of an improved ratio of N:digestible OM in the total diet (Allden, 1981) and an improved duodenal N status (Egan and Moir, 1965).

Ruminal fermentation. Ruminal ammonia-N concentrations showed a time X treatment interaction (P<.0001) which suggests that treatment differences were dependent on sampling time (Figure 7). Ruminal ammonia-N concentrations peaked 2 h after supplementation and increased linearly (P<.0001) with added RDP supplementation. Ammonia-N is a primary end product of ruminal protein degradation and should be elevated when RDP sources such as soybean meal are consumed.

Ruminal ammonia-N concentrations remained below .25 mg/dL for the control and 175 g RDP treatments for most of the day. Slightly higher concentrations of ruminal ammonia-N (2.3, 1.8 and 7.5 mg/dL) were reported by Barton and Hibberd (1984) for steers fed prairie hay (4.9% CP) and receiving no RDP (control), 173 g RDP or 435 g RDP, respectively. Guthrie and Wagner (1988) also reported that ruminal ammonia-N concentrations were low (.46 mg/dL for unsupplemented control and .52 mg/dL for 174 g RDP). Similar concentrations of ruminal

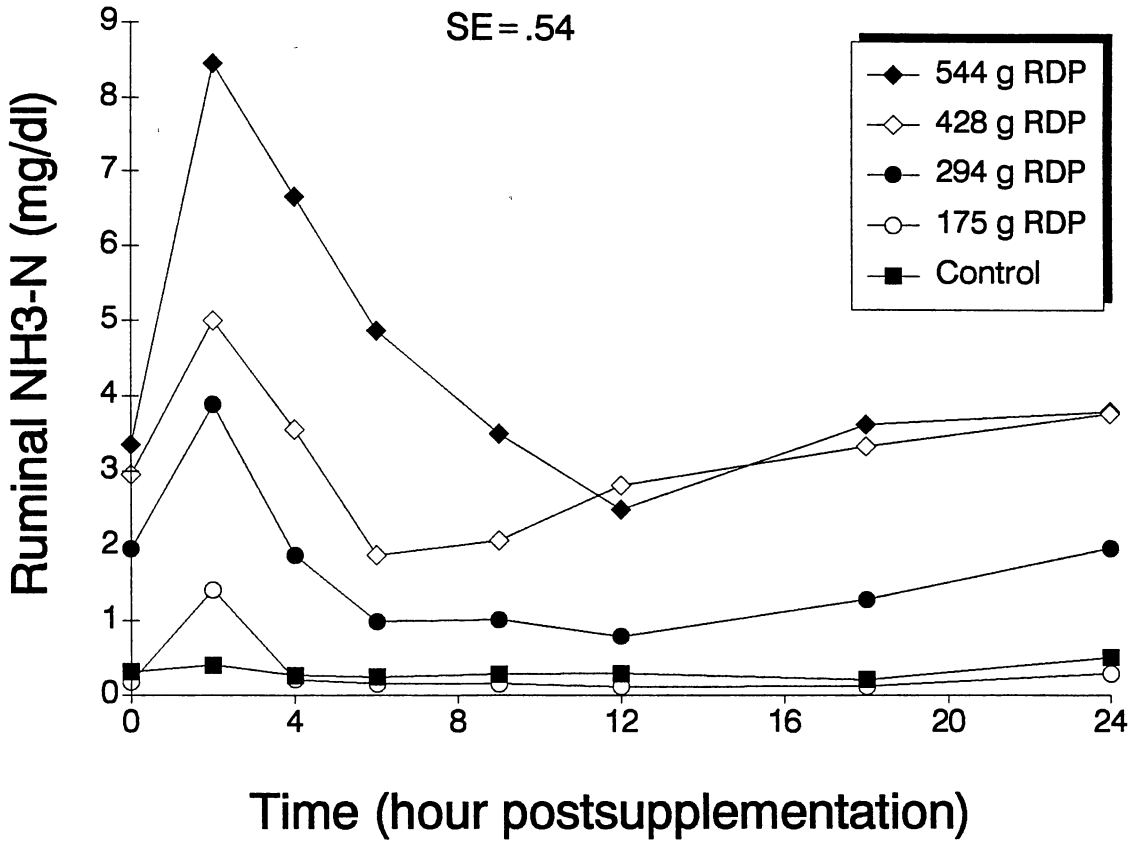

Figure 7. Ruminal ammonia-N concentrations in beef cows fed low quality native grass hay as affected by incremental levels of supplemental ruminally degraded protein (RDP).

ammonia-N for controls and low levels of RDP may be the result of rapid and extensive utilization of ammonia-N for synthesis of microbial protein (Adams and Kartchner, 1984). When 418 or 544 g RDP were fed, ruminal ammonia-N concentrations were sustained above 2 mg/dL for most of the day. Ruminal ammonia-N concentrations from 2 to 5 mg/dL are suggested for maximum microbial protein synthesis (Satter and Slyter, 1974). Microbial N flow in our study, however, peaked when concentrations of ammonia-N were well below 2 mg/dL.

Organic matter content of isolated microbial cells tended (P=.12) to increase linearly with added RDP (Table 8). Ash and N concentrations of microbial isolates were lower than averages of 20% ash and 8.2% N reported for ruminal bacteria (Smith, 1975). Composition of microbial isolates closely resemble values reported by Stokes et al. (1988). Both N and nucleic acid content increased (linear, P=.0001) with added RDP. Although microbial OM flow decreased with RDP supplementation, the increased N content (Hespell and Bryant, 1979) and increased RNA:N ratio (Bergen et al., 1982) of isolated microbial cells suggest that ruminal microbes were in a more rapid phase of growth as the faster liquid and particle dilution rates would imply. Lower N content of bacteria with a N deficiency (energy excess) has been proposed previously (Hespell and Bryant, 1979). Deficiency of N at the lower levels of supplemental RDP probably increased storage of carbohydrate in microbes, thus greater concentrations of N in microbial isolates were detected at higher levels of supplemental RDP.

Compared to the control, in situ rates of hay OM (P<.0001) and NDF (P=.0002) digestion were increased by supplementation (Table 9). Supplementation increased ruminal ammonia-N which would be expected to stimulate fiber digestion by cellulolytic bacteria (Bryant, 1979). Incremental levels of supplemental RDP increased the rate of in situ hay OM (P<.0001) and NDF (P=.0002) digestion linearly. A plot of these data suggest that the rate of in situ OM (P=.001, r=.82) and NDF (P=.01, r=.71) digestion were dependent upon the concentration of ruminal ammonia-N (Figure 8). Supply of peptides and amino acids would have increased with incremental levels of RDP. Therefore, ruminal ammonia-N should not be considered as the only factor responsible for this increased rate of digestion. In situ OM disappearance at 96-h was

TABLE 8. COMPOSITION OF RUMINAL MICROBIAL ISOLATES IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
Microbial isolates, %										
OM	78.73	84.36	86.38	85.40	87.46	1.127	.0001	.12	.99	.25
N	5.37	6.08	6.96	7.39	7.85	.184	.0001	.0001	.28	.56
Nucleic acid	2.97	3.84	4.85	5.98	6.51	.312	.0001	.0001	.46	.61
Nucleic acid-N	.48	.62	.79	.97	1.06	.051	.0001	.0001	.46	.61
Nucleic acid-N, % of microbial-N	8.98	10.25	11.27	13.18	13.44	.565	.0004	.0007	.51	.34

[a]Control vs average of supplemental treatments.
[b]Linear response to increased level of ruminally degraded protein (control omitted).
[c]Quadratic response to increased level of ruminally degraded protein (control omitted).
[d]Cubic response to increased level of ruminally degraded protein (control omitted).

TABLE 9. IN SITU HAY OM AND NDF DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
Rate of digestion, %/h[e]										
OM	1.22	1.39	1.56	1.64	1.88	.056	.0001	.0001	.59	.35
NDF	1.59	1.86	2.13	2.22	2.68	.109	.0002	.0002	.42	.29
Lag time, h[f]										
OM	7.68	6.60	7.05	8.12	5.47	1.060	.47	.98	.32	.17
NDF	8.44	6.57	6.79	7.77	4.34	1.289	.18	.34	.18	.39
OM disappearance, %										
Hours of incubation										
6	15.85	16.61	17.48	19.41	17.62	.337	.0001	.002	.0003	.003
12	20.35	21.18	27.36	28.41	30.07	.575	.0001	.0001	.0002	.03
24	28.63	35.57	37.38	41.32	42.07	.804	.0001	.0001	.52	.15
48	49.00	52.87	57.61	59.64	63.30	.682	.0001	.0001	.43	.16
96	69.25	72.33	73.89	74.11	73.96	.498	.0001	.03	.10	.66
NDF disappearance, %										
Hours of incubation										
6	21.72	22.59	23.71	25.47	24.24	.563	.0009	.01	.04	.16
12	26.11	26.64	34.15	35.15	37.24	.770	.0001	.0001	.0007	.03
24	35.00	43.12	45.16	50.20	50.98	.900	.0001	.0001	.48	.08
48	58.48	63.51	68.95	73.40	75.61	1.003	.0001	.0001	.91	.09
96	81.31	85.51	86.53	87.14	86.47	.626	.0001	.21	.19	.76

[a]Control vs average of supplemental treatments.
[b]Linear response to increased level of ruminally degraded protein (control omitted).
[c]Quadratic response to increased level of ruminally degraded protein (control omitted).
[d]Cubic response to increased level of ruminally degraded protein (control omitted).
[e]Rate of digestion was estimated from the slope of the regression of the natural logarithm of residual OM and NDF over time.
[f]Lag time = (4.605-extrapolated residue content at time zero)/rate of digestion.

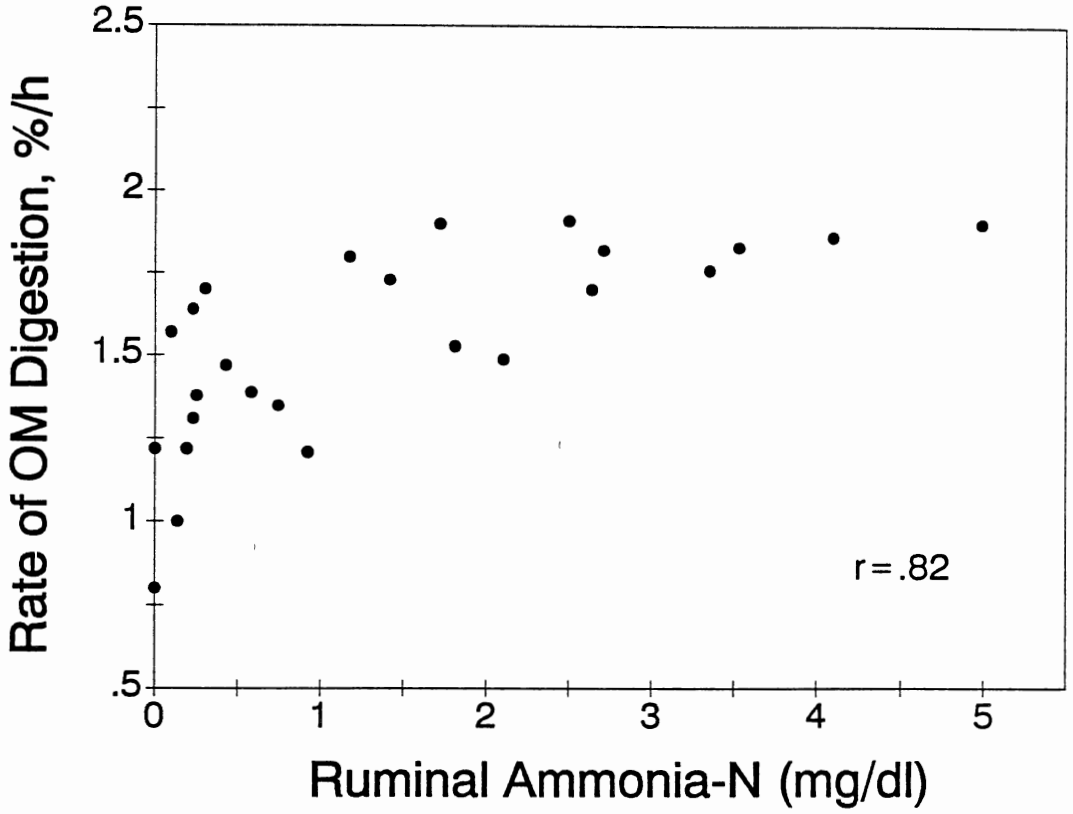

Figure 8. Relationship between rate of OM digestion and ruminal ammonia-N (linear model R^2 = .51; quadratic model R^2 = .54).

greatest (quadratic, P=.10) with 428 g RDP and tended to be lower with 544 g RDP, although rate of digestion was faster for the 544 g RDP than for the 428 g RDP supplement. Because of this relationship, it is unclear if higher levels of RDP would increase in situ degradation rates further. At the highest level of supplemental RDP, the reduced lag time may be the result of hastened onset of microbial digestion (Mertens, 1982).

Compared to the control, supplementation increased (P=.0002) total VFA concentrations in ruminal fluid (Table 10). Supplementation increased the molar proportions of butyrate (P<.0001) and valerate (P=.01) at the expense of acetate (P=.03). In previous studies, supplemental protein has increased total VFA concentrations whereas molar proportions of acetate have been reduced (Stokes et al., 1988; DelCurto et al., 1990a). In contrast, McCollum and Galyean (1985) reported that total VFA were not changed despite a decrease in the molar proportions of acetate. Neither propionate concentration (P=.86) nor the acetate/propionate ratio (P=.43) were altered by supplementation. Protein supplements have either increased (McCollum and Galyean, 1985; Judkins et al., 1987; Stokes et al., 1988) or, in agreement with these data, have not altered the molar proportions of propionate (DelCurto, 1990a).

Incremental quantities of RDP in this study did not alter either the total VFA concentration or the acetate/propionate ratio (Table 10). Acetate tended (P=.12) to decrease linearly as supplemental RDP was increased, whereas butyrate increased (linear, P=.005). Concentrations of the branched chain VFA, isovalerate (P=.03) and valerate (P=.15) the former being derived from amino acid catabolism, increased linearly and may have influenced ruminal fermentation via stimulated activity of cellulolytic bacteria (Dehority et al., 1967).

Ruminal pH was higher for the control (P<.0001) than for the supplemented diets (Figure 9). Lower ruminal pH with supplementation probably resulted from greater VFA concentrations. Due to enhanced ruminal fermentation, one might expect that ruminal pH should decline with increasing quantities of RDP. Mean ruminal pH, however, declined quadratically (P=.02) to 6.24 and then increased to 6.35 as 544 g RDP was fed, perhaps due to the higher amount of ammonia present in ruminal fluid. Rates of fiber digestion may be reduced by low pH in the rumen (Mertens

TABLE 10. VFA PROFILES AND MEAN RUMINAL pH IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
Total, mmol/ml	88.08	122.21	111.77	123.78	123.01	5.385	.0002	.49	.31	.11
Acetate, mol/100 mol	62.20	60.14	59.35	59.86	57.74	1.061	.03	.12	.47	.34
Propionate, mol/100 mol	21.10	20.94	21.86	21.62	20.66	.886	.86	.75	.23	.90
Isobutyrate, mol/100 mol	2.52	2.58	2.28	1.33	2.57	.520	.57	.62	.11	.18
Butyrate, mol/100 mol	9.95	11.08	11.23	11.60	11.91	.220	.0001	.005	.68	.73
Isovalerate, mol/100 mol	2.72	2.52	2.85	2.76	3.73	.369	.56	.03	.33	.31
Valerate, mol/100 mol	1.50	2.74	2.43	2.81	3.39	.403	.01	.15	.21	.75
Acetate/propionate	2.96	2.89	2.76	2.82	2.81	.160	.43	.77	.66	.68
Ruminal pH	6.50	6.41	6.37	6.24	6.35	.029	.0001	.02	.02	.01

[a]Control vs average of supplemental treatments.
[b]Linear response to increased level of ruminally degraded protein (control omitted).
[c]Quadratic response to increased level of ruminally degraded protein (control omitted).
[d]Cubic response to increased level of ruminally degraded protein (control omitted).

