2018-06-01

# Plane of Energy Nutrition on Blood Metabolites, Milk Production and Lamb Growth in Friesian Sheep

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# Effects of Nutritional Modification in Pseudo and Ruminant Livestock

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A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

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Small ruminant species are utilized for their diverse products including meat, dairy products, and wool. Effective and humane management are essential to maintain high production rates and comfortable animals. To attain this objective, managers needs to have an extensive knowledge of husbandry techniques, understanding of physiological processes, and familiarity with nutritional requirements. We examined the effects of varying feed components on two different ruminal species. In Chapter 1, we conducted a study to evaluate the effects of a low metabolizable energy (LME) and high metabolizable energy (HME) diet on twenty-two Friesian/Lacuane cross ewes and lamb nutritional status. Effects on milk production during early lactation stages and the growth of the neonatal lambs were also investigated. We anticipate energy levels will have an effect on milk production and lamb growth. Our results indicate that ewes on the LME diet produced more milk with higher concentrations of fat though this group maintained lower body condition. We concluded that neither the HME nor the LME diet met the needs of the sheep due to the shift in nutrient partitioning towards milk production rather than allocating nutrients to maintaining both body condition and milk production. Limited energy requirements are further evidenced by the decline in back fat (BF) for both the HME and LME groups for the duration of the study. We determined the degradation parameters of grass hay supplemented with soybean meal (SBM) and the effects of SBM on compartment 1 (C1) ammonia and volatile fatty acid (VFA) concentrations in alpacas. Our findings show that the degradation rate was not different for dry matter (DM), but it was for crude protein (CP) ($P<0.05$). With this data it can be concluded that SBM can be a CP supplement when the diet is insufficient to improve microbial yield. It should be noted that care should be taken to avoid causing a protein-energy imbalance. The results of these two studies indicate shifts in nutrients availability and changes in feeding strategies can affect both the health of the animal and their subsequent offspring.

Keywords: Alpaca, Friesian sheep, in situ digestibility, soybean meal, VFA

ACKNOWLEDGMENTS

I must first thank my husband, Zachary, who never ceased to provide me with unwavering positivity and comfort. He told me that he never doubted that I could accomplish this goal and his constant source of love and patience got me through many tearful nights. Supportive is a huge understatement and I could not have done it without him. Thank you!

I would also like to express gratitude to my parents David and Heidi Smith. My father, David, told me the world was mine, I just had to go get it. His words of encouragement pushed me to obtain this higher degree. My mother, Heidi, is my personal cheerleader and her love and support got me through countless bouts of self-doubt, fatigue, and discouragement. I am forever grateful for her example of perseverance in the face of adversity.

Finally, I would like to thank my chair, Todd F. Robinson, for the endless hours he spent answering my questions, talking me through details, editing my drafts, and making sure I was learning as much as possible. Without his guidance, knowledge, and patience, I would not have been able to complete this degree.

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# CHAPTER 1

## Plane of Energy Nutrition on Blood Metabolites, Milk Production and Lamb Growth in Friesian Sheep

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## ABSTRACT

This study was conducted to evaluate the effects of a low metabolizable energy (LME) and high metabolizable energy (HME) diet on twenty-two Friesian/Lacuane cross ewe milk production and nutritional status of the ewes and their lambs. Ewes were housed in a common paddock with *ad libitum* water and were fed alfalfa hay at 3% of body weight (BW). On day 100 of gestation, ewes were divided into metabolizable energy (ME) groups and fed alfalfa hay and rolled corn that provided either 80% low metabolizable energy (LME) or 140% high metabolizable energy (HME) of recommended ME requirement based on published NRC (2007) values for 70 kg ewes carrying twins, nursing twins and producing 1.5 to 2.9 kg milk/day. The treatment period was from the last trimester of gestation (approximately six weeks) to six weeks after parturition. Lamb treatments included HME, LME and lambs artificially reared (AR) on goat's milk. Body weight and backfat (BF) were monitored and recorded weekly for each ewe while weekly lamb BW was obtained for the duration of the study. Blood samples were collected weekly from the ewes beginning at their third trimester and concluding six weeks post parturition. Lamb blood samples were also collected weekly for six weeks post birth. Blood metabolites including, glucose, plasma urea nitrogen (PUN), creatinine, total protein (TPP) and triglycerides were analyzed to assess the nutritional status of both ewes and lambs. In addition, weekly milk samples for each ewe were analyzed for butter fat, protein, lactose, milk urea nitrogen (MUN), somatic cell count (SCC), and solids-not-fat (SNF). Ewe body weight was

not effected by treatment. There were differences in BF with the HME group having more BF than the LME group. A weekly effect was noted for ewe blood components glucose, PUN, and TPP. Milk fat percentage, daily fat produced, and lactose were affected by energy treatments. The LME group displayed both higher BF percentages and daily fat in milk while the HME group had higher concentrations of milk protein and lactose percentages. Lamb weight showed weekly and treatment affects for all three lamb groups (AR, HME, and LME) with the HME group weighing the most by the end of the experiment. Concentrations of plasma glucose, PUN, and creatinine resulted in differences with the HME group having the highest concentration of each component. Our results indicate that perinatal nutrition effects both the ewe and lamb as well as milk production. Because of the lower energy intake of the LME group, we see that nutrient partitioning occurs enabling the ewe to allocate energy towards growth of the fetus and to produce enough milk to sustain growth of the lamb post placental drop. This partitioning of energy came at the expense of body condition for the LME group, and to a lesser extent to the HME group, in order to produce adequate milk for the offspring. It is also evident, that neither diet met animals' energy nutrient requirements as both BW and BF declined for each group post parturition.

## INTRODUCTION

Animal production is a fast growing and evolving industry. The Food and Agriculture Organization of the United Nations acknowledges that livestock contribute 40% of the global value of agricultural output and in addition, support the livelihoods and food security of almost a 1.3 billion people (Food and Agriculture Organization of the Unites States, 2014). In 2014, milk production reached about 168 million tonnes of milk and of that 3.2% of total milk

production came from sheep (*Ovis aries*), goats (*Capra aegagrus*), and bison (*Bison bison*); (Food and Agriculture Organization of the United States, 2014). Dairy sheep of the Friesian breed production is not as well established in the United States as it is in Europe and surrounding countries. In the United States, sheep are typically utilized for their meat and wool products.

Pregnant ewes undergo a gestation period of five months (approximately 150 days) and lactate from four to five months in commercial herds. During gestation and lactation, the endocrine system of the ewe distributes nutrients throughout the body to support the fetus and thereby causes major changes affecting metabolic processes (Charismiadou et al., 2000). In this way, prenatal ewe nutrition is vital to both the mother and potential offspring (Charismiadou et al., 2000). Consequently, animal performance is contingent on the intake of metabolizable and digestible nutrients (Mertens, 1994). Deficiency or overabundance of nutrients will affect the animal's productive capabilities. Though some ewes may be fed what is considered the appropriate mixed diet based on ewe breed, size, gestational stage, and milk production needs (NRC, 2007), there is a possibility that fetal growth could be reduced because of a placental limitation on the supply of nutrients (Mellor, 1983). When this occurs, nutrient restriction during gestation shift nutrient partitioning towards the uterus to foster growth of the fetus (Celi et al., 2008). Additionally, underfeeding pregnant sheep can result in a variety of adverse effects on fetal and newborn lambs such as affecting placental size, growth of the fetus, fetal fat reserves allocation for use after birth, udder development as well as colostrum and milk production (Mellor, 1983 and 1988). Likewise, metabolism can vary due to the amounts and ratios of absorbed nutrients, as well as the individual, and the interaction of, biochemical pathways (Mertens, 1994). With the multitude of interactions occurring, it is important to keep in

mind that differences in breed, diet, environment and management can also affect nutrient requirements and utilization by the animals (Galvani et al., 2008).

Our objective was to evaluate the effects of a low metabolizable energy (LME) and high metabolizable energy (HME) diet on Friesian ewe and lamb nutritional status including analysis of blood components, weight, and body condition scores (BCS). In addition, we determined the effects of ME on milk production during early lactation. We expected the energy level fed to the ewes will have an effect on milk production and lamb growth. Lambs are subdivided into three categories: those that are offspring from the HME fed ewes, those that are offspring from the LME fed ewes, and a small portion that are unable to nurse from mother and are fed goat milk make up the artificially reared (AR) group. Analysis and evaluation of ME is reviewed to better understand the energy effects on these high milk producing sheep.

## METHODS

### *Animals, location and experimental design*

Twenty-two Friesian/Lacuane cross ewes, between the ages of 2 and 5 years, were bred to East Friesian rams under the approval of the BYU IACUC (#16-1103). Pregnancy was confirmed by ultrasound initially, then by blood analysis (Utah Veterinary Diagnostic Laboratory, Logan, UT). Each ewe was identified by a farm ear tag as well as federal scrapies ear tag. Three days after birth, each lamb was fitted with a farm tag and scrapies tag. One day prior to beginning the experiment (third trimester), ewes were vaccinated with a commercial 8-way product (Ultrabac 8®, Zoestis Animal Health, Parsppany, NJ, USA) and dewormed with a broad-spectrum anti-parasitic (Valbazen®, Zoestis Animal Health, Parsppany, NJ, USA).

Treatment groups were fed either an 80% (LME) or 140% (HME) of recommended ME requirement based on the Small Ruminant NRC (2007) values for 70 kg ewes with high milk production in late gestation and early lactation. For the gestation period the NRC ME requirement was 3.50 and 6.12 MCal for the LME and HME groups. The lactation period NRC ME target was 4.38 and 7.67 MCal.

