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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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).

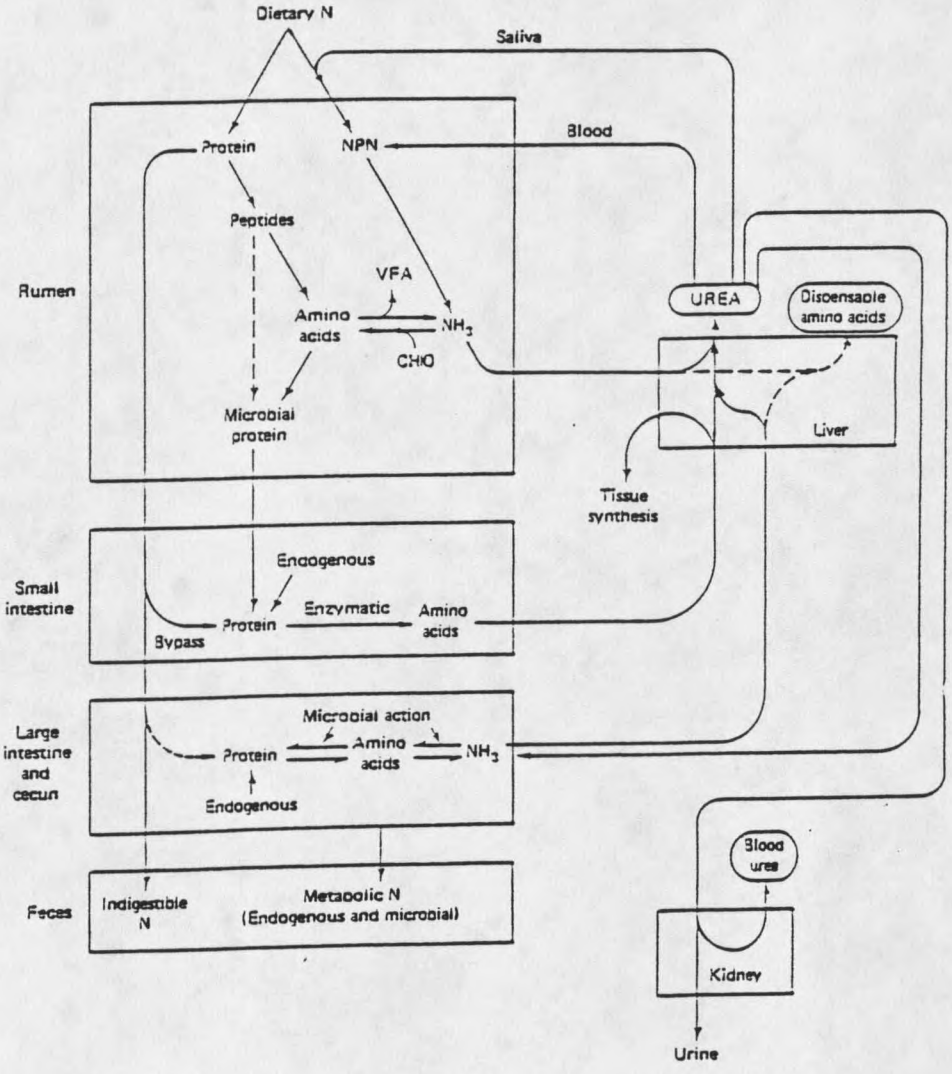


Figure 1. Pathways of digestion, absorption and metabolism of nitrogen in the ruminant.

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.

Table 2. Ewe nutrient requirements[a]

Physiological state of ewe	CP, g/d	ME, Mcal/kg
Maintenance	113	2.4
Flushing	164	3.8
1st 15 wks gestation	130	2.8
Last 4 wks gestation		
130-150% lambing rate	193	3.8
180-225% lambing rate	214	4.4
Lactation suckling twins, 1st 6-8 wks.	420	6.6
Lactation suckling twins, last 4-6 wks.	334	5.9

Protein and ME requirements of a 70 kg ewe.
Adapted from Nutrient Requirements of Sheep, NRC (1985a).

Recently, Kelly et al. (1989) found that moderate to severe undernutrition of ewes bearing twins during gestation induced significant levels of fetal mortality. They also reported that 9 of 93 Merino ewes bearing twins and fed below maintenance from d 30 of gestation to parturition lost 1 or both fetuses between d 30 and 95 of gestation, and a further 3 of 66 remaining ewes had fetal losses between d 95 and 140 of gestation.

Thomas et al. (1989) reported that ewes supplemented with grain on alternate days while grazing winter range gained less weight and had less lambs born per ewe exposed to the ram. They speculated that lower reproductive rate in those fed on alternate days may be related to reproductive wastage.

It is generally assumed that ewes in good body condition at mating can lose

some weight in the 2nd and 3rd months of gestation (Wallace, 1948). However, during the last 4 weeks of the gestation CP requirements increase (Table 2) and are dependent on ewe demands in terms of expected lambing rate. Proper feeding in late gestation can offset some of the effects of undernourishment in mid-gestation and reasonable lamb birth weights can be obtained, but postpartum ewe weight and body condition may be depleted (Orr and Treacher, 1990b).

The rapid increases in the gravid uterine weight (Robinson et al., 1977) close to lambing, may be a contributing factor to the observation of lower feed intakes during weeks 16 to 20 when fetal growth and development increases rapidly (Orr and Treacher, 1984, 1989, 1990a). Forbes (1968) measured uterine volumes of sheep at various stages of gestation and estimated the volumes of the reticulo-rumen contents, intestinal contents and abdominal fat. He reported uterine volume increased steadily as gestation progressed and rumen volume was not depressed until the last 5 weeks of gestation. This is in contrast to mid-gestation when the differences in fetal demand are less and rumen volume is not restricted by uterine development and conceptus products.

At parturition CP requirement increases dramatically to accommodate the demands of lactation, however in some instances it cannot be accommodated. A ewe suckling twins during the first 6-8 weeks of lactation experiences the greatest CP demand of any physiological state (Table 2). If body energy reserves are sufficient and CP supplementation is adequate, milk production yields can meet demands.

Prevention of a CP deficit in ewes experiencing a negative energy balance during gestation or lactation requires a supplement that will provide a source of EP (Robinson, 1987). Within the ewes body during an energy deficient state, a source of EP can fill a CP deficit. Ewe CP needs may be deficient if ME is low, causing reduced microbal CP synthesis. By supplying a source of bypass CP and sufficent ME the quantity of microbial and EP reaching the small intestine will supply enough AA to meet the requirements for fetal growth and development and the ewe's needs.

Digestion and Metabolism Trials

Using growing lambs, Merchen et al. (1987) studied the effect of supplemental CP sources, urea (U), SBM or corn gluten meal (CGM) on protein digestibility and N balance. They found protein source had no effect on nutrient digestibility or N balance. They speculated that the supply of key limiting AA may not have been increased enough with CGM to elicit a response.

Digestibility and nutrient flow determinations were made with sheep fitted with abomasal cannulae and fed 4 different supplements containing varying proportions of fish meal (FM) and silage (Cody et al., 1990). Increased flow of AA (as indicated by NAN flow) to the small intestine on the supplemented diets was due to EP from FM. These results were expected because the total CP and rumen degradable CP were well in excess of the requirements. Silage digestibility was not affected by the addition of EP due to a low feed intake level and a constant forage to supplement ratio.

Klusmeyer et al. (1990) used Holstein cows fitted with ruminal and duodenal cannulas to investigate the effects of source (SBM vs. CGM) and amount of CP (11.0% vs. 14.5%) on ruminal fermentation and passage of nutrients to the small intestine. They reported duodenal N was not affected by supplemental CP source or level of CP. Feeding CGM did not affect total N or non-NH_3 N (NAN) flows to the small intestine because flow of non-NH_3 nonmicrobial N (NANMN) was increased by an average of only 24 g/d per cow and microbial N flow was decreased by an average of 30 g/d per cow compared with feeding SBM diets. They speculated that EP comprised only a small proportion of duodenal N and failed to increase passage of N to the small intestine.

