INFLUENCE OF COLOSTRUM FROM SAFFLOWER SUPPLEMENTED EWES ON LAMB COLD TOLERANCE AND LAMB GROWTH

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Brenda Lee Robinson

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Brenda Lee Robinson

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Dr. Rodney W. Kott

Approved for the Department of Animal and Range Sciences

Dr. Bret Olson

Approved for the Division of Graduate Education

Dr. Carl A. Fox

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ABSTRACT

Five hundred, ninety-seven and 643 white face range ewes were used in a 2 year study, respectively, in a 3-way factorial arrangement to determine effect of supplemental linoleic oil on lamb serum metabolites, thermogenesis and lamb growth. During the last 45 ± 4 days of gestation ewes were group fed a daily supplement of either 0.23 kg·ewe^{-1}·d^{-1} whole safflower seed (SS) or 0.34 kg·ewe^{-1}·d^{-1} whole barley (C). Colostrum was collected from each supplement treatment and pooled according to treatment. At parturition, twin born lambs received either SS or C pooled colostrum. One h postpartum, lambs were placed in a 0^{o}C dry cold chamber for 30 min. and lamb rectal temperature was recorded at one min intervals. Blood samples were taken prior to and after cold exposure. Lamb weights were taken at birth, turnout and weaning for growth. There was no difference ($P > 0.10$) in body temperatures between lambs born to ewes supplemented with SS vs. C or between lambs given pooled colostrum form SS or C supplemented ewes. At 0 minutes lambs born to SS supplemented ewes had lower glucose ($P = 0.05$), total protein ($P = 0.05$) and NEFA ($P = 0.003$) and higher BUN ($P = 0.03$) than lambs from C supplemented ewes. Lambs from SS supplemented ewes had lower NEFA ($P = 0.01$) after cold exposure (30 minutes). Total protein concentration was similar in year 1 but lower ($P < 0.01$) in year 2. Concentrations of αGP were higher in lambs from SS supplemented ewes at both 0 and 30 min in year 1. Lambs from SS ewes had higher ($P < 0.01$) blood αGP levels at both 0 and 30 min in year 1 than in year 2. No differences ($P > 0.30$) were detected between lambs from SS ewes vs. C for changes in any blood metabolites measured during cold stress period. No differences ($P > 0.12$) were detected for average date of birth, number of lambs born, number of lambs present at turnout and weaning or for weight of lambs at turnout or weaning for ewes receiving either SS or C supplements.

CHAPTER 1

INTRODUCTION

Lamb mortality is one of the leading causes of lowered productivity in sheep operations. Previous research at Montana State University (Safford and Hoversland, 1960; Kott et al., 1998) suggests that in a typical shed lambing operation, 23% of all lambs born die between birth and weaning. Half of these lambs died during the first 45 days of life. Starvation and weather interact to be the major cause of mortality in lambs less than 30 days of age. At parturition, the survival of the newborn lamb depends on its ability to adapt to its new environment (Alexander, 1962a). Neonatal lambs and calves have well developed thermogenic mechanisms, including both shivering thermogenesis in muscle tissue and non-shivering thermogenesis (Alexander and Williams, 1968). In advanced thermoregulators, of which lambs and calves are typical examples, the ability to utilize non-shivering thermogenesis in BAT is vital to prevent hypothermia in the critical period of time directly following birth. Stott and Slee (1987) estimated that by improving the lambs ability to tolerate cold condition by as little as 7 minutes could result in a 9% decrease in post lambing mortality. Hypothermia is expected in a proportion of newborn lambs in exposed windy conditions if the ambient temperature were to fall below 23^{o}C (Alexander, 1962a). Alexander (1961) and Hamadeh et al. (2000) both saw an initial rise in temperature without shivering prior to a decrease in lamb body temperature associated with cold stress, concluding that the heat production could be attributed to non-shivering thermogenesis.

Previous research (Nedergaard et al., 1983; Lammoglia et al., 1999a; Encinias et al., 2004) indicates that supplementing the diet of dams with a high linoleic and linolenic acid supplement just prior to parturition can affect cold tolerance in their offspring. Therefore, the objectives of this study will be to evaluate the effects of supplementing late gestating ewes with a high linoleic acid diet on lamb thermogenesis and the resulting colostrum on the cold tolerance of newborn lambs.

CHAPTER 2

LITERATURE REVIEW

Lamb Survival

Lamb mortality is one of the leading causes of lowered productivity in sheep operations. A three year study conducted at Montana State University by Safford and Hoversland (1960) reported 13.9 and 23.5% mortality in lambs between birth and 45 days of age and birth to weaning, respectively. The average age of mortality for lambs dying within the first 45 days of age was 5.9 days. Fifty-six percent of these deaths were within 3 days of birth and 73% within 5 days. Kott and Thomas (1987) reported that during the years of 1967 to 1984, the average yearly lamb mortality in Montana ranged from 17.3% to 28.8%, averaging 23%. Rowland et al. (1992) monitored four typical spring, shed-lambing western range sheep operations in Colorado. They found that lamb losses ranged from 8.2 to 12.2%. Although mortality values reported were smaller than the previously reported Montana studies, the overall mortality was probably similar due to the fact that in the Colorado study, mortality was only monitored during the lambing period (approximately one to one and a half months). In each of the four flocks, mortality monitoring was stopped 10 days after the last ewe lambed. These researchers reported 50% of lamb mortality occurred during parturition or within 24 h after parturition. Greater than 85% of these lamb deaths occurred in those born to ewes having 2 or more lambs. These results are similar to those reported by other workers (Moule, 1954; Matthews and Ogden, 1957; Gates, 1977, Nass, 1977; Rook, 1997).