During the first two trimesters of gestation, ewes were housed in a common paddock with *ad libitum* water and alfalfa hay (*Medicago sativa*; see Table 1-1). On day 100 of gestation (approximately the beginning of the last trimester), ewes were randomly divided into ME treatment groups to ensure that ewe ages were equally represented in the two treatments, with 11 ewes per diet.

Ewes were housed in two dry lot pens with access to shelter bedded with straw and *ad libitum* water. Ewes gave birth in these pens and soon after, ewes and lambs were moved into a barn for sample collection. Ewes and lambs were sampled as outlined below. If a ewe had triplets (15 ewes), quadruplets (1 ewe), or it was determined she was unfit to raise a lamb, the lamb or lambs were moved to the artificially reared (AR) lamb group after receiving colostrum. After parturition, ewes with lambs were moved to cribs as outlined below. Lambs in the AR group were fed 60 ml of sheep colostrum at three 4-hr intervals. They were then fed 75 ml of fresh goat milk four times per day until d-7. From d-8 to d-17 lambs were fed 120 ml four times per day. From d-15 to d 21 lambs were fed 240 ml three times per day. From d-22 to d-42 lambs were fed 480 ml twice daily. Goat milk fed to AR lambs was analyzed for milk components as outlined below (Table 1-4).

*Sampling*

From day 100 of gestation to parturition (approximately day 145), ewes were fed daily (0700 hr) treatment diets (see Table 1-1 and Table 1-2). Ewes were weighed weekly at 1000 hr on a platform scale while lambs were weighed on a portable scale until large enough (3000 g) to use the platform scale. Body condition score was determined by backfat (BF) depth over the 12$^{th}$ rib (industry standard) using A-mode ultrasound (Preg-Alert Pro, Renco, Golden Valley, MN, USA). Blood was drawn (5 ml) weekly using a syringe and needle from the jugular vein of each ewe. The site of extraction was shaved and cleaned with alcohol prior to the blood draw.

At parturition, ewes were weighed, BF measured, and blood drawn post placental drop prior to putting the ewe and lambs into crib pens. At birth, lambs were weighed and 2ml of blood drawn prior to nursing and being put into the cribs with their mother, or in the case of the AR lambs, into the lamb nursery. Ewes and lambs remained in the cribs for three days while they "mothered-up" (i.e., socially bond). After that time, ewes and lambs were reintroduced back with the treatment group.

From parturition to six-weeks postpartum, weekly weights, BF, and blood samples were collected from the ewes and weight and blood samples from the lambs. Plasma was harvested by centrifugation, aliquoted and stored for analysis. Twenty-four hr milk production was measured by holding the lambs from the ewes for 24 hr (0700 to 0700) three days after weight, blood and BF samples were collected. Milk weight was measured and a 20ml sample collected in Dairy Herd Improvement Association (Rocky Mountain DHIA, Logan, UT, USA) vials.

*Assays*

Plasma from both ewes and lambs was analyzed using colorimetric assay kits for glucose, urea nitrogen, creatinine, total plasma protein, and triglycerides using TECO kits (TECO Diagnostics, Anaheim, CA, USA). Analysis was conducted at Brigham Young University (Provo, UT, USA). Weekly milk samples for each ewe were analyzed for butter fat, protein, lactose, urea nitrogen, and somatic cell count. Analysis was conducted at the Rocky Mountain DHIA (Logan, UT, USA).

*Statistical Analysis*

Statistical analysis was conducted with the proc Mixed module in SAS (SAS Inst., Inc., Cary, NC). Fixed main effects included ME treatment and week, while animal was random to account for repeated measures. Least square means for treatment and week were determined to be significant at $P<0.05$. Model comparisons included weekly ME treatment comparisons for the response variables weight, BF, metabolites, milk production and milk composition. Main effect comparisons were also made between prepartum, parturition and post parturition. Treatment and week main effect comparisons were analyzed for the lamb response variables and expressed as least square means and determined significant at $P<0.05$.

## RESULTS

Three ewes did not complete the study due to sickness ($n = 1$) or death ($n = 2$). The LME group had eight sets of twins, and three sets of triplets, and one ewe that had quadruplets. Within the HME group, one ewe gave birth to a single lamb, other produced seven sets of twins, and two sets of triplets. The LME groups produced 29 lambs while the HME group produced 21 lambs. Eleven lambs were moved to the AR group. Three of the AR lambs were from the

LME group while the remaining eight were from the HME group. Hay and corn grain were completely consumed by the ewes of both groups, leaving no feed residual, therefore feed intake for each group is that presented in Table 1-2.

*Ewes*

Body weight (Figure 1-1) was not different between treatment groups. As expected, there was a sharp drop in weight at parturition for all ewes, with a difference of 20 kg between pre and post parturition. Pre-parturition weights increased from 75.2 kg to 84. 5 kg for the LME group and from 76.1 kg to 84.2 kg for the HME group. After parturition there was no change in weight for either group, with postpartum weights averaging 68.7 and 67.2 kg for LME and HME respectively. Back fat (Figure 1-3), showed a difference by treatment. The LME group backfat steadily decreased from week -6 (3.0 mm) to week +6 (1.8 mm), while HME ewes increased from 3.3 to 3.6 mm between week -6 to week -3 then decreased to 2.7 mm at week -1 and maintained until week +4 where they decreased to 2.4 at week +6.

Weekly blood metabolites are presented in Table 1-3 and a comparison of weight, and blood metabolites of ewes during pre- and post-parturition is presented in Table 1-5. There was no treatment effect for plasma glucose concentration however, there was a week effect. For both the HME and LME groups, the week of parturition differed from the weeks prior and post parturition with a concentration of 9.43 mmol/l and 10.19 mmol/l; respectively. The average concentration of glucose for sheep in general has been reported to range from 2.78 to 4.44 mmol/l (3.80 ± 0.33; Kaneko et al., 1997). The average pre-partum concentration for this study was 3.91 and 4.32 mmol/l for LME and HME respectively and 3.80 and 3.77 mmol/l postpartum.

Plasma urea nitrogen varied significantly between treatment groups where LME PUN concentrations steadily increased from 4.3 mmol/l at week -5 to 7.0 mmol/l at week -1. During this period HME concentrations fluctuated between 3.3 to 3.7 mmol/l. At parturition, both HME and LME were similar at 4.5 mmol/l. Postpartum concentrations averaged 4.3 and 4.9 mmol/l HME and LME. There were no differences in creatinine for treatment or week. Prepartum concentrations were 72.9 and 67.9 µmol/l for LME and HME, 77.0 and 69.9 µmol/l at parturition and 69.4 and 74.3 µmol/l postpartum.

Total plasma protein displayed a weekly effect in both the HME and LME groups. From week -5 to week -1 LME TPP decreased from 68.6 to 57.4 g/l, while at this same time, HME fluctuated between 73.2 to 65.3 mmol/l. At parturition, both HME and LME groups increase to 73.6 and 69.3 mmol/l. The LME group remained at this level for the remainder of the experiment. At week +1 HME levels drop to 50.9 mmol/l before increasing to an average of 64.0 mmol/l for the remainder of the experiment. Prepartum triglyceride levels are 0.247 and 0.275 mmol/l for LME and HME, increasing to 0.280 and 0.291 mmol/l at parturition then dropping to 0.195 and 0.192 mmol/l.

*Milk Data*

There was no difference in treatment groups or week effect for milk yield (Table 1-4), averaging 1.665 kg/d for HME and 1.779 kg/d for LME. Fat percentage was different for treatment effect, 2.53% for HME and 4.43% for LME. Average daily milk fat was also only different between treatment groups, 44.8 g/d for HME and 87.7 g/d for LME. Milk protein percent was different for week effect where HME decreased from 4.93 to 4.44% and no change noted for LME (average 4.75%). Daily milk protein was not different between treatment or week

and averaged 75.0 and 85.0 g/d for HME and LME respectively. Milk lactose percent was different between treatment, 5.43% for HME and 5.12% for LME. No differences were noted for daily lactose, averaging 89.9 and 90.3 g/d for HME and LME respectively. No differences were determined for solids not fat % (SNF) or daily production or for MUN. Milk samples and weights were corrected for energy based on the equation (Hemme, 2017).

ECM = (0.327 x milk kg) + (12.95 x fat kg) + (7.2 x protein kg). Where EMC is energy-corrected milk. ECM was not different for treatment, week, or the interaction of the two variables. From week 1 to week 6 for both treatment groups declined with a difference of 0.56 g LME, and 1.00 g HME. The average for the two groups are (5.02 g LME), (3.95 g HME).

*Lambs*

Lamb weight (Figure 1-1) increased steadily for 6 weeks post parturition with lambs in the HME group increasing from 4.625 kg to 15.288 kg, a 231% increase. LME lamb weights increased from 4.180 kg to 13.221 kg (216% increase), while the AR weights increased from 4.256 kg to 12.300 kg (189% increase) over the six-week period. There is no difference in birth weight across the treatments and treatment differences do not become evident until weeks 5 and 6, where the HME group weights were 2.052 and 2.988 kg greater than the LME and AR.

Lamb blood metabolite analysis is presented in Table 1-6. There is no treatment effect for glucose concentrations (see Figure 1-2) between the lamb groups. However, there is a weekly difference for blood glucose between week zero (at birth; from 2.37 to 3.48 mmol/l) increasing to between 8.32 to 9.57mmol/l at week 2 then decreasing to between 5.0 to 5.26 mmol/l at week 6. Plasma urea nitrogen was significant for only the weekly effect where at birth levels are 4.8, 4.9 and 5.5 mmol/l for LME, HME and AR respectively. The LME and HME

PUN levels increased to 6.4 mmol/l at week 1, while the AR group dropped to 2.6 mmol/l. Week 2 to 6 PUN levels fluctuated between 2.00 to 3.89 mmol/l.