Hussein et al. (1991b) indicated that replacing SBM with fish meal (FM) in lamb diets decreased ruminal CP degradation from 72.1% to 54.5%. They reported that FM altered the nature of NAN at the duodenum by contributing more dietary N escaping ruminal microbial degradation. This agrees with Lindberg (1984), who reported that dietary CP degradation was decreased from 75% to 54% when FM replaced a highly degraded protein source (ie., rapeseed meal) in lamb diets. These results suggest that FM can be used efficiently in manipulating AA flow to the small intestine of the ruminant animal.

Production Trials

Gestation. Chittenden et al. (1935) reported that production of gestating Rambouillet ewes grazing dormant winter range supplemented with either corn, cottonseed cake or a 25% protein pellet was similar to those not supplemented.

They concluded that ewe body condition score and the type of winter range available for grazing did affect the supplementation benefits.

Darroch et al. (1950) studied the effect of supplementation of Columbia ewes prebreeding and post-breeding. They reported that flock fertility (number of lambs born) increased 9% and fleece weight increased .17 kg/d per ewe by supplementing prebreeding and during early gestation as compared to only during early gestation or only during late gestation.

Hoversland et al. (1956) reported that BW of 2-yr-old ewes increased as the CP content of winter range supplement increased from 11.2 to 19.3 to 28.5 to 36.4% crude protein. However, the group that received the 11% CP pellet produced an equal amount of lamb and wool at a lower concentrate cost. Therefore, the group supplemented at the low level was the most profitable. They also concluded that nutrient requirements for 2-yr-old ewes were higher than older ewes, above those required for maintenance, reproduction, lactation and wool production.

Clanton (1957) reported that supplementation of gestating ewes with a 25% protein pellet (barley and cottonseed meal) at a rate of .12 kg/d improved lamb weaning weights and profits over that of corn or no supplement. Van Horn et al. (1959) reported that gestating ewes grazing winter range gained or lost weight in direct proportion to the amount of barley based supplement fed. They reported that it was profitable to supplement 2 out of 3 years on winter range.

Investigating the metabolic responses of crossbred ewes to increasing

quantity of CP during late gestation, Lynch and Jackson (1983) fed diets containing either 7, 9 or 12% CP. Ewes fed the 9% CP diet had greater feed intake, which resulted in improved BW, higher daily gains and higher lamb survivability at 30 d of age compared to ewes fed 7 or 12% CP diets. They reported that ewes fed the 7% CP diet had impaired liver function.

Working with Rambouillet ewes in Texas on an accelerated and conventional production system, Huston (1983) fed a cottonseed meal (CSM), sorghum grain and molasses supplement to ewes at low, medium, high and low-high levels. He reported that supplemental feed tended to decrease forage intake and slightly increase digestible organic matter intake, suggesting a substitution effect. He concluded, that energy intake was low during the study possibly caused by a decrease in intake. In contrast, Harris et al. (1989) reported supplementing .15 kg of a barley based supplement had no effect on forage DMI, although supplemented ewes had higher N intakes than control ewes.

Hoaglund (1989) reported that supplementation of gestating ewes with SBM improved ewe nutritional status compared to U fed ewes. Additional intake of CP from BM further improved ewe nutritional status, as demonstrated by improved BW and body condition score changes, and supported by blood metabolite profiles. She reported that enhanced nutritional status was probably related to improved microbial activity and/or increased CP reaching the small intestine.

Ewes in mid-gestation fed a supplement containing BM and a low concentration of nonstructural carbohydrates had improved ewe nutritional status

compared to those fed SBM or urea. Padula (1990) reported lambs born to BM supplemented ewes had heavier weaning weights and tended to be heavier than U supplemented ewes.

Kenney and Roberts (1984) supplemented ewes fed poor quality hay, during late gestation and early lactation, with either lupin, oat or wheat grains. They reported that gestating ewes fed lupins performed very well, ewes supplemented wheat performed poorly and ewes supplemented with oats were intermediate. They speculated that ewes supplemented with lupins performed best because lupin was high in CP and ME, low hay intake may have been associated with the poor performance of ewes fed wheat. The CP analysis of the supplements were 31.2, 15.0, and 16.0%, respectively.

Lactation Gonzalez et al. (1984) fed supplements containing either low, medium or high concentrations of FM to Finn X Dorset ewes during the first week of lactation. They found CP concentration in milk increased as CP supplement level increased and ewe tissue losses of protein decreased as increasing amounts FM were supplemented. They surmised that at relatively high ME intakes there are responses in milk yield to EP supplements due to an increased incremental efficiency of protein utilization.

Loerch et al. (1985) found that ewe milk production was improved by supplementation with EP (blood and meat-bone meal). They suggested that EP sources increased the supply of limiting AA to the small intestine.

Working with lactating ewes, Sheehan and Hanrahan, (1989) compared

SBM and FM CP sources and level of CP intake (low, 247 g/d; med, 315 g/d; high, 382 g/d). No difference in lamb growth rate or milk yield due to protein type was detected. However, protein level had a significant linear effect on both milk yield and lamb growth rate. This response may have been due to the increased quantity of essential AA available for absorption from the small intestine. The lack of response when supplying EP may be attributed to the maximization of microbial protein synthesis to meet production demands in the SBM supplemented ewes. Therefore, enhanced AA availability by providing EP in the form of fish meal yielded no differences.

Ely et al. (1991), comparing diets of lactating ewes supplemented with SBM or distillers dried grains with solubles (DDGS), reported ewes consuming SBM exhibited greater DM and CP digestion rates. Replacing 100% of the SBM with DDGS in a lactation diet reduced ewe weight loss and increased milk fat percentage, but lowered milk production and subsequent daily gain of lambs, and efficiency of converting milk components to lamb gain. Therefore, complete replacement of SBM with DDGS did not provide a more desirable nutritional status for lactating ewes.

Growth Fattet et al. (1984) reported that overfat animals on straw diets supplemented with EP (FM), while losing fat, gained lean tissue so that live weight and empty body weight were maintained. Whereas, animals on a sole diet of straw, while maintaining live weight, still lost empty body weight fat and lean tissue. They suggested that the lambs have the ability to mobilize body fat and continue

protein accretion provided there are available AA for tissue accretion and fat for mobilization. Vipond et al. (1989) reported similar results in experiments designed analogously. They reported that fish meal and rumen degradability of 30% of the EP is required, because the degradable fraction contributes to CP supply by making up for the deficit of rumen degradable CP in the straw basal diet.

Using an 85% corn diet, Sun et al. (1991) supplemented lambs with 3 levels of ruminal escape protein, (REP; low, med and high), by varying the proportions of SBM, corn gluten, BM and feather meal. They reported that varying the REP level of high-corn diets did not improve performance by finishing lambs. However, it was suggested REP supplementation may have had greater effects on performance with diets lower in concentrate or total CP or containing grain with CP of higher ruminal digestibility than corn. They concluded that REP supplementation would be more appropriate nutritional management for animals consuming a roughage based diet.

Wool Follicle Development

Follicle development occurs in three stages, pre-trio, trio and post-trio (Figure 3). The pre-trio stage commences on d 35-40 of prenatal life and the surface of the skin is usually covered by day 55-60 with primary follicles (McFadden, 1967). The average duration of this stage in any region of the fetus is about fifteen days. During the trio stage commencement, small lateral follicles appear on either side of the most advanced follicles established in the pre-trio stage. Establishing these follicles usually requires fifteen days. Trio stage

development generally begins by day 75 of prenatal life, but may begin as early as day 55, and is finalized by day 90 of prenatal life. To this point only primary follicles have developed.

The post-trio stage, third and final in the follicle development processes is distinguished by the appearance of small follicles, called secondary follicles, between the central and lateral primaries which were primaries previously established in the pre-trio and trio stages, respectively. These newly developing, secondary follicles consistently occupy positions opposite the side of the primary follicle, which contain the sudoriferous and sebaceous glands, accessory components of the primary follicle (see Figure 2).

