The effects of substituting blood meal for soybean meal on nutritional status of gestating ewes
by Bradley Joseph Schloesser

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University

Abstract:
These studies were designed to determine the influence of substituting soybean meal (SBM) with blood meal (BM) on the nutritional status of gestating ewes fed grass hay diets, progeny wool follicle development and N presented to the duodenum. In Exp. 1, forty Targhee ewes were allotted randomly to 5 groups (n=8) and supplemented with either none (HAY); SBM; 2/3 SBM: 1/3 BM; 1/3 SBM:2/3 BM; or BM. Hay analysis on a DM basis for CP, NDF and ADF was 8.0, 64.0 and 45.7%, respectively. Soybean meal, BM or their-combinations provided 22 g of dietary CP daily. Diets were formulated to be isocaloric and isonitrogenous. Dietary treatments had no (P>.05) influence on ewe BW or body condition score changes and ewes gained an average of 7.7 kg of BW during the 84-d experiment. Wool production and ewe and lamb weights were not influenced (P>.05) by dietary treatments. Blood metabolite concentrations (total protein, albumin, blood urea N, creatinine, glucose and free fatty acids) were monitored during the experiment and differences (P<.05) were detected. However, all values were within the normal range for sheep and not of biological importance. Lamb wool follicle development was similar (P>.05) for all treatment groups, follicle density per mm2 and secondary to primary follicle ratios were 22.4 and 10.8, respectively. In Exp. 2, ruminal and duodenal cannulated wethers were arranged in a 3 X 5 Iatin square. Ruminal ammonia concentrations and microbial N as a percentage of nonammonia N presented to the duodenum were greater for HAY supplemented wethers than BM-fed wethers. However, N flow to the duodenum and available for absorption were similar (P>.05) across all treatment groups. In summary, substituted HAY CP from SBM, BM or their combinations did not enhance ewe nutritional status or progeny production. This response was due to similar quantities of N being presented to the small intestine. It appears that HAY alone satisfied ewe protein requirements during gestation.

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7-26-91
Date

Verl M. Thomas
Chairperson, Graduate Committee

Approved for the Major Department

7-26-91
Date

L C. Dayn
Head, Major Department

Approved for the College of Graduate Studies

August 9, 1991
Date

Henry J Parsons
Graduate Dean

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ABSTRACT

These studies were designed to determine the influence of substituting soybean meal (SBM) with blood meal (BM) on the nutritional status of gestating ewes fed grass hay diets, progeny wool follicle development and N presented to the duodenum. In Exp. 1, forty Targhee ewes were allotted randomly to 5 groups (n=8) and supplemented with either none (HAY); SBM; 2/3 SBM:1/3 BM; 1/3 SBM:2/3 BM; or BM. Hay analysis on a DM basis for CP, NDF and ADF was 8.0, 64.0 and 45.7%, respectively. Soybean meal, BM or their combinations provided 22 g of dietary CP daily. Diets were formulated to be isocaloric and isonitrogenous. Dietary treatments had no (P>.05) influence on ewe BW or body condition score changes and ewes gained an average of 7.7 kg of BW during the 84-d experiment. Wool production and ewe and lamb weights were not influenced (P>.05) by dietary treatments. Blood metabolite concentrations (total protein, albumin, blood urea N, creatinine, glucose and free fatty acids) were monitored during the experiment and differences (P<.05) were detected. However, all values were within the normal range for sheep and not of biological importance. Lamb wool follicle development was similar (P>.05) for all treatment groups, follicle density per mm^2 and secondary to primary follicle ratios were 22.4 and 10.8, respectively. In Exp. 2, ruminal and duodenal cannulated wethers were arranged in a 3 X 5 latin square. Ruminal ammonia concentrations and microbial N as a percentage of nonammonia N presented to the duodenum were greater for HAY supplemented wethers than BM-fed wethers. However, N flow to the duodenum and available for absorption were similar (P>.05) across all treatment groups. In summary, substituted HAY CP from SBM, BM or their combinations did not enhance ewe nutritional status or progeny production. This response was due to similar quantities of N being presented to the small intestine. It appears that HAY alone satisfied ewe protein requirements during gestation.