Blood creatinine is not significant between treatments, but was for week with levels higher at birth ranging from 204.2 to 157.9 μmol/l. The levels decrease to an average of 55 μmol/l at week 2 and remain constant at this level for the remainder of the experiment. Total plasma protein was different for both treatment and week. At birth, the levels are 49.2, 42.2 and 46.8 g/l for HME, LME and AR respectively. The remainder of the experiment the levels fluctuated between 53 and 67 g/l, with LME most often being the highest and AR the lowest. Triglyceride concentrations were not different between treatments or weeks, ranging between 0.367 to 0.774 mmol/l.

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## DISCUSSION

Dietary restrictions during gestation can affect the metabolic processes of ruminant animals and their subsequent offspring (Celi, 2008). This is evident in the contrast between the two diet regimes compared in this experiment. We documented changes due to diet in the body weight, body condition, and milk production. Milk production, for instance, can be increased through the utilization of energy-rich diets which reduce the mobilization of energy from body reserves (Cannas, 2013). Diet regimes we implemented were kept simple to minimize the sources of metabolizable energy.

### *Ewes*

Body weights of the two treatment groups were not different. Addah et al., (2017) suggest that sheep are able to compensate for sudden changes in nutrient restriction by increasing the efficiency of nutrient absorption and utilization, even though the sudden shift initially

reduces average daily gain. Previous studies such as Mora et al., (1996) indicate that for a limited duration, ruminants have the ability to cope with moderate levels of malnutrition. The short period the experimental diets were fed may be the reason there is no difference between the weights of the ewes. Though there were no differences between weights of our two treatment groups, there is a difference in BF during the study. We used BF as a body condition estimate where thicker BF would indicate body energy reserves. It is well documented that body fat reserves are first accrued internally, then inter-muscularly and then as BF. These fat stores provide the animal with reserves that can be utilized to maintain substrate for required energy needs. When energy balance is negative lipolysis provides energy substrates to meet the body's energy needs. The first store of lipid to be utilized when energy is limited is BF. Though there is a numerical decrease in BF from the initiation of the study (approximately 0.5 mm for both treatments), statistical differences do not become apparent until after parturition when the energy needs of lactation overcome the dietary supply. The sharpest decline of prepartum body condition for both groups is from week -3 to parturition suggesting that energy levels prior to parturition may not adequate to meet the energy demands placed on the ewe for fetal growth and the preparation for lactation. The postpartum decline of BF for the LME ewes is further evidence of the effects of limiting energy requirements on lactating ewes. Similarly, the HME diet does not seem to meet the energy demands of lactation as BF decreased at week +4.

Blood metabolite determinations provide an understanding of how various factors can influence general nutritional status. The combination of these blood metabolites provides information on how administered treatments affect metabolic processes. In the case of this experiment, how does dietary energy level affect nutritional status of Friesian ewes and lambs. Burton et al., (2003) and Celi et al., (2008) report glucose responses in pre and postpartum

alpacas and goats show a spike in blood glucose at parturition, similar to the response noted in our sheep. Leat (1974) also observed an increase in the glucose concentration in ewe plasma within 2 to 3 days prior to parturition followed by a decrease in levels 20 days postpartum. The period between late gestation and early lactation is an intense metabolic transition phase from providing nutrients to the fetus to lactogenesis (Burton et al., 2003). Bell (1995) concluded that the gravid uterus absorbs 30 to 50% of the ewe's glucose supply, thus putting stress on the ewe to maintain required glucose levels. Homeorhetic hormones (e.g., glucocorticoids, growth hormone, prolactin and estradiol) interact with homeostatic hormones (e.g., insulin and cortisol) to regulate glucose levels (Tucker, 1985). The glucose spike at parturition could be a result of the timing of sample collection. In the case of this experiment, with the fetus born, the large amount of maternal glucose going to the fetus has stopped and the glucose regulation has not yet adjusted. The ewe now must transition glucose to the needs of lactation.

When protein intake is in excess or energy is limiting, urea N levels will increase due to the catabolism of amino acids either for fat stores or for energy needs. Increases in protein and energy can increase retention of nitrogen in growing ruminant animals and may result in an increase of PUN (Tur et al., 2017). Recycling PUN via the rumen is important for microbial growth and function and improves nitrogen utilization (Wang et al., 2012), thus urea N not taken up by the rumen is excreted in the urine. Excess concentrations of urea in the blood can affect multiple physiological processes including production of milk, immune function, embryo survivability and reproductive efficiency (Dominic et al., 2014). Several studies have stated that PUN serves as an indirect indicator of the energy or protein levels in the diet in conjunction with levels of energy required (Ramin et al., 2010). In this study, PUN levels for the LME group increased from approximately 4.5 to 7.0 mmol/l between week -5 and parturition, while the

HME group PUN remained relatively constant between 3.5 and 4.0 mmol/l. This indicates the LME ewes may have shifted to protein catabolism to meet energy needs brought on by the dietary energy restriction.

Triglyceride concentrations in the blood provide an indication of fat metabolism, where low levels indicate malnutrition and high levels obesity. Prepartum triglyceride levels are higher compared to postpartum indicating there is a shift in nutrient status and that the postpartum ewes have a higher energy demand than prepartum. This is brought out in the NRC (2007), where lactating ewes require higher energy than gestating ewes. What these metabolites in conjunction with the body condition indicate is that the requirements published in the NRC (2007) may be inadequate for these dairy sheep breeds.

*Milk*

Due to its nutritional composition, sheep milk contains more nutrients and a larger supply of total solids than cow or goat milk (Recio et al., 2009). Sheep milk provides a source of proteins, lipids, calcium and phosphorous while balancing a similar quantity of carbohydrates, fat, and proteins (Recio et al., 2009). Several factors affect milk yield including lactation length, environmental factors, feed quality and availability, and genetics to name a few (Collier et al., 2017; Pulina et al., 2007). Lactose synthesis controls water secretion; thus, lactose determines milk volume osmotically (Miglior et al., 2006). Additionally, protein and fat concentrations in milk are largely determined by dietary protein, VFA production in the rumen and the water content driven by lactose (Henao-Velasquez et al., 2014).

Energy requirements for lactation are determined by energy corrected milk (ECM; Milis, 2008). The equation to determine ECM takes into account the percent of fat and protein as well

as volume. Somewhat surprisingly, the LME group has a higher milk yield and higher concentration of milk fat than the HME group, but when expressed on an ECM basis there are no differences between the groups. Cannas et al., (2013) found that ewes fed lower amounts of non-fiber carbohydrates (NFC) produced more milk than those fed more NFC. Additionally, those on the high diet were able to partition ME towards fat deposits rather than milk secretion (Roche et al., 2008). A comparable result occurred in this study where the ewes in the LME group produced more milk than those in the HME group. Like the ewes in the Cannas et al., (2013) study, this may be a result of ME being shuttled towards fat reserves rather than milk production. Ewes in the HME group had on average higher backfat than those in the LME group, 3.0 and 2.5 mm respectively, supporting this assumption. Cannas further suggests that higher milk production in sheep with lower NFC could be the result of a more marked partitioning of dietary energy towards milk synthesis (Cannas et al., 2013). These results indicate that ewes fed 140% ME (HME) allocated more energy towards maintaining high body conditions (e.g., fat stores) than towards milk production. Whereas those fed 80% ME (LME) did not have the resources to maintain higher body conditions, they redirect resources towards milk production to sustain the growing lamb.

Milk fat varies greatly during the lactation period, between ewes, between daily milking, between milking sheep breeds, and due to season and climate (Milis, 2008; McDonald et al., 1995). This variation can be due in part to concentration of volatile fatty acids (VFAs) which originate in the rumen. Specific VFA's such as acetate, propionate, and butyrate, are bi-products of microbiota that provide the main source of energy for ruminants and have an individualized profile per animal. The proportion and type of volatile fatty acids produced in the rumen depend on the substrate metabolized and the species of bacteria present (Dijkstra,

1994). Our study did not focus on VFAs, however a difference in milk fat concentration may be attributed to differing levels and composition of VFAs. Acetate and butyrate are the primary precursors for milk fat synthesis whereas propionate is glucogenic (Bergman, 1990; Urrutia and Haveratine, 2017). In goats, Eknæs and Skeie (2006) and Eknæs et al., (2006) concluded that milk yield was not only affected by the mobilization of tissue energy, but it also affected milk composition; specifically the fatty acid profile.

Other factors that affect milk fat concentration include energy balance of the ewes, neutral detergent fiber (NDF) fraction in forages consumed, NFC, as well as particle size of the feed, amount of feed consumed, and the fatty acid composition of dietary fat supplements (Pulina, 2006). Goetsch et al., (2011) states that forage source has an impact on milk fat that is independent of energy intake. Milk fat synthesis is stimulated via diets rich in digestible fiber, likely through the enhanced supply of acetate to the mammary gland (Cannas et al., 2013). Milk fat yield in goats was found to be greater for diets consisting of higher forage (60 to 65%) content (Álvarez et al., 2007; Ngwa et al., 2009).

Unlike the findings of Alvarez et al., (2007) and Ngwa et al., (2009), this study found that milk fat was higher for ewes in the LME group. Goetsch et al., (2011) stated that forage source, rather than energy intake, has an impact on milk fat concentration. The LME sheep in our study were limit fed the alfalfa in addition to the corn grain to achieve a level of 80% ME. The HME group was fed more than the required dry matter intake (NRC, 2007) and no hay refusal was noted, leading us to conclude that even the HME were limit fed to some degree. Amount of hay (forage) provided for each treatment group could still be too low for Eastern Friesian causing no difference in milk yield or milk fat concentrations.