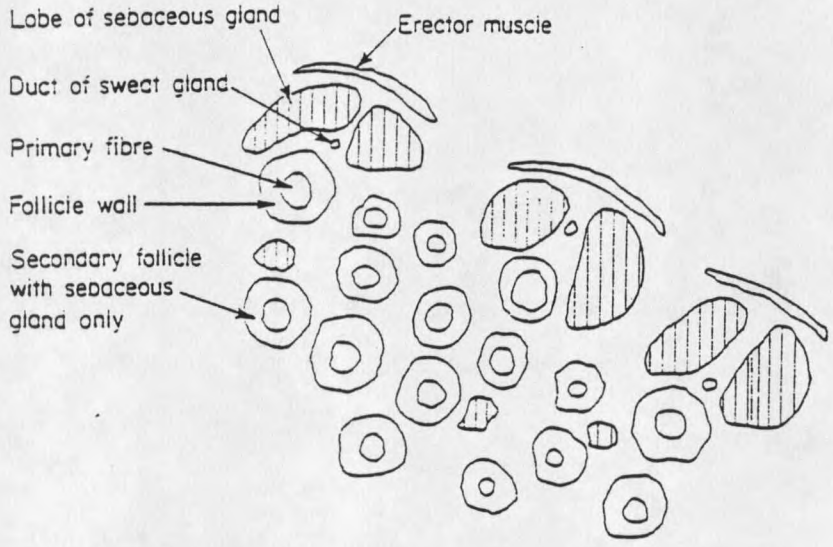
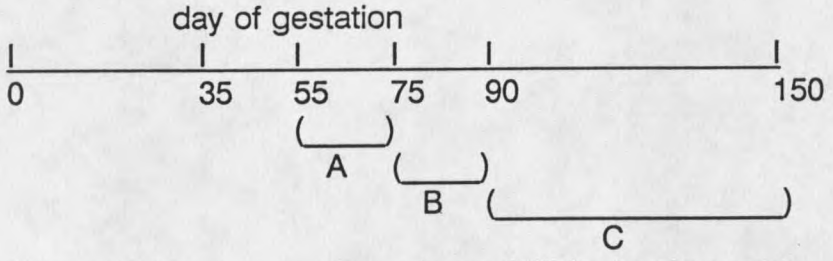


Figure 2. Adapted from a diagram of a typical follicle group in an adult sheep, as seen in horizontal section. (From Ryder, 1960.)

Secondary follicle formation begins after trio stage development is complete and by day 120 of gestation development is rapid and intensive overcrowding of the interstitial tissue results. Developmental stages of secondaries follow those for primaries as described by Lyne (1957) and McFadden (1967). Secondary follicles cover the fetal body by parturition, 40 days after the primaries have covered the skin and ceased initiation.



A = pre-trio, primary follicle development
B = trio, primary follicle development
C = post-trio, secondary follicle development

Figure 3. Wool follicle development scheme in utero.

Factors on Wool Follicle Development

Environment influences the quality and quantity of wool produced. Environmental influences prenatally may permanently restrict the ability of sheep to grow wool. Detrimental factors can occur in utero or in early postnatal life and may be dependent on or independent of feed conditions (Corbett 1979). Everitt (1967) reported adverse nutrition under controlled grazing conditions permanently affected wool production. He found that severe undernutrition of ewes late in pregnancy reduced their progeny's wool follicle population and adversely affected

live weight and wool production over 3 years. Conversely, Denney et al. (1988) reported that imposing low levels of nutrition on grazing ewes in late pregnancy had no significant effect on progeny wool production. They did indicate, however, that fewer secondary follicles were mature at weaning in the progeny and grease fleece weight was reduced at one year of age but not at two or three years of age when postnatal undernutrition was imposed.

The number of follicles initiated in the skin during foetal life has a direct bearing on the size of wool fibers eventually produced (Moore et al., 1989). Schinckel (1955) suggested that the greatest proportion of the secondary follicle population may be initiated before birth. Since follicle initiation and fibre specification are temporally distinct processes, both parameters must be determined at, or prior to, the earlier developmental event, i.e. initiation (Moore et al., 1989).

Nutrition has an important influence on the rate of maturation of the secondary follicle population (Schinckel 1955; Short 1955). Lyne (1964) reported that the restriction of nutrient intake reduced the fiber output of secondary follicles. Increased wool production of sheep fed a high protein diet resulted in increased fiber length and diameter (with the exception of the fine wool Merino), presumably reflecting an increase in essential AA supply to the intestines (Hemsley and Reis 1984).

Wool growth is highly dependent on the quantity of AA available (Reis and Schinckel 1961; 1963). Thus increases in wool growth rate in response to

ingestion of a protein supplement may be indicative of its bypass protein content. Carrico et al. (1970) however reported that groups of sheep may differ substantially in their response to protein supplements. They concluded when AA requirements are exceeded other factors became more important in limiting wool growth.

The ultimate capacity of a sheep to produce wool is influenced by nutrition during follicle development. Marston (1955) working with sheep stunted by malnutrition reported that expression of genetic potential for wool production is not possible when poor nutrition is imposed during fetal and early postnatal life. He found the malnutrition caused a permanent limitation on the subsequent capacity of sheep to produce wool by reducing the number of follicles developed.

Johnson et al. (1988) indicated plane of nutrition of the ewe during gestation is an important factor determining whether or not lambs reach their genetic potentials for density (mm^2) and secondary to primary follicle (S/P) ratio (Table 3). The supply of nutrients to the fetus during late gestation has been shown to affect the extent of initiation and probable degree of branching of the secondary follicles. The degree to which these follicles mature to produce fibers is affected by the level of nutrition of the lamb during early life. A reduction in the number of follicles initiated due to limited nutrients during prenatal life is considered permanent. However, there is some disagreement among researchers as to whether limited nutrition during early postnatal life inhibits follicle maturation or only delays the process.

Table 3. Wool follicle density per mm^2 and S/P ratio of sheep from a mid-side biopsy sample.

Breed	Age of sheep	S/P ratio	Density[a] mm^2	Source
Rambouillet	at birth	10.52	Primary 12.97	Ruttle and Sorensen 1965
Merino	24 months	20.7	67.8	Williams and Winston 1987
Corriedale	7-8 months	10.8	31.1	Carter and Clarke 1957b
Suffolk	11-12 months	4.8	20.4	Carter and Clarke 1957b
Peppin Medium Merino	12-14 months	19.0	79.8	Carter and Clarke 1957a
Targhee	at birth	4.2	34.2	Abouheif et al. 1984
Rambouillet	at birth	7.5		Shetaewi and Ross 1987
Merino	13-15 months		80.3	Moore et al. 1989
Southdown-Merino	5-24 months	8.0	28.8	Lyne 1961
Medium Wool Merino			50.6	Young and Chapman 1958
Strong Wool Merino			34.4	Young and Chapman 1958
Medium Wool Merino	at birth	20.1	94.2	Short 1955
S. Aust. Merino	12 weeks	14.01	70.84	Everitt 1967
Medium Wool Merino	5-6 months	13.9	58.0	Short 1955

[a]Follicle density equals primary + secondary unless otherwise specified.

The primary effect of restricted feed intake during prenatal life on mature wool production is a reduction in total follicle number (Schinckel and Short, 1961). Narayan (1960) suggested that limiting prenatal nutrition adversely affects the growth of secondary follicles. He reported that any adverse condition influencing the initiation or maturation of secondary follicles, even to a small extent, will tilt the balance in favor of primary follicles and the adult fleece will then be composed of fibers with a higher percentage of primary than secondary follicles. The net result would be a lower density of wool fibers and lighter fleece produced by the animal.

Everitt (1967) reported significant wool follicle population differences in lambs subjected to different levels of prenatal nutrition. He indicated that total follicle populations of lambs born to ewes fed well in late pregnancy exceeded their ill fed mates by over nine million follicles.

Research results have emphasized the important relationship between nutrition and follicle numbers, and hence the effect of nutrition on fibre production. The plane of nutrition of the ewe during mid and late pregnancy when the secondary follicles are developing in the fetus is important to subsequent life time wool production of progeny. If insufficient nutrition is provided at these stages of a ewe's biological cycle, the lifetime wool production may be affected (Harmsworth and Day, 1979). Likewise, nutrition of the lamb during its first few months of life when the secondary follicles are maturing and coming into production is a critical period.