Kott and Thomas (1987) reported that the major causes of lamb mortality in Montana are predators (35.5%) and weather (30.6%). Previous Montana researchers (Safford and Hoversland, 1960) found that 72% of all lamb losses could be classed into five major categories; pneumonia (16%), starvation (13.8%), stillbirths (14.3%), dysentery (11.8%), and no visible lesions (15.8%). More recently, Rowland et al. (1992) reported that the leading causes of newborn lamb death in four western Colorado shed-lambing operations were starvation, dystocia, stillbirth (unknown cause), and infectious diseases. These results are similar to those reported by other research in Montana (Jordan et al., 1985) and in other parts of the western United States (Gates, 1977; Gee et al., 1977; Nass 1977; Shelton and Wade 1979).

Hyperthermia

At parturition, the survival of the newborn depends on its ability to rapidly adapt to its new environmental conditions. In many cases cold and starvation interact to comprise the major cause of death (Slee, 1977). Cold and starvation can account for up to half of all death losses (Alexander, 1962b and Houston and Maddox, 1974). Eales et al. (1982) defined the time periods between birth and 5 hours of age (early) and between 12 and 36 hours of age (late) as two periods of high risk from hypothermia.

The major cause of hypothermia during the early time period is excessive heat loss. Newborn lambs are saturated in fetal fluid and are highly susceptible to hypothermia (Alexander, 1962a). Eales et al., (1982) stated that in the late period with older lambs, hypothermia is largely the result of depressed heat production associated

with depleted energy reserves and starvation. In their study, early hypothermia accounted for 24% of hypothermic lambs. The remainder became hypothermic between 12 and 36 h of age as the limited energy reserves present at birth were depleted. Cold exposure may not only deplete body energy reserves by increasing energy demands but also prevent the replenishment of reserves by reducing mobility and inhibiting the suckling drive. Thus cold exposure can accelerate death by starvation as well as cause acute hypothermia (Alexander and Williams, 1968). A viable lamb must possess sufficient energy reserves via brown fat at birth and then nurse relatively soon after birth.

A lamb's ability to regulate its body temperature is well developed at birth (Alexander, 1962a). Stott and Slee (1987) estimated that by improving the lambs ability to tolerate cold conditions by as little as 7 minutes could result in a 9% decrease in post lambing mortality. Hyperthermia is expected in a proportion of newborn lambs in exposed windy conditions if the ambient temperature falls below 23^{o}C (Alexander, 1962a). The lower critical body temperature or temperature at which an increased metabolic rate is necessary to maintain body temperature of dried newborn lambs (Alexander, 1961) and calves (Vermorel et al., 1989) appears to be around 22^{o}C. Alexander (1961) found newborn lambs were able to sustain body temperature in ambient temperatures as low as -5^{o}C, by increasing heat production 2-3 times basal levels. Alexander (1961) and Hamadeh et al. (2000) both saw an initial rise in temperature without shivering prior to a decrease in lamb body temperature associated with cold stress, concluding that the heat production could be attributed to non-shivering thermogenesis.

The cold stress/starvation complex is also a major issue in the beef cattle industry. Calf losses have a serious negative impact on the economic viability of the cow/calf producer and the beef industry as a whole (Bellows et al., 1987). In 1995, ranchers in Montana lost approximately 23,000 calves because of severe weather (Stockgrowers Newsletter, 1996). Hypothermia is a problem faced by neonatal calves in regions that experience cold inclement weather during calving (Olson et al., 1980; Robinson and Young, 1988). Total neonatal mortality has been estimated to be approximately 9%, with about 7% of this mortality attributable to cold stress (Bellows et al., 1987).

Lamb Body Temperature Regulation

At parturition, the survival of the newborn lamb depends on its ability to adapt to its new environment (Alexander, 1962a). Neonatal lambs and calves have well developed thermogenic mechanisms, including both shivering thermogenesis in muscle tissue and non-shivering thermogenesis (Alexander and Williams, 1968).

Non-shivering Thermogenesis – Brown Adipose Tissue

The primary organ used for the production of thermoregulatory heat by non-shivering thermogenesis in mammals is brown adipose tissue (BAT) or brown fat (Nicholls and Locke, 1984; Stott and Slee, 1985). In newborn lambs non-shivering thermogenesis fueled by BAT is responsible for up to half the total heat production induced by cold conditions (Alexander, 1979). In advanced thermoregulators, of which lambs and calves are typical examples, the ability to utilize non-shivering thermogenesis

in BAT is vital to prevent hypothermia in the critical period of time directly following birth.