*MUN and Milk Protein*

Urea nitrogen is a waste product derived by the breakdown of protein, is formed in the liver, circulates in the blood and is excreted in the urine. Blood urea concentrations rapidly equilibrate with body fluid pools such as the mammary gland. In the secretory cells of the mammary gland, urea moves into the milk and becomes a non-protein nitrogen component of milk (Cannas, 1998; Gustafsson and Palmquist, 1993). MUN and blood urea nitrogen concentrations are used to evaluate diets fed to ruminants because they are considered to be adequate indicators of protein metabolism and intake (Jelinek et al., 1996; Roseler et al., 1993). Though there are no differences between MUN or PUN in our study, at week three and four (when there were high concentrations of MUN in the HME group; 13.5 mmol/l and 13.3 mmol/l), milk production decreased. Weeks three and four for the HME group were the only times MUN concentrations exceeded 12.6 mmol/l of MUN concentration. This could be a result of the alfalfa hay crude protein level. To keep the diets simple and easily manageable, diets we used were not isonitrogenous while CP was slightly below requirement for the LME group and well above requirement for the HME group.

Lactose concentrations were higher for the HME group throughout the 6-week collection period, with overall means of 5.43% and 5.12% for HME and LME respectively. Henao-Velasquez et al (2014), stated that lactose levels differ daily in milk production and differ in concentration of fat, milk urea nitrogen, glucose availability and somatic cell count. Our findings were similar in that lactose levels followed a similar curve as the MUN levels, with a low concentration of both components at week one, a slight increase through subsequent weeks, and finally a decrease at week five. ECM was not significant for treatment, week or the interaction.

*Lambs*

*Birth Weight*

Fetus growth during the third trimester varies due to ewe nutritional status during late gestation (Robinson, 1980). The fetus however, does increase by nearly 75% during this time. Late gestation is recognized as a highly energy-inefficient physiological process for ruminants due partly to the high expenditure of energy for maintenance and growth of the fetus (Kiani, 2006; Lodge and Heany, 1970). Birth weight of lambs was not different across our three treatments. The AR group was a result of lambs born to ewes from both ME treatments who were unable to raise the lamb on their own. Because they were evenly a result of both groups coming from sets of twins or triplets, no differences were noted in birth weights between the groups. Lamb weight increased from birth to the end of our experiment (six weeks), with the HME group showing great weight gain despite no difference in milk yield, milk composition or ECM between the treatment groups. Ewes in this study were fed the alfalfa hay *ad libitum* prior to the initiation of the experiment. The lack of difference between birth weights of the two ME groups may be attributed to the LME utilizing feed more efficiently as explained by Lu et al., (2005) or due to fat reserves in the ewes that were able to compensate for the restriction in energy intake, or both.

*Blood Metabolites*

Plasma glucose levels were different at parturition and weeks two and three displaying a weekly effect but no treatment effects. Glucose concentrations for each group were as follows: AR (6.08 mmol/); HME (7.00 mmol/l); LME (6.70 mmol/l), nearly twice the normal values published by Kaneko et al., (1997) (2.78-4.44 mmol/l). Milk lactose and

other glucogenic compounds have been shown to increase blood glucose (Rauprich et al., 2000).

One week after parturition, there was a sharp increase in the concentration of PUN for the LME and HME groups. At week two, the HME group and the AR group continued to exhibit high concentration of PUN, though the LME group's levels were significantly lower. Kirk and Walker (1976) stated that neonatal sheep have difficulty excreting urea the first few days of life. However, lambs become more efficient as they age, as evidenced by the PUN concentration beginning to level out six weeks post-birth similar to alpaca cria (Burton et al., 2003).

Creatinine levels were higher overall in the HME group than in the LME and AR groups. There was a drop in creatinine levels one week following parturition for all three groups after parturition and concentrations leveled out without a significant increase. Burton et al., (2003) described how metabolic pathways are adjust to new sources of substrate during this first week of life. Protein mobilization and kidney function may account for the high levels of creatinine during the first week of life (Burton, 2003).

## CONCLUSIONS

The results of this study demonstrate that perinatal and post-natal nutrition affects ewe and offspring metabolic processes and milk production. Energy restriction in the LME group resulted in nutrient partitioning allowing ewes in the LME group to produce milk that facilitate lamb growth such that they grew at a rate similar to those from the HME group. It is evident that ewes and offspring can adapt to restricted diets, although body condition will be compromised so that adequate milk is produced for the lambs. The simple diet regime we implemented to meet the needs of the ewes during three different stages of life (late gestation,

parturition, and lactation) was deficient, as evidenced by declining BF of the ewes within six weeks of parturition. This indicated a redistribution of body reserves for lactation. Further research is needed to refine energy requirements of Friesian milking sheep.

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# FIGURES

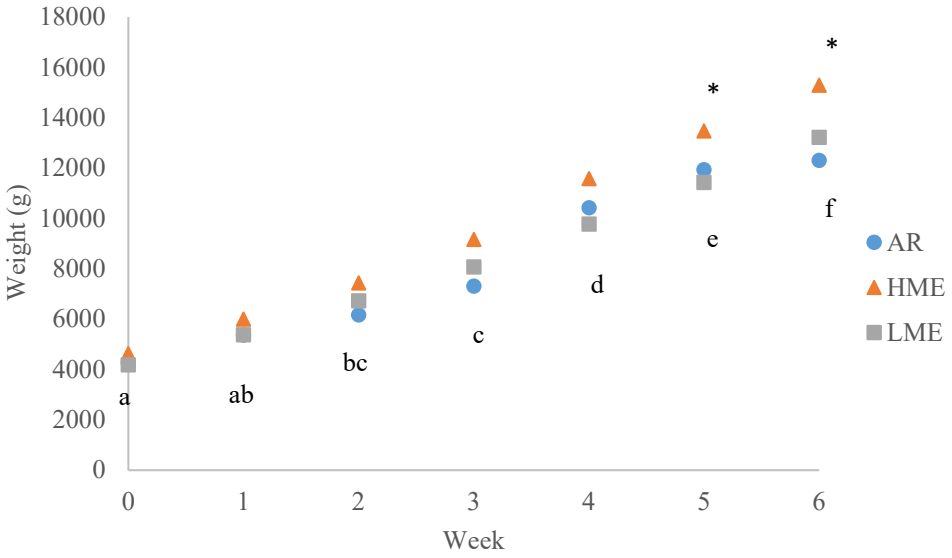


Figure 1-1. Lamb weight changes from birth to six-weeks of age for artificially reared (AR), high energy (HME) and low energy (LME) lambs. Asterisk (*) indicates difference (P<0.05) between HME group and the other two (LME and AR), while "abcdef" indicate differences (P<0.05; SEM 2.47) between weeks.

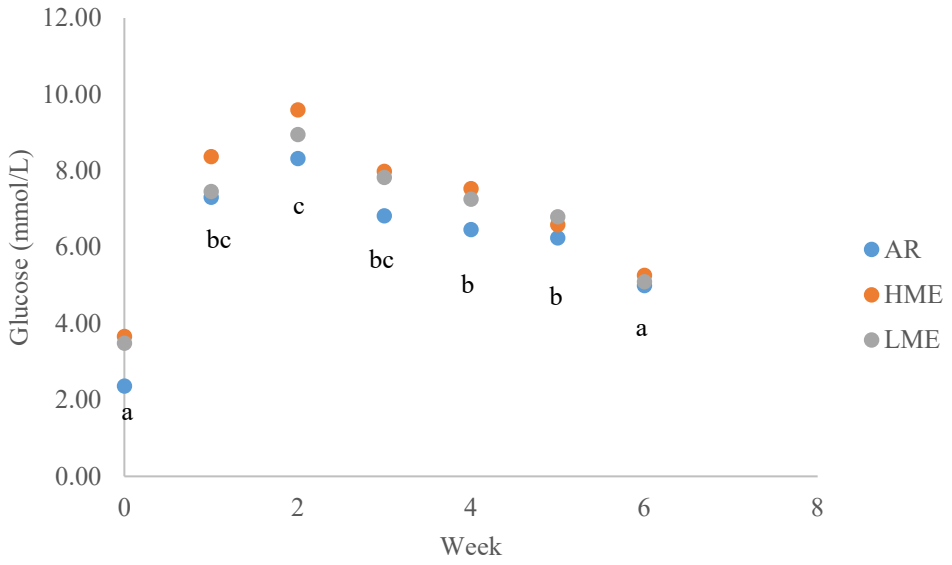


Figure 1-2. Effects of maternal energy intake (80% vs. 140%) on blood glucose concentrations of lambs naturally reared on dams (HME=140%; LME=80%) or artificially reared (AR) on goats milk. Week means with differing "abc" are different at P<0.05 (SEM 0.52). There are no treatment differences.