Quantity of wool grown by a sheep depends upon the number of wool follicles growing a fiber and rate of growth of each fiber. The number of wool follicles on sheep is genetically determined but may be modified by nutrition (Black and Reis, 1979). Ryder and Stephenson (1968), reported that increasing density of wool fibers is the most promising way to increase fleece weight without changing quality.

EXPERIMENTAL PROCEDURES

Experiment 1

In 1990, 40 pregnant Targhee (avg. BW = 63 kg) ewes bred to a Targhee ram were allotted randomly to one of five dietary treatments (n = 8) in an 84-d experiment initiated approximately on d 55 and concluding on d 139 of gestation. Treatments were: control, (no supplemental protein) (HAY); soybean meal (SBM); 2/3 SBM:1/3 blood meal (BM), (2S:1B); 1/3 SBM:2/3 BM, (1S:2B); or BM. Ewes were individually penned and fed once daily at 0700 in 1.2 x 1.5 m pens. At 1600 ewes were released from confinement and allowed access to water in a group setting. Diets and orts were weighed at each feeding and recorded at 1600 daily.

Supplements were formulated to provide 45 g/d CP, which was approximately 40% of the daily CP intake (NRC, 1985a), of this, 22 g of the supplemental CP component was provided by SBM, BM or their combinations. The quantity of supplemental protein provided was desired to be equivalent to that supplied by a typical supplement fed to gestating ewes grazing winter range (V.M Thomas, personal communication). Isonitrogenous and isocaloric supplements (Table 4) were formulated to provide 90% of the CP and 100% of the ME NRC (1985a) requirements for pregnant ewes the first 15 wks of gestation, while varying in EP content. Consumption of supplement and nutrient intakes for each treatment

group are given in Table 5. Half of the daily hay diet was chopped, the other half pelleted with the supplement, they were fed separately. The nutrient composition of the smooth brome (*Bromus inermis*) hay was 7.3% CP, 1.99 Mcal/kg of ME, 58.3% neutral detergent fiber (NDF) and 41.6% acid detergent fiber (ADF).

Table 4. Ingredient and nutrient composition of supplements (DM basis)

	Supplement				
Item	HAY	SBM	2S:1B	1S:2B	BM
DM, %	91.2	91.8	92.3	92.1	92.5
Ingredient, %					
Grass hay	93.9	80.9	81.7	82.1	82.6
Soybean meal		10.4	6.9	3.5	
Blood meal			1.8	3.6	5.4
Peanut oil		2.7	3.3	4.2	5.2
Bentonite	2.9	2.9	2.9	2.9	2.9
Potassium chloride	1.2	.9	1.0	1.2	1.3
Gypsum	.8	.7	.8	.8	.8
Monosodium phosphate	.8	.5	.6	.7	.7
Salt-trace mineral mix[a]	1.0	1.0	1.0	1.0	1.0
Nutrient composition					
CP, %	9.1	10.5	10.3	10.5	10.9
NDF, %	48.2	47.0	48.2	47.9	47.7
ADF, %	33.6	33.5	34.0	33.6	33.6
ADIN, %[b]	.04	.02	.02	.02	.02
Sulfur	.20	.25	.28	.30	.30
Phosphorous	.33	.36	.38	.36	.32
Calcium	.80	.65	.59	.61	.67
ME, Mcal/kg[c]	1.86	2.16	2.15	2.17	2.19

[a]Composition of salt-trace mineralized salt: NaCl, 98%; Zn, .35%; Mn, .28%; Fe, .175%; Cu, .035%; I, .007%; Co, .007%.
[b]ADIN=acid detergent insoluble nitrogen.
[c]Calculated from NRC (1985a) values.

Fasted (12 h) BW were recorded at the beginning and end of the experiment. Palpable body condition scores (Russel et al., 1969) were determined by two technicians when ewe weights were obtained.

Wool fiber length (mm rule) was monitored by dye banding with Durafur Black R[1] and measured as described by Langlands and Wheeler (1968).

Table 5. Ingredient and nutrient intake

	Supplement				
	HAY	SBM	2S:1B	1S:2B	BM
DM intake					
Hay, kg/hd/d	.73	.72	.72	.72	.71
Supplement, kg/hd/d	.56	.44	.46	.48	.45
Total, kg/hd/d	1.29	1.16	1.18	1.20	1.16
Nutrient intake					
CP, g/hd/d	115.2	111.7	108.6	111.2	110.5
ME, Mcal/hd/d	2.5	2.4	2.4	2.5	2.4

Blood samples were obtained at 28-d intervals starting at the beginning of the experiment, (Period 1, d 0-28; Period 2, d 29-56; Period 3, d 57-84). Blood samples were collected via jugular puncture (Lindsay, 1978) just before feeding (semi fasted state). Serum samples were analyzed for total protein (TP), albumin (ALB), glucose (GLU), blood urea nitrogen (BUN) and creatinine (CRT) using a semi-automated analyzer[2]. Free fatty acid (FFA) concentrations were measured

[1]Icianz Pty. Ltd., Imperial Industries Chemical Limited, Frankfurt, Germany.

[2]Ames Pacer Analyzer, Miles Laboratory, Inc. Elkart, IN.

from whole blood samples using the NEFA-C kit[3] (Bauman, 1988).

At the conclusion of the 84-d feeding period, which was approximately one week prior to parturition, ewes were allowed ad libitum access to a grass-alfalfa hay mixture, plus .45 kg/hd/d of barley grain. Individual grease fleece weights were obtained 4 d after the conclusion of the experiment. Data collected included birthdate, type of birth, weight, sex, lamb weaning weights ewe weight at weaning. Ewes were fed .67 kg/hd/d barley and allowed ad libitum access to a grass-alfalfa hay during the first three weeks of lactation. Ewes and lambs then grazed on improved pasture consisting of smooth brome (*Bromus inermis*) and crested wheatgrass (*Agropyron cristatum*) until weaning.

Six mm diameter skin biopsies were obtained from mid-side sites when lambs were 100 d of age. Histological and measurement techniques were used to determine follicle density and the ratio of secondary to primary follicles (Maddocks and Jackson 1988).

Immediately following the skin biopsy each specimen was placed in 10 ml glass container containing 5 ml of 10% buffered formalin solution. The skin samples were sent to the Montana Vet Diagnostic Lab for tissue processing. Procedures for skin tissue processing involved placement in a plastic embedding cassette, with acquisition number written on cassette. Tissue was fixed in Lillies Neutral Buffered Formalin (NBF) for at least 24 h at room temperature without vacuum, pressure or agitation.

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[3]Biochemical Diagnostics, Inc. Brentwood, N.Y.

Following fixation in NBF, the cassette containing each individual sample was processed in an Auto Technicon[4]. The processing solution and time schedule was:

80% ethanol - two hours - room temp

95% ethanol - one hour - room temp

100% ethanol - two hours - room temp

100% ethanol - two hours - room temp

100% ethanol - two hours - room temp

100% ethanol - two hours - room temp

Propar[5] - one hour - room temp

Propar - half hour - room temp

Propar - half hour - room temp

Paraffin Para Plast-X-tra[6] - one hour - 59°C

Paraffin Para Plast-X-tra - one hour - 59°C

After paraffin infiltration, tissue samples were taken off the processor and embedded in paraffin molds using a Tissue Tech Embedding Center[7]. Once the blocks (plastic cassette containing the fixed skin tissue embedded in paraffin) cooled and solidified they were taken from the stainless steel paraffin molds and

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[4]Shandon Inc., Pittsburg, PA.

[5]Anatech, LTD., Battle Creek, MI.

[6]Oxford Labware, Div. of Sherwood Medical, St. Louis, MO.

[7]Shandon Inc., Pittsburg, PA.

placed on ice to keep cold.

The blocks were faced and allowed to set and soak on the ice for five minutes. The blocks were cut at 8 μ on a Standard American Optical 820 Rotary Microtome[8]. Ribbons were floated on a 43°C waterbath and backed upon standard 25 X 75 mm glass slides. Slides were then dried in an oven at 59°C for one h until no water droplets remained on the slide.