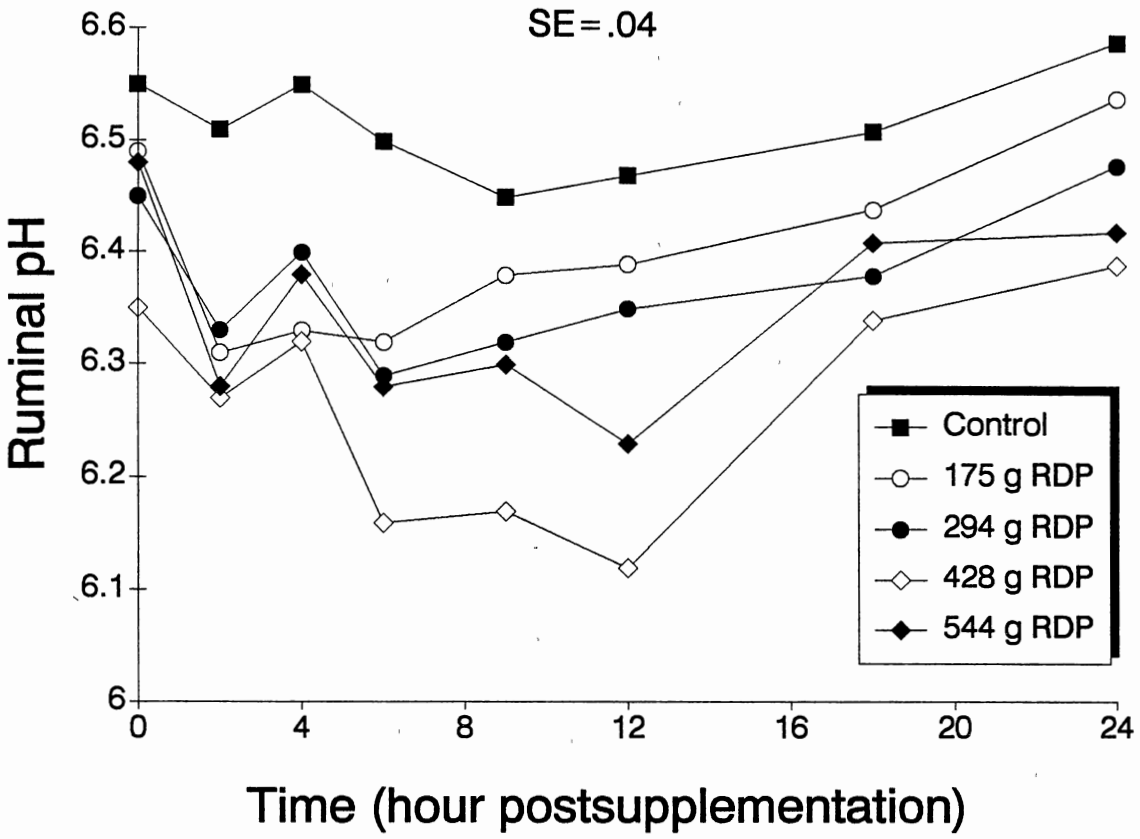

Figure 9. Ruminal pH in beef cows fed low quality native grass hay as affected by incremental levels of supplemental ruminally degraded protein (RDP).

and Ely, 1982); however, in this study the lowest ruminal pH corresponded with the maximum rate and extent of ruminal fermentation. Differences in ruminal pH were relatively small and pH remained above 6.0; therefore, lower pH should have had no deleterious effect on rate of digestion.

To conclude, the quantity of RDP required to maximize hay intake was more than the quantity of RDP required to maximize microbial protein synthesis. Consequently, ruminal digestion was optimized at higher concentrations of ruminal ammonia than was microbial growth, suggesting that attached microbes may either require more ammonia or be exposed to a lower ammonia concentration than the free-floating microbes (Hoover, 1986). Thus, potential fermentability of the forage probably placed an upper limit on microbial growth (Bergen et al., 1982). Hay intake was maximized with 428 g RDP. The cause for this plateau in hay intake is unclear. Above 428 g RDP, additional supplemental RDP was utilized as an energy substrate by microbes. Lower 96 h in situ hay digestion for 544 g RDP than for 428 g RDP might support this conjecture of competition. The 544 g RDP supplement was formulated entirely with soybean meal whereas the 428 g RDP supplement contained 40% soybean hulls. A major portion (50%) of OM in the 544 g RDP supplement was composed of protein, whereas the 428 g RDP supplement contained 37% protein (OM basis). Energy derived from supplemental soybean meal would be expected to be utilized less efficiently for microbial growth than energy from supplemental soybean hulls (Demeyer and Tamminga, 1987). Consequently, the 544 g RDP supplement may have reduced intake and microbial protein yield because of an improper balance between supplemental protein and energy. Digestible OM intake for the 428 g supplement was 5.25 kg while the TDN requirement for these cows was only 4.59 kg (NRC, 1984). Consequently, above 428 g RDP, energy requirements for the cow probably were satisfied which may have caused hay intake to decline. Most studies refer to the positive influence of increased ratios of digestion products (small intestinal protein absorption/true ruminal digestion) on forage intake. Egan (1966) reported that duodenal infusion of casein reduced feed intake of sheep. In that study, the ratio of absorbed nutrients (protein/energy) may have influenced intake (Egan, 1977). In our

study, small intestinal protein absorption was greatest with 544 g RDP. Additional protein absorbed in the small intestine would be catabolized as an energy source which further limited intake. In addition, greater flow of specific amino acids to the duodenum may have suppressed intake via feedback inhibition on the brain (Forbes, 1988).

Ruminally degraded protein appears to be a key factor that limits utilization of low quality forage. Forage utilization was enhanced by RDP supplementation, due to an increase in ruminal ammonia-N which increased rate of OM disappearance. A logical conclusion is that supplementation of low quality forage with urea could provide ammonia-N and benefit forage utilization. Ammonia-N, however, may not be the only factor that benefits forage utilization. Soybean meal and other natural RDP sources are more slowly degraded than urea plus microbes require peptides, amino acids and BCFA that are devoid in urea. Positive associative effects of supplementation were the result of increased digestion and passage. The quantity of supplemental protein required to maximize energy intake was 88.6 g supplemental RDP/kg digestible OM intake. With an estimate of energy intake, this value might be used to predict the RDP requirement of cattle consuming low quality forage. For example, if a cow requires 5 kg of TDN, supplementation should provide 443 g of RDP. This computes to a feeding rate of 615 g/d of soybean meal.

The quantity of supplemental protein required to maximize forage utilization was 580 g (428 g RDP). Total daily intake of CP was 912.5 g (hay plus supplement) which is 43% higher than the CP requirement for a 545 kg gestating beef cow but 4.4% less than the same cow's requirement during lactation (NRC, 1984). Although this level of CP appears to be adequate for lactating cows, actual RDP requirements may be greater due to the partitioning of N toward the mammary gland during lactation. In addition, the digestibility of forage protein (19.7% ADIN), may have limited the value of protein in this forage. Consequently, protein supplementation of gestating beef cows based on NRC requirements and CP estimates of dormant forage may not maximize intake and utilization of low quality forage.

Implications

This study illustrates the powerful influence that supplemental ruminally degraded protein exerts on the intake and utilization of low quality grass hay. Because protein is an expensive component of supplementation programs, producers seldom will feed too much protein; however, protein intake above NRC requirements increased utilization of low quality forage. Approximately 90 g of supplemental ruminally degraded protein was required per kg of digestible organic matter intake. This ratio might be used in supplementation programs for estimating the quantity of supplemental ruminally degraded protien required for targeted levels of performance.

CHAPTER IV

RUMINALLY UNDEGRADED PROTEIN FOR BEEF COWS FED LOW QUALITY GRASS HAY

Abstract

Five mature beef cows fitted with ruminal, duodenal and ileal cannulae were utilized in a 5 x 5 Latin square design to determine the effect of ruminally undegraded protein (RUP) supplementation on hay utilization and intake, site and extent of digestion and duodenal N flow. Cows with free choice access to coarsely chopped native grass hay (4.3% CP) were supplemented with two levels of total protein (400 or 600 g/d) containing two proportions (28 or 50%) of RUP. Supplements were formulated with blends of soybean hulls and soybean meal. Blood meal and corn gluten meal were added to formulate the 50% RUP supplements. Intake of hay OM and digestible OM were greater (P=.008) for 600 g CP supplements. Proportion of RUP did not alter (P=.56) hay OM intake or digestible OM intake. Duodenal flows of feed N (P=.001) and microbial N (P=.03) were greater for 600 g CP supplements, but did not differ between levels of RUP. Total tract particle passage rate tended (P=.38) to decrease and ruminal fill tended to increase (P=.19) with the 50% RUP supplements. The higher level of supplemental CP increased ruminal ammonia-N concentrations and rate of in situ digestion of OM (P=.02). Ruminal ammonia-N declined when RUP was increased to 50% of the supplemental protein; however, rate of in situ digestion of OM was not (P=.42) altered. Microbial efficiency, total VFA and acetate/propionate were not affected by either the level of CP or proportion of RUP. Increasing the proportion of RUP from 28% to 50% did not alter forage utilization, digestible OM intake or duodenal N flow so RUP supplementation would not be expected to benefit cattle performance.

Key Words: Beef Cattle, Grass Hay, Protein Supplementation, Protein Degradation

Introduction

Fall calving beef cows grazing dormant native grass (< 4% CP) require supplements to maintain productivity. Most range supplements are composed of cottonseed meal or soybean meal blended with low-protein or cereal grain byproduct feeds. Formulation of these supplements usually is based on total protein, with protein characteristics such as ruminal degradation being disregarded. The ruminal degradation of N from high protein feedstuffs varies widely (NRC, 1985). Consequently, supplements formulated with low ruminally degraded protein feeds may limit utilization of forage by cellulolytic bacteria (Hibberd and Martin, 1990).

With low quality forage diets, microbial N flow may not satisfy the protein requirements of lactating beef cows (Orskov, 1982; NRC, 1985) even when ruminal fermentation is maximized (Scott, 1992a). Feedstuffs rich in RUP might augment microbial protein flow to the small intestine. Production studies have illustrated that RUP can either augment (Miner and Petersen, 1989; Miner, et al., 1990) or replace (Hibberd et al., 1988) a portion of the ruminally degraded protein (RDP) fraction of supplements. If RUP can replace a portion of the RDP fraction of supplements without adversely affecting forage utilization, then nitrogen and amino acid flow to the duodenum is being manipulated. In addition, if dietary manipulation of RUP can optimize the profile amino acids flowing to the duodenum, total protein intake might be reduced without decreasing cow performance (Stanton et al., 1983). The objective of this experiment was to determine the effect of RUP supplementation at two levels of supplemental protein on forage utilization and intake, site and extent of digestion, ruminal fermentation and composition of duodenal-N in beef cows fed low quality grass hay.

Materials and Methods

Five mature, nonpregnant Limousin x Angus/Hereford beef cows (509 kg empty body weight) fitted with a permanent ruminal, and double-L type cannulas in the proximal duodenum

and distal ileum (Streeter, et al. 1991) were randomly allocated to five treatments in 5 x 5 Latin square. Cows were housed in individual pens (4.7 x 2.3 m; concrete-slatted floors) in an environmentally controlled barn. Coarsely chopped (5-cm screen) native grass hay and fresh water were available free choice. Native grass hay harvested in July, contained 4.3% CP (Table 11). The control supplement consisted of 108 g mineral plus 58 g dried molasses (as-is) and was used to assess the digestibility of unsupplemented hay. The remaining four treatments supplied two levels (400 or 600 g) of total protein with two proportions (28 or 50%) of RUP. Supplements were formulated by blending soybean meal with soybean hulls (Table 12). To formulate supplements, ruminal degradation of soybean meal and soybean hull protein was estimated at 72% (NRC, 1985). Supplemental energy supply (1,360 g TDN/d) was equalized with soybean hulls (TDN estimated at 75%) to prevent confounding effects between supplemental protein and energy. A blend consisting of 70% CP from blood meal and 30% CP from corn gluten meal was added to produce the 50% RUP supplements. Blood meal (82% RUP) and corn gluten meal (55% RUP) were utilized because of their complementary amino acid profiles (NRC, 1985). Calcium, phosphorus, trace mineralized salt and vitamin A were added to meet the requirements of a 550 kg lactating beef cow (NRC, 1984). Sodium sulfate was included to maintain a nitrogen:sulfur ratio of 12:1 in the supplement. In addition, dairy flavors were added to enhance the palatability of unpelleted supplements.

Feeding, sampling, and laboratory analyses were conducted as described by Scott (1992a) with the following exceptions. Due to a large amount of fines contained in the hay, fresh hay amounting to 4.5 kg plus the previous day's consumption was offered immediately after supplements were consumed. Hay, hay refusals, supplements and digesta were analyzed for nitrogen by KjelTec 1030 Auto Analyzer[1]. A washing machine was used to wash in situ bags postincubation. Bags were washed a total of four times (each wash and spin cycle lasted 5 min). Cows were weighed on d 21 during ruminal evacuation. Evacuated ruminal liquid was separated from solid by a mop squeezer. The liquid portion was passed through a 2-mm screen.

1Tecator Company; Tecator AB, Box 70, S-263 01 Hoganas, Sweden.

TABLE 11. CHEMICAL COMPOSITION OF NATIVE GRASS HAY[a]

Item	% (DM basis)
OM	93.16
CP	4.30
NDF	75.20
Acid insoluble ash	4.24
Lignin	5.37

[a]Major species include Andropogon gerardi, Schizachyrium scoparium, Panicum vergatum and Sorghastrum nutans.

TABLE 12. COMPOSITION, NUTRIENT SUPPLY AND FEEDING RATE OF SUPPLEMENTS PROVIDING TWO LEVELS OF PROTEIN AND TWO PROPORTIONS OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP	
Item	Control	28% RUP[a]	50% RUP	28% RUP	50% RUP
Feed composition, % (DM basis)					
Soybean hulls		60.20	71.34	31.36	47.99
Soybean meal		29.40	4.14	58.07	20.36
Blood meal			7.30		10.90
Corn gluten meal			6.71		10.01
Molasses	34.08	4.00	4.00	4.00	4.00
Dicalcium phosphate	37.16	3.44	3.44	3.44	3.44
TM salt[b]	28.11	2.50	2.50	2.50	2.50
Sodium sulfate	.02	.38	.49	.55	.71
Dairy flavors	.03	.03	.03	.03	.03
Vitamin A (30,000 IU/g)	.63	.05	.05	.05	.05
Nutrient, % DM					
CP[c]	3.0	22.2	22.2	33.3	33.3
TDN[d]	22.2	73.9	72.0	75.8	72.9
Intake, g/d					
DM	160	1,840	1,840	1,860	1,840
CP					
Total[c]	7	416	405	606	599
RDP[e]	4	300	202	436	300
RUP[a]	3	116	202	170	300
TDN[d]	38	1,363	1,325	1,410	1,344

[a]RUP = Ruminally undegraded protein.

[b]Trace mineralized salt contained 92% NaCl, .25% Mn, .20% Fe, .033% Cu, .007% I, .005% Zn and .0025% Co.

[c]Actual analysis.

[d]Estimated from NRC (1984).

[e]Estimated from NRC (1985), RDP of soybean hulls assumed equal to soybean meal.