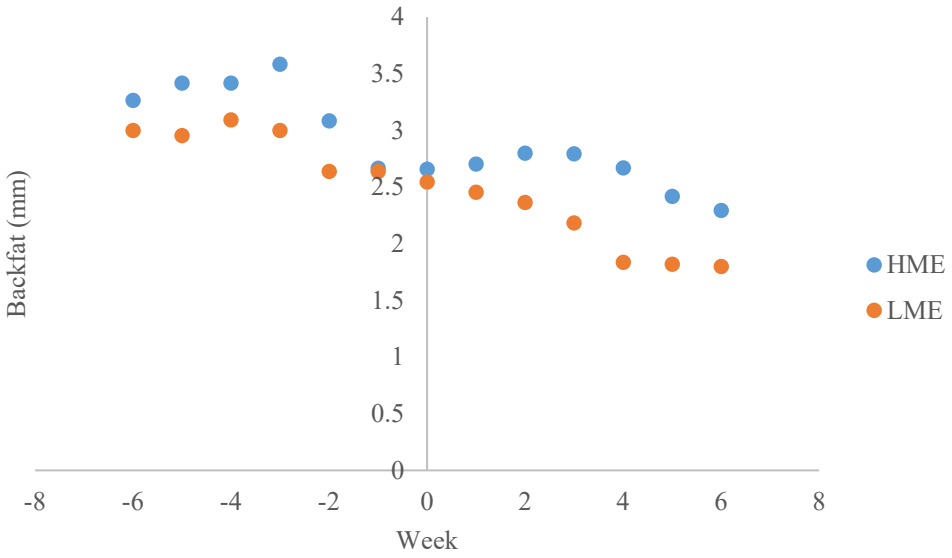


Figure 1-3. Backfat measurements of ewes fed at 80% (LME) or 140% (HME) of NRC (2007) energy requirement. Treatment and week differences are significant at P<0.05 (SEM 0.22).

# TABLES

Table 1-1. Chemical composition of the two diet components (alfalfa and corn) expressed on a percent dry matter basis.

| | Alfalfa | Corn |
|---|---|---|
| Crude protein | 21.4 | 8.9 |
| NDF | 37.1 | 11.0 |
| ADF | 27.9 | 4.1 |
| Lignin | 7.4 | 1.1 |
| NFC | 28.3 | 73.8 |
| Starch | 0.6 | 66.0 |
| Fat | 2.7 | 4.5 |
| Ash | 10.5 | 1.8 |
| NEL, Mcal/kg | 1.39 | 2.07 |

[a]Expressed on dry matter basis

Table 1-2. The diet formulation of both the LME and HME groups adjusted for two stages in life: gestation and lactation.

| | Gestation | | Lactation | |
|---|---|---|---|---|
| | LME | HME | LME | HME |
| DMI, kg/d | 1.59 | 2.10 | 1.95 | 2.58 |
| Alfalfa, kg/d | 1.350 | 1.786 | 1.459 | 1.932 |
| Corn, kg/d | 0.236 | 0.314 | 0.486 | 0.645 |
| NFC, % | 35.1 | 35.1 | 39.6 | 40.8 |
| NDF, % | 33.1 | 33.2 | 30.5 | 30.5 |
| Protein intake, g/d | 310 | 410 | 355 | 470 |

Table 1-3. Ewe weight, backfat and blood metabolites of dairy sheep fed at 80% or 140% of energy requirement for six weeks prior, at, and six weeks post parturition.

| | | Weeks from Parturition | | | | | | | | | | | | | SEM | P<0.05[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diet[a] | -6 | -5 | -4 | -3 | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | | TRT | Week | TxW |
| Weight, | LME | 76.1 | 79.1 | 81.9 | 83.8 | 85.6 | 84.2 | 66.9 | 66.6 | 69.5 | 68.6 | 67.0 | 65.0 | 66.1 | 2.47 | ns | + | ns |
| kg | HME | 75.2 | 78.1 | 80.0 | 81.5 | 84.2 | 84.5 | 67.6 | 69.7 | 69.7 | 65.8 | 65.3 | 65.3 | 64.5 | | | | |
| Backfat, | LME | 3.0 | 3.0 | 3.1 | 3.0 | 2.6 | 2.6 | 2.5 | 2.5 | 2.4 | 2.2 | 1.6 | 1.8 | 1.8 | 0.22 | + | + | ns |
| mm | HME | 3.3 | 3.4 | 3.4 | 3.6 | 3.1 | 2.7 | 2.7 | 2.7 | 2.7 | 2.8 | 2.7 | 2.4 | 2.4 | | | | |
| Glucose, | LME | 3.20 | 3.31 | 3.89 | 4.14 | 4.55 | 4.43 | 10.18 | 3.18 | 3.75 | 3.84 | 4.22 | 4.34 | 3.65 | 0.51 | ns | + | ns |
| mmol/l | HME | 3.81 | 3.91 | 4.13 | 4.76 | 4.49 | 4.81 | 9.43 | 4.10 | 3.05 | 3.56 | 3.97 | 3.72 | 4.22 | | | | |
| UreaN, | LME | 4.80 | 4.33 | 5.26 | 6.35 | 6.84 | 7.02 | 4.55 | 3.44 | 3.54 | 3.78 | 4.84 | 5.00 | 5.01 | 1.28 | ns | ns | ns |
| mmol/l | HME | 4.04 | 3.77 | 3.44 | 3.73 | 3.89 | 3.30 | 4.59 | 3.48 | 4.87 | 4.54 | 4.81 | 5.90 | 5.18 | | | | |
| Creatinine, | LME | 67.2 | 72.3 | 85.3 | 73.2 | 66.7 | 73.2 | 77.0 | 70.7 | 65.3 | 67.5 | 68.9 | 70.0 | 74.9 | 4.42 | ns | ns | + |
| µmol/l | HME | 64.3 | 67.8 | 63.0 | 67.2 | 69.1 | 75.4 | 69.9 | 72.9 | 72.7 | 75.4 | 69.9 | 79.7 | 75.3 | | | | |
| TPP, | LME | 68.0 | 68.6 | 66.7 | 60.7 | 64.0 | 57.4 | 69.3 | 66.0 | 62.2 | 64.9 | 59.0 | 69.5 | 69.6 | 3.2 | ns | + | ns |
| g/l | HME | 73.2 | 73.3 | 68.1 | 65.3 | 66.6 | 65.3 | 73.6 | 50.9 | 65.4 | 62.7 | 65.9 | 66.2 | 70.2 | | | | |
| Triglycerides, | LME | 0.240 | 0.255 | 0.254 | 0.264 | 0.243 | 0.227 | 0.280 | 0.194 | 0.174 | 0.203 | 0.199 | 0.216 | 0.184 | 0.018 | ns | + | ns |
| mmol/l | HME | 0.210 | 0.275 | 0.308 | 0.275 | 0.316 | 0.265 | 0.291 | 0.185 | 0.166 | 0.227 | 0.178 | 0.210 | 0.187 | | | | |

[a]LME = low energy diet; HME = high energy diet.
[b]Symbol + indicates significance.

Table 1-4. Milk yield and milk composition of dairy ewes fed diets providing 80% or 140% of energy requirement.

| | | Week from Parturition | | | | | | P<0.05[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Diet[a] | 1 | 2 | 3 | 4 | 5 | SEM | TRT | Week | TxW |
| Milk yield | LME | 1783 | 1838 | 1813 | 1886 | 1573 | 260 | ns | ns | ns |
| g/d | HME | 1667 | 1785 | 1657 | 1565 | 1650 | | | | |
| Milk Fat, | LME | 5.00 | 3.95 | 3.94 | 4.52 | 4.74 | 0.61 | + | ns | ns |
| % | HME | 3.34 | 2.72 | 2.44 | 2.16 | 2.01 | | | | |
| Milk Fat, | LME | 94.3 | 84.3 | 80.6 | 101.2 | 77.9 | 19.6 | + | ns | ns |
| g/d | HME | 64.3 | 46.4 | 42.2 | 35.4 | 35.6 | | | | |
| Milk Protein, | LME | 5.00 | 4.63 | 4.67 | 4.62 | 4.85 | 0.13 | ns | + | ns |
| % | HME | 4.93 | 4.62 | 4.53 | 4.45 | 4.44 | | | | |
| Milk Protein, | LME | 89.7 | 85.9 | 84.6 | 87.2 | 76.6 | 12.4 | ns | ns | ns |
| g/d | HME | 81.8 | 79.9 | 73.7 | 68.5 | 70.8 | | | | |
| Milk Lactose, | LME | 5.03 | 5.08 | 5.29 | 5.06 | 5.16 | 0.11 | + | ns | ns |
| % | HME | 5.27 | 5.54 | 5.51 | 5.41 | 5.43 | | | | |
| Milk Urea, | LME | 4.89 | 5.25 | 5.21 | 5.29 | 5.57 | 0.48 | ns | ns | ns |
| mmol/l | HME | 4.71 | 5.86 | 6.75 | 6.64 | 5.82 | | | | |
| ECM[b] | LME | 5.36 | 5.14 | 4.99 | 5.61 | 4.80 | 0.92 | ns | ns | ns |
| | HME | 4.33 | 3.88 | 3.57 | 3.22 | 3.33 | | | | |

[a]LME = low energy diet; HME = high energy diet

[b]ECM: energy corrected milk = (0.327 x milk pounds) + (12.95 x fat pounds) + (7.2 x protein pounds).

[c]Symbol + indicates significance.

Table 1-5. Weight and blood metabolite comparisons of pre- and post-parturition ewes fed 80 or 140% required energy intake.

| | | Stage | | | | P<0.05 | | |
|---|---|---|---|---|---|---|---|---|
| | Diet[a] | Pre | Parturition | Post | SEM | TRT | Stage | TxP |
| Weight, kg | LME | 81.8 | 66.8 | 67.2 | 5.11 | ns | + | ns |
| | HME | 80.7 | 67.5 | 68.7 | | | | |
| Backfat, mm | LME | 2.89 | 2.55 | 2.05 | 0.12 | + | + | ns |
| | HME | 3.24 | 2.66 | 2.71 | | | | |
| Glucose, mmol/l | LME | 3.92 | 10.19 | 3.80 | 0.22 | ns | + | ns |
| | HME | 4.32 | 9.43 | 3.77 | | | | |
| Urea N, mmol/l | LME | 5.75 | 4.57 | 4.25 | 0.79 | ns | ns | + |
| | HME | 3.68 | 4.57 | 4.75 | | | | |
| Creatinine, mg/dL | LME | 0.825 | 0.871 | 0.785 | 0.028 | ns | ns | + |
| | HME | .768 | 0.791 | 0.840 | | | | |
| TPP, g/l | LME | 64.2 | 69.1 | 64.9 | 1.4 | ns | + | ns |
| | HME | 69.3 | 74.2 | 63.8 | | | | |
| Triglycerides, mmol/l | LME | 0.247 | 0.280 | 0.195 | 0.009 | ns | + | ns |
| | HME | 0.275 | 0.291 | 0.192 | | | | |

[a]LME = low energy diet; HME = high energy diet.
[b]Symbol + indicates significance.