Newborn ruminants produce 40 to 50% of their body heat through nonshivering thermogenesis fueled by BAT (Alexander and Williams, 1968; Stott and Slee, 1985). Brown adipose tissue is more thermogenic than any other tissue by about a factor of ten (per unit of mass). Brown adipose tissue is located between the scapulas, at the nap of the neck along the major vessels in the thorax and abdomen and in other scattered locations in the body (Ganong, 1999). In normal healthy newborn lambs almost all of the adipose tissue, amounting to about 1.5% of body weight is BAT (Alexander and Bell, 1975). In lambs dying from hypothermia, fat deposits are depleted so that available fat is virtually exhausted (Alexander, 1962b). The unique structure and development of BAT contribute to its importance in heat regulation for neonates such as lambs, calves, and humans. Its primary function is to generate body heat. In contrast to white adipose cells (fat cells), which contain a single lipid droplet and provide lipid fuel for tissues remote form itself, BAT cells contain numerous smaller droplets. Brown adipose tissue cells are highly specialized for non-shivering thermogenesis, containing higher numbers of mitochondria and have more capillaries than typical white adipose tissue (Cannon and Nedergaard, 2004). Brown adipose tissue proliferation and differentiation is β-andenergic mediated, while mature adipose cells form in response to thyroid hormone T_3 (Aihaud et al., 1992).

The generation of heat via nonshivering thermogenesis in BAT is due to the presence of a unique enzyme uncoupling protein-1 (UCP-1) which separates the

metabolic reactions of oxidative phosphorylation from ATP synthesis and mitochondrial respiration releasing energy as heat (Nicholls and Locke, 1984; Casteilla et al., 1987). This uncoupling protein is exclusive to BAT were it is mostly regulated by norepinephrine and thyroid hormones. Protons are actively pumped out of the mitochondria by the electron transport chain. Energy is stored as a protein gradient across the mitochondrial inner membrane. This energy is used to synthesize ATP when the protons flow back across the membrane, down their concentration gradient. In BAT, heat is produced by signaling the mitochondria to allow protons to run back along the gradient without ATP production. This is allowed since an alternative return route for the protons exists through an uncoupling protein in the inner membrane. This protein, known as uncoupling protein 1 (thermogenin), facilitates the return of the protons after they have been actively pumped out of the mitochondria by the electron transport chain. This alternative route for protons uncouples oxidative phosphorylation and the associated energy is released as heat (Cannon and Nedergaard, 2004). With the loss of BAT, neonates loose the ability to generate heat through non-shivering thermogenesis (Alexander, 1962b; Alexander and Williams, 1968). Research with rats have shown that BAT contains a high iodothyronine 5' deiodinase activity (Leonard et al., 1983) indicating that this tissue can convert thyroxin (T_4) to the active thyroid hormone 3,3',5-triiodothyronine (T_3). Brown adipose issue contains nuclear T_3 receptors and T_3 plays a role in the regulation of the transcription of gene coding for the uncoupling protein (Bianco et al., 1988).

The heat generating power of brown adipose tissue was estimated (Slee et al., 1987) to be approximately 380 W/kg. Assuming that BAT is about 1.5% of body weight, a newborn lamb with a birth weight of 4.7 kg would contain about 70 g BAT giving a heat output of 28 W or 6 W/kg. Metabolic trials (Slee et al., 1987) show an average metabolic rate increase of 7 W/kg from the resting level of 6 W/kg (when BAT is presumably inactive) to 13 W/kg after stimulation. The potential for non-shivering thermogenesis in calf BAT (Alexander et al., 1975) appears to be similar to that in lambs (Alexander and Williams 1968), both producing a 2 to 3 fold increase in metabolic rate.

An increase in BAT weight plus specific enzymatic and morphological changes occur predominantly between day 120 of gestation and parturition in sheep (Alexander, 1978; Klein et al., 1983). Mitochondrion begin to increase in number within BAT cells at 80-90 d of gestation and continue until term (Klein et al., 1983). In calves, Landis et al. (2002) reported that at 96 d prepartum BAT had little accumulation of mitochondrion and the mitochondria present were small. At 24 d prepartum mitochondria were large with cristae and at 14 d prepartum mitochondria were elongated with differentiated cristae. Cristae are folds in the inner membrane of mitochondria important in the development of BAT because they are indicators in the surface area of the inner membrane of the mitochondria. An increase in this inner membrane increases the potential for UCP-1 within the mitochondria, which increases the thermogenic capacity of the cell (Landis et al., 2002).

Shivering Thermogenesis

Shivering thermogenesis, a significant source of heat in cold-exposed adults, has usually been dismissed as an insignificant source of heat in the newborn (Taylor, 1960; Brück, 1961; Hull and Segall, 1965). Hull and Segall (1965) concluded that shivering in the new-born rabbit occurred only when heat production was near maximum. Brück and Wünnenberg (1965) estimated that shivering supplied only approximately 10% of the total heat production in newborn guinea-pigs exposed to air at 8^{o}C.

Factors Affecting Thermogenesis

The lamb's ability to regulate body temperature after birth is affected by other factors including ingestion of colostrum, birth type, breed, and birth weight. Colostrum intake has been shown to have a positive affect on new born lambs and their ability to tolerate cold exposure (Hamadeh et al., 2000). The survival of lambs during the first h after birth depends on energy supply from body reserves and colostrum intake (Eales and Small, 1981; Clarke et al., 1997). Alexander (1961) found that the ingestion of warm milk increased heat production in newborn lambs, but did not have an affect on 3 d old lambs fasted for 12 h. He concluded that 3 d old lambs had already reached their peak metabolism shortly after birth and that ingesting warm milk did not provide enough calories to fuel an increase in body metabolism. Eales and Small (1981) reported a 17 to 20% increase in the metabolic rate of new born lambs when fed colostrum prior to cold stress. In research conducted by Sampson and Slee (1981), lamb response to cold was dependent on breed due to an effect on skin thickness, coat depth, birth weight and, litter size. However, Hamadeh et al. (2000) found that breed and sex were poor indicators of

cold tolerance in lambs when they compared Rambouillet and Targhee lambs. This research did find that single born lambs had higher body temperatures than twin born lambs. Therefore, Hamadeh et al. (2000) concluded the lambs ability to tolerate cold environments was influenced more by birth type than breed.