Following completion of the Latin square, duplicate dacron bags[2] (5 X 10 cm; 53±10μm pore size) containing 1.1 g (as-is) ground (1-mm screen) SBH, SBM and each supplement plus unground CGM and BM were placed in a cow receiving the 600 CP/28% RUP supplement. To reduce fluctuations in ruminal ammonia-N concentrations, the cow received one-half of her daily supplement at 0800 and 1600 on d 1 and at 0800 on d 2. One-fourth of her daily supplement was fed at 1600 on d 2 and at 0200, 0800, and 1200 on d 3. Bags were placed sequentially within the rumen and removed simultaneously at 1600 on d 3. Bags were incubated for times representing 3, 6, 9, 12, 18, 24 and 48 h of digestion and were washed until the effluent was clear; bags were dried (80^{o} C for 24 h). Rates of DM and N disappearance were estimated from the slope of the regression of the natural logarithm of DM and N residues over time.

Statistical analyses. One cow, which became ill during period three, was replaced during subsequent periods; her data were deleted from period three. Intake, flow and digestibility data were subjected to least squares analyses of variance with a model that included period, animal and treatment. Orthogonal contrasts were used to compare the control vs all supplements plus effects of CP level (400 vs 600 g), RUP proportion (28 vs 50%) and the CP X RUP interaction. When the CP X RUP interaction was deemed significant ($P<.05$) treatment differences were detected by t-test. Ruminal data were analyzed as a split plot over time with effects of period, cow, and treatment tested with period X treatment X cow; treatment X hour was tested with the residual. A repeated measures analysis was conducted to determine an adjusted probability value for treatment X hour (Huynh and Feldt, 1976). Treatment effects were tested with the previously listed contrasts.

Results and Discussion

Supplement characteristics. Supplements were formulated to provide either 400 or 600 g/d of total CP. The 400 g CP supplements supplied 416 and 405 g/d of total CP (by analyses) whereas the 600 g CP supplements supplied 606 and 599 g/d of total CP (by analyses) for 28%

2Ankom, Fairport, NY.

and 50% proportions of RUP, respectively (Table 12). Pepsin insoluble N (PIN; % of N) of supplements was similar within level of protein but was slightly higher for 50% RUP supplements (Table 13). In situ rate of DM and N degradation were not significantly different within 50% RUP supplements (Table 13). Different rates of degradation within 28% RUP supplements can be attributed to a faster rate of DM and N degradation for soybean meal than for soybean hulls (Table 14). Pepsin insoluble N (% of N) content of feeds was greatest for soybean hulls (22.22%) and corn gluten meal (11.20%) whereas soybean meal and blood meal contained less than 7% PIN (Table 14). Rankings for disappearance of N from in situ incubations at 48 h were close to the rank of RDP values reported by NRC (1985). In situ incubation of supplements verify the low rate of ruminal N degradation of CGM, BM (Table 14) and 50% RUP supplements (Table 13). Nitrogen disappearance at 24-h of incubation was lower (30.11% lower for 400 CP and 28.54% lower for 600 CP) for 50% RUP than for 28% RUP supplements.

OM intake and digestion. The OM digestibility of unsupplemented hay was 58.7%; hay OM was consumed at 1.2% BW (Table 15). Supplementation increased (P=.0001) hay OM and total OM intake. Increased hay intake afforded by supplementation did not (P=.38) result from improved hay digestibility. Increased (P=.0001) digestible OM intake with supplementation resulted from an increase (P=.02) in the total tract OM digestibility due to addition of digestible OM (1.3 kg TDN) contained in the supplement. The 400 g CP/28% RUP supplement increased digestible OM intake by 73% which is lower than the two-fold increase in digestible OM intake reported by Scott (1992a) who fed an identically formulated supplement. These trials utilized the same cows and hay from the same barn. Although the hay used in this study had been stored an additional year, the average concentration of lignin was 2% lower and NDF was 5% lower than the previous study. Digestibility was 12.3% higher (58.6 vs 46.3%) and intake was .3% higher (.8 vs 1.1% BW) in the second study; these differences might be attributed to concentrations of lignin and NDF (Van Soest, 1982). Supplementation increased (P=.0001) true ruminal OM disappearance, corrected for microbial OM flow, compared to the control (Table 16). There was a tendency (P=.14) for true ruminal OM disappearance, expressed as a percentage of OM intake, to

TABLE 13. IN SITU DRY MATTER AND NITROGEN DISAPPEARANCE FROM SUPPLEMENTS SUPPLYING TWO LEVELS OF PROTEIN AND TWO PROPORTIONS OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
N, %	3.63	3.67	5.32	5.34				
Pepsin insoluble N, % of N	15.51	16.03	9.76	11.54				
DM disappearance, %								
Hours of incubation								
0	33.51	27.40	36.92	30.16	.380	.001	.0001	.44
3	30.92	32.28	47.09	36.71	1.403	.01	.003	.38
6	46.38	37.43	55.67	44.14	2.048	.02	.008	.56
9	60.18[c]	49.91[d]	74.48[e]	54.15[d]	1.136	.001	.0002	.002
12	70.18[c]	56.14[d]	81.84[e]	62.86[f]	.879	.0005	.0007	.05
18	79.98	66.67	89.06	73.36	.721	.0004	.0001	.17
24	90.63	79.39	93.96	80.06	.693	.04	.0001	.13
48	95.82[c]	87.82[d]	96.81[c]	85.58[d]	.574	.34	.0001	.05
Rate of DM digestion, %/h	8.82[c]	5.61[d]	10.54[e]	5.66[d]	.361	.07	.0004	.08
N disappearance, %								
Hours of incubation								
0	17.85	16.27	18.95	20.77	1.538	.14	.9432	.33
3	35.06	22.69	38.40	28.32	1.664	.05	.0025	.53
6	46.38	31.40	49.27	34.43	2.509	.30	.0040	.98
9	65.52	34.08	78.49	44.29	1.860	.003	.0001	.50
12	83.82	51.63	90.38	55.65	.795	.003	.0001	.19
18	86.97[c]	48.57[d]	93.97[e]	60.59[f]	.685	.0002	.0001	.02
24	91.48	55.08	95.32	60.66	1.037	.01	.0001	.45
48	94.20	64.09	96.54	68.00	1.459	.10	.0001	.62
Rate of N digestion, %/h	10.13[c]	2.54[d]	13.24[d]	3.10[c]	.224	.0012	.0001	.005

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RDP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c,d,e,f]Means within a row lacking a common superscript letter differ ($P<.05$).

TABLE 14. IN SITU DRY MATTER AND NITROGEN DISAPPEARANCE FROM SOYBEAN HULLS (SBH), SOYBEAN MEAL (SBM), CORN GLUTEN MEAL (CGM) AND BLOOD MEAL (BM)

Item	SBH	SBM	CGM	BM	SE
Nitrogen, %	1.95	7.96	10.93	14.65	
Pepsin insoluble N, % of N	22.22	6.06	11.20	3.09	
DM disappearance, %					
Hours of incubation					
0	18.28^{a}	35.47^{b}	18.19^{a}	13.82^{c}	.739
3	23.73^{a}	46.31^{b}	25.11^{a}	15.03^{c}	.954
6	30.01^{a}	56.72^{b}	25.54^{c}	15.70^{d}	.882
9	42.23^{a}	78.75^{b}	35.04^{a}	15.87^{c}	2.057
12	53.44^{a}	92.61^{b}	41.65^{c}	15.33^{d}	1.620
18	69.56^{a}	97.34^{b}	46.26^{c}	16.02^{d}	1.190
24	82.70^{a}	98.41^{b}	50.83^{c}	14.84^{d}	1.479
48	94.87^{a}	98.88^{b}	65.95^{c}	13.95^{d}	.301
Rate of DM digestion, %/h	7.22^{a}	18.36^{b}	2.15^{c}	-.01^{d}	.367
N disappearance, %					
Hours of incubation					
0	29.27^{a}	16.67^{b}	11.60^{b}	11.70^{d}	1.555
3	34.10^{a}	32.15^{a}	17.36^{b}	9.93^{c}	.598
6	40.02^{a}	44.69^{b}	17.19^{c}	12.24^{d}	.880
9	51.56^{a}	73.06^{b}	24.44^{c}	10.89^{d}	1.969
12	63.08^{a}	92.70^{b}	29.46^{c}	12.44^{d}	1.429
18	68.74^{a}	97.22^{b}	32.72^{c}	12.79^{d}	1.469
24	70.82^{a}	97.79^{b}	38.45^{c}	16.45^{d}	1.619
48	83.55^{a}	98.09^{b}	56.58^{c}	16.59^{d}	.654
Rate of N digestion, %/h	4.19^{a}	18.39^{b}	1.48^{c}	.30^{d}	.221

^{a,b,c,d}Means within a row lacking a common superscript letter differ ($P < .05$).

TABLE 15. HAY AND TOTAL OM INTAKE AND DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
Intake										
Hay OM, kg/d	5.4	7.2	7.7	8.4	8.3	.32	.0001	.008	.56	.34
Hay OM, % BW	1.18	1.49	1.59	1.70	1.71	.059	.0001	.006	.35	.39
Total OM, kg/d	5.4	8.8	9.3	10.0	9.9	.32	.0001	.008	.56	.35
Total OM, % BW	1.18	1.83	1.92	2.04	2.05	.050	.0001	.008	.36	.44
Digestible OM, kg/d	3.2	5.5	5.6	6.2	6.0	.18	.0001	.003	.89	.33
Digestible OM, % BW	.69	1.14	1.16	1.27	1.25	.033	.0001	.003	.90	.40
Digestibility, % of intake										
Hay OM	58.7	57.6	56.7	58.2	57.4	1.42	.38	.58	.52	.97
Total OM	58.7	62.7	61.2	63.0	61.8	1.28	.02	.69	.26	.92

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

TABLE 16. SITE AND EXTENT OF OM DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
Intake, g/d	5,437.8	8,819.7	9,269.8	10,008.9	9,915.1	323.31	.0001	.008	.56	.34
Flow, g/d										
Duodenal	2,461.8	4,266.4	4,605.3	4,856.2	4,863.7	264.94	.0001	.09	.49	.48
Feed	1,998.0	3,522.6	3,749.1	4,005.9	3,993.7	237.03	.0001	.10	.63	.57
Microbial	463.8	743.8	856.2	850.2	870.0	50.16	.0001	.19	.18	.30
Ileal	2,298.6	3,687.9	3,775.3	4,016.1	4,126.7	235.80	.0001	.12	.66	.96
Fecal	2,252.7	3,363.0	3,677.0	3,785.0	3,874.0	205.67	.0001	.11	.31	.53
Disappearance, g/d										
Apparent ruminal	2,976.0	4,553.3	4,664.5	5,152.7	5,051.4	189.42	.0001	.01	.99	.52
True ruminal	3,439.8	5,297.2	5,520.7	6,002.9	5,921.4	189.81	.0001	006	.69	.37
Post-ruminal	209.1	903 3	928.3	1,071.1	989.7	76.13	.0001	.11	.69	.43
Small intestinal	163.2	578.5[d]	830.0[d]	840.1[d]	737.0[d]	108.6	.0002	.38	.47	.08
Hindgut	45.9	324.8	98.3	231.0	252.7	88.51	.06	.70	.23	.13
Total tract	3,185.1	5,456.6	5,592.8	6,223.8	6,041.1	182.35	.0001	.003	.89	.33
Digestibility, % of intake										
Apparent ruminal	54.8	52.1	51.2	52.4	51.9	1.79	.11	.75	.66	.89
True ruminal	63.0	60.5	60.2	60.7	60.6	1.69	.14	.86	.91	.96
Post-ruminal	3.9	10.6	10.0	10.6	9.9	.72	.0001	.93	.34	.88
Small intestinal	4.1	7.6	9.6	9.2	8.0	1.40	.007	.99	.76	.21
Hindgut	-.2	3.0[d]	.4[c]	1.4[cd]	1.9[cd]	.94	.06	.96	.24	.08
Total tract	58.7	62.7	61.2	63.0	61.8	1.28	.02	.69	.26	.92
Digestibility, % entering segment										
Small intestinal	9.2	16.5	20.3	19.6	17.0	2.83	.007	.96	.81	.20
Hindgut	-1.6	4.7	-.8	2.2	3.1	2.56	.14	.77	.34	.17
OM digestibility, % of total tract digestion										
Apparent ruminal	93.3	82.9	83.5	82.9	83.9	1.40	.0001	.89	.56	.89
True ruminal	107.4	96.5	98.5	96.3	98.0	1.23	.0001	.75	.13	.90
Post-ruminal	6.6	17.1	16.5	17.1	16.1	1.40	.0001	.89	.56	.89
Small intestinal	7.0	11.8	15.5	14.7	13.0	2.44	.02	.95	.68	.22
Hindgut	-.3	5.2[d]	1.0[c]	2.4[cd]	3.2[cd]	1.56	.05	.82	.25	.08

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c,d]Means within a row lacking a common superscript letter differ (P<.05).

be 4% greater for the control than for the protein supplemented diet. Unsupplemented hay probably was retained in the rumen for a longer period of time which increased the percentage of ruminal disappearance. Supplementation increased (P=.0001) total OM, feed OM and microbial OM flow from the rumen.

Within supplements, the higher level of CP increased (P=.008) daily hay OM intake by 750 g (Table 15). Total OM intake (hay plus supplement) also was increased (P=.008) by the 600 g CP supplements. Supplements containing 50% RUP did not affect (P=.56) hay OM intake or total OM intake. The intake response to RUP supplementation of beef cows has not been evaluated. Previously, Miner and Peterson (1989) reported that fecal output was greater with RUP supplementation although this response might be expected because RUP was added to supplements providing equal RDP levels. Although added RDP increased intake of low quality grass hay (Scott, 1992a), these data suggest that RUP can replace approximately 22% of supplemental RDP without depressing hay OM intake.

Digestible OM intake increased (P=.003) by .6 kg when the 600 g CP supplements were fed, although, total tract OM digestibility was not improved (P=.69; Table 15). The 50% RUP supplements tended (P=.26) to reduce total tract OM digestibility, but digestible OM intake was not (P=.90) affected. Supplemental TDN supply, which was slightly higher for 28% RUP supplements, may have partially increased total tract digestibility. Nonetheless, substituting a portion of RUP for RDP did not depress digestible OM intake.

True ruminal OM disappearance increased (P=.006) with 600 g CP supplements (Table 16). Maximum disappearance corresponded with the greatest intake of hay OM. Consequently, greater ruminal disappearance with 600 CP supplements was a function of increased intake rather than improved digestibility. The proportion of supplemental RUP did not decrease (P=.69) true ruminal OM disappearance. Higher proportions of supplemental RUP, however, tended (P=.13) to increase the percentage of total tract disappearance occurring in the rumen (% of total tract OM disappearance).

The 600 g CP supplements increased duodenal OM (P=.09) and feed OM (P=.10) flow (Table 16). The higher proportion of supplemental RUP did not alter (P=.63) duodenal feed OM flow. Microbial OM flow tended (P=.18) to increase with higher levels of both CP and RUP. Within 28% RUP supplements, microbial OM flow was greater for the 600 g CP supplement. This contradicts data reported by Scott (1992a) who fed supplements formulated similar to 28% RUP supplements and reported an inverse relationship between microbial OM flow and quantity of supplemental RDP. Postruminal OM disappearance (small intestinal plus hindgut) was greater (P=.11) for 600 g CP supplements. Scott (1992a) reported that postruminal OM digestion increased with higher levels of RDP due increased passage rate.

Fiber intake and digestion. Intake of NDF tended (P=.17) to increase with 50% RUP supplements (Table 17). The 50% RUP supplements were formulated with larger quantities of soybean hulls (Table 12); thus, greater intake of NDF without a concomitant increase in hay OM intake reflects the higher concentrations of NDF contained in RUP supplements. The percentage of total tract NDF digestion was not affected by either the supplemental level of CP (P=.86) or proportion of RUP (P=.44). Ruminal NDF disappearance (P=.01) was greater for 600 g CP supplements. Consequently, hindgut NDF disappearance tended (P=.15) to be reduced with 50% RUP supplements due to more extensive digestion in the small intestine.