After drying, the slides were placed in a rack on a Shandon Automatic Varistain 24-2 Stainer[9]. Slides were stained using a regular Hematoxylin and Eosin staining schedule. The staining routine follows:

Number of Changes	Fluid	Time
3	Pro Par	4 min. each
3	100% ethanol	30 sec. each
2	distilled H_2O	30 sec. each
1	Gill's #2 Hematoxyl[10]	3 min.
2	running H_2O	30 sec. each
1	Scotts Tap Water Substitute Concentrate[11]	2 min.
1	running H_2O	2 min.

[8]American Optical Co., Sci. Inst. Div., Buffalo 15, N.Y.

[9]Shandon Inc., Pittsburg, PA.

[10]Fisher Scientific, Orangeburg, N.Y.

[11]Sigma Diagnostics, St.Louis, MO.

1	80% ethanol	1 min.
1	95% ethanol	1 min.
1	Eosin (1% alcohol soln)	1 min.
2	95% ethanol	30 sec. each
2	100% ethanol	1 min. each
4	Pro Par	1 min. each

Slides were then cover slipped (#1 thickness) using Refrax[12] mounting medium, labeled and allowed to dry.

Counts of the follicle population were made at 100 X on a microprojector. An area 100 mm^2 was marked on a recording sheet and used to define the counting boundaries. Follicles, both primary and secondary, falling within the counting boundaries, were counted using a standard convention for structures covered by marginal lines i.e., those which were crossed by the left hand and top margins of the area are included in the count, while those on the right hand and bottom were excluded.

Primary follicles are defined by recognition of the accessory structures, sweat gland, sebaceous gland, arrector pili muscle and finally by position in the follicle group, secondary follicles may have an associated sebaceous gland (Figure 2). Primary and secondary follicles were counted separately, as well as being included in the total. Ten fields were counted, total "apparent" follicle density and secondary to primary (S/P) ratio were calculated.

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[12]Anatech, LTD., Battle Creek, MI.

However, to derive a "true" figure for density it was necessary to compare the area of the histological specimen on which the count was performed with the area of the original sample, presumed to be the area of the trephine (6 mm). Specimen shrinkage was accounted for using the following formula: Correction factor = area of specimen divided by area of trephine (Maddocks and Jackson, 1988) Average shrinkage for all samples was 8.6 percent.

Ewe BW, and condition score changes, fiber length, grease fleece weight, lamb birth and post-treatment ewe and lamb weights were analyzed using GLM procedures of SAS (1987). Main effects included treatment, ewe age, lamb birth type and their interactions. Birth date was included as a covariate in the least squares model. Lamb weight analysis included lamb sex in the model. Serum metabolite concentrations were analyzed by multivariate analysis of variance (SAS, 1987). The repeated measures procedure was used to account for the repeated sampling of the same ewe in each period. For all models pairwise comparisons of least squares means were performed using T-tests with the Bonferroni inequality (Snedecor and Cochran, 1967).

Experiment 2

Three rumen and duodenal fistulated wethers were used in a 3 X 5 latin square design to determine the influence of substituting SBM with BM on N flow to the small intestine. Wethers were housed in metabolism pens and received a different supplement (Table 5) in each of the five (14 d) collection periods. Wethers were fed a grass-alfalfa hay mixture at 2% of BW and isocaloric and

isonitrogenous supplements (Table 4) for a 12 d adjustment period at 0700 and 1900 hrs. At each feeding, wethers were intra-ruminally administered gelatin capsules containing 2.5 g chromium oxide to monitor digesta flow. On d 12 duodenal extrusa samples were collected at 0, 3, 6, 9 and 12 after the 0700 feeding, measured for pH and individual 60 ml samples frozen (-20C) for later determination of DM, ash content and ammonia N.

Duodenal samples were freeze dried, ground through a Wiley mill (2 mm screen) with macro Kjeldahl N (AOAC, 1984) analyses conducted. Chromium concentration was determined on duodenal extrusa using the procedures described by Fenton and Fenton (1979). On d 14, rumen extrusa was collected 0, 3, 6, 9 and 12 h after the 0700 feeding to measure pH, and bacteria were isolated as described by Smith and McAllan (1974). Purine concentrations of microbial pellets were determined to estimate microbial synthesis (Zinn and Owens, 1986). Additional calculated variables were total N and nonammonia N (NAN) as a percent of N intake. Nonammonia nonmicrobial N (NANMN) and microbial N as a percent of NAN was also determined. Diets were analyzed for DM and total N (% N X 6.25=CP) using macro kjeldahl N analysis (AOAC, 1984). Acid detergent fiber and NDF fiber were determined by procedures described by Van Soest and Robertson, (1980).

Treatments were arranged in a 3 X 5 latin square with five repeated observations on each animal within each period. Dependent variables were analyzed by multivariate repeated measures analysis using the GLM procedure of

SAS (SAS, 1987). Calculated variables were analyzed using analysis of variance, first with treatment, animal and period as effects in the model and again changing treatment to a continuous variable according to supplemental escape protein levels. For all models pairwise comparisons of least squares means were performed using T-tests with the Bonferroni inequality (Snedecor and Cochran, 1967).

RESULTS

Experiment 1

Dietary treatments had no (P = .72) influence on ewe weight change or BCS changes (Table 6) during the 84 d experiment. Ewes gained an average of 7.7 kg of BW and body condition declined 0.4 units from an initial average of 3.4 BCS during the experiment.

Table 6. Least squares means for effect of protein source on ewe weight and body condition score.

	Supplement					
Item	HAY	SBM	2S:1B	1S:2B	BM	SE[a]
Weight, kg						
Initial	66.7	63.4	59.1	63.0	62.7	2.0
Final	74.5	71.2	67.7	69.3	70.1	2.1
Change	7.9	7.7	8.6	6.4	7.8	1.0
Body condition						
Initial	3.5	3.4	3.2	3.5	3.5	.2
Final	3.0	2.9	2.9	2.9	3.0	.2
Change	-.3	-.4	-.3	-.6	-.4	.15

[a]SE = standard error of least squares means

Serum metabolite concentrations PROT, ALB, BUN, CRT, GLU and FFA were determined during the experiment (Table 7). Protein treatments had no effect (P = .35) on serum PROT concentrations. Serum ALB concentrations were not influenced (P = .25) during P1. Ewes fed 2S:1B; 1S:2B and BM had greater

($P < .01$) serum ALB concentrations in P2 than ewes fed HAY or SBM. In P3, all protein supplemented ewes had greater ($P < .01$) serum ALB concentration than ewes fed HAY. Protein treatments had no effect ($P = .35$) on BUN during P1 and P2. However, in P3, all protein supplemented ewes had greater ($P < .05$) BUN concentration than ewes fed HAY. Creatinine metabolite concentrations during P1 were similar ($P = .75$) for all treatment combinations. During P2 CRT concentrations were greater ($P < .05$) for SBM supplemented ewes than BM with HAY and SBM + BM combinations being intermediate. During P3 CRT concentrations were greater ($P < .05$) for SBM supplemented ewes than 1S:2B with HAY, 2S:1B and BM supplemented ewes being intermediate. Ewes fed BM + SBM combinations had greater ($P < .05$) GLU concentrations than HAY supplemented ewes and SBM fed ewes were intermediate during P1, while during P2 GLU concentrations were similar ($P = .19$) for all treatment combinations. Ewes fed 2S:1B had greater ($P < .01$) GLU concentrations than 1S:2B, BM or HAY during P3 with no differences ($P = .08$) detected between 2S:1B and SBM or HAY and SBM. Free fatty acid concentrations in P1 were greater ($P < .05$) for BM supplemented ewes than SBM or SBM + BM combinations with HAY being intermediate. During P2 ewes supplemented with BM had higher ($P < .05$) FFA concentrations than 2S:1B with HAY, SBM and 1S:2B intermediate. Serum FFA concentrations during P3 were similar ($P = .41$) for all treatment combinations.

Table 7. Least squares means for effect of protein source on serum metabolite concentrations.