Sampson and Slee (1981) tested the ability of lambs of 10 different breeds to resist hypothermia by immersing lambs in a progressive cooling water bath. Cold resistance was defined as the time in minutes after initiation of cold bath treatment that the lambs body temperature drops to 35^{o}C. The average cold resistance ranged from a high of 98 ± 4.3 (mean SE) and 89 ± 4.6 min for Cheviot and Welsh Mountain lambs, respectively, to a low of 36 ± 4.1 and 38 ± 5.3 min for Soay and Finnish Landrace lambs, respectively. The Tasmanian Merino (genetically the breed most closely related to the type of sheep used in this trial) had a cold tolerance of 45 ± 5.6 minutes. Slee and coworkers (1987) used this procedure to evaluate cold tolerance in 48 lambs from lines previously selected for high and low cold resistance (24 lambs per line). Lambs from the high selected line had higher cold tolerances than those from the low line. The average cold resistance of lambs tested in their trial was 37.7± 8.1 (mean SD). Clarke et al. (1997 and 1998) exposed newborn lambs from either a normal delivery or a cesarean delivery to a warm (30^{o}C) or a cold (15^{o}C) environment for 6 hours post birth. They reported that lambs body temperature rose immediately after exposure and than declined from 2 to 6 h.

Prepartum nutritional stress may have an adverse effect on the neonatal lamb and calf's ability to produce heat making the newborn more susceptible to environmental effects of cold and or wet weather. Fetal nutrient availability during late gestation seems

to have a differential effect on brown fat development relative to fetal growth (Carstens et al., 1997; Clarke et al., 1997). This seems to be particularly true in species where placental transport of fatty acids is low (i.e. sheep and rat) and is independent of its effects on fetal growth (Alexander, 1978). Prepartum energy restriction of ewes reduced proportional weights of peritoneal adipose tissue (predominant brown adipose tissue depot) by 17% in single and 24% in twin fetuses (Alexander, 1978). Stevens et al. (1990) found that intravenous glucose supplementation of sheep fetuses from day 115 of gestation to term increased proportional weights of peritoneal adipose tissue 47% and fetal weight 18%. Energy restriction in cows during the last trimester reduces heat production in calves by 9.9% (Ridder et al., 1991). Corah et al. (1975) observed that pregnant cows fed 70% of their calculated energy requirements during the last ninety days of gestation produced calves with increased incidence of mortality at or near birth (3% vs. 10% for heifers and 10% vs. 19% for cows on high vs. low energy diet, respectively). Maternal under nutrition during the last 10 days in ewes reduced the body lipid concentrations of lambs at term by approximately 50% (Mellor and Murray, 1985). Lammoglia et al. (1999b) found that feeding heifers supplemental fat during late gestation increased glucose concentrations in the newborn calf resulting in a favorable response in body temperature in the cold-stressed newborn. In this trial, 22 crossbred, primiparous heifers (11 per treatment) were fed either a low or high fat diet during the last trimester of pregnancy. After birth calves were placed in a 0^{o}C cold chamber and rectal temperatures were obtained at 10-minute intervals. Rectal temperatures were affected by diet x time of cold exposure. Lammoglia et al. (1999b) suggested that this

increased fat availability had a positive effect on heat generation in the newborn during prolonged periods of cold stress.

In a two year study, Encinias et al. (2004) fed late gestating ewes 45 d prepartum a high linoleic diet in the form of cracked safflower seed. They reported beneficial effects of supplementation with high linoleic safflower seed during gestation. They reported higher number of lambs from the low oil supplement died from starvation and pneumonia. Lambs from the ewes receiving the high oil diet had greater survivability to weaning. This agrees with studies done with rats where diets high in linoleic acid resulted in increased BAT activity and overall thermogenesis (Schwartz et al., 1983; Nedergaard et al., 1883). Lammoglia et al. (1999b) found that calves born to heifers supplemented with safflower seeds during late gestation were able to maintain body temperature longer when exposed to cold. Encinias et al. (2004) did not see a difference in BAT weight in euthanized lambs from ewes fed the high linoleic diet vs. the low linoleic diet, and suggested that the mechanism by which high linoleic safflower seed acts on gestating ewes does not seem to be increased BAT stores. These authors proposed that positive benefits were due to an increase in the thermogenic capacity of BAT rather than its quantity. This is in agreement with Alexander and Bell (1975), who reported no relationship in lambs between the dissectible amount of BAT and the maximal thermogenic response to cold. Results from Encinias et al. (2004) suggest feeding high linoleic safflower seed to ewes during the last 45 d of gestation increases lamb survivability at parturition with no changes in ewe body weight or condition, which suggests an economic benefit from supplementation.