Nitrogen intake, flow and digestion. Supplementation increased (P=.0001) hay N intake, total N intake and duodenal N flow above the control (Table 18). Compared to the control, 400 and 600 g CP supplements increased duodenal N flow by 2.4 and 3-fold, respectively. Supplementation increased (P=.0001) feed N flow by 241% and microbial N flow by 116%. These data illustrate the dramatic impact that supplementation has on duodenal N status of cattle consuming low quality forage.

Duodenal-N flow was 140 g and 171 g for the 400 g and 600 g levels of CP, respectively (Table 18). The estimate of N flow for 600 g CP supplements is 16% and 11% above the daily protein requirements for a 545 kg gestating and lactating beef cow (NRC, 1984), respectively. The 600 g CP supplements increased (P=.002) duodenal N flow 22% above 400 g CP supplements.

TABLE 17. SITE AND EXTENT OF NDF DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
Intake, g/d	4,399.6	6,682.8	7,209.2	7,356.5	7,512.2	251.01	.0001	.04	.17	.40
Disappearance, g/d										
Ruminal	2,838.9	4,079.0	4,336.8	4,563.5	4,586.0	134.84	.0001	.01	.28	.33
Postruminal	-108.7	194.1	171.3	85.8	122.9	46.53	.0002	.06	.87	.46
Small intestinal	-191.4	-117.9	97.7	-114.9	5.9	135.98	.25	.71	.20	.69
Hindgut	82.7	312.0	73.5	200.7	116.9	112.94	.41	.73	.15	.44
Total tract	2,730.2	4,273.1	4,508	4,649.2	4,708.9	137.76	.0001	.03	.27	.47
Digestibility, % of intake										
Ruminal	65.0	61.9	61.5	63.1	62.5	1.15	.03	.29	.66	.94
Postruminal	-2.9	2.9	2.2	1.2	1.4	.54	.0001	.02	.53	.34
Small intestinal	-3.5	-1.0	1.8	-.6	.2	1.77	.06	.70	.29	.53
Hindgut	.6	3.9	.3	1.8	1.1	1.43	.40	.60	.13	.26
Total tract	62.1	64.8	63.7	64.3	63.9	1.09	.08	.864	.44	.69
Digestibility, % entering site										
Small intestinal	-9.1	-1.7	5.0	-.7	.2	4.63	.05	.39	.39	.47
Hindgut	.1	7.2	-1.4	2.8	1.8	3.66	.49	.86	.18	.24
Digestibility, % of total digestion										
Ruminal	104.8	95.2	96.5	98.2	97.7	.95	.0001	.03	.68	.32
Postruminal	-4.8	4.7	3.5	1.8	2.3	.95	.0001	.03	.68	.32
Small intestinal	-5.9	-1.8	2.7	-1.4	.6	2.88	.05	.75	.25	.62
Hindgut	1.1	6.5	.9	3.2	1.7	2.33	.40	.55	.12	.31

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

TABLE 18. SITE AND EXTENT OF N DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
N intake, g/d	37.8	117.3	118.0	155.5	154.8	2.87	.0001	.0001	.99	.78
Supplemental	1.2	66.6	64.8	97.0	95.8	.74	.0001	.0001	.061	.62
Hay	36.6	50.7	53.2	58.5	59.0	2.60	.0001	.01	.54	.65
Flow, g/d										
Duodenal-N	57.4	139.1	140.0	169.7	171.7	8.89	.0001	.002	.86	.94
Feed-N	23.7	71.9	69.0	89.7	93.0	5.43	.0001	.001	.97	.52
Microbial-N	31.9	62.3	67.0	73.6	73.2	3.99	.0001	.03	.56	.46
Ammonia-N	1.9	4.9	4.0	6.4	5.5	.42	.0001	.002	.04	.96
Pepsin insoluble-N	28.7	59.3	61.2	70.8	70.3	3.84	.0001	.01	.84	.72
Ileal-NAN[c]	26.2	56.7	62.5	68.2	76.8	3.91	.0001	.003	.07	.68
Fecal-NAN	27.6	52.5	59.1	61.9	67.7	2.84	.0001	.004	.04	.87
NAN disappearance, g/d										
Apparent ruminal	-19.7	-21.8	-22.0	-14.2	-16.9	7.28	.89	.33	.83	.84
True ruminal	14.1	45.4	49.0	65.8	61.8	3.97	.0001	.0006	.96	.28
Postruminal	27.9	81.6	76.9	101.4	98.5	5.86	.0001	.0020	.49	.86
Small intestinal	29.4	77.4	73.4	95.1	89.4	5.88	.0001	.007	.39	.87
Hindgut	-1.5	4.2	3.5	6.3	9.1	2.37	.009	.08	.64	.40
Total tract	10.1	64.8	59.0	93.5	87.1	2.34	.0001	.0001	.02	.88
NAN digestibility, % of N intake										
Apparent ruminal	-54.7	-19.0	-16.0	-6.3	-8.9	4.86	.0001	.04	.96	.51
True ruminal	34.5	38.0	41.9	43.0	40.4	3.83	.11	.61	.86	.34
Postruminal	75.7	70.3	64.0	64.2	62.8	3.53	.01	.25	.25	.43
Small intestinal	80.2	67.0	61.3	60.6	57.5	3.90	.0005	.16	.25	.71
Hindgut	-4.4	3.4	2.6	3.6	5.2	1.64	.0004	.34	.77	.41
Total tract	26.2	55.6	51.4	62.0	57.5	1.35	.0001	.0003	.005	.91
NAN digestibility, % entering segment										
Small intestinal	53.6	58.5	54.5	59.1	54.8	1.40	.04	.74	.008	.90
Hindgut	-6.8	6.3	4.4	7.8	9.2	2.87	.0005	.22	.93	.52
Microbial efficiency[d]	8.9	11.9	11.9	12.0	12.2	.75	.002	.80	.89	.93
Nitrogein efficiency[e]	154.7	119.0	116.0	106.3	108.9	4.86	.0001	.04	.96	.51

[a]Probability levels for: Control vs all supplements; CP = 400g CP vs 600g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c]NAN = nonammonia-N.

[d]grams microbial N flow/kg true ruminal OM digestion.

[e](grams duodenal NAN flow/grams N intake) X 100.

Flow of feed N (P=.001), microbial N (P=.03) and ammonia-N (P=.002) to the duodenum was increased by the higher level of supplemental CP. Stokes et al. (1988) also reported similar increases in duodenal N flow when soybean meal was fed at .12% and .24% BW to steers. Scott (1992a), however, reported that microbial N flow was not increased above 294 g of supplemental RDP, even though hay intake increased linearly. Consequently, incremental levels of supplemental RDP failed to increase duodenal microbial N flow despite higher ruminal ammonia-N concentrations and greater hay intake. Therefore, energy availability apparently limited ruminal fermentation and subsequent microbial protein synthesis (Bergen et al., 1980). In this study, microbial protein synthesis was increased with higher levels of supplemental CP because hay was more digestible. The ratio of supplemental RDP to digestible OM intake for the 600 g CP/28% RUP supplement was 70 which is lower than the 88.6 g RDP/kg digestible OM intake previously reported to maximize forage utilization (Scott, 1992a). Because hay OM digestibility was 12 percentage units higher in this study, feeding higher quantities of supplemental RDP might be expected to stimulate microbial protein synthesis and digestible OM intake.

Flow of duodenal N (P=.86), feed N (P=.97), and microbial N (P=.56) were not altered by the proportion of supplemental RUP (Table 18). Similarly, Schloesser et al. (1992) reported that neither feed N flow nor bacterial N flow were affected (P=.05) by substitution of blood meal for soybean meal for ewes fed grass hay containing 8% CP. Scott (1992a) reported that the quantity of supplemental RDP required to maximize microbial N synthesis was less than the quantity of RDP required to maximize hay intake. Therefore, duodenal N flow may be limited by microbial protein synthesis with low quality forage diets supplemented with RDP. We anticipated that flow of undegraded supplemental N from 50% RUP supplements would increase N flow to the small intestine. If ruminal hay N degradability was not affected by supplements and microbial N flow estimates are correct, then these data suggest that extent of ruminal degredation of supplemental N was not different among RUP supplements. Consequently, estimates of ruminal N degradability for blood meal and corn gluten meal (NRC, 1985) may be inappropriate for low quality forage diets. Nitrogen degradation rates in situ with these diets, however, were lower (P<.0001) for 50%

RUP than for 28% RUP supplements (Table 14). Alternately, the failure of higher RUP supplements to increase duodenal feed N flow relative to microbial N flow could be explained by an inflated microbial N flow to the duodenum. Because blood meal contains RNA, we hypothesized that a portion of undegraded blood meal RNA flowed to the duodenum and contributed to the RNA attributed to microbes. To correct for undegraded blood meal RNA, ruminal N survival of blood meal (82%; NRC, 1985) was multiplied by its RNA content. Blood meal, however, contained less than 2% RNA and was included in the higher RUP supplements only at low levels (7 and 11%). Consequently, the contribution of indigestible RNA from blood meal could cause an overestimation in microbial N flow by only 1 to 2 g/d.

Duodenal ammonia-N flow was decreased ($P=.04$) with the 50% RUP supplements (Table 18). Scott (1992a) indicated that duodenal ammonia-N flow increased as RDP was supplemented. Microbial efficiency (microbial N flow/kg true ruminal OM disappearance) was not altered ($P>.80$) by the level of supplemental CP or RUP. Other studies have indicated that protein supplementation of low quality forages does not alter microbial efficiency (Stokes et al. 1988; Scott, 1992a).

True ruminal and small intestinal disappearance of nonammonia nitrogen (NAN) was greater ($P<.007$) for 600 g CP supplements (Table 18). Total tract NAN disappearance was increased ($P=.0001$) by 46% (28.4 g) for 600 g CP supplements. Supplements containing 50% RUP decreased ($P=.02$) total tract NAN disappearance. The proportion of supplemental RUP, however, did not alter the quantity of NAN disappearance at the other segments. Although PIN flow to the duodenum was not altered by proportion of RUP, digestibility of NAN (expressed as the percentage entering the small intestine) was lower ($P=.008$) for 50% RUP supplements. Perhaps protein digestibility of blood meal and corn gluten meal was lower than expected. Pepsin insoluble N (% of N) content of supplements and feeds (Table 13, 14), however, suggest that decreased small intestinal digestibility of 50% RUP treatments cannot be attributed blood meal and corn gluten meal. Thus, the absence of a hay intake response to 50% RUP supplements may be due to reduced availability of amino acid N from RUP feedstuffs. Neither intake nor digestion

were depressed, however, for 50% RUP supplements. Although small intestinal N digestibility was lower (P=.08) for 50% RUP supplements, amino acid flow may have differed.

Passage rate and fill. Supplementation increased (P=.002) total tract particle passage rate (Table 19). Because supplementation did not increase digestibility, increased intake can be attributed partially to an increased passage rate. Supplementation increased ruminal fluid dilution rate (%/h, P<.0001) and decreased retention time (P<.0001). Ruminal fluid volume (P=.0003) and % BW (P=.02) also were increased by supplementation. A faster ruminal dilution rate obtained with supplements probably are responsible for the increased microbial effiency observed with supplemental CP (Owens and Isaacson, 1977).

The higher level of supplemental protein did not (P=.72) increase total tract particle passage rate (Table 19) even though total OM intake was 1 kg higher. Increased intake with protein supplements has been associated more often with faster rates of passage (McCollum and Galyean, 1985; Guthrie and Wagner, 1988; Scott, 1992a) than with no change in rate of passage (Fleck et al., 1988). The higher proportions of supplemental RUP tended (P=.38) to reduce particle passage rates. Consequently, when OM or NDF digestion was expressed as a percentage of total digestion, 50% RUP supplements shifted the site of digestion toward the rumen. Within level of CP, increased microbial OM flow was associated with slower passage rate. Scott (1992a) also reported the same relationships when incremental levels of supplemental RDP were fed. The explanation for these relationships, however, is unclear. Duodenally infused casein increased ruminal fill above infusions of urea/glucose in forage fed steers eventhough fecal output was not altered (Garza-F et al., 1991). Similarly, higher RUP supplements tended (P=.19) to increase ruminal DM fill (% BW) without altering fecal output. Greater fill for higher RUP supplements was offset by slower passage rates (%/h); consequently, intake was not altered by proportion of RUP.

Neither the level of CP nor the proportion of RUP altered (P>.50) ruminal fluid dilution rate, retention time or volume (Table 19). In contrast, slowed fluid dilution rate and increased ruminal fluid volume was reported by Miner and Petersen (1989) for RUP supplements compared

TABLE 19. PASSAGE RATES AND RUMINAL FILL IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
Total tract										
Particle passage rate, %/h	1.37	2.73	2.56	2.95	2.56	.335	.002	.72	.38	.70
Ruminal										
DM fill, % BW[c]	1.85	2.11	2.28	2.23	2.26	.076	.0004	.50	.19	.29
Ruminal fluid										
Dilution rate, %/h[d]	5.44	8.20	8.04	8.06	6.35	.391	.0001	.83	.86	.57
Retention time, h[d]	18.88	12.76	12.82	13.67	12.50	.875	.0001	.74	.53	.49
Volume, % BW[c]	11.40	12.20	12.62	12.45	12.22	.385	.02	.83	.78	.34

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c]Estimated from ruminal evacuation.

[d]Estimated with cobalt·EDTA.

to soybean meal. Microbial efficiency should increase with faster fluid dilution rate (Owens and Isaacson, 1977). In our study, ruminal fluid dilution rate was not altered by level of CP or proportion of RUP, and microbial efficiency was not changed by diet.

Ruminal fermentation. Ruminal ammonia-N concentrations showed a time X treatment interaction (P=.0001) indicating that treatment differences depended on sampling time (Figure 10). Concentrations of ammonia-N were not detectable in ruminal fluid from control cows. These relatively low ruminal ammonia-N concentrations reflect the low N content of mature grass hay and rapid incorporation of feed N into microbial protein (Scott, 1992a).

Ruminal ammonia-N concentrations peaked two h after supplementation and declined thereafter (Figure 10). Ruminal ammonia-N concentrations remained below 1 mg/dL for the 400 g CP supplements for most of the day. Concentrations of ruminal ammonia-N were higher (P=.0001) for 600 than for 400 g CP supplements at all sampling times, except 24 h. A reduced rate and extent of in situ N degradation probably lowered ruminal ammonia-N concentrations of the 50% RUP supplemented diets. Similarly, Scott (1992a) reported that ruminal ammonia-N were increased by supplemental RDP.