Table 1-6. Effects of maternal energy intake (80% vs. 140%) on weight and blood metabolites of lambs naturally reared on dams or artificially reared on goats milk for six weeks post birth.

| | | Weeks from Birth | | | | | | | | P<0.05[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diet[a] | 0 | 1 | 2 | 3 | 4 | 5 | 6 | SEM | TRT | Week | TxW |
| Weight, kg | LME | 4180 | 5378 | 6722 | 8076 | 9771 | 11426 | 13221 | 520 | + | + | ns |
| | HME | 4625 | 5997 | 7442 | 9158 | 11575 | 13478 | 15288 | | | | |
| | AR | 4256 | 5360 | 6161 | 7304 | 10416 | 11933 | 12300 | | | | |
| Glucose, mmol/l | LME | 3.48 | 7.46 | 8.95 | 7.83 | 7.25 | 6.80 | 5.10 | 0.52 | ns | + | ns |
| | HME | 3.66 | 8.37 | 9.59 | 7.99 | 7.53 | 6.59 | 5.26 | | | | |
| | AR | 2.37 | 7.30 | 8.32 | 6.82 | 6046 | 6.24 | 5.00 | | | | |
| Urea N, mmol/l | LME | 4.91 | 6.39 | 2.00 | 1.98 | 2.40 | 3.26 | 3.65 | 0.64 | ns | + | + |
| | HME | 4.83 | 6.44 | 2.43 | 2.25 | 2.93 | 3.62 | 3.89 | | | | |
| | AR | 5.54 | 2.61 | 2.93 | 2.17 | 2.85 | 2.92 | 3.25 | | | | |
| Creatinine, mmol/l | LME | 157.9 | 74.1 | 46.0 | 64.4 | 49.1 | 57.9 | 57.9 | 11.5 | ns | + | ns |
| | HME | 204.2 | 111.8 | 56.2 | 68.4 | 58.8 | 55.2 | 54.0 | | | | |
| | AR | 177.7 | 59.3 | 59.1 | 74.5 | 56.5 | 57.9 | 65.8 | | | | |
| TPP, g/l | LME | 42.2 | 63.9 | 55.9 | 59.7 | 57.8 | 57.9 | 66.8 | 2.3 | + | + | + |
| | HME | 49.2 | 62.4 | 58.7 | 62.0 | 58.0 | 56.2 | 58.4 | | | | |
| | AR | 46.8 | 54.3 | 53.3 | 56.5 | 53.0 | 53.0 | 56.7 | | | | |
| Triglycerides, mmol/l | LME | 0.552 | 0.576 | 0.500 | 0.638 | 0.774 | 0.561 | 0.405 | 0.066 | + | + | ns |
| | HME | 0.554 | 0.531 | 0.596 | 0.580 | 0.668 | 0.598 | 0.570 | | | | |
| | AR | 0.432 | 0.498 | 0.463 | 0.498 | 0.531 | 0.367 | 0.470 | | | | |

[a]LME = low energy diet; HME = high energy diet; AR = artificially reared lambs.
[b]Symbol + indicates significance.

# CHAPTER 2

## Digestion of Soybean Meal in Alpacas

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## ABSTRACT

The objective of this experiment was to determine the degradation parameters of grass hay (GH) supplemented with soybean meal (SBM) and to determine the effects of SBM on compartment 1 of the rumen (C1) ammonia (NH3-N) and volatile fatty acid (VFAs) concentrations in alpacas. Four C1 fistulated adult male alpacas (*Vicugna pacos*) (7±1.5 years old; 61±5 kg body weight; BW) were housed in metabolism crates and received water *ad libitum* during the treatment periods of this study. The GH and SBM treatments were fed at 0700 every day. Treatment periods were for 14 days in which GH or SBM treatments were randomly allocated to each alpaca. On day 14 volatile fatty acids (VFAs), pH and NH3-N were determined at 1, 3, 6, 10, 14, 18 and 24 hrs post feeding. C1 degradation of GH and SBM was determined with the alpacas being fed GH only and the samples incubated for 0, 2, 4, 8, 14, 24, 48 and 72 hrs. Dry matter (DM) and crude protein (CP) degradation were determined and divided into three categories: *a* = immediately soluble; *b* = non-soluble but degradable; and *u* = non-degradable/unavailable, potential extent of degradation (PE), degradation rate (*c*), effective degradation (ED) and kp (passage rate) = 5.5%·hr-1). Total DM intake was different between the two treatments ($P<0.05$), while CP intake was increased (72.5 to 191.0 g/d) with the addition of SBM. SBM NH3-N level was greater than GH. Total VFA concentration was not different with the inclusion of SBM, but for percent composition a shift was noted away from acetate (Ac) towards propionate and butyrate (Pr, Bu). DM fraction *a* was not different between GH and

SBM, while there was for fraction *b.* CP fractions a and b were different. Degradation rate was not different for DM, but was for CP. From these data we learned that SBM can be a CP supplement when the diet is deficient to improve microbial yield, but care should be taken to avoid causing a protein-energy imbalance.

## INTRODUCTION

Soybean meal (SBM) is a solid product resulting from the oil extraction process from whole soybeans. More than 99 percent of soybean meal today is produced through the solvent-extraction process in which the dehulled, conditioned flakes of soybeans are washed in a countercurrent manner with hexane. The solvent solubilizes the lipid material in the soybeans and the hexane-oil mixture is separated through a series of steps. After evaporation of the oil-rich extract, the "spent flakes" of soybeans are toasted, then dried and cooled. Soybean meal is used primarily as a filler and protein source in animal diets. The typical composition of soybean meal produced through the solvent-extraction process is 90% dry matter, 44.0% crude protein, 0.5% ether extract, 7.0% crude fiber, and 6.0% ash.

Studies of the effects of soybean meal on many animals have been performed, but the digestion kinetics and characteristics have not yet been determined in alpacas (*Vicugna pacos*). Alpaca digestive systems are comprised of three compartments similar to that of the ruminant species, they are called compartment 1 (C1), compartment 2 (C2), and compartment 3 (C3). The use of soybean meal in animal diets has been used to alter levels of amino acids in the diet that are typically deficient in grains and grain by-products (Swick et al., 1995; Swick, 1998). The increased protein content in the diet should be

beneficial to the microflora of the alpaca since the additional protein and energy available to the rumen microflora increases the production of VFAs from the fermentation of these components.

It has been shown that pseudo-ruminant species produce and absorb VFAs from the digestive tract (Bergman, 1990). Engelhardt and Sallmann (1972) showed that large quantities of VFA were absorbed in the C1 and C2 of guanaco (*Lama guanicoe*), particularly in the glandular saccule region. Engelhardt et al., (1979) found in llamas that absorption of water, sodium and VFA's also occurred in the C3. Species differences between camelids and ruminants occur in rumen volume, dilution rates and absorption rate of VFA's which affects volatile fatty acid concentrations (Abbas et al., 1995; Elsden, 1946).

Rumen microbial nitrogen requirement for protein synthesis is met by ammonia, free amino acids, and peptides obtained from degradation of dietary CP and recycled CP (Boucher et al., 2007). Camelids are also known to recycle nitrogen at a higher rate, although feed protein degradation is not different between camelids, sheep and goats (Jouany et al., 1995). Due to the camelid's higher rate of nitrogen recycling, it is assumed that urea supplementation would be detrimental to the C1 ecosystem because of the readily available nitrogen. As such, SBM is a good candidate as a CP supplement because of its slower degradation rate observed in other species. The objective of this experiment was to determine the degradability of grass hay (GH) supplemented with SBM and the C1 NH3-N and VFA production associated with the addition of SBM in alpacas.

## METHODS

Four adult male alpacas ($\bar{x}$ = 7±1.5 years old; 61±5 kg BW) were housed in metabolism crates during the treatment periods of this study. Each alpaca had previously been instrumented

with a C1 fistula, as outlined by Robinson et al. (2013). Care of these animals followed animal use and care guidelines (FASS, 2010) provided by the The Camelid Center Animal Use Committee. When not collecting samples, the alpacas were walked for 30 minutes daily. Alpacas were fed a mixed-grass hay forage (orchard grass (Dactylis glomerata); meadow bromegrass (Bromopsis biebersteinii); smooth bromegrass (Bromus inermis)) for thirty days prior to the experiment allowing them to acclimate to this GH forage. During this period, the alpacas were fed ad libitum at 0700, and water was offered ad libitum. Dry matter intake (DMI) was measured during the last seven days of the acclimation. This research was conducted in two trials: in situ degradation and determination of C1 VFA concentrations of GH and GH supplemented with 333g DM soybean meal (SBM).