CHAPTER 3

MATERIALS AND METHODS

Objectives and Hypotheses

The objective of this study was to evaluate the effects of feeding a supplement containing high levels of linoleic acid (high oil) in the form of safflower seeds during late gestation and subsequent colostrum consumption on lamb cold tolerance and lamb survivability and growth to weaning. The three hypotheses used to test these objectives were; hypothesis 1 (H_{o1}); there will be no difference in lamb cold tolerance for those lambs born to ewes on a high oil supplement versus those born to ewes on a low oil supplement, hypothesis 2 (H_{o2}); there will be no difference in lamb tolerance to cold between those given colostrum pooled from ewes supplemented with high oil and those lambs given colostrum pooled from ewes fed a low oil supplement, and hypothesis 3 (H_{o3}); there will be no difference in lamb survivability and growth to weaning for those born to ewes on a high oil supplement versus those born to ewes on a low oil supplement.

Montana State University General Sheep Management Protocol

According to routine MSU sheep management, the ewe flock was brought in from winter range, directly prior to shearing, generally in early March. While on winter range ewes were supplemented with 0.15 $kg \cdot ewe^{-1} \cdot d^{-1}$ of a 20% protein supplement. Shearing occured in mid-March, after which animals were kept in a dry lot situation with ad libidum access to hay and a daily supplement of 0.23 $kg \cdot ewe^{-1} \cdot d^{-1}$ barley. At the onset of

lambing, ewes were moved into lambing drop lots where they were monitored by lambing personnel until parturition. Upon parturition, ewes and lambs were removed from the drop lot and placed in a 1.5 m^2 lambing jug. Ewe ID, ewe breed, lamb sex, lamb weight, date, and time of birth were recorded. At the time of weighing, lamb navels were trimmed, dipped in tincture iodine and lambs were checked to make sure they have suckled. Barring any complications, ewes and lambs remained in the lambing jug for 24 h, at which time they were moved to small mixing pens according to birth type. Twin mixing pens contained five ewes and ten lambs and single mixing pens contained ten ewes and ten lambs. Ewes and lambs remained in the first mixing pens for 5-7 days before being moved to a larger group of mixing pens containing thirty lambs and their dams per pen. After another 5-7 days they were moved to a larger pasture where single and multiple born lambs and their mothers were maintained in separate groups. Ewes were allowed ad libidum alfalfa hay plus a daily supplement of barley (0.23 $kg \cdot ewe^{-1} \cdot d^{-1}$ for ewes with single lambs and 0.45 $kg \cdot ewe^{-1} \cdot d^{-1}$ for ewes with twins). The two groups remained separate until they were turned out on summer range at the end of May when lambs were approximately 30-35 days of age. Ewes and lambs remained on mountain range until weaning at the end of August, when lambs were approximately 115-120 d of age. Ewes were then turned back out to pasture until they were sorted into breeding groups in mid-October and placed in a dry lot pen. Rams were turned into assigned pens for single sire mating. After 20 days, assigned rams were removed, ewes were put in a small pasture as one flock and black face terminal sires were turned in for cleanup breeding for another 20 days. Fifteen days prior to breeding, ewes were nutritionally

flushed by supplementing with 0.11 – 0.23 $kg \cdot ewe^{-1} \cdot d^{-1}$ of grain or protein supplement or in some years, turned into alfalfa hay field aftermath. After breeding, ewes were turned onto winter range and fed a protein supplement until shearing.

Ewe Feed Treatments

In a two year study conducted in 2001 and 2002, approximately one month prior to lambing, gestating ewes maintained at the Montana State University's Red Bluff Research Station located near Norris, Montana were randomly allocated within breed (Rambouillet, Targhee and Columbia) and age (2 to 6 years) into two groups so that each group had a similar number of each breed and ages. A treated group was supplemented with safflower seed (SS) while a control group received supplemental barley (C). The number and average age of ewes used in this trial are listed in Table 1.

Table 1. Number of ewes by treatment and year

	Year 1		Year 2	
	Control	Safflower	Control	Safflower
Lamb Cold Tolerance Trial				
N	10	10	9	12
Age	4.4	4.1	4.6	4.0
SE	0.36	0.36	0.38	0.33
Lamb Production Trial				
N	292	305	316	327
Age	3.29	3.25	3.49	3.39
SE	0.07	0.07	0.07	0.07

Safflower supplemented ewes were group fed 0.23 $kg \cdot ewe^{-1} \cdot d^{-1}$ whole safflower seeds. The safflower seeds provided 127 g of supplemental oil (Table 2) or based on an estimated total intake of 2.27 $kg \cdot ewe^{-1} \cdot d^{-1}$ (adapted from NRC, 2007) the equivalent to 4% of the total diet. Previous research at Montana State University (Kott et al, 2003 and Boles et al, 2005) suggests that in feedlot rations that intake and performance of lambs are not adversely affected by diets containing up to 6% supplemental oil. Leupp et al. (2006) reported no affect on supplementing canola seeds to provide 4% of the diet DM as crude fat on forage intake and diet digestibility in steers consuming high forage diets. In addition the safflower seed supplement provided 36.9 g of crude protein (CP) and 2.44 $Mcal \cdot kg^{-1}$ of metabolizable energy (ME). Centennial Safflower seed (Bergman et al, 2001) that contained 44% oil with a fatty acid composition of 79.6% linoleic acid, 10.8% oleic acid, and 7.7% saturated fatty acid were used. Whole barley was fed to the control group at the rate of 0.34 $kg \cdot ewe^{-1} \cdot d^{-1}$. Supplemental barley provided an additional 36.4 g of CP and 2.06 $Mcal^{-1} \cdot kg$ of ME (Table 2). The amount of supplemental barley was designed to provide approximately the same amount of supplemental CP and ME provided by the safflower supplement.