INTRODUCTION

Throughout Montana native range provides much of the forage base for gestating ewes during the winter because of the absence of snow cover. Early work by Van Horn et al. (1959) reported it was beneficial to supplement gestating ewes grazing southwestern Montana winter range 2 out of 3 years. Harris et al. (1989) found that pregnant ewes grazing winter range did not receive adequate protein nutrition, and protein appeared to be more limiting than energy. However, these studies did not evaluate ruminally undegraded protein (escape protein; EP).

Hoaglund (1989) reported that pregnant ewes fed straw diets supplemented with blood meal (BM) had improved N balance, ewe weight and body condition score (BCS) changes and wool growth in comparison to those fed urea (U) or soybean meal (SBM). They speculated that EP reaching the small intestine in BM ewes was the primary reason for improved metabolism and performance. Padula (1990) evaluated the influence of ruminally undegraded protein and nonstructural carbohydrates (NSC) on ewe nutritional status during mid-gestation. Their data suggested that diets containing a low concentration of NSC yielded the greatest quantity of microbial N reaching the small intestine when ewes were supplemented

with BM, in comparison to SBM or U. However, the optimum level of BM in supplements for ewes fed low quality roughages was not determined.

The research reported herein consisted of two phases. A study designed to determine the influence of substituting SBM with BM at varying levels on the nutritional status and progeny wool follicle development of ewes fed grass hay diets during mid-gestation. Phase II consisted of a dose titration metabolism trial with cannulated wethers to characterize ruminal microbial protein production, EP and microbial efficiencies of the diets fed in phase I.

LITERATURE REVIEW

Protein Metabolism In Ruminants

Proteins are the fundamental components of all structures in the organism, therefore, animal performance and production are dependent upon the amount and turnover of proteins at various sites (Riis, 1983). The total of all protein (CP) catabolism and anabolism processes is CP metabolism. The general pathways of nitrogen digestion, absorption and metabolism are depicted graphically in Figure 1 by Maynard and Loosli (1979). The dotted lines indicate routes which are used but are probably quantitatively small.

Rumen Metabolism of Dietary Protein

The most important characteristic of dietary CP the extent it is ruminally degraded (Orskov, 1982). Fermentation in the forestomach involves degradation of ingested feed and nutrients (Tamminga, 1979). Ruminal fermentation leads to a complex system of CP use in ruminants (Baldwin and Denham, 1979). Fermentation in the reticulo-rumen is responsible for the utilization of complex polysaccharides and the degradation and synthesis of protein (Asplund, 1975).