		Supplement					
Metabolite	Period	HAY	SBM	2S:1B	1S:2B	BM	SE[a]
PROT, g/dl	1	5.7	5.6	5.6	5.5	5.8	.1
	2	6.0	6.2	6.2	6.0	6.4	.2
	3	5.3	5.3	5.4	5.4	5.5	.2
ALB, g/dl	1	3.4	3.4	3.6	3.4	3.5	.06
	2	3.4^{b}	3.5^{b}	4.0^{c}	3.7^{c}	3.7^{c}	.06
	3	2.9^{b}	3.2^{c}	3.4^{d}	3.2^{cd}	3.3^{cd}	.07
BUN, mg/dl	1	13.6	13.5	15.1	14.0	13.6	.7
	2	17.4	18.7	16.6	18.4	17.0	.8
	3	11.9^{b}	16.6^{c}	16.2^{c}	17.0^{c}	15.1^{c}	1.1
CRT, mg/dl	1	1.33	1.33	1.36	1.26	1.37	.06
	2	1.53^{bc}	1.72^{b}	1.52^{bc}	1.49^{bc}	1.46^{c}	.08
	3	1.52^{bc}	1.64^{b}	1.49^{bc}	1.34^{c}	1.47^{bc}	.08
GLU, mg/dl	1	47.3^{b}	50.7^{bc}	52.1^{c}	51.7^{c}	52.9^{c}	1.3
	2	41.9	42.8	47.7	46.4	47.6	2.1
	3	37.0^{b}	42.6^{bc}	46.5^{c}	41.5^{b}	39.4^{b}	2.3
FFA μ Eg/l	1	540.6^{bc}	527.6^{b}	509.7^{b}	461.6^{b}	774.4^{c}	93
	2	645.5^{bc}	623.2^{bc}	552.1^{b}	650.2^{bc}	853.2^{c}	103
	3	738.8	1052.3	967.7	809.6	962.4	125

[a]SE = standard error of least squares means
[b,c,d]Means in same row with different superscripts differ ($P < .05$).

Protein supplementation had no effect (P > .05; Table 8) on fleece weight or fiber length. Lamb wool follicle ratios and densities at 100 days of age were similar (P > .05; Table 8). Average S/P ratio and follicle density (no./mm^2) were 10.8 and 22.7, respectively. Ewe and lamb BW following parturition (60.4 and 4.2 kg, respectively) were not influenced (P > .05) by prepartum dietary treatments (Table 9).

Table 8. Least squares means for effect of protein source on ewe fleece weight, fiber length and progeny wool follicle traits.

	Supplement					
Item	HAY	SBM	2S:1B	1S:2B	BM	SE[a]
Fleece weight, kg	4.4	4.0	3.9	4.0	4.0	.3
Fiber length, mm	16.5	16.9	15.5	16.4	16.8	.9
S/P follicle ratio	11.1	10.8	10.4	11.2	10.7	.68
Follicle density per mm^2	22.1	24.4	21.5	23.1	22.5	1.02

[a]SE = standard error of least squares means

Table 9. Least Squares Means for Effect of Protein Source on Post-Lambing Ewe and Lamb Weights (kg).

	Supplement					
Item	HAY	SBM	2S:1B	2B:1S	BM	SE[a]
Ewe weight						
Post-lambing	62.5	62.1	57.4	58.4	61.5	2.3
Weaning	69.9	64.8	65.6	66.7	66.2	1.6
Lamb weight						
Birth	4.5	4.3	3.9	4.0	4.2	.21
Weaning	22.0	22.5	23.5	22.6	20.3	3.8

[a]SE = standard error of least squares means

Experiment 2

In the metabolism trial N intake was not affected when wethers were fed the different sources of supplemental protein or HAY (Table 10). Feeding additional EP also did not affect ($P > .05$) N flow, NAN, NANMN, or microbial N flows (grams per day) to the duodenum. However, NANMN and microbial N as a percent of NAN were different ($P < .05$). For each unit increase in EP supplement NANMN increased ($P < .01$) by .57 percent.

Ruminal NH_3 concentrations were lower ($P < .05$) for BM supplemented wethers than all other treatment combinations. As ruminal NH_3 concentrations declined microbial N presented to the duodenum also tended to decline, as did microbial N, % of NAN. Ruminal pH was similar ($P = .11$) for all treatment groups. However, duodenal pH, for BM was greater ($P < .05$) than SBM supplemented wethers with HAY and SBM + BM combinations intermediate in response.

Table 10. Least squares means for effect of protein source on intake of N and passage of nitrogenous compounds in gastrointestinal tract of wethers.

	Supplement					
Item	HAY	SBM	2S:1B	1S:2B	BM	SE[a]
Intake, N g/d*	16.57	18.08	17.90	18.10	18.54	
Ruminal pH	6.57	6.70	6.64	6.57	6.68	0.05
Ruminal Ammonia	13.3[b]	12.3[b]	12.5[b]	10.8[b]	9.6[c]	0.72
Flow to duodenum,						
Duodenal pH	3.49[b,c]	3.37[b]	3.60[b,c]	3.55[b,c]	3.68[c]	0.06
Total N, g/d	23.21	20.19	25.38	21.46	19.58	2.91
Total N, % N intake	132.2	116.4	140.6	122.1	106.7	12.4
Ammonia N, g/d	1.34	1.15	1.31	1.15	1.23	.22
NAN, g/d	21.9	19.0	24.1	20.3	18.4	2.7
NAN, % N intake	124.8	104.8	119.1	119.6	114.2	13.3
NANMN, g/d	10.9	9.3	12.1	12.0	12.1	2.1
NANMN, %NAN	49.0[b]	49.9[b]	54.2[b,c]	56.6[c]	57.4[c]	1.7
Microbial N, g/d	10.68	9.02	9.43	9.31	8.80	1.13
Microbial N, % of NAN	51.0[b]	50.1[b]	45.8[bc]	43.4[c]	42.6[c]	1.7
Microbial N g/kg DM	8.93	7.60	8.00	7.96	7.51	.84
Microbial N g/kg OM	12.50	11.28	11.98	12.35	10.30	.89

[a]SE = standard error of least squares means
[bc]Means in same row with different superscripts differ (P < .05).
*Arithmetic mean

DISCUSSION

Crude protein supplements had no effect on ewe BW change. This response was probably due to ME intake at NRC (1985a) recommended levels and similar quantities of N being presented to the small intestine. Total N presented to the duodenum was similar among CP supplements in Exp. 2.

Wethers fed the HAY diet in Exp. 2 had greater ruminal NH_3 concentrations and more microbial N as a percentage of NAN presented to the duodenum than BM-fed wethers. This suggests that ruminal requirements for CP synthesis were probably met by nutrients supplied in HAY alone. Ruminal NH_3 concentrations were greater than the minimal requirement recommended by Satter and Roffler (1975) for optimal CP synthesis on all diets. Therefore, one would not anticipate increased ruminal CP synthesis by substituting N from either SBM or BM for HAY nitrogen. These data agree with Klusmeyer et al. (1990), who reported that microbial N flow to the duodenum and efficiency of microbial growth were not enhanced by substituting EP from corn gluten meal (CGM) for SBM in Holstein cows. They suggested that no response may have been due to only a small portion of the total dietary CP being provided by SBM or CGM.

Increased escape of dietary CP does not always increase production. Polan (1988) reported that CP sources less degradable than SBM reduce microbial

growth rate. Because EP may be poorly digested postruminally, the balance of AA available for absorption from the small intestine may be reduced and limit animal production (Owens and Bergen, 1983). Conversely, if microbial CP is the only CP source 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 EP is desired (NRC, 1985a). As already indicated NAN of non-microbial origin (EP) increased and microbial N decreased as a percentage of total N presented to the duodenum when the concentration of supplemental BM increased.

Type of forage fed may influence the response to EP. Hoaglund (1989) and Padula (1990) found that when ewes were fed straw based diets nutritional status was improved by supplementation with EP. In our experiments however, supplementing with EP did not enhance nutritional status. This may have been due to the higher quality of the basal forage fed in our trials as compared to the straw in their experiments. In our experiment the base diet (grass hay) contained 8.0% CP compared to around 4.5% CP in straw for both Hoaglund (1989) and Padula (1990).