Ruminal ammonia-N concentrations for the 600 g CP/28% RUP supplement remained above 1.6 mg/dL throughout the day (Figure 10). Both the 400 g CP/28% RUP and 600 g CP/50% RUP supplements supplied the same quantity of RDP, but ruminal ammonia-N was higher for the 600 g CP/50% RUP supplement. The additional 284 g of supplemental RUP increased ruminal ammonia-N above that produced by a supplement providing a similar quantity of RDP. The higher ruminal ammonia-N concentration for the 600 g CP/50% RUP supplement was higher probably due to nitrogen recycling, although nitrogen efficiency (duodenal flow/intake) was greater for the 400 g CP/28% RUP supplement. Alternatively, supplemental N was depleted more rapidly in situ from the 400 g CP/28% RUP supplement (Table 13). Consequently, feed protein may have been incorporated into microbial protein more rapidly; this would reduce ruminal ammonia-N concentrations. Lower concentrations of ruminal ammonia-N

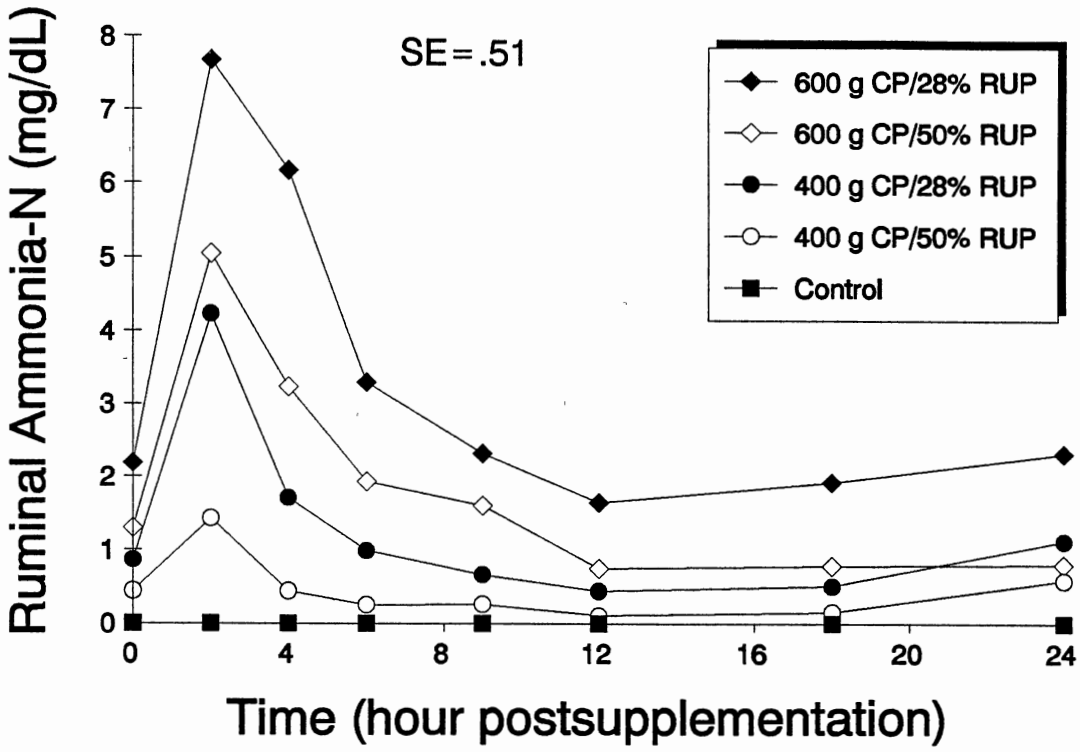

Figure 10. Ruminal ammonia-N concentrations in beef cows fed native grass hay as affected by supplemental level of protein and proportion of ruminally undegraded protein (RUP).

for 50% vs 28% RUP supplements were associated with slower passage rates, so salivary recycling probably was lower with the lower RUP diets.

Concentrations of OM, N, nucleic acid-N and the nucleic acid-N/microbial-N ratio in microbial isolates (Table 20) and in microbial isolates reported by Scott (1992a) were dependent upon the quantity of supplemental RDP. The proportion of supplemental RUP influenced the composition of microbial isolates to a greater degree than did the level of supplemental CP. Microbial OM concentrations were not affected (P=.30) by level of CP, however, there was a tendency (P=.19) for microbial OM to be lower for 50% RUP supplements. The 600 g CP supplements increased (P=.02) N, nucleic acid and nucleic acid-N concentrations in microbial isolates. In contrast, nitrogen (P=.01), nucleic acid and nucleic acid-N (P=.0001) were reduced with the 50% RUP supplements. The nucleic acid-N/microbial-N ratio tended (P=.12) to be greater for 600 g CP supplements but was reduced (P=.0005) for 50% RUP supplements. Scott (1992a) suggested that higher microbial-N and nucleic acid-N/microbial-N ratios were associated with microbes in a more efficient phase of growth and depositing less polysaccharide.

Disappearance of OM from in situ bags at each hour of incubation was greater (P<.02) and rate of hay OM degradation was faster (P=.0001) when supplemental protein was fed (Table 21). The 400 g CP/50% RUP supplement provided the least supplemental RDP (200 g) and produced the slowest rate of digestion. The percentage of OM disappearing at 6, 24, 48 and 96-h of incubation was greater (P<.05) for the 600 g CP supplements. The increased (P=.02) rate of digestion with 600 g CP supplements probably is due to higher ruminal ammonia-N concentrations. Scott (1992a) reported a positive relationship between ruminal ammonia-N and rate of digestion. The 50% RUP supplements decreased (P<.05) the percentage of OM disappearing at 24, 48 and 96-h of incubation. Despite these numeric differences, the lower ruminal ammonia-N concentrations for 50% RUP supplements did not significantly depress (P=.21) rate of hay OM digestion. The explanation for similar rates of digestion, within level of CP, even though ruminal ammonia-N concentrations were different is unclear. Either recycled N may have impacted ruminal fermentation to a greater degree for 50% RUP supplements or greater

TABLE 20. COMPOSITION OF RUMINAL MICROBIAL ISOLATES IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
Microbial isolates, %										
Organic matter	79.51	85.89	84.29	86.93	85.48	1.193	.0003	.30	.19	.94
Nitrogen	5.46	7.33	6.61	7.66	7.23	.202	.0001	.02	.01	.43
Nucleic acid	3.89	8.22	5.78	8.81	7.07	.387	.0001	.02	.0001	.32
Nucleic acid-N	.58	1.24	.87	1.32	1.06	.058	.0001	.02	.0001	.32
Nucleic acid -N, % of microbial-N	10.75	16.74	13.09	17.14	14.62	.674	.0001	.12	.0005	.35

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

TABLE 21. IN SITU OM DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
OM disappearance, %										
Hours of incubation										
6	18.43	18.85[cd]	19.41[de]	20.70[f]	19.87[e]	.314	.0001	.0001	.65	.01
12	20.69	22.54[c]	23.71[cd]	26.03[d]	22.48[c]	1.337	.02	.32	.34	.04
24	27.41	34.58	32.94	39.39	37.92	.825	.0001	.0001	.04	.90
48	40.77	55.55	51.76	59.32	57.92	1.351	.0001	.0001	.04	.30
96	62.04	72.68	70.70	74.32	72.79	.925	.0001	.02	.04	.78
Rate of digestion										
OM, %/h[g]	.78	1.48	1.25	1.60	1.59	.098	.0001	.02	.21	.22

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c,d,e,f]Means within a row lacking a common superscript letter differ ($P < .05$).

[g]Rate of digestion was calculated from the slope of the regression of the natural logarithm of residual OM over time.

concentrations of nonprotein OM may have provided microbes with a more readily available source of energy. Miner and Petersen (1989) supplemented 200 g/d of RDP in the form of soybean meal, soybean meal/blood meal or soybean meal/urea/corn gluten meal and reported that RUP supplements increased the rate of in situ NDF digestion. Ruminal ammonia-N concentrations in their study were higher for RUP supplements. This would be similar to the comparison of our 400 g CP/28% RUP supplement to our 600 g CP/50% RUP supplement, wherein RDP supply was equalized but additional RUP increased ruminal ammonia-N and rate of digestion. Miner and Petersen (1989) suggested that RUP supplements were degraded at a slow rate over a long period of time, thereby supplying more amino acids or certain carbon skeletons to enhance fiber digestion.

Protein supplementation increased (P=.003) total ruminal VFA concentration (Table 22). Molar proportions of both acetate (P=.09) and isobutyrate (P=.02) were greater with 50% RUP than 28% RUP supplements. An increased acetate proportion might be attributed to a reduced rate of supplemental DM degradation (Table 13). Within level of supplemental CP, forage utilization was similar despite lower ruminal ammonia-N for the higher proportions of RUP. This might be the result of an increase (P=.02) in the isobutyrate proportion which may have stimulated the activity of cellulolytic bacteria when higher proportions of RUP were fed (Dehority et al., 1967). The proportion of butyrate tended (P=.18) to increase with more CP. Similarly, Scott (1992a) reported that butyrate increased at the expense of acetate with incremental levels of supplemental RDP. Supplemental level of CP or RUP did not alter (P>.31) total VFA concentrations, molar proportions of propionate or the acetate/propionate ratio.

Ruminal pH showed a signficant (P=.05) time x treatment interaction, therefore ruminal pH is presented graphically (Figure 11). Ruminal pH was higher (P=.0001) with the control than with protein supplemented diets. High ruminal pH (6.61) coupled with low ruminal ammonia may reflect the slow rate of fermentation for unsupplemented forage (Scott, 1992a). Supplements containing 600 g CP tended to reduce (P=.13) ruminal pH. Added RDP, contained in 28% RUP supplements, decreased (P=.0001) ruminal pH at all hours compared to 50% RUP supplements.

TABLE 22. VFA PROFILES IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
Total, mmol/ml	91.20	116.90	129.27	127.96	132.84	9.783	.003	.40	.36	.66
Acetate, mol/100 mol	69.31	67.31	69.03	67.70	68.14	.539	.04	.38	.09	.33
Propionate, mol/100 mol	18.01	18.28	17.16	17.96	17.74	.756	.76	.84	.35	.50
Isobutyrate, mol/100 mol	1.41	1.05	1.93	1.35	1.68	.254	.71	.92	.02	.24
Butyrate, mol/100 mol	9.91	11.05	10.60	11.17	11.10	.257	.001	.18	.29	.40
Isovalerate, mol/100 mol	.88	.88	.56	.88	.45	.363	.61	.85	.28	.88
Valerate, mol/100 mol	.48	1.08	.72	.94	.88	.199	.05	.93	.28	.39
Acetate/propionate	3.90	3.76	4.07	3.90	3.92	.174	.921	.99	.31	.33

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

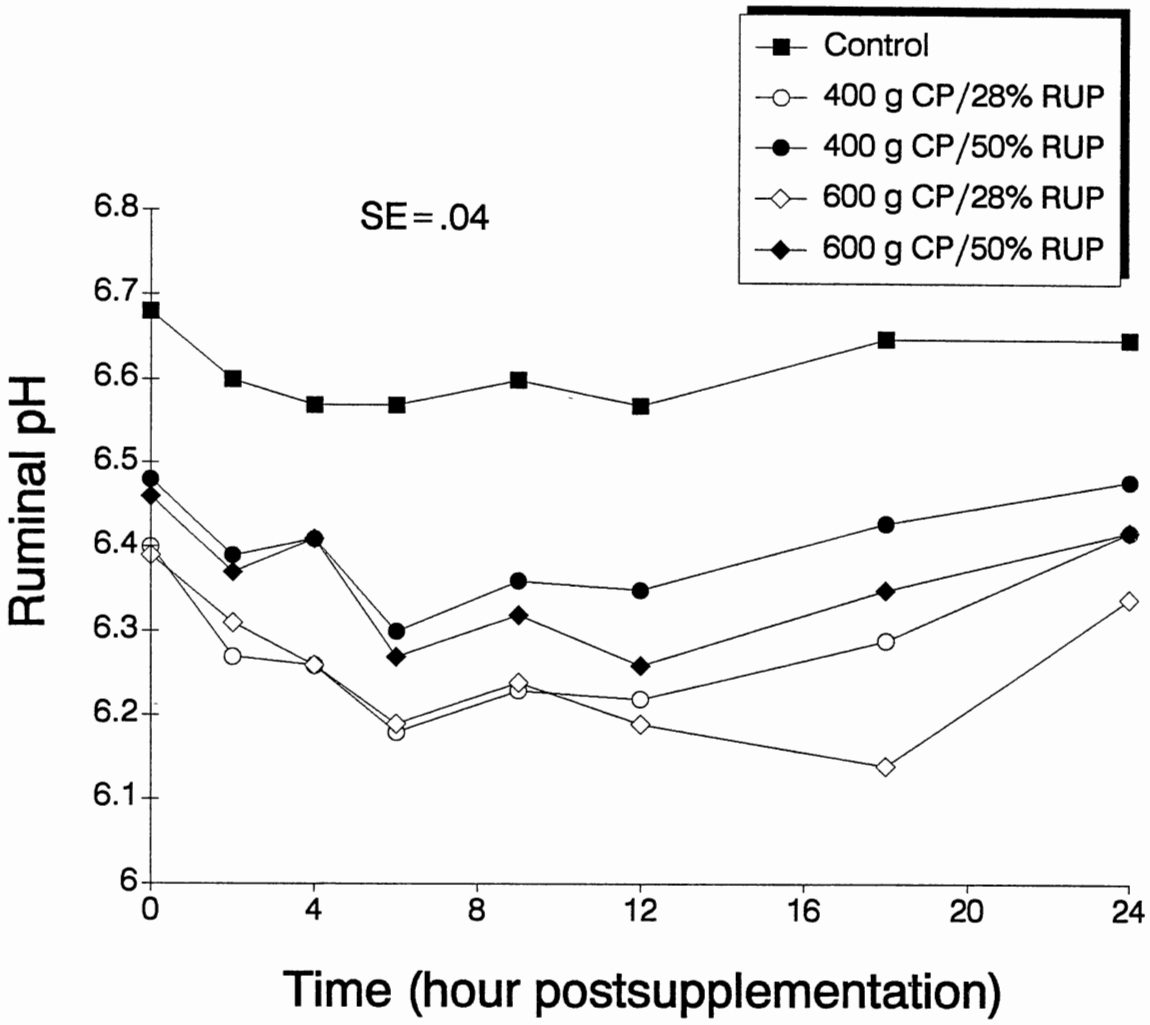

Figure 11. Ruminal pH in beef cows fed low quality native grass hay as affected by supplemental level of protein and proportion of ruminally undegraded protein (RUP).

Similar to our comparison of 400 g CP/28% RUP and 600 g CP/50% RUP, Miner and Petersen (1989) showed that ruminal pH was decreased and rate of digestion was faster when RUP was added to 200 g soybean meal.

Supplements containing 50% RUP supplied approximately half as much RDP as 28% RUP supplements; therefore, ammonia-N should have limited ruminal fermentation of 50% RUP supplemented diets (Scott, 1992a). In this study, in situ rate of hay OM degradation and true ruminal OM disappearance were not dependent on the proportion of supplemental RUP despite the lower ruminal ammonia-N concentrations with the 50% RUP supplements. Although ruminal ammonia-N and in situ degradation of supplemental N confirm that ruminal degradation was lower for 50% RUP than 28% RUP supplements, composition of duodenal N flow suggests that NRC (1985) values used to calculate ruminal protein degradation were incorrect. It is plausible that ruminal kinetics afforded by low quality forage diets increased ruminal N degradation relative to NRC estimates.

Compared to the control, both low and high RUP supplements increased digestible OM intake and duodenal N flow. Their modes of action, however, appear to be different. With 28% RUP supplements, increased intake was a function of an increased passage rate. In contrast, 50% RUP supplements may have increased intake via increased ruminal fill. Increased NAN flow has been suggested to increase forage intake without a concomitant increase in the rate or extent of digestion (Egan and Doyle, 1985). They suggested that greater intake was accomplished via enhanced fill. Although duodenal NAN flow was not affected by the proportion of RUP, the amino acid profile of duodenal chyme could have been altered. If intestinal amino acid profiles were enhanced for 50% RUP supplements, then increased ruminal fill may be the result of meeting specific amino acid deficiencies (Forbes, 1988). Alternatively, if blood urea supply limits saliva flow and forage intake, digested RUP might prove more useful than an equal amount of RDP.

This study emphasizes the dramatic positive associative effect that supplemental digestible fiber and protein have on the intake and utilization of low quality native grass hay. Increasing the quantity of supplemental CP from 400 to 600 g/day increased intake of CP and

digestible OM by 234 and 607 g/day, respectively. Therefore, milk production potentially could be increased by 2.5 kg and 3.9 kg due to greater intake of CP and TDN, respectively (NRC, 1984). These additional nutrients should ameliorate body weight and condition losses for fall calving cows grazing dormant native grass pastures.