Table 2. Nutrient composition of supplements fed to ewes (ewe/d basis)

	Supplement	
	Barley	Safflower Seed
Intake		
kg (as fed)	0.34	0.23
kg (DM)	0.30	0.21
DM (%)	89	93
Oil (g)[a]		126.6
CP (g)	36.4	36.9
ME (Mcal/kg)	2.06	2.44

[a] Added oil at 4% of total dry matter intake (total dry matter intake estimated at 2.27 $kg^{-1} \cdot hd^{-1} \cdot d$)

Ewes were group fed assigned supplements daily. Supplement feeding for the entire ewe flock was initiated 30 days prior to the estimated start of lambing. The average time that ewes received the assigned supplements in the trial was 45 ± 4 days. Both barley and safflower seeds were fed in a whole form, daily in bunks. In addition, ewes had ad libidum access to long stem alfalfa hay containing approximately 14% crude protein. All animal procedures were approved by the Montana State University Institutional Animal Care and Use Committee (Protocol #AA-030).

Lamb Cold Tolerance Trial

Before data collection began, colostrum was randomly collected from ewes in the flock having lambed within 12 hours. Colostrum was pooled according to supplement group and stored at -20^{o}C. Pooled colostrum from analysis for SS and C groups are listed in Table 3. Although colostrum samples from year 2 appeared to have higher nutrient content than year 1, nutrient values for safflower and control colostrum were similar within year.

Table 3. Composition of pooled colostrum by ewe supplement (Safflower vs. Barley) and year.

	Year 1		Year 2	
	Control	Safflower	Control	Safflower
Fat, %	12.36	12.6	12.18	11.48
Protein, %	18.91	20.36	21.54	21.04
Lactose, %	3.06	3.08	3.96	3.14
Solids, %	36.67	38.36	40.24	37.89
Solids Not Fat (SNF), %	24.29	25.74	28.02	26.34

Ewes bearing twins were randomly selected from the two supplement groups at parturition. Twins of similar birth weight having not suckled from the ewe and not being exposed to ambient temperatures below the lower critical temperature of 22^{o}C (Alexander, 1961; Alexander, 1962a) were used for this study. A total of 20 ewes in year 1 and 21 ewes in year 2 were utilized. Average time for ewes on supplement to parturition was 41 d ± 4.

Lamb Management

At parturition, lambs were muzzled to prevent nursing and placed in a warm, (1.5 m^2) lambing jug maintained above the lower critical temperature (22.8^{o}C; Alexander, 1962a), remaining with the ewe for 30 min to allow for maternal recognition. After 30 min lambs were processed according to MSU lambing protocol where they were sexed, weighed and the umbilical cord was trimmed and dipped in iodine. Lambs were then bled via jugular puncture using a non-hepranized 10 ml vacutainer blood collection tube and needle. Lambs were assigned a colostrum treatment, safflower colostrum (SC) or barley colostrum (BC) and colostrum was administered via an esophageal tube at

15mg/kg of body weight. Lambs were then placed back in jug with the dam for another 30 min. At 1 hr of age, lambs were removed from their assigned jug and prepared for cold exposure. Individual lambs were fitted with a rectal temperature sensor connected to a mini-logger series 2000 (Mini Mitter Company, Inc., Surviver, OR). Twin lambs were placed in holding crates (183 cm^2) and two temperature values were recorded prior to cold exposure to obtain a basal temperature for each lamb. Lambs were then placed into a 0^oC, dry cold environmental chamber (0.9 cubic m) at which the 30 minute cold exposure period was initiated. Rectal temperatures were recorded by the min-logger at one minute intervals and logged onto an attached computer. After cold exposure, lambs were removed from the cold chamber. At this time, a final blood sample was collected via jugular puncture. Lambs were then returned to their dams in the warmed lambing jug for approximately 1 hr, before being returned to the flock lambing system.

Sample Analysis

Blood samples were allowed to coagulate at room temperature before being centrifuged for 20 minutes at 1000 x g to obtain serum. Serum was decanted into 12 x 75 mm plastic serum tubes, capped and stored frozen at -20^oC to be analyzed for glucose (GLU), cholesterol (CHOL), total protein (TP), blood urea nitrogen (BUN), non-esterified fatty acids (NEFA), cortisol, alpha-1-acid glycoprotein (αGP), triiodothyrone (T_3), and thyroxine (T_4) and T_3:T_4 ratio. Non-esterfied fatty acids were assayed using a NEFA-C kit (Wako Chemicals USA, Inc, Richmond, VA) as described in Hamadeh et al. (2000). Blood urea nitrogen , glucose, cholesterol, and total protein were assayed using specific Flex reagent cartridges (Catolog No. DF21, DF39A, DF27, DF73) on a

Dimension clinical system (DADE Behring, Inc., Newark, DE). Concentrations of BUN and glucose were determined using a bichromatic (340 and 383 nm) rate technique. Cholesterol concentrations were determined in serum samples using a polychromatic (540, 452, 700 nm) endpoint technique. Cortisol, T_3, and T_4 concentrations were assayed by a solid-phase RIA kit (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA) (Berardinelli et al., 1992). Alpha-1-acid glycoprotein was analyzed using an Ovine α_1-Acid Glycoprotein (α_1AG) measurement kit (Cardiotech Services, Inc., Louisville, KY).