*In Situ digestibility trial*

This trial followed the thirty-day acclimation period to GH. During this trial, alpacas were fed GH at 0700 and 1900 to provide a steady state of digestion (Vanzant et al., 1998). DMI was determined during the end of the thirty-day acclimation period and was divided in half for the twice-daily feeding. DMI was calculated using feed refusal (i.e., non-ingested GH) gathered daily that was weighed and dried for each animal. Grab samples of the GH fed were dried throughout both trials and used to determine the quantity of DM fed. From this value for DM fed, the dried refusal was subtracted and daily DMI calculated. Daily DMI values were used to statistically determine the treatment DMI for each treatment.

In situ substrate samples included GH and SBM, and each substrate was ground through a Wiley Mill (Aurthur H. Thomas Co., Philadelphia, PA) with a 2mm screen. Four samples of each substrate were prepared by weighing 5 g of each, then placing them into 10cmx20cm Dacron bags (50μm pore; Ankom Technology, Macedon, NY). Dacron bags of

substrate were soaked in water (39˚C) for 20 min, prior to incubation, to reduce lag time associated with wetting. Substrates were incubated for 0, 2, 4, 8, 14, 24, 48 and 72 hrs. Bags were placed into the C1 of each alpaca and removed at the same end time. Upon removal, the bags were placed in ice water to halt further microbial digestion. Additionally, each bag was rinsed until the water ran clear (~15 min). The 0-hr samples were soaked and rinsed as outlined without incubation. Following rinsing, bags were dried at 50˚C for at least 48 hrs to a dried weight.

Residual material from in situ incubation was analyzed for DM and CP. CP concentration was determined using a LECO combustion N analyzer (LECO TruSpec, St. Joseph, MI, USA). N values determined by the LECO analyzer were then converted to CP, using the standard conversion factor of 6.25. Total DM and CP degradation were divided into three pool fractions: *a* = immediately soluble; *b* = the non-soluble but degradable; and *u* = the undegradable/unavailable fraction. Fraction calculations were performed as outlined by Nilsen et al., (2015). Effective C1 disappearance (ED) of DM and CP were determined as described by Ørkov and McDonald (1979) as a + b x (c/(c + kp)), where c the disappearance rate (%·hr-1) and kp is the passage rate (5.5%·hr-1; Nilsen et al., 2015).

*Volatile Fatty Acid Trial*

Two treatments consisted of grass hay (GH), and GH supplemented with 333g dry matter (DM) soybean meal (SBM). The level of SBM inclusion was targeted to triple the total CP intake. Alpacas were fed at 0700 each day. Each treatment period was for 14 days. The SBM supplement was fed first and was consumed within 15 min of feeding. Hay was fed upon completion of SBM consumption. Diurnal C1 VFA samples were collected on day 14 and processed as outlined by Oldham et al., (2013) at 1, 3, 6, 10, 14,

18 and 24 hrs post feeding. Samples were immediately processed for VFA analysis and the supernatant frozen for future analysis (Oldham et al., 2013). In addition, 8ml of strained C1 fluid was added to 2 ml of 25% metaphosphoric acid, mixed and frozen for future analysis of NH3 (Chaney and Marbach, 1962). The remaining C1 sample was used for pH determination using a pH meter (Corning 340, Tewksbury, MA, USA) with a combination probe.

*Statistical Analysis*

The in situ DM and CP degradation parameters were determined by fitting the data to the nonlinear regression model of Ørkov and McDonald (1979) using Proc NLIN of SAS (SAS, 2002). The GLM procedure (SAS, 2002) was used to determine the treatment effects of the degradation estimates. Least square means for treatments were determined using unadjusted *t* tests and a level of significance at $P<0.05$. The diurnal VFA and pH data were analyzed using a linear mixed model with treatment, time, and the interaction as main effects with time treated as a repeated measure (Littell et al., 1998). The SAS (SAS, 2002) procedure MIXED was used for these calculations and a probability of $P<0.05$ was considered significantly different. Least square means for levels of the treatment/time factors were calculated and compared using unadjusted *t* tests.

## RESULTS

The chemical composition of the feeds used in this experiment are presented in Table 2-1. The DM degradation parameters for GH and SBM are found in Table 2-2 and the patterns are presented in Figure 2-1. Fraction *a* DM degradation (Fig. 2-1, panels A and B) was not different

between treatments. The *b* fraction was different between GH and SBM where GH was greater than SBM. Potential extent (PE) of DM degradation, based on the sum of fraction *a* and *b*, was greater for GH than SBM, while CP PE was not. The rate of DM degradation was not different between the treatments at 8.1 and 10.3 %·hr-1 for GH and SBM, respectively. Crude protein degradation rate (Fig. 2-1, panel C and D) was greater for SBM by 350% at 1.9%·hr-1 for GH and 8.6 %·hr-1 for SBM. Using acid detergent insoluble ash as a marker, Nilsen et al., (2015) calculated kp from four alpacas to be 0.0549 %·hr-1 ± 0.0173 fed the same GH as used in this experiment. We used this derived value of (0.0549) to determine effective degradation (ED). Dry matter ED was the same for GH and SBM at 51.5% and 54.9% respectively, while CP ED was greater for SBM than GH at 49.8% and 63.5% respectively.

Dry matter intake during the VFA trial was not different for GH and SBM at 944 g/d and 878 g/d, while the inclusion of SBM did decrease the DM hay intake to 545 g/d. The SBM treatment CP intake, as we planned, was 195.5 g/d, higher than GH of 71.4 g/d. The C1 diurnal pH was not different between the sampling times or between the two treatments. The mean treatment pH (see Table 2-3) was different where GH and SBM were 6.81 and 6.65, respectively. The SBM NH3-N (Table 2-3; 9.28 mg/dL) was 164% greater than GH at 3.52 mg/dL. Volatile fatty acid concentrations and proportions (Table 2-3) were not significant between the diurnal samples collected, so the data are not presented. No differences were noted between the treatment total VFA concentrations (63.1 and 67.7 mmol/l). The Ac and Bu molar concentrations were not different between the GH and SBM treatments, while Pr increased from 12.0 mmol/l for GH to 14.7 mmol/l for SBM. Expressed on a percentage basis, Ac was higher for GH at 72.4% than SBM at 69.5%. The inclusion of SBM increased the percentage of Pr and Bu above the GH treatment. The acetate:propionate ratio was not different.

# DISCUSSION

Camelid digestive processes include a longer particle retention time (Heller et al., 1986), greater volatile fatty acid absorption and nitrogen recycling than other ruminants (Rübsamen and Engelhardt, 1979; Engelhardt et al., 1984). These digestion dynamics are why the camelid system is unique from true ruminants. Retention time of llamas is 50% longer than sheep fed the same diet (Lemosquet et al., 1996). Nitrogen recycling is an important component of the camelid's protein metabolism. Farid et al., (1979) concluded that nitrogen conservation is due to a decrease in fecal and urine N excretion. Comparing sheep and llama renal urea N excretion, Hinderer and Engelhardt (1975) showed llama's excretions were lower than sheep because urea N turnover in llamas was 3% versus 12% in sheep. Protein supplements are added to increase dietary nitrogen. Soybean meal is one of the most commonly used protein supplements in the United States animal industry. The addition of supplemented N improves microbial activity and fermentation (Mahouachi et al., 2003). While N supplementation can be beneficial, N utilization by the C1 microbiome is dependent on dietary carbohydrate content. Volatile fatty acid, NH3-N concentrations and buffering components all contribute to the physio-chemical stability of the C1 environment where all of these factors result in more efficient microbial activity. Efficient microbial activity equates to an increase in VFA production for energy usage by the animal. Improvement of N efficiency is dependent upon an understanding of C1 N sequestration, protein digestion, degradation and intestinal absorption.

The soluble *a* DM fraction degradation of soybean meal by alpacas in our study were similar to those found by Gonzales et al., (2002), while the *b* fraction and kp were different. Gonzales further showed that the degradation of similarly processed SBM from

different sources are different and concluded that processing of the SBM influences rumen availability. Soybean meal degradation has not studied in alpacas. Several studies have looked at SBM degradation in a number of other species. Marghazini et al., (2013) showed in Nili-Ravi buffalo (Bubalus bubalis) CP degradation of SBM was 16.5 and 71.8% for fractions *a* and *b*, with a degradation rate of 0.178 h-1. In sheep fed an alfalfa/concentrate diet, Kamalak et al., (2005) found SBM DM degradation to be 25.1 and 56.5% for fractions *a* and *b* respectively with a degradation rate of 0.06 h-1. For the same sheep SBM CP degradation fractions *a* and *b* were 33.4 and 51.3% with the same degradation rate. Huntington and Givens (1997) showed in cattle and sheep SBM degradation of 26 and 72% for fractions *a* and *b* with a degradation rate of 0.078 h-1. Though the species are different, the results are similar and variation may be accounted for by Gonzales et al., (2002)'s findings. These findings are also in agreement with Prigge et al., (1984) who concluded that degradation of feeds is similar for mature ruminants.