Grease fleece weight and longitudinal wool growth were similar among treatment groups as would be expected when ewes were in a positive nutritional state. Because ewes were in a positive nutritional state and CP supplementation did not increase the quantity of intestinal N available for absorption, no wool response was expected.

Progeny wool follicle development was similar regardless of protein source fed during gestation. Lyne (1964) reported that severe nutrient restriction during gestation was required to reduce secondary follicle development, as did other researchers (Narayan, 1960; Schnickel and Short, 1961). Everitt (1967) indicated that lambs born to ewes well-fed in late gestation, had greater wool follicle densities in comparison to lambs born to nutrient restricted ewes. No difference in birth weight was detected in our study. Therefore, nutrients available for development of primary and secondary follicles were probably adequate. Lamb secondary follicle development was probably not restricted in our experiment probably because AA supply was adequate.

Protein source had no effect on post-lambing or weaning time ewe and lamb weights. Because CP supplementation did not increase the quantity of intestinal N, no difference in weights was expected. Our data agree with those of Hoaglund (1989) and Padula (1990) who also reported similar post-lambing and weaning results when they fed an EP source to ewes during mid-gestation.

In summary, substituted hay CP with 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. Moderate quality hay provided sufficient CP for optimal microbial CP production and for progeny follicle development. These data suggest that a grass-hay containing 8% CP was sufficient to support adequate ewe BW gain during gestation and substitution of hay CP with SBM and or BM CP was not required.

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APPENDIX

Table 11. Analysis of variance for effect of protein source on ewe initial weight.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	205.69	51.42	1.83	.15
Fetal number (FN)	1	6.02	6.02	0.21	.65
Ewe age	1	471.26	471.26	16.75	<.01
Treatment*FN	4	32.40	8.10	0.29	.88
Error	27	759.42	28.13		

Table 12. Analysis of variance for effect of protein source on ewe final weight.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	179.04	44.76	1.39	.26
Fetal number (FN)	1	39.31	39.31	1.22	.28
Ewe age	1	552.06	552.06	17.12	<.01
Treatment*FN	4	89.96	22.49	0.70	.60
Error	27	870.84	32.25		

Table 13. Analysis of variance for effect of protein source on ewe weight change.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	15.08	3.77	0.52	.72
Fetal number (FN)	1	12.55	12.55	1.74	.20
Ewe age	1	10.55	10.55	1.46	.24
Treatment*FN	4	53.95	13.49	1.87	.14
Error	27	194.48	7.20		

Table 14. Analysis of variance for effect of protein source on initial BCS.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.20	0.05	0.22	.92
Ewe age	1	0.75	0.75	3.34	.08
Fetal number	1	0.12	0.12	0.54	.47
Parturition date	1	<.01	<.01	0.03	.87
Treatment*Age	4	1.01	0.25	1.12	.37
Error	26	5.84	0.22		

Table 15. Analysis of variance for effect of protein source on final BCS.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.16	0.04	0.26	.90
Ewe age	1	0.01	0.01	0.07	.79
Fetal number	1	0.32	0.32	2.04	.16
Parturition date	1	0.57	0.57	3.63	.07
Treatment*Age	4	0.44	0.11	0.70	.60
Error	26	4.05	0.16		

Table 16. Analysis of variance for effect of protein source on ewe BCS change.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.33	0.08	0.48	.75
Ewe age	1	0.47	0.47	2.77	.11
Fetal number (FN)	1	0.33	0.33	1.93	.18
Treatment*FN	4	0.50	0.12	0.73	.58
Error	27	4.59	0.17		

Table 17. Analysis of variance for effect of protein source on total protein (period 1).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.496	0.124	1.14	.36
Ewe age	1	0.175	0.108	1.61	.22
Fetal number (FN)	1	0.051	0.051	0.47	.50
Treatment*FN	4	0.381	0.095	0.88	.49
Error	27	2.927	0.108		

Table 18. Analysis of variance for effect of protein source on total protein (period 2).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.546	0.136	0.78	.55
Ewe age	1	0.887	0.887	5.04	.03
Fetal number (FN)	1	0.601	0.601	3.41	.08
Treatment*FN	4	0.454	0.114	0.65	.64
Error	27	4.752	0.176		

Table 19. Analysis of variance for effect of protein source on total protein (period 3).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.187	0.047	0.25	.90
Ewe age	1	0.460	0.460	2.51	.13
Fetal number (FN)	1	0.877	0.877	4.78	.04
Treatment*FN	4	0.344	0.086	0.47	.76
Error	27	4.957	0.184		

Table 20. Analysis of variance for effect of protein source on albumin (period 1).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.146	0.036	1.40	.26
Ewe age	1	0.149	0.149	5.74	.02
Fetal number (FN)	1	<.001	<.001	0.01	.93
Treatment*FN	4	0.115	0.029	1.11	.37
Error	27	0.699	0.026		

Table 21. Analysis of variance for effect of protein source on albumin (period 2).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	1.240	0.310	10.42	<.01
Ewe age	1	<.001	<.001	0.03	.87
Fetal number (FN)	1	0.138	0.138	4.65	.04
Treatment*FN	4	0.103	0.026	0.87	.50
Error	27	0.803	0.030		

Table 22. Analysis of variance for effect of protein source on albumin (period 3).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.836	0.209	6.38	<.01
Ewe age	1	0.204	0.204	6.23	.02
Fetal number (FN)	1	0.218	0.218	6.66	.01
Treatment*FN	4	0.186	0.047	1.42	.25
Error	27	0.884	0.030		

Table 23. Analysis of variance for effect of protein source on BUN (period 1).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	12.09	3.02	0.97	.44
Ewe age	1	0.59	0.59	0.19	.67
Fetal number (FN)	1	8.73	8.73	2.82	.11
Treatment*FN	4	12.89	3.22	1.04	.41
Error	27	83.74	3.10		

Table 24. Analysis of variance for effect of protein source on BUN (period 2).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	22.59	5.65	1.14	.36
Ewe age	1	34.45	34.45	6.97	.01
Fetal number (FN)	1	1.25	1.25	0.25	.62
Treatment*FN	4	16.89	4.22	0.85	.50
Error	27	133.40	4.94		

Table 25. Analysis of variance for effect of protein source on BUN (period 3).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	103.04	25.76	3.36	.02
Ewe age	1	130.92	130.92	17.09	<.01
Fetal number (FN)	1	3.95	3.95	0.52	.48
Treatment*FN	4	33.49	8.37	1.09	.38
Error	27	206.82	7.66		

Table 26. Analysis of variance for effect of protein source on creatinine (period 1).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.047	0.011	0.48	.75
Ewe age	1	0.037	0.037	1.51	.23
Fetal number (FN)	1	0.005	0.005	0.20	.66
Treatment*FN	4	0.189	0.047	1.93	.14
Error	27	0.662	0.025		

Table 27. Analysis of variance for effect of protein source on creatinine (period 2).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.316	0.079	1.68	.18
Ewe age	1	0.040	0.040	0.85	.36
Fetal number (FN)	1	0.111	0.111	2.36	.14
Treatment*FN	4	0.036	0.009	0.19	.94
Error	27	1.266	0.047		

Table 28. Analysis of variance for effect of protein source on creatinine (period 3).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.307	0.077	1.62	.20
Ewe age	1	0.004	0.004	0.08	.78
Fetal number (FN)	1	0.004	0.004	0.08	.78
Treatment*FN	4	0.134	0.034	0.71	.59
Error	27	1.280	0.047		

Table 29. Analysis of variance for effect of protein source on glucose (period 1).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	120.63	30.15	2.84	.04
Ewe age	1	149.96	149.96	14.13	<.01
Fetal number (FN)	1	17.69	17.69	1.67	.21
Treatment*FN	4	152.90	38.22	3.60	.02
Error	25	275.95	10.61		

Table 30. Analysis of variance for effect of protein source on glucose (period 2).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	195.48	48.87	1.64	.20
Ewe age	1	233.01	233.01	7.81	<.01
Fetal number (FN)	1	187.23	187.23	6.28	.02
Treatment*FN	4	50.50	12.63	0.42	.79
Error	25	775.37	29.82		

Table 31. Analysis of variance for effect of protein source on glucose (period 3).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	336.00	84.00	2.34	.08
Ewe age	1	369.82	369.82	10.30	<.01
Fetal number (FN)	1	191.53	191.53	5.33	.03
Treatment*FN	4	48.56	12.14	0.34	.85
Error	25	933.76	35.91		

Table 32. Analysis of variance for effect of protein source on FFA (period 1).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	423704	105926	1.84	.15
Ewe age	1	185599	185599	3.23	.08
Fetal number (FN)	1	4121	4121	0.07	.79
Treatment*FN	4	137763	34441	0.60	.67
Error	27	1551236	57453		

Table 33. Analysis of variance for effect of protein source on FFA (period 2).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	368571	92142	1.30	.30
Ewe age	1	4838	4838	0.07	.80
Fetal number (FN)	1	330392	330392	4.65	.04
Treatment*FN	4	259918	64979	0.92	.47
Error	27	1916712	70989		

Table 34. Analysis of variance for effect of protein source on FFA (period 3).