Statistical Analysis

Temperature data were analyzed using the repeated measures procedure of SAS (SAS Inst., Inc., Cary, NC). The model included the effects of ewe treatment, lamb colostrum treatment, year, ewe treatment x year, lamb colostrum treatment x year and ewe treatment x lamb colostrum treatment x year. Blood metabolite data were analyzed using GLM procedures of SAS (SAS Inst., Inc., Cary, NC). The model for blood metabolite concentrations prior to cold exposure (0 min) included the effects of ewe treatment, year and the interactions between ewe treatment x year. Blood metabolite concentrations post cold exposure (30 min) and for concentration change during cold exposure were analyzed using the same model as used for temperature data. Lamb colostrum treatment was not included in the model at 0 min as colostrum treatments were applied after that blood sample was collected. Differences between individual means were detected by least significant differences procedure.

In this analysis, lamb was the experimental unit. Although ewes were group fed their experimental diets previous work (Taylor et al., 2002) concluded that when sheep were hand fed supplements, approximately one half of the ewes consumed from 80 to 120% and 90% consumed between 50 and 150% of the target intake.

Lamb Survivability and Growth Trial

Data collected for lamb growth and survivability was obtained following MSU production protocol. Lamb weights, sex, birth type, breed, and birthday were recorded at lambing. Lamb body weights were recorded again at turnout, May 26, 2001 and May 26, 2002 (average 33 days of age) before animals were turned out to summer pasture and again at weaning, August 21, 2001 and August 27, 2002 (average 124 days of age).

Statistical Analysis

Data was analyzed using GLM procedures of SAS (SAS Inst., Inc., Cary, NC), with ewe as the experimental unit. The model included the effects of ewe treatment, year, breed and age of dam with all appropriate interactions. Differences between individual means were detected by least significant differences procedures.

CHAPTER 4

RESULTS AND DISCUSSION

Lamb Cold Tolerance Trial

Temperature

The affects of year, ewe treatment and lamb treatments are depicted in Figures 1, 2, and 3, respectively. There was an interaction between time and year (Figure 1; $P = 0.005$) and time and lamb treatments (Figure 2; $P = 0.06$). No other interactions were detected ($P > 0.10$). Lamb body temperature was affected ($P < 0.001$) by time of cold exposure. Lamb body temperatures increased after cold exposure and peaked at around 17 minutes of exposure and leveled off or were declining at 30 minutes (Figure 1). This indicates that cold exposure did stimulate brown adipose tissue metabolism and associated heat production. This would also indicate that brown adipose heat production was beginning to diminish by 30 minutes. This is supported by previous work at MSU conducted by Hamadeh et al. (2000) and Dafoe et al. (2008). Initial body temperatures were 39.03 ± 0.17 vs. 39.11 ± 0.17 and peaked at 39.28 ± 0.17 vs. 39.48 ± 0.17 (min 17) for years 1 vs. 2, respectively. Temperature values found in this study were similar to those reported by Hamadeh et al. (2000) for lambs receiving colostrum. In contrast, our values are generally higher than those reported by Dafoe et al. (2008) where cold exposure treatments were imposed prior to colostrum administration. Hamadeh et al. (2000) found that lambs receiving colostrum had higher body temperature than those receiving no colostrum. Temperature levels reported for cold exposed lambs not

receiving colostrum were similar to temperature values reported by Dafoe et al. (2008). Non-shivering thermogenesis in BAT is activated by cold exposure via noradrenaline (Symonds et al., 1992) resulting in increases in rectal temperature of about 1°C (Alexander and Williams, 1968; Slee et al., 1987).

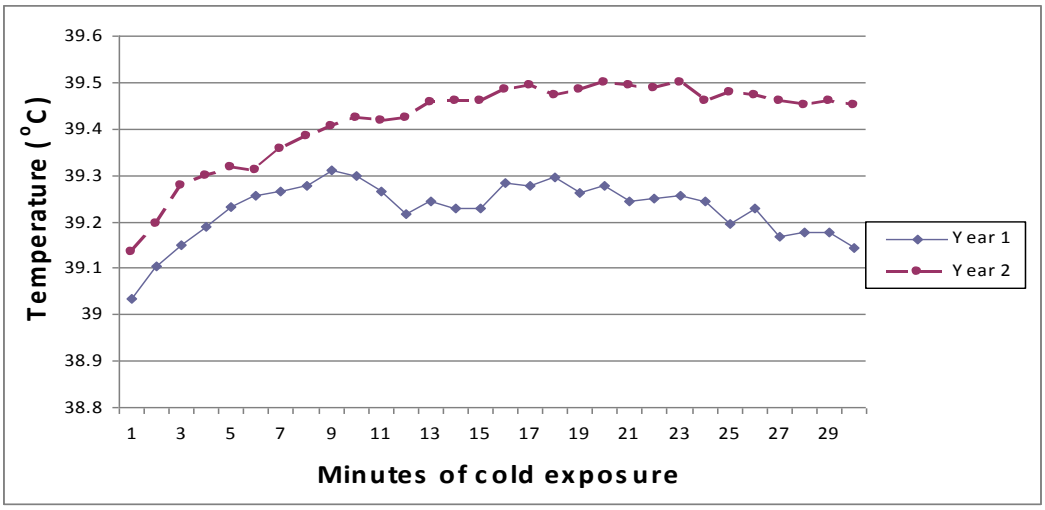

Figure 1. Least squares means of rectal temperatures of newborn lambs by year over time