Substitution of approximately one-fourth of supplemental RDP with RUP did not increase the utilization of hay, digestible OM intake or duodenal N flow. Consequently cattle performance would not be expected to increase. In addition, RUP supplements often cost more than RDP supplements; therefore these data imply that substituting a portion of RUP for RDP in supplements for low quality forage does not appear to be beneficial. For a majority of evaluated parameters, the interaction of CP level by RUP proportion was not significant which suggests that responses due to the proportion of supplemental RUP were independent of supplemental protein level. Supplemental CP, not RUP, appeared to be the primary factor of interest. Decreased intake for 400 g CP/50% RUP supplements compared to 600 g CP/28% RUP supplements suggests that substituting a portion of RUP for RDP in supplements for low quality forage will not reduce total supplemental protein requirements. Within 28% RUP supplements, however, microbial protein synthesis appeared to be limited by RDP and ruminal ammonia supply. If hay digestibility were lower, a higher proportion of supplemental RUP may have spared total supplemental protein because microbial protein synthesis might have been maximized at a lower level of supplemental RDP. The ratio of RDP to digestible OM intake was lower than the optimum value of 88.6 observed by Scott (1992a). Therefore, additional RDP may have stimulated ruminal fermentation and increased digestible OM intake. Further research is required to develop a better understanding of the requirements for supplemental RDP and RUP for low quality forage diets.

Implications

Rate of in situ digestion of nitrogen suggested that the addition of RUP to range supplements decreased the rate and extent of ruminal protein degradation. But in vivo, an increased supply of ruminally undegraded protein, did not affect energy intake or duodenal N

flow. Therefore, when economically justified, small concentrations of blood meal/corn gluten meal blends might be substituted for a portion of ruminally undegraded protein when formulating low-starch/digestible fiber supplements. Digestible nitrogen supply, not ruminally undegraded protein, appeared to limit intake and digestion. Further research is required to determine whether ruminally undegraded protein is needed. Furthermore, NRC estimates for ruminally undegraded protein concentrations in feedstuffs may overestimate in vivo protein escape with low quality forage diets due to an extremely slow rate of passage.

CHAPTER V

RUMINALLY UNDEGRADED PROTEIN FOR FALL CALVING BEEF COWS GRAZING DORMANT TALLGRASS PASTURES

Abstract

Fall calving beef cows (n=72) grazed dormant tallgrass pastures for 104 d (December to March) and received digestible fiber supplements containing two levels of protein (400 and 600 g CP) and two proportions of ruminally undegraded protein (28 and 50% RUP). All supplements were formulated with soybean meal and soybean hulls. A blend of blood meal and corn gluten meal was added to formulate 50% RUP supplements. Supplemental energy supply was equalized at 1.4 kg TDN/d. The higher level of protein decreased (P=.004) body weight and condition losses. The proportion of RUP did not alter (P=.78) body weight change. Although differences were small, cows receiving 50% RUP supplements lost body condition faster (P<.04) than cows fed supplements containing 28% RUP. Within 400 g CP, cows fed 50% RUP supplements lost .24 units more body condition in 104 days than cows receiving 28% RUP supplements. Calves suckling cows fed 600 g CP gained 6.8 kg more weight (P<.0001) than calves whose dams were fed 400 g CP. Within the 600 g CP supplements, 28% RUP resulted in greater (P<.05) milk production and faster (P<.0001) calf weight gain. Mean milk protein (% of wet matter) was increased (P<.09) with the higher CP supplements. Ruminal ammonia-N (2 to 4 h postsupplementation) was greater (P<.0003) with the higher level of CP but was decreased (P<.01) as the quantity of RUP in the supplement was increased from 28% to 50%. In summary, greater quantities of supplemental CP increased cow herd performance but supplemental RUP did not benefit the productivity of lactating beef cows.

Key Words: Beef Cattle, Native Grass, Protein Supplementation, Protein Degradability

Introduction

Fall calving beef cows grazing dormant native grass pastures (<4% CP) require extensive supplementation to maintain productivity. Most range supplements are composed of cottonseed meal or soybean meal blended with cereal grain or low-protein byproduct feeds. Formulation of these supplements are based on total protein, while protein characteristics are often ignored. Because the ruminal N degradation of protein feedstuffs varies (NRC, 1985), supplements possessing high quantities of ruminally undegraded protein (RUP) may deprive cellulolytic bacteria of ruminal ammonia-N . Consequently, high levels of supplemental RUP may reduce the utilization of low quality forage (Hibberd and Martin, 1990). Ruminal fermentation and utilization of low quality forage, however, was not decreased when RUP composed up to 50% of supplemental protein (Scott, 1992b).

Production studies with beef cattle illustrated that RUP can either augment (Miner and Petersen, 1989; Miner et al., 1990) or replace (Hibberd et al., 1988) a portion of the ruminal degradable protein (RDP) fraction in supplements for low quality forage diets. Incorporation of RUP into both lactating dairy cow rations (Orskov, 1982) and lactating beef cow supplements (Hibberd et al., 1988) has increased milk production. Hibberd et al. (1988) illustrated that milk production and calf weight gain increased while body weight and condition losses were decreased when protein from blood meal replaced 50% of total supplemental protein. Consequently, RUP may reduce total supplemental protein requirements (Stanton et al., 1983).

Scott (1992b) fed supplements containing two proportions (28 and 50%) of RUP at two levels of total protein (400 and 600 g CP) to cows consuming low quality grass hay. The proportion of RUP did not alter digestible OM intake or the composition and flow of duodenal N. Thus, the objective of this study was to evaluate supplements formulated by Scott (1992b) on cow herd productivity.

Materials and Methods

Fall-calving cows (n=72; average calving date October 1) were fed one of four supplements in a 2 x 2 factorial design. Cows were assigned to treatments based on calving date, body weight and condition. Pelleted (.61 cm) supplements provided two levels of total protein (400 g and 600 g CP/d) with two proportions of ruminally undegraded protein (28% and 50% RUP). Supplements were formulated with blends of soybean meal and soybean hulls (Table 23). Ruminal degradation of soybean meal protein was estimated at 72% (NRC, 1984) to formulate supplements. Supplemental energy was formulated to supply a similar quantity of TDN which ranged from 1.3 to 1.5 kg TDN/d; energy supply was equalized with soybean hulls (TDN estimated at 75%) to prevent confounding effects between supplemental protein and energy. A blend consisting of 60% CP from blood meal and 40% CP from corn gluten meal was added to formulate the 50% RUP supplements. Blood meal (82% RUP) and corn gluten meal (55% RUP) were utilized because of their complementary amino acid profiles (NRC, 1985). Supplemental calcium, phosphorus, trace mineralized salt and vitamin A was formulated to meet requirements. In addition, sodium sulfate was included to maintain a supplemental nitrogen:sulfur ratio of 12:1 and dairy flavors were added to enhance the palatability of supplements. Cows were individually fed their respective weekly allotment of supplement five days per week (M, T, W or Th, F and S). Cows were moved to a different native grass pasture on day 45 of the 104 day study.

To equalize fill, cows were fed 2.3 kg cottonseed meal for one week prior to and for one week following the end of the study. These initial and final weights were used to evaluate treatment effects over the entire length of the 104 day study. Cow weights and body condition scores also were evaluated at two week intervals. Body condition (1=emaciated, 9=obese) was the average of three independent scores. Calves were weighed 5 h following separation from the dam.

Diet samples were collected at four week intervals by 5 esophageally cannulated steers. Esophageal collection bags were constructed with a closed bottom to prevent leaching of soluble

TABLE 23. COMPOSITION, FEEDING RATE AND NUTRIENT SUPPLY OF SUPPLEMENTS PROVIDING TWO LEVELS OF PROTEIN AND TWO PROPORTIONS OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP	
Item	28% RUP[a]	50% RUP	28% RUP	50% RUP
Feed composition, % (DM basis)				
Soybean hulls	68.52	77.98	43.25	57.39
Soybean meal	22.78	.19	48.57	14.80
Blood meal		6.55		9.79
Corn gluten meal		6.02		8.99
Molasses	3.00	3.00	3.00	3.00
Dicalcium phosphate	2.97	3.43	2.31	3.00
TM salt[b]	2.25	2.25	2.25	2.25
Sodium sulfate	.39	.49	.53	.68
Vitamin A (30,000 IU/g)	.06	.06	.06	.06
Dairy flavors	.03	.03	.03	.03
Nutrient, % DM				
CP[c]	19.7	19.4	27.6	28.4
TDN[d]	74.1	72.0	76.3	73.2
Intake, g/d				
DM	2,002	2,002	2,002	2,002
CP				
Total[c]	394	394	552	569
RDP[e]	284	197	398	285
RUP[a]	110	197	154	284
TDN[d]	1,483	1,441	1,527	1,344

[a]RUP = Ruminally undegraded protein.

[b]Trace mineralized salt contained 92% NaCl, .25% Mn, .20% Fe, .033% Cu, .007% I, .005% Zn and .0025% Co.

[c]Actual analysis.

[d]Estimated from NRC (1984).

[e]Estimated from NRC (1985), RDP of soybean hulls assumed equal to soybean meal.

compounds from the forage. The diet samples were immediately placed on ice, frozen (-15^{o} C) and lyophilized. Diet samples were analyzed for dry matter, ash (AOAC, 1975), crude protein (N x 6.25; KjelTec 1030 Auto Analyzer)[a] and neutral detergent fiber (Goering and Van Soest, 1970). Nutrient content of forage was expressed on an OM basis.

Milk production was estimated by the weigh-suckle-weigh procedure (Totusek et al., 1973) at four week intervals. Calves, removed from the dams at 0800, were allowed to suckle at 1300 to begin the milk production study. Additional sucklings were at 2130 and at 0630 and 1300 the following day. Daily milk production estimates were calculated as the sum of the last three milk productions.

Ruminal fluid samples (collected from 2 to 4 h postsupplementation) were obtained at four week intervals from 32 cows (8 per treatment). Ruminal fluid was collected via a vacuum pump with a suction strainer attached to a stomach tube. Fluid was processed and analyzed as reported by Scott (1990a). In conjunction with ruminal sampling, milk was stripped (20 ml) from udders and placed on ice prior to analysis for concentrations of fat and protein.

Statistics. Rate of body weight and body condition change was calculated by regressing biweekly changes on day of the trial. Cow and calf performance, milk composition and production and ruminal ammonia-N data were analyzed by least squares procedures with a model that included calf sex, cow age, level of protein, proportion of RUP, CP level X RUP proportion interaction plus calving date as a covariate. Rates were analyzed by the same model. A repeated measures analysis was conducted to determine an adjusted probability value for treatment X sampling day for milk parameters and ruminal ammonia-N. When the CP level X RUP proportion interaction was deemed significant ($P<.10$), treatment differences were detected by t-test.

Results and Discussion

Forage quality. Crude protein content of esophageal diet samples from pasture 1 declined from 4.6% on December 8 to 3.2% on January 17 (Table 24). Due to limited forage availability, all cattle were relocated to pasture 2 on January 23 (day 45). The CP content of

TABLE 24. CHEMICAL COMPOSITION OF DIET SAMPLES FROM DORMANT TALLGRASS PASTURES

	Pasture 1		Pasture 2		
Chemical component	Day 0	Day 45	Day 62	Day 90	Day 104
	- - - - - - - - - - - - - - -		% OM basis		- - - - - - - - - - - - - - -
CP[a]	4.6 ± .21	3.2 ± .29	4.3 ± .34	4.2 ± .23	4.4 ± .25
NDF[a]	85.6 ± 1.75	84.6 ± .42	80.2 ± 2.89	83.2 ± 2.26	84.4 ± 1.53

[a]Mean (n=4) ± SD.

pasture 2 was relatively stable (average 4.3%). Concentrations of NDF ranged from 80.2 to 85.6% throughout the 104-d study.

Cow performance. The 600 g level of protein supplementation decreased (P=.004) losses of body weight and condition by 11.6 kg and .26 units, respectively (Table 25). Through day 45, cows receiving the 600 g CP/50% RUP supplement tended to lose (P>.05) the least weight (Figure 12). By day 62, however, weight loss was similar within 600 g CP supplements. Rates of body weight (P=.0006) and condition (P=.002) loss were lower with 600 g than 400 g CP supplements. Differences in body condition, due to level of CP, were detected by d 45 of the trial (Figure 13). The higher CP level supplements probably increased energy intake and duodenal N flow (Scott, 1992b), which decreased loss of body weight and condition compared to the lower level of supplemental CP. Reproductive performance should have been improved due to the enhanced nutritional status afforded by higher levels of supplemental CP (Short et al. 1990).

The proportion of supplemental RUP did not alter (P=.78) body weight change. Scott (1992b) reported that 50% RUP supplements did not decrease energy intake or duodenal N flow; therefore, no difference in cow weight change due the higher proportion of supplement RUP was expected. Nevertheless, although differences were small, feeding 50% RUP supplements increased both the total (P=.08) and rate (P=.04) of body condition loss. Orskov (1982) suggested that lactating dairy cows fed RUP, when in negative energy balance, may mobilize more body reserves for milk synthesis (Orskov, 1982). Hibberd et al. (1988) reported that cow weight and condition score losses of lactating beef cows were reduced when protein from blood meal supplied 50% of supplemental protein. Initially, cows receiving 400 g CP/50% RUP supplements depleted body reserves more rapidly (Figure 13); therefore, those cows lost more body condition (.91 units) during the study.

Calf performance and milk parameters. A CP level by RUP proportion interaction (P=.06) was observed for milk production (Table 26). The 600 g CP/28% RUP supplement resulted in the highest (P<.05) milk production. Milk production was relatively low and no differences (P>.10) were detected among the remaining supplemental treatments. Total weight gain of calves also

TABLE 25. BODY WEIGHT AND CONDITION OF BEEF COWS GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Weight, kg								
Initial	470.2	453.4	458.5	456.9	8.92	.64	.31	.40
Final	399.6	381.6	397.1	398.9	7.75	.34	.31	.20
Change	-70.6	-71.9	-61.4	-58.0	3.84	.004	.78	.55
Rate, kg/d	-.81	-.82	-.68	-.70	.041	.0006	.43	.72
Body condition, units								
Initial	5.14	5.13	5.14	5.10	.067	.79	.72	.89
Final	4.48	4.22	4.64	4.54	.110	.03	.11	.47
Change	-.67	-.91	-.50	-.56	.086	.004	.08	.30
Rate, units/d	-.006	-.008	-.004	-.005	.0008	.002	.04	.43

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

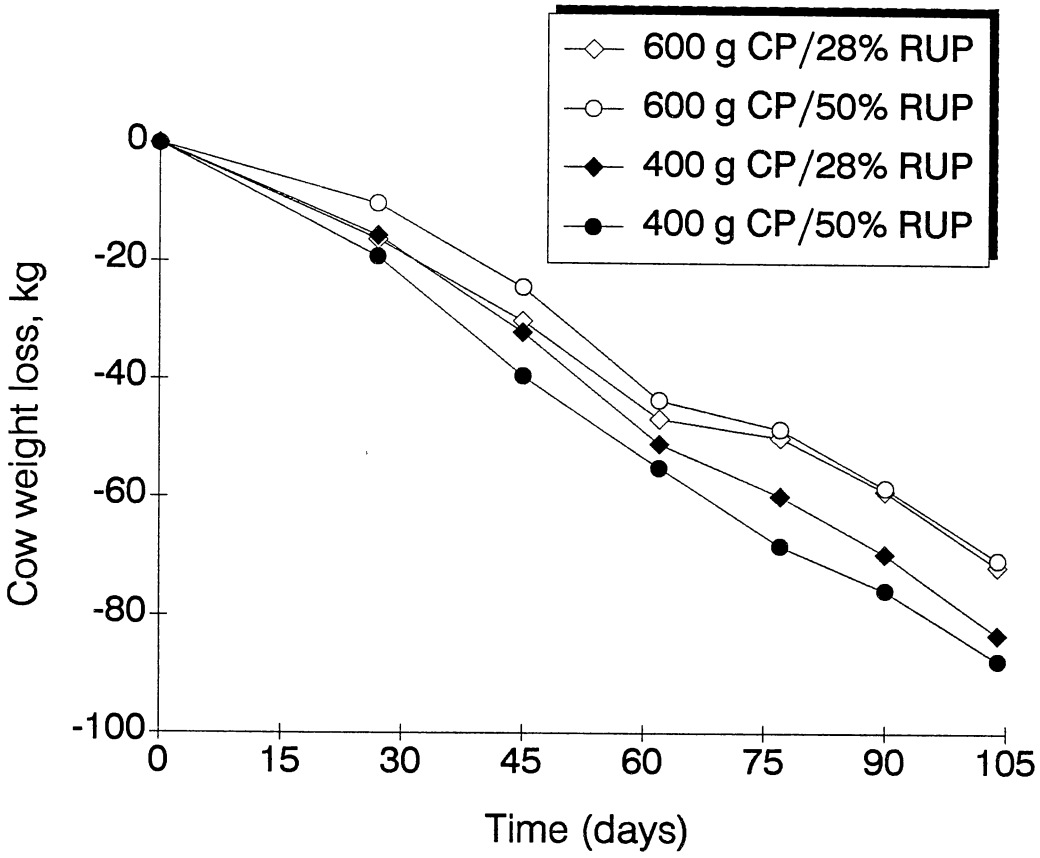

Figure 12. Cow weight loss in response to supplemental level of protein and proportion of ruminally undegraded protein (RUP).