Effective degradation for the GH was higher as a percentage than that noted by Stevens et al., (2014). They estimated the kp to be between 2 and 4%, while data from Nilsen et al., (2015) fed the similar hay to that used in this study measured kp to be 5.49%. On a DM basis there was no difference between GH and SBM, but on a CP basis SBM ED was greater than GH. The inclusion of SBM in the present study to GH shows an effect on the production of NH3-N and VFA composition with no effect on pH. The results were an increase in NH3-N and a change in the proportion of VFA from Ac to Pr and Bu. Lourenco et al., 2013 showed in sheep fed meadow grass hay that the inclusion of SBM increased rumen NH3-N by 130%. This increase in NH3-N was in conjunction with no change in rumen pH, %Pr or total VFA, but a lower %Ac and increased Bu and Ac/Pr were noted. Steers that consumed fescue straw with SBM

supplementation at similar inclusion as our study, Cappellozza et al., (2013) saw no difference in pH, total VFA, VFA composition or the Ac/Pr ratio. Raboisson et al., (2012) fed rumen cannulated steers SBM at 1 and 2% BW and concluded that an increase in rumen NH3-N was a result of the rapid degradation of the SBM. SBM protein and carbohydrate components are reported to produce a rapid increase in VFA after consumption (Sauvant et al., 2002). Jouany et al., (1995) concluded that the buffering capacity of camelids was better under acidic conditions and that VFA absorption was partially responsible. The C1 pH is closely tied to VFA production and absorption. Darlis et al., (2000) found goats and sheep fed rice straw supplemented with SBM had 118 and 110 mm total VFA, respectively. Acetate, propionate and butyrate percentages were different between the two species and were 79.1 and 75.8%, 15.6 and 18.0%, 5.3 and 6.0%, respectively. The effects of SBM on NH3-N, VFA and pH vary and may be due to the forage quality being supplemented. Taminga (1983) stated that solubility, susceptibility of microbial proteases and residence time in the rumen are factors that affect degradation of protein. Our findings suggest that the total diet must be looked at to assure SBM supplementation will be advantageous.

Mahouachi et al., (2003) stated that, rumen N-NH3 less than 50 mg/l will have a limiting effect on microbial synthesis (Satter and Slyter, 1974). Jouany et al., (1995) demonstrated in camelids and sheep fed low quality hay that, although camelids have a higher N recycling, feed protein degradation to N-NH3 is not different between the two species. They found N-NH3 concentration in the foregut was lower in the camelids having lower N-NH3 concentrations in the foregut than that found in sheep was due to faster passage of the fluid phase, faster rate of absorption across the stomach wall or a higher uptake by the microbial population. Dulphy et al., (1997) concluded that the higher water turnover rate found in llamas, as compared

to sheep, may in fact increase cellulolytic activity because of the rapid passage of substances that could hinder microbial growth.

## CONCLUSIONS

Data from this study provide the degradation patterns of SBM and GH which can be used to enhance our understanding of protein-energy deficiencies of alpacas. Further research needs to be conducted to determine the level of SBM that can foster optimal microbial growth. The CP fraction degradation and rate kinetics of SBM for alpacas is of interest because of their unique digestive system. Carmalt (2000) presents a case for protein-energy malnutrition syndrome in alpacas. The quality of the diet is believed to be the cause of this syndrome, where either CP or energy are lacking and interacting with the efficient use of the other. Hall and Huntington (2008) discussed nutrient synchrony, where simultaneous provision of carbohydrates (energy) and protein improve microbial functions in the rumen.

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FIGURES

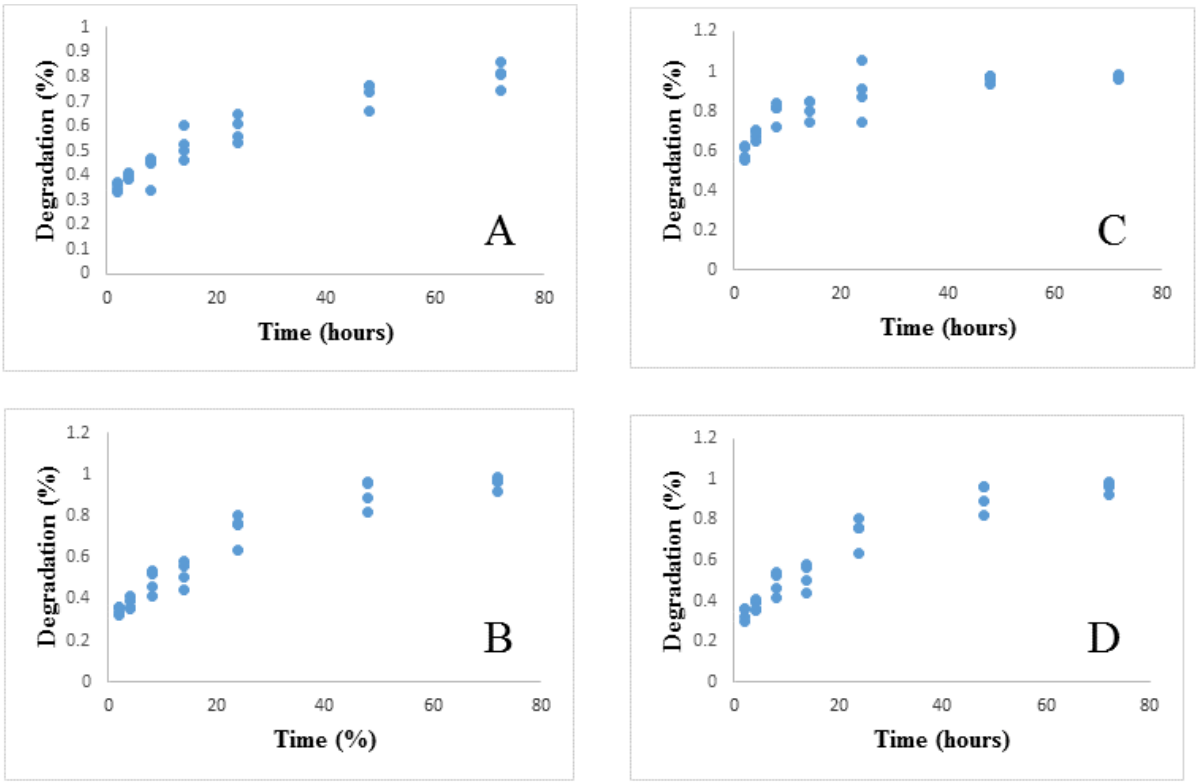


Figure 2-1. Dry matter degradation pattern for grass hay (Panels A) and soybean meal (Panel B) and crude protein degradation for grass hay (Panel C) and soybean meal (Panel D).

# TABLES

Table 2-1. Composition of feed[a] for both grass and soybean meal (SBM) presented in percentages with the supplementation presented in grams.

| | Grass | SBM |
|---|---|---|
| Dry matter, % | 93.0 | 91.6 |
| Crude protein, % | 11.4 | 51.6 |
| NDF, % | 56.8 | 11.4 |
| ADF, % | 34.0 | 7.2 |
| Lignin, % | 4.8 | 0.9 |
| Fat, % | 2.6 | 1.7 |
| Ash, % | 8.8 | 8.4 |
| Non-fiber carbohydrate[b], % | 23.5 | 27.7 |
| Starch, % | 1.1 | ND |
| Calcium, % | 0.38 | 0.27 |
| Phosphorus, % | 0.24 | 0.87 |
| Potassium, % | 2.16 | 2.40 |
| | | |
| Supplement fed | | |
| Dry matter fed, g | | 333 |
| Crude protein fed, g | | 172 |

[a]Analysis of duplicate samples were performed by Dairy One Forage Lab using wet chemistry procedures.

[b]Fraction determined by calculations. NFC = 100–CP–NDF–Fat–Ash–Bound protein.

Table 2-2. Dry matter and crude protein degradation kinetics for grass hay and soybean meal (SBM).

| | DM | | | CP | | |
|---|---|---|---|---|---|---|
| | Grass Hay | SBM | SEM | Grass Hay | SBM | SEM |
| *a* | 24.0 | 30.5 | 2.51 | 35.3[d] | 25.7[c] | 1.34 |
| *b* | 50.0[b] | 37.3[a] | 0.91 | 57.2[c] | 66.3[d] | 2.44 |
| *c*, %/h$^{-1}$ | 8.1 | 10.3 | 1.5 | 1.9[c] | 8.6[d] | 1.6 |
| Potential extent, % | 74.0[b] | 67.8[a] | 1.87 | 92.5 | 92.0 | 2.42 |
| Effective degradation, % | 51.5 | 54.9 | 1.67 | 49.8[c] | 63.5[d] | 4.14 |

[ab]Row means within DM with differing superscripts are significantly different at P<0.05. [cd]Row means within CP with differing superscripts are significantly different at P<0.05.

[*]*a* = immediately soluble; *b* = the non-soluble but degradable; *c* = degradation rate based on the equation $a + b(1 - e^{-ct})$ Ørkov and McDonald (1979). Potential extent is the sum of *a* + *b* and effective degradation is calculated as $a + (b \times c/(c + k_p))$, where $k_p$ is 0.0549.

Table 2-3. Dry matter intake, $NH_3$-N, concentration and composition of volatile fatty acids in the C1 of alpacas fed grass hay and grass hay soybean meal (SBM).

| | Treatment | | |
|---|---|---|---|
| | Grass hay | SBM | SEM |
| Dry matter intake, g/d | 944 | 878 | 74.0 |
| Hay DM intake, g/d | 944[b] | 545[a] | 74.0 |
| Crude protein intake, g/d | 72.5[a] | 191[b] | 5.69 |
| NDF intake, g/d | 380[a] | 342[a] | 33.3 |
| pH | 6.81 | 6.65 | 0.06 |
| $NH_3$-N, mg/dl | 3.52[a] | 9.28[b] | 0.55 |
| Acetate, mmol/l | 45.7 | 46.9 | 2.26 |
| Propionate, mmol/l | 12.0[a] | 14.7[b] | 0.92 |
| Butyrate, mmol/l | 5.44 | 6.11 | 0.31 |
| Total, mmol/l | 63.1 | 67.7 | 3.27 |
| AC/PR | 3.87 | 3.37 | 0.43 |
| Acetate, % | 72.4[b] | 69.5[a] | 0.84 |
| Propionate, % | 19.4[a] | 21.5[b] | 0.78 |
| Butyrate, % | 8.58[a] | 9.03[b] | 0.11 |

[ab]Row means with differing superscripts are significantly different at $P<0.05$.