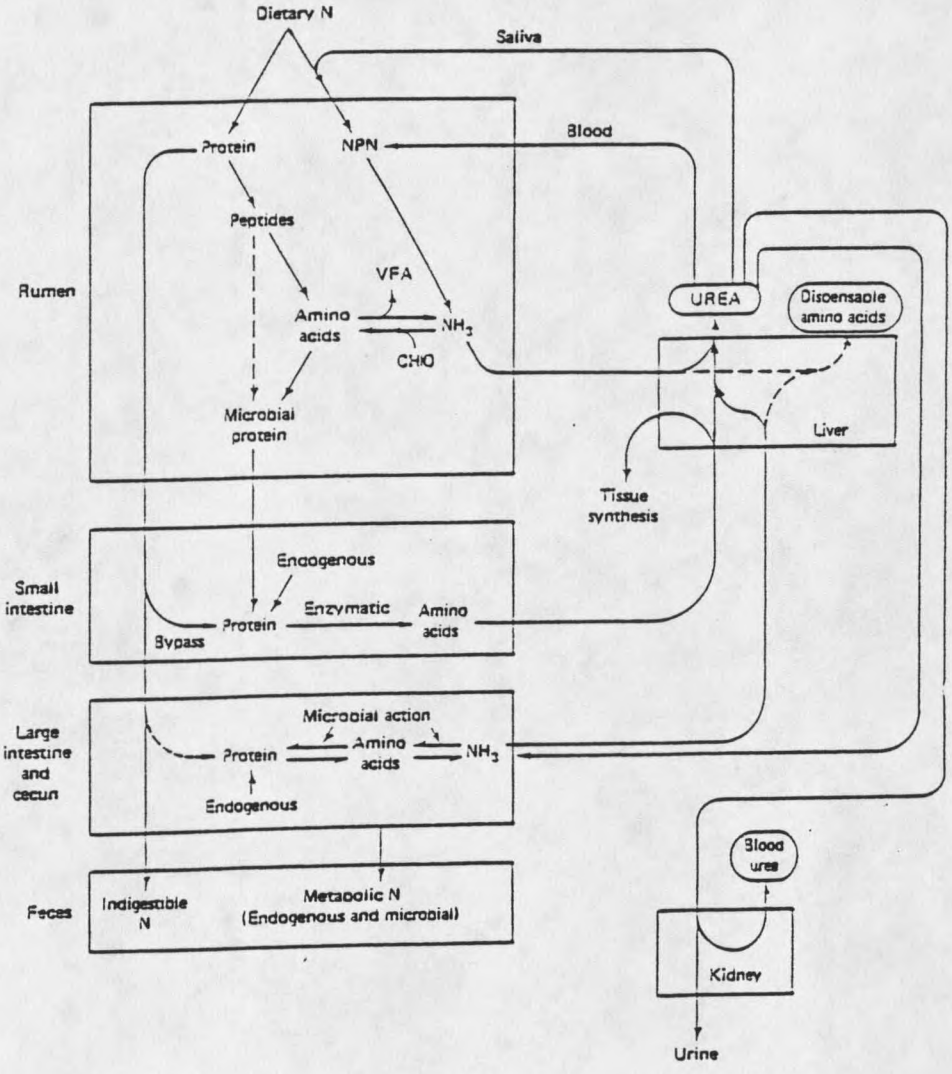

Figure 1. Pathways of digestion, absorption and metabolism of nitrogen in the ruminant.

Protein consumed by a ruminant may be degraded by both bacteria and protozoa and part of the ingested CP remains intact to pass out of the rumen. Microbial competition can have an effect on ruminal environment and CP degradation. Jouany et al. (1988) reported that protozoa not only prey on bacteria, but they also change the ruminal environment and CP metabolism.

Protein degradation involves two steps: (1) hydrolysis of the peptide bond (proteolysis) to produce peptides and amino acids (AA); and (2) deamination and degradation of AA (NRC, 1985b). Hydrolysis of peptides to AA appears to be the rate-limiting step in CP degradation (Annison et al., 1959; Lewis, 1962; Russell et al., 1983).

Proteolytic enzymes appear to be associated primarily with the bacterial cell wall, with a small amount of cell-free activity probably resulting from cell lysis (Allison, 1970). Although, Blackburn and Hobson, (1962) and Allison, (1970) reported that proteolytic activity of rumen microorganisms is not greatly altered by diet. Recent experimental results indicate that diet can have an effect on CP degradation in the rumen. Proteolysis may be altered through changes in pH (Loerch et al., 1983; Thonney and Hogue, 1985; Hussein and Jordan, 1991a) and changes in bacterial numbers or types (NRC, 1985b). Amino acids are either used for microbial growth, or degraded to ammonia and fatty acids. Amino acids are rapidly degraded in the rumen, and therefore only small quantities of free AA would be available for absorption or passage from the reticulo-rumen (NRC, 1985b).

Estimation of ruminal CP degradation is difficult because it is hard to distinguish between endogenous, microbial and dietary protein (NRC, 1985b). Protein degradation is measured by a variety of methods. In vivo measurements via a cannula in the rumen, abomasum or small intestine require the use of an indigestible marker and collection of subsamples (Zinn et al., 1980). Samples are strained, centrifuged and analyzed for indigestible markers (chromic oxide) with spectrophotometry to estimate the passage rate of digesta. By taking samples at varying locations along the gastrointestinal tract estimates can be made concerning the available protein, synthesis rate of CP and degradation of feed and microbial protein.

In situ procedures involve the use of dacron or nylon bags that are suspended in the rumen. Contained in the bags are measured forage, concentrate or supplement samples. Samples are exposed to the rumen environment, and microbes can enter and exit the bags and degrade the feedstuff within. The not degraded in the bag cannot leave the bag due to small pore size. When the time allowed for degradation has lapsed the bag and remaining contents are removed and CP disappearance is then calculated. Mehrez and Orskov (1977a) suggested the in situ technique is acceptable for CP degradability determinations.