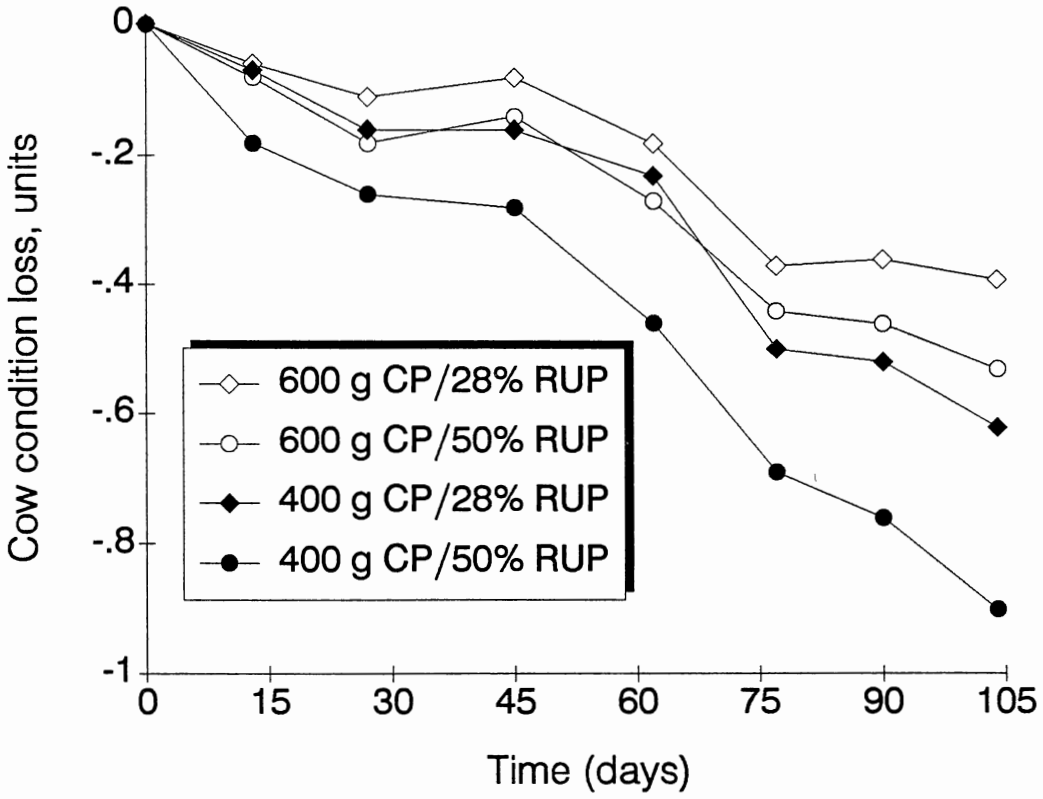

Figure 13. Cow condition loss in response to supplemental level of protein and proportion of ruminally undegraded protein (RUP).

TABLE 26. MILK PRODUCTION OF COWS AND BODY WEIGHT GAIN OF CALVES GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Milk, kg/d	4.01[c]	4.18[c]	4.52[d]	4.20[c]	.132	.04	.55	.06
Calf weight, kg								
Initial	99.1	100.4	96.3	97.3	.88	.0007	.17	.86
Final	137.0[e]	138.4[e]	144.0[f]	139.2[e]	1.24	.002	.17	.01
Change	38.0[g]	38.0[g]	47.7[h]	41.9[i]	.66	.0001	.0001	.0001
Rate, kg/d	.38	.37	.47	.41	.018	.0004	.08	.18

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c,d]Means within a row lacking a common superscript differ (P<.05).

[e,f]Means within a row lacking a common superscript differ (P<.01).

[g,h,i]Means within a row lacking a common superscript letter differ (P<.0001).

showed an interaction (P<.0001) for supplemental CP level by proportion of RUP (Table 26) although expressed on a rate of gain basis no interaction was detected. Calves suckling cows fed the 600 g CP/28% RUP supplement gained the most (P<.0001) weight. Calves whose dams received the 600 g CP/50% RUP supplement gained more weight (P=.01) than calves from dams supplemented with 400 g CP supplements. No difference (P>.10) in weight gain was detected between proportions of RUP for calves from dams receiving 400 g CP supplements. Calves whose dams received 400 g CP/50% RUP supplements, however, gained more weight through d 77 than calves from cows fed 400 g CP/28% RUP supplements (Figure 14). This response suggests that milk production was increased (P=.36) above 400 g CP/28% RUP supplements, which would substantiate the greater loss of body condition for 400 g CP/50% RUP supplements.

After day 0, higher levels of supplemental CP increased (P<.10) milk protein concentrations (Table 27). The lactating beef cows utilized in this study were in negative energy balance as illustrated by body weight and condition losses. Consequently, higher levels of supplemental protein probably increased milk protein concentration due to an improvement in N status. Milk fat concentration was not consistently altered by supplemental level of CP or proportion of RUP (Table 27). Scott (1992b) reported that ruminal acetate concentration was increased (P=.09) for 50% RUP supplements; however, milk fat concentrations were not altered by proportion of supplemental RUP in this study.

Ruminal ammonia-N. Ruminal ammonia-N concentrations obtained 2 to 4 h postsupplementation were higher (P=.0003) for supplements providing 600 g CP (Table 28) although an interaction with RUP was detected on day 27. Within level of CP, 50% RUP supplements generally produced lower (P<.05) concentrations of ruminal ammonia-N (Table 28). Supplements in this study supplied three different levels of supplemental ruminally degraded protein (RDP). The 400 g CP/28% RUP and 600 g CP/50% RUP supplements supplied the same quantity of RDP. Although RDP supply was formulated to be equal within those supplements, ruminal ammonia-N concentrations were greater for the 600 g CP/50% RUP supplement. Scott (1992b) reported similar ruminal ammonia-N patterns when similar supplements were fed to cows

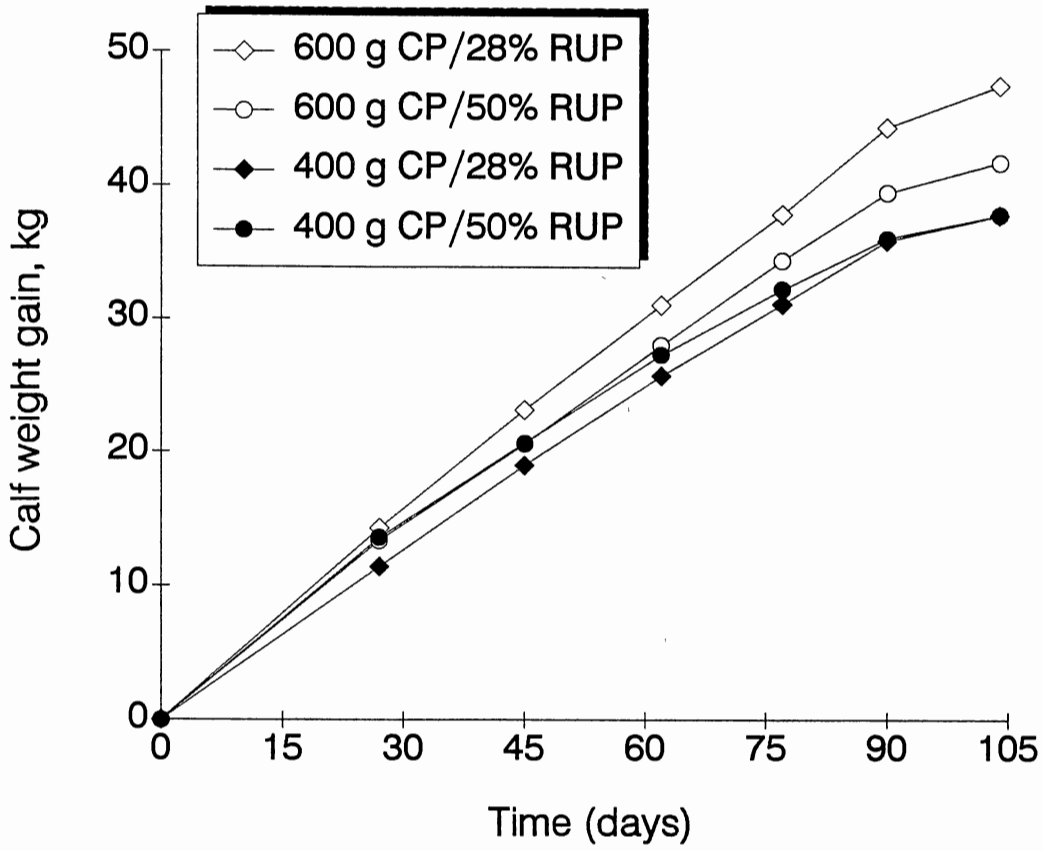

Figure 14. Calf weight gain in response to supplemental level of protein and proportion of ruminally undegraded protein (RUP).

TABLE 27. CONCENTRATIONS OF PROTEIN AND FAT IN MILK FROM COWS GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Day	---------- Milk protein, % ----------							
0	3.13	3.27	3.24	3.30	.088	.42	.25	.63
27	3.25	3.23	3.45	3.57	.098	.09	.56	.69
62	3.31	3.27	3.40	3.51	.098	.08	.69	.43
90	3.32	3.22	3.40	3.52	.085	.02	.88	.21
104	3.33	3.20	3.46	3.45	.083	.02	.39	.52
Day	---------- Milk fat, % ----------							
0	2.46	3.34	2.40	3.43	.461	.98	.04	.86
27	2.91	2.73	2.63	3.00	.406	.99	.82	.49
62	2.92	2.20	2.62	2.49	.271	.99	.12	.27
90	1.64	1.78	1.81	1.94	.265	.80	.89	.71
104	2.70[c]	2.01[cd]	1.88[d]	2.28[cd]	.266	.28	.58	.05

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c,d]Means within a row lacking a common superscript letter differ (P<.05).

TABLE 28. RUMINAL AMMONIA-N CONCENTRATIONS IN COWS GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Day[c]	---------- ruminal ammonia-N, mg/dL[d] ----------							
0	4.38	3.67	4.44	4.04	.856	.79	.50	.85
27	3.19[d]	1.31[e]	7.00[f]	3.48[d]	.490	.0001	.0001	.0001
62	3.09	2.42	6.24	4.21	.584	.0001	.03	.24
90	2.49[e]	1.64[e]	6.70[f]	4.17[g]	.510	.0001	.003	.10
104	3.37	2.12	6.36	4.17	.657	.0003	.01	.45

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c]Day of trial by concentration of ruminal ammonia interaction ($P<.05$).

[d]Ruminal fluid collected 2 to 4 hours postsupplementation.

[e,f,g]Means within a row lacking a common superscript letter differ ($P<.05$).

in confinement. Within RUP proportions, ruminal ammonia-N increased because the higher protein supplements increased the supply of RDP (Scott, 1992b).

In summary, providing 200 g more supplemental protein to lactating beef cows grazing low protein range grass decreased loss of body weight and condition and increased calf weight gains. Scott (1992b) reported that higher proportions of supplemental RUP did not alter digestible OM intake or duodenal N flow in dry beef cows fed supplements formulated similarly and fed at the same rate. Although differences were relatively small and cow body weight was not altered, lactating cows fed 400 g CP/50% RUP supplements lost the most body condition. Furthermore, 50% RUP supplements limited the weight gain of calves whose dams received 600 g CP. In this study, physiological effects of lactation, environmental temperatures and increased energy demands of grazing probably influenced digestive function, metabolism and subsequent performance of cows relative to data reported by Scott (1992b) who used dry, barren cows housed in an environmentally controlled building. These data suggest that increasing the proportion of RUP in range supplements does not improve the productivity of lactating beef cows. In contrast, Hibberd et al. (1988) reported that replacement of 22% of the supplemental RDP fraction with RUP increased cow and calf productivity. In this study, replacing 22% of the RDP with RUP was not beneficial. Finally, supplements that incorporate high concentrations of RUP are more expensive and cannot be economically justified with concflicting performance data.

Implications

Digestible fiber supplements that provided an additional 200 g CP (600 g CP vs 400 g CP) decreased body weight and condition loss of lactating beef cows grazing dormant forage and increased weight gain of their calves. Manipulating the supplemental RDP to RUP ratio, however, did not improve performance. Therefore, the inclusion of RUP feeds into supplements for lactating beef cows grazing low quality forage cannot be justified at current feedstuff prices. These data both agree and conflict with results from other studies; further research is required to resolve this discrepency.

CHAPTER VI

SUMMARY AND CONCLUSIONS

RDP study. Utilization of low quality forage diets was limited due to low N content and poor digestibility. Supplementation increased microbial N synthesis and duodenal N flow by an average of 143% and 167%, respectively. Thus ruminally degraded protein appears to be the primary factor that limits N status of cattle consuming low quality forage diets. Supplementation with ruminally degraded protein increased digestible OM intake by an average of 138%. This study illustrates the powerful impact that supplemental RDP exerts on the intake and utilization of low quality grass hay. An increase in ruminal ammonia-N concentration enhanced fermentability and stimulated microbial activity. The quantity of RDP required to maximize hay intake was less than the quantity of RDP required to maximize microbial protein synthesis. Hence, fermentability of the forage probably placed an upper limit on microbial growth (Bergen et al., 1982).

Hay intake was maximized with 428 g RDP; higher amounts depressed hay intake. The cause for this decrease in hay intake is unclear. The surplus RDP presumably was utilized as an energy substrate by microbes and may have upset the balance between supplemental protein and energy. Digestible OM intake for the 428 g supplement was 5.25 kg versus an estimated TDN requirement of 4.59 kg (NRC, 1984). Consequently, above 428 g RDP, energy requirements probably were satisfied thus causing hay intake to decline. In this study, small intestinal protein absorption was greatest with 544 g RDP. Greater flow of specific amino acids to duodenum may have suppressed intake via feedback inhibition on the brain (Forbes, 1988).

The quantity of supplemental protein required to maximize forage utilization was 580 g (428 g RDP). Total daily intake of CP was 912.5 g (hay plus supplement); this is 43% higher than the CP requirement for a 545 kg gestating beef cow and only 4.4% below the cow's requirement

during lactation (NRC, 1984). Consequently, protein supplementation of gestating beef cows based on NRC requirements may be inadequate to maximize intake and utilization of low quality forage. Because protein is an expensive component of supplementation programs, producers seldom will feed too much protein; nevertheless, NRC requirements for protein intake were suboptimal for utilization of low quality forage. The quantity of supplemental protein required to maximize energy intake was 88.6 g RDP/kg digestible OM intake. This value might be used to predict the RDP requirement of cattle consuming low quality forage. If a cow requires 5 kg of TDN, about .6 kg/d of soybean meal would be required with a forage containing below 6% CP. This value may prove useful to estimate the need for supplemental ruminally degraded protein for cattle consuming low quality forage diets.