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	426873	106718	1.03	.41
Ewe age	1	148	148	0.00	.97
Fetal number (FN)	1	176697	176697	1.70	.20
Treatment*FN	4	236048	59012	0.57	.69
Error	27	2809247	70989		

Table 35. Analysis of variance for effect of protein source on fleece weight.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	0.70	0.17	0.44	.78
Age	1	<.01	<.01	0.01	.92
Fetal number (FN)	1	1.01	1.01	2.56	.12
Initial weight	1	0.63	0.63	1.59	.22
Treatment*Age	4	0.72	0.18	0.45	.77
Treatment*FN	4	1.21	0.30	0.76	.56
Error	22	8.70	0.40		

Table 36. Analysis of variance for effect of protein source on wool growth.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	7.03	1.76	0.43	.79
Age	1	18.55	18.55	4.51	.05
Fetal number (FN)	1	8.77	8.77	2.13	.16
Initial weight	1	1.20	1.20	0.29	.59
Treatment*Age	4	16.71	4.18	1.02	.42
Treatment*FN	4	26.54	6.63	1.61	.21
Error	22	90.47	4.11		

Table 37. Analysis of variance for effect of protein source on progeny secondary to primary follicle ratio.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	3.45	0.86	0.21	.93
Ewe age	1	6.76	6.76	1.65	.21
Parturition date	1	59.75	59.75	14.60	<.01
Treatment*Ewe age	4	16.84	4.21	1.03	.40
Error	40	163.72	4.09		

Table 38. Analysis of variance for effect of protein source on progeny follicle density.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	39.00	9.75	1.13	.36
Ewe age	1	3.57	3.57	0.41	.52
Parturition date	1	63.80	63.80	7.41	.01
Treatment*Ewe age	4	148.71	37.18	4.32	<.01
Error	40	344.58	8.61		

Table 39. Analysis of variance for effect of protein source on ewe weight at parturition.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	132.22	33.05	0.96	.45
Fetal number (FN)	1	9.35	9.35	0.27	.61
Ewe age	1	285.21	285.21	8.29	<.01
Parturition date	1	83.07	83.07	2.41	.13
Treatment*FN	4	28.61	7.15	0.21	.93
Error	26	894.79	34.42		

Table 40. Analysis of variance for effect of protein source on ewe weight at weaning.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	92.61	23.15	1.48	.24
Lambs weaned (LW)	1	41.28	41.28	2.64	.12
Ewe age	1	67.94	67.94	4.34	.05
Wt. at weaning	1	363.29	363.29	23.21	<.01
Treatment*LW	4	28.61	7.15	0.21	.93
Error	23	359.98	15.65		

Table 41. Analysis of variance for effect of protein source on lamb birth weight.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	3.01	0.75	1.67	.17
Sex	1	0.02	0.02	0.05	.82
Birth type	2	16.54	8.27	18.28	<.001
Parturition date	1	4.50	4.50	9.94	<.01
Treatment*Sex	4	5.72	1.43	3.16	.02
Error	56	25.34	0.45		

Table 42. Analysis of variance for effect of protein source on lamb weaning weight.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Treatment	4	36.52	9.13	0.10	.98
Ewe age	3	828.15	276.05	2.96	.04
Sex	1	0.05	0.05	0.00	.98
Type of rearing	2	611.08	305.54	3.28	.05
Treatment*Sex	4	147.31	36.83	0.39	.81
Error	44	4103.43	93.26		

Table 43. Analysis of variance for effect of protein source on rumen pH.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	0.212	0.106	18.80	<.01
Treatment	4	0.039	0.010	1.73	.31
Period	4	0.096	0.024	4.27	.09
Error	4	0.023	0.006		

Table 44. Analysis of variance for effect of protein source on rumen ammonia.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	0.26	0.13	0.10	.91
Treatment	4	24.57	6.14	4.73	.08
Period	4	6.97	1.74	1.34	.39
Error	4	5.20	1.30		

Table 45. Analysis of variance for effect of protein source on duodenal pH.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	0.64	0.318	31.02	<.01
Treatment	4	0.15	0.037	3.58	.12
Period	4	0.12	0.030	2.91	.16
Error	4	0.04	0.010		

Table 46. Analysis of variance for effect of protein source on duodenal nitrogen.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	296.65	148.33	6.90	.05
Treatment	4	48.98	12.25	0.57	.70
Period	4	126.41	31.60	1.47	.36
Error	4	85.99	21.50		

Table 47. Analysis of variance for effect of protein source on duodenal nitrogen, percent of N intake.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	11314	5657	10.94	.02
Treatment	4	575	144	0.28	.88
Period	4	2957	739	1.43	.37
Error	4	2068	517		

Table 48. Analysis of variance for effect of protein source on ammonia nitrogen.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	1.212	0.606	4.91	.08
Treatment	4	0.070	0.017	0.14	.96
Period	4	0.203	0.051	0.41	.79
Error	4	.494	0.123		

Table 49. Analysis of variance for effect of protein source on nonammonia nitrogen.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	259.99	129.99	6.60	.05
Treatment	4	46.47	11.62	0.59	.69
Period	4	126.21	31.55	1.60	.33
Error	4	78.72	19.68		

Table 50. Analysis of variance for effect of protein source on nonammonia nitrogen, % of N intake.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	9935	4967.8	11.08	.02
Treatment	4	527	132.0	0.29	.87
Period	4	2754	688.5	1.54	.34
Error	4	1793	448.4		

Table 51. Analysis of variance for effect of protein source on nonammonia nonmicrobial nitrogen.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	142.36	71.17	6.18	.06
Treatment	4	15.26	3.82	0.33	.85
Period	4	26.11	6.53	0.57	.70
Error	4	46.08	11.52		

Table 52. Analysis of variance for effect of protein source on nonammonia nonmicrobial nitrogen, as % of nonammonia nitrogen.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	243.81	121.90	15.81	.01
Treatment	4	165.67	41.42	5.37	.07
Period	4	26.02	6.51	0.84	.56
Error	4	30.85	7.71		

Table 53. Analysis of variance for effect of protein source on microbial nitrogen.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	18.22	9.11	2.83	.17
Treatment	4	5.35	1.34	0.42	.79
Period	4	19.41	4.85	1.51	.35
Error	4	12.89	3.22		

Table 54. Analysis of variance for effect of protein source on microbial nitrogen, as % of nonammonia nitrogen.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	243.81	121.90	15.81	.01
Treatment	4	165.67	41.42	5.37	.07
Period	4	26.02	6.51	0.84	.56
Error	4	30.85	7.71		

Table 55. Analysis of variance for effect of protein source on microbial nitrogen per kg of dry matter.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	4.30	2.15	1.20	.39
Treatment	4	3.11	0.78	0.43	.78
Period	4	11.90	2.98	1.66	.32
Error	4	7.18	1.79		

Table 56. Analysis of variance for effect of protein source on microbial nitrogen per kg of organic matter.

Source of variation	Degrees of Freedom	Sums of Squares	Mean Squares	F-Ratio	PR > F
Animal	2	2.76	1.38	0.68	.56
Treatment	4	7.92	1.98	0.97	.51
Period	4	9.84	2.46	1.20	.43
Error	4	8.17	2.04		