A common in vitro method for estimating CP degradability involves incubation of a test feed with rumen fluid and a control buffer solution (McDougal, 1948). Measurement of resulting ammonia production following incubation and

fermentation gives an estimate of digestion characteristics, but does not include rate of passage estimates because it is conducted in a test tube.

Protein presented to the small intestine for digestion in ruminants is from two sources, dietary CP that escapes ruminal degradation and microbial CP synthesized by the microbial population in the rumen. Dietary CP that is not digested in the rumen has been described as bypass or escape protein (Owens and Bergen, 1983). The effective use of combinations of CP sources with complementary AA profiles to alter the quantity and profile of AA supplied to the host animal depends on satisfying the following criteria: 1) ruminal microbial CP synthesis must be maintained by including a dietary source of ruminally degraded CP or N to provide ammonia (NH_3) N and other products of CP breakdown to the microbial population and 2) complementary CP must constitute a major portion of dietary CP and be resistant to ruminal degradation yet available in the small intestine for digestion to AA (Cecava et al., 1990). Table 1 presents estimates of the percentage of ruminally undegraded protein for selected feedstuffs.

Dietary CP that escapes ruminal degradation and flows to the omasum consists of two fractions: 1) CP that resists microbial attack in the rumen; and 2) CP that evades microbial attack in the rumen and passes to the omasum without thoroughly mixing with ruminal contents. Protein flushed out of the rumen at feeding time and passing through the esophageal groove would fall into this category. The term "undegraded" protein is most suited to the first fraction, while "bypass" would be more suited to the second fraction.

Table 1. Estimates of percentage of ruminally undegraded protein in feedstuffs.

Feedstuffs	Undegraded Protein %[a]
Blood meal	82
Brewers dried grains	49
Corn gluten meal	55
Feather meal	71
Fish meal	78
Meat & bone meal	49
Rapeseed meal	28
Soybean meal	35
Sunflower meal	26

[a] Adapted from Nutrient Requirements of Dairy Cattle, NRC (1989).

Microbial Protein

Microbial flow (quantity of microbes exiting the rumen over time) must be accounted for to estimate microbial growth and efficiency (NRC, 1985b). Microbial flow is dependent on ruminal flow or turnover of ruminal contents and is important for maintenance requirements of ruminal microorganisms. Maintenance requirements vary with microbial species (Hespell and Bryant, 1979), but, maintenance cost increases with low growth rates, thus, a decrease in efficiency of energy fermented. Polan (1988) reported that external or dietary factors affect ruminal flow, such as amount, quality and length of forage, fermentation rates, processing and total feed intake.

Microbial CP leaving the rumen ultimately provides a source of AA for the host animal as the CP is digested and absorbed in the small intestine. Microbial flow from the rumen can meet 50 percent or more of the AA requirements of ruminants in various states of production (Orskov, 1982). In order for microbial CP to leave the rumen it must first be synthesized by the ruminal microbial population. Microbial access to CP seems to be the most important factor influencing CP degradation in the rumen (NRC, 1985b).

The organisms comprising the microbial population in the rumen have been described by Hungate (1966) and Russell and Hespell (1981). In general the microflora is comprised of bacteria, protozoa (Hungate, 1966), spirochetes (Paster and Canale-Parola, 1982) and fungi (Bauchop, 1981).

Substrates required for microbial synthesis include energy, NH_3, AA, vitamins and minerals (Hungate, 1966). Conflicting ruminal NH_3 concentration required for optimal microbial growth have been reported. Microbial function was not limited by ruminal NH_3 concentrations in a continuous culture until levels fell below 3 to 5 mg/100 ml (Satter and Roffler, 1975; Hogan, 1975). However, Lodman et al. (1990) reported no differences in range cow productivity when ruminal NH_3 concentrations ranged from 1.0 to 2.2 mg/dl.