RUP studies. Supplements containing 50% RUP supplied half as much RDP as 28% RUP supplements, therefore ammonia-N should have limited ruminal fermentation of 50% RUP supplemented diets (Scott, 1992a). In contrast, in situ rate of hay OM degradation and true ruminal OM disappearance were independent of the proportion of supplemental RUP despite lower ruminal ammonia-N concentrations for 50% RUP supplements. Postruminal digestion of CP and recycling of N to the rumen may explain this discrepancy. Within 28% RUP supplements, microbial protein synthesis was limited by RDP supply. The ratio of RDP to digestible OM intake was lower than requirements estimated by Scott (1992a); therefore, additional RDP may have stimulated ruminal fermentation and increased digestible OM intake. Although ruminal ammonia-N and in situ degradation of N confirmed that ruminal degradation of 50% RUP supplements was less than for 28% RUP supplements, composition of duodenal N flow suggests that NRC (1985) values underestimated ruminal degradability, probably due to very long ruminal retention times with low quality forage diets. Although both levels of RUP increased digestible OM intake and duodenal N flow, their modes of action may have been different. With 28% RUP supplements, increased intake was a function of passage. In contrast, cows fed 50% RUP supplements had slower passage rates (%/h) but similar intake and fecal output. Therefore, slower passage must have been offset via increased fill. Although duodenal NAN was not different between proportions

of RUP, the composition of duodenal amino acid flow should have differed. Our study was not designed to critically evaluate ruminal fill. Rumens were evacuated only once per cow per treatment. Personal observation indicates that cows do not eat the same quantity every day (i.e., 1.8% BW every day). To account for diurnal and daily variation in intake, ruminal volume needs to be several times.

Increasing the quantity of supplemental CP from 400 to 600 g/day increased intake of CP and digestible OM by 234 and 607 g/day, respectively. Thereby, milk production potentially could be increased by 2.5 kg and 3.9 kg due to greater intake of CP and TDN, respectively (NRC, 1984). In the production study, these additional nutrients reduced losses in body weight and condition for fall calving cows grazing dormant native grass pastures. Substitution of approximately one-fourth of supplemental RDP with RUP, did not depress the utilization of hay, digestible OM intake or duodenal N flow of dry cows in confinement. However, performance data does not concur when similar supplements were fed to lactating cows grazing low quality pasture. Cows receiving low CP-high RUP supplements lost the most body condition while calves whose dams were fed high CP-low RUP supplements gained the most weight. These data implied that substituting a portion of RUP for RDP in supplements for low quality forage would not reduce total supplemental protein requirements nor benefit cow herd productivity. Conflicting performance data strongly suggests that further research is required.

In conclusion, manipulating ratios of supplemental RDP and RUP should enhance our understanding of the ruminal dynamics of cattle consuming low quality forage diets. Feeding value of low quality forage is not always accurately assessed by routine procedures. Further research is needed to pinpoint RDP and RUP requirements at various concentrations of hay N and hay digestibility.

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APPENDIX

ACCESSORY DATA TABLES

TABLE 29. HAY AND TOTAL OM INTAKE AND DIGESTION IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
Intake										
Hay OM, kg/d	3.8	5.3	6.4	7.5	7.2	.38	.0001	.002	.08	.38
Hay OM, % BW	.79	1.03	1.26	1.45	1.41	.077	.0001	.002	.10	.59
Total OM, kg/d	3.9	7.0	8.0	9.1	8.7	.38	.0001	.003	.08	.38
Total OM, % BW	.81	1.36	1.58	1.76	1.71	.076	.0001	.004	.10	.63
Digestible OM, kg/d	1.8	3.8	4.5	5.2	5.1	.21	.0001	.0004	.05	.33
Digestible OM, % BW	.38	.74	.89	1.01	1.00	.039	.0001	.0002	.06	.53
Digestibility, % of intake										
Hay OM	46.3	46.6	49.3	52.2	51.9	1.50	.05	.02	.36	.62
Total OM	46.6	54.9	56.5	58.1	58.6	1.33	.0001	.05	.71	.86

[a]Control vs average of supplemental treatments.
[b]Linear response to increased level of ruminally degraded protein (control omitted).
[c]Quadratic response to increased level of ruminally degraded protein (control omitted).
[d]Cubic response to increased level of ruminally degraded protein (control omitted).

TABLE 30. RUMINAL AMMONIA-N (mg/dL) IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Hour postsupplementation	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
0	.31	.17	1.96	2.95	3.36	.544	.004	.0001	.21	.93
2	.40	1.42	3.89	5.00	8.46	.544	.0001	.0001	.37	.13
4	.26	.21	1.88	3.56	6.66	.544	.0001	.0001	.19	.56
6	.24	.15	.99	1.88	4.87	.544	.005	.0001	.05	.40
9	.28	.15	1.02	2.08	3.51	.544	.02	.0001	.60	.95
12	.29	.11	.79	2.81	2.49	.544	.04	.0002	.36	.13
18	.21	.12	1.29	3.35	3.64	.544	.002	.0001	.42	.27
24	.51	.29	1.98	3.78	3.81	.544	.002	.0001	.13	.44

[a]Control vs average of supplemental treatments.

[b]Linear response to increased level of ruminally degraded protein (control omitted).

[c]Quadratic response to increased level of ruminally degraded protein (control omitted).

[d]Cubic response to increased level of ruminally degraded protein (control omitted).

TABLE 31. RUMINAL pH IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Hour postsupplementation	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
0	6.55	6.49	6.45	6.35	6.48	.042	.03	.45	.04	.10
2	6.51	6.31	6.33	6.27	6.28	.042	.0001	.35	.87	.39
4	6.55	6.33	6.40	6.32	6.38	.042	.0001	.72	.94	.13
6	6.50	6.32	6.29	6.16	6.28	.042	.001	.15	.08	.06
9	6.45	6.38	6.32	6.17	6.30	.042	.001	.04	.03	.05
12	6.47	6.39	6.35	6.12	6.23	.042	.0001	.0001	.09	.005
18	6.51	6.44	6.38	6.34	6.41	.042	.01	.56	.11	.58
24	6.59	6.54	6.48	6.39	6.42	.042	.005	.02	.32	.46

[a]Control vs average of supplemental treatments.

[b]Linear response to increased level of ruminally degraded protein (control omitted).

[c]Quadratic response to increased level of ruminally degraded protein (control omitted).

[d]Cubic response to increased level of ruminally degraded protein (control omitted).

TABLE 32. DUODENAL AND ILEAL pH IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY INCREMENTAL LEVELS OF SUPPLEMENTAL RUMINALLY DEGRADED PROTEIN

		Ruminally degraded protein, g/d					Probability			
Item	Control	175	294	428	544	SE	Control[a]	Linear[b]	Quad[c]	Cubic[d]
pH										
Duodenal	2.48	2.31	2.33	2.31	2.44	.045	.009	.06	.22	.35
Ileal	7.84	7.82	7.85	7.83	7.86	.019	.81	.33	.86	.27

[a]Control vs average of supplemental treatments.

[b]Linear response to increased level of ruminally degraded protein (control omitted).

[c]Quadratic response to increased level of ruminally degraded protein (control omitted).

[d]Cubic response to increased level of ruminally degraded protein (control omitted).

TABLE 33. RUMINAL AMMONIA-N IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Hour postsupplementation	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
0	-.05	.86	.44	2.17	1.30	.513	.009	.01	.11	.52
2	-.33	4.23	1.43	7.68	5.05	.513	.0001	.0004	.004	.90
4	-.12	1.71	.44	6.17	3.23	.513	.03	.006	.09	.44
6	-.04	.99	.25	3.29	1.93	.513	.09	.03	.25	.71
9	-.02	.66	.27	2.32	1.60	.513	.06	.02	.35	.77
12	.00	.44	.11	1.64	.75	.513	.05	.01	.08	.36
18	-.02	.51	.16	1.92	.78	.513	.01	.003	.02	.16
24	-.01	1.12	.59	2.32	.80	.513	.008	.06	.02	.16

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

TABLE 34. RUMINAL pH IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Hour postsupplementation	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
0	6.68	6.40	6.48	6.39	6.46	.041	.006	.86	.31	.95
2	6.60	6.27	6.39	6.31	6.37	.041	.001	.93	.15	.57
4	6.57	6.26	6.41	6.26	6.41	.041	.004	.98	.03	.98
6	6.57	6.18	6.30	6.19	6.27	.041	.0001	.85	.08	.67
9	6.60	6.23	6.36	6.24	6.32	.041	.001	.88	.15	.74
12	6.57	6.22	6.35	6.19	6.26	.041	.001	.34	.19	.66
18	6.65	6.29	6.43	6.14	6.35	.041	.0002	.07	.02	.57
24	6.65	6.42	6.48	6.34	6.42	.041	.005	.28	.31	.88

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

TABLE 35. DUODENAL AND ILEAL pH IN BEEF COWS FED LOW QUALITY NATIVE GRASS HAY AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

		400 g CP		600 g CP			Probability[a]			
Item	Control	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	Control	CP	RUP	CP x RUP
pH										
Duodenal	2.20	2.38	2.33	2.38	2.39	.034	.0001	.19	.51	.35
Ileal	7.79	7.76	7.71	7.78	7.72	.016	.002	.13	.0001	.69

[a]Probability levels for: Control vs all supplements; CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

TABLE 36. BODY WEIGHT AND WEIGHT CHANGE OF BEEF COWS GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Body weight, kg								
Day								
0	492.5	478.8	482.4	482.0	9.43	.7113	.4576	.4820
27	476.9	459.7	466.2	471.8	9.05	.9398	.5212	.2123
45	460.5	439.5	452.4	457.8	8.28	.5419	.3504	.1150
62	441.7	424.0	435.9	438.6	8.47	.6018	.3784	.2291
77	432.8	410.8	432.6	433.8	8.42	.1785	.2211	.1732
90	423.2	403.3	423.5	423.7	8.19	.2087	.2340	.2231
104	409.4	391.2	410.9	411.7	7.92	.1690	.2743	.2353
Weight change, kg								
Day								
27	-15.6[cd]	-19.1[c]	-16.2[cd]	-10.2[d]	2.77	.1354	.6648	.0921
45	-32.0[cd]	-39.3[c]	-30.0[cd]	-24.2[d]	3.25	.0104	.8184	.0473
62	-50.8	-54.9	-46.5	-43.3	3.78	.0395	.9022	.3425
77	-59.7	-68.0	-49.7	-48.2	4.12	.0006	.4178	.2356
90	-69.3	-75.5	-58.8	-58.2	3.85	.0006	.4713	.3800
104	-83.1	-87.6	-71.4	-70.3	4.54	.0021	.7107	.5360

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c,d]Means within a row lacking a common superscript letter differ ($P < .05$).

TABLE 37. BODY CONDITION AND CONDITION CHANGE OF BEEF COWS GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Body condition, units								
Day								
0	5.14	5.13	5.14	5.10	.067	.7882	.7171	.8924
13	5.07	4.95	5.08	5.02	.060	.5421	.1498	.6469
27	4.99	4.87	5.03	4.92	.058	.4313	.0668	.8752
45	4.99	4.84	5.05	4.96	.077	.2398	.1328	.7240
62	4.91	4.67	4.95	4.83	.084	.2302	.0329	.4417
77	4.64	4.44	4.77	4.66	.098	.0821	.1228	.6258
90	4.62	4.36	4.77	4.64	.108	.0505	.0765	.5675
104	4.52	4.23	4.74	4.57	.108	.0120	.0331	.5690
Condition change, units								
Day								
13	-.07	-.18	-.06	-.08	.043	.2012	.1384	.3900
27	-.16	-.26	-.11	-.18	.044	.1454	.0567	.6763
45	-.16	-.28	-.08	-.14	.055	.0499	.0937	.5086
62	-.23	-.46	-.18	-.27	.050	.0197	.0023	.1439
77	-.50	-.69	-.37	-.44	.068	.0065	.0616	.4035
90	-.52	-.76	-.36	-.46	.080	.0049	.0372	.3774
104	-.62	-.90	-.39	-.53	.083	.0007	.0135	.3970

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

TABLE 38. BODY WEIGHT AND WEIGHT CHANGE OF CALVES SUCKLING COWS GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Body weight, kg								
Day								
0	99.1	100.4	96.3	97.3	2.46	.2266	.6191	.9496
27	110.5	114.1	110.6	110.8	2.84	.5722	.5075	.5354
45	118.8	121.1	119.4	118.0	2.99	.7657	.7932	.4456
62	124.8	127.8	127.4	125.4	3.22	.9738	.8706	.4377
77	130.3	132.7	134.3	131.8	3.34	.6307	.9956	.4571
90	135.1	136.6	140.8	136.9	3.44	.3746	.7269	.4221
104	137.0	138.4	144.0	139.2	3.47	.2592	.6223	.3728
Weight change, kg								
Day								
27	11.4[c]	13.6[d]	14.3[d]	13.4[d]	.70	.0512	.3461	.0244
45	19.0[c]	20.7[c]	23.2[d]	20.6[c]	.91	.0238	.6290	.0221
62	25.8[c]	27.4[c]	31.1[d]	28.1[cd]	1.22	.0135	.5680	.0582
77	31.22	32.3	38.0	34.5	1.50	.0030	.4082	.1228
90	36.0	36.2	44.5	39.6	1.67	.0005	.1493	.1211
104	38.0	38.0	47.7	41.9	1.82	.0003	.1116	.1101

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c,d]Means within a row lacking a common superscript letter differ ($P < .05$).

TABLE 39. PASSAGE RATES AND FECAL OUTPUT OF COWS GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Total tract passage rate, %/h								
Liquid[c]	5.89	6.01	6.94	5.80	.566	.40	.36	.23
Particle[d]	3.11	2.85	3.02	3.26	2.933	.53	.95	.36
Fecal output[e]								
g/d	5,116[f]	4,691[g]	5,048[f]	5,120[f]	138.0	.13	.21	.04

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

[c]Liquid passage rate estimated from Co concentrations in feces. Cobalt was dosed by mixing 1 g Co·EDTA (in solution with 50 ml water) with Yb-labeled hay.

[d]Particle passage rate estimated from Yb concentrations in feces. Ytterbium labeled (1 g Yb on 250 g hay) hay was prepared by immersion and fed to cows.

[e]Fecal output estimated by feeding pelleted supplements containg 10 g chromic oxide. Fecal output was determined by the ratio of chromium in composite fecal samples and supplements.

[f,g]Means within a row lacking a common superscript letter differ ($P<.05$).

TABLE 40. CONCENTRATIONS OF LACTOSE AND NONFAT SOLIDS IN MILK FROM COWS GRAZING DORMANT NATIVE GRASS PASTURES AS AFFECTED BY SUPPLEMENTAL LEVEL OF PROTEIN AND PROPORTION OF RUMINALLY UNDEGRADED PROTEIN

	400 g CP		600 g CP			Probability[a]		
Item	28% RUP[b]	50% RUP	28% RUP	50% RUP	SE	CP	RUP	CP x RUP
Day	---------- Milk lactose, % ----------							
0	5.27	5.19	5.32	5.21	.078	.59	.21	.82
27	4.95	5.05	5.25	5.15	.206	.31	.99	.64
62	5.04	5.06	5.24	5.25	.158	.20	.92	.96
90	5.18	5.14	5.20	5.26	.088	.42	.94	.53
104	5.14	5.07	5.24	5.18	.106	.32	.55	.97
Day	---------- Milk nonfat solids, % ----------							
0	9.01	9.08	9.18	9.13	.106	.59	.21	.82
27	8.84	8.80	9.33	9.15	.190	.03	.56	.70
62	8.98	8.96	9.26	9.38	.164	.03	.74	.66
90	9.12	8.99	9.23	9.40	.010	.01	.83	.12
104	9.09	8.91	9.33	9.27	.118	.01	.31	.59

[a]Probability levels for: CP = 400 g CP vs 600 g CP; RUP = 28% RUP vs 50% RUP; CP x RUP = CP by RUP interaction.

[b]RUP = Ruminally undegraded protein.

VITA

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