Using automatic continuous feeders to provide whole barley fortified with urea to sheep, Mehrez et al. (1977b) reported that ruminal NH_3 concentrations less than 20 to 25 mg/100 ml limited microbial growth. Hume et al. (1970) observed maximum microbial growth when ruminal NH_3 concentration reached

approximately 9 mg/100 ml. In contrast, Miller (1973) working with dairy cows, found a considerably higher value of approximately 29 mg/100 ml required for maximum microbial growth. Results of a more recent in vivo study (Okorie et al., 1977) indicated that maximal protein synthesis was achieved when the rumen NH_3 concentration reached 5 mg/100 ml; an observation consistent with the in vitro results of Satter and Slyter (1974).

Stern and Hoover (1979) reviewed numerous studies conducted to determine microbial CP synthesis. Production was expressed as grams CP synthesized per 100 g organic matter digested (OMD) or per 100 g dry matter digested (DMD) in the rumen. Estimates of microbial CP synthesis reported in sheep were 13.3 g N/100 g OMD when fed a semipurified diet + urea (Hume et al., 1970), 12.5 g N/100 g DMD when consuming hay (Mathison and Milligan, 1971) and 9.9 g N/100 g DMD for cattle fed grass + soybean meal (Kropp et al, 1977). Stern and Hoover (1979), also reported that microbial CP synthesis required an adequate supply of N to allow for optimal fermentation to occur and that energy availability must then be balanced with N concentration for maximal efficiency of microbial growth. In several studies (McCarthy et al., 1989; Rooke and Armstrong, 1989; Cecava et al., 1990) feeding CP sources susceptible to ruminal degradation increased microbial CP efficiency and microbial N flow to the small intestine compared with CP more resistant to ruminal degradation.

Efficiency of microbial growth and yield are affected by the quantity of organic matter (OM) fermented in the rumen (Rohr et al., 1986). Ruminal

fermentability of OM can be influenced by altering the forage:concentrate ratio of the diet (Sniffen and Robinson, 1987). They reported that maximum bacterial yield is achieved at about 70% forage in diets, with reductions in yield at forage levels lower.

Decreasing ruminal degradation of dietary CP does not always increase production. Polan (1988) reported CP sources less degradable than SBM, when fed as the major N source, reduced microbial growth. Because EP may be poorly digested postruminally, the balance of AA available for absorption from the small intestine may be poor (Young et al., 1981; Owens and Bergen, 1983). Conversely, if microbial CP is the only CP reaching the small intestine, animal production may not be maximal (Satter et al., 1977). Presentation to the small intestine of a mixture of microbial and complementary dietary CP is desired (NRC, 1985a).

Small Intestine Metabolism of Nitrogen

Amino acids available for absorption from the small intestine are supplied by microbial and/or escape protein. Ruminants have the same requirements for AA as nonruminants (Black et al., 1957; Downes, 1961). In ruminants however, the relationship of dietary AA supply with tissue requirements has been difficult to define because of the intervention of the CP digestive and synthetic functions in the rumen (NRC, 1985a). Also, AA requirements are difficult to quantify because of the variability in requirements for various production functions.

As discussed in other sections, factors that affect microbial CP production and ruminal degradation of dietary CP can modify N supply presented to the small

intestine. Oldham and Tamminga, (1980) reported that in sheep consuming either concentrate or forage diets, duodenal N flow ranged between 10.5 and 12.5 g nonammonia nitrogen (NAN) per Mcal metabolizable energy (ME) consumed; illustrating energy consumption is a major determinant of the quantity of N presented to the small intestine.

The quantity of N exiting the abomasum can range from 30 to 100% microbial protein and 0 to 70% undegraded dietary protein (Smith, 1975). The chemical composition of intestinal N was described by Oldham and Tamminga (1980) as essential AA (EAA), 35%; nonessential AA (NEAA), 30%; amides, 4%; nucleic acids, 11%; NH_3, 6%; and an unknown fraction, 14 percent. Amino acid composition of intake CP (EAA:NEAA ratio) can influence the balance of AA available for absorption.

The uptake of AA occurs in the sheep jejunum and ileum against a concentration gradient requiring metabolic energy (Johns and Bergen, 1973). The highest rate of AA disappearance in situ from the digesta in the small intestine occurs in the mid-jejunum (Ben-Ghedalia et al., 1974). Apparent absorption of NAN and AA between the duodenum and ileum can be used to estimate disappearance rates. Fraser et al. (1990) reported N retention will increase in response to increasing absorbed AA supply from the intestines at levels equal to the protein N lost in passage of digesta across the rumen. Small intestine absorption rates are 65% and 70% for NAN and AA respectively, of the intake N presented to the duodenum (NRC, 1985b). Tamminga (1980) reported that

apparent absorption of total N is usually 5% lower than that of amino acids.

Calculation of true N absorption requires correction for the endogenous N that is not reabsorbed from the small intestine. Swanson (1982) identified endogenous CP entering the small intestine in the form of enzymes, bile, mucus, serum albumin, lymph, epithelial cells, and other degradable products from the lining of the gastrointestinal tract. Nolan (1975) characterized the input of NAN (g/d) to the small intestine of sheep as undegraded intake protein, 6.5; bacterial CP, 10.3; and intestinal secretions, 17.0. Endogenous losses for sheep were 0.10 of the N supply to the proximal duodenum (Lu et al., 1981; Merchen and Satter, 1983).

Tissue Metabolism of Nitrogen

A substantial part of most AA absorbed from the small intestine are apparently metabolized in the absorption process (Tamminga and Oldham, 1980). There appears to be no preference for either EAA or NEAA absorbed from sheep intestine (Tagari and Bergman, 1978).

Individual tissue requirements for essential nutrients may differ from those of the whole body (Harris et al., 1990). For example, if EAA composition of each tissue in the sheep is compared with AA available from ruminal microbial CP then, the limiting AA for growth of the carcass is histidine while that for skin and wool are the sulfur containing AA, cystine and methionine (Storm and Orskov, 1983). If one tissue has a significant protein turnover and consumes large amounts of a single AA then the remaining AA flux may be imbalanced for other tissues and low

efficiencies for production result, plus high AA oxidation (Harris et al., 1990).

Sheep demands of skin products for the S-amino acids (combined with the fractional CP synthesis rate of 10-20% for skin; Attaix et al. 1988) probably create the greatest potential for such imbalances for other body components. Preston and Leng (1987) listed AA as the second-limiting nutrient behind energy for puberty, conception, pregnancy and lactation. Whitelaw et al. (1986) working with lactating dairy cows identified methionine to be the limiting AA for milk production. Thus, AA are identified as the first-limiting nutrient ahead of energy.

Tissue consumption of AA is in a constant state of flux. For example, as energy and nitrogen intakes decrease from above maintenance to below maintenance, there is an adaptive decrease in rates of both whole body CP synthesis and degradation (Harris et al., 1990).

The ability to make adaptive responses in whole body CP metabolism is dependant on the rapid fluxes of CP during synthesis and degradation relative to the flux into CP gain or loss (Harris et al., 1990). Pregnancy and lactation may result in increased turnover of muscle CP but the major factor responsible for loss of muscle CP in pregnancy seems to be an increased rate of degradation (Vincent and Lindsay, 1985).

Growth, development and metabolism are influenced by metabolic hormones. Protein and polypeptide hormones regulate cell function by binding to a cell-membrane-specific receptor that controls the activity of the enzyme, adenylate cyclase, which catalyzes the conversion of ATP to cAMP and

pyrophosphate (Reeves, 1987). For example, Garlick and Grant (1988) enhanced the responsiveness of muscle CP synthesis to insulin by providing the EAA leucine. These implications suggest that AA signal hormones in the area of CP metabolism.

Response of Pregnant Ewes to Supplemental Protein

Protein Requirements

Ewe nutritional requirements, as for all classes of livestock, have typically separated maintenance from production. One phase of the ewe's production cycle is gestation. This is a time during the ewe's life when proper nutrition is critical to the overall success of her reproductive performance (Botkin et al., 1988).

Protein and energy requirements (Table 2) increase during the flushing period, then decline during the first 15 weeks of gestation. During the third and fourth weeks of gestation (implantation stage), there is a strengthening of the bond between the embryo cotyledons and the maternal caruncles, and adequate nutrition will facilitate a strong bond (Robinson, 1983). Many of the nutritionally provoked deaths occurring between days 15 and 30 after mating arise from low nutrient intake and poor condition following mating (Robinson, 1983). Doney and Gunn (1981) reported providing a constant supply of nutrients to the gestating ewe and the developing ova or fetus may minimize decreases in reproductive performance.