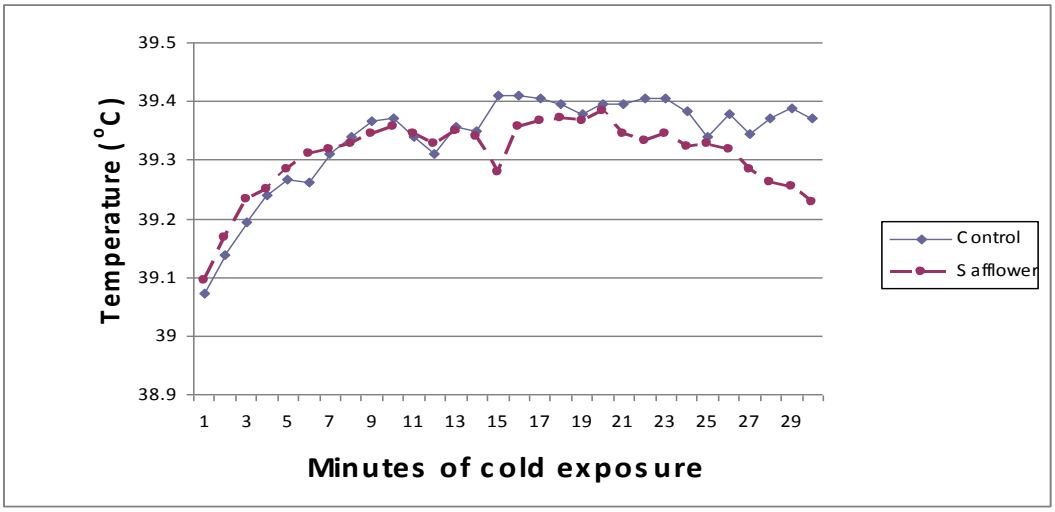

Figure 2. Least squares means of rectal temperatures of newborn lambs by ewe supplement treatment over time.

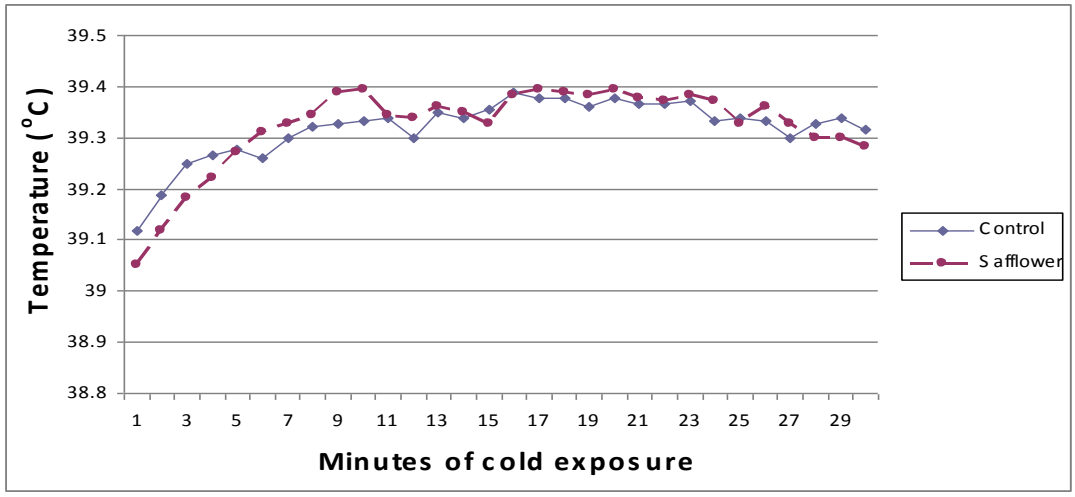

Figure 3. Least squares means of rectal temperatures of newborn lambs by lamb colostrum treatment over time

There was no difference ($P > 0.10$) in body temperatures over time between lambs born to ewes supplemented with safflower seeds vs. control (Figure 2) or between lambs given pooled colostrum form safflower seed or control supplemented ewes (Figure 3). In our study lambs born to ewes fed safflower seeds had body temperatures similar in magnitude to those born from ewes fed a control diet in the Dafoe et al. (2008) study. These researchers found that lambs born to ewes fed safflower seeds had lower body temperatures throughout cold exposure compared to lambs from control ewes. Hamadeh et al. (2000) reported that lambs that received colostrum prior to cold stress had higher body temperatures than those not receiving colostrum. This suggests that the apparent negative affects reported by Dafoe et al. (2008) from safflower seed supplementation are diminished by colostrum consumption.

Serum Metabolites

Blood metabolites of lambs prior to (0 min) and after (30 min) cold exposure are reported in Tables 4 through 7. Interactions were detected between ewe treatment and year for total protein at 30 min ($P = < 0.01$) and for ewe treatment and year for αGP at both 0 min and 30 min ($P < 0.01$) and thus ewe treatment comparisons for total protein at 30 minutes (Table 6) and αGP (Table 7) were made within year. No other interactions were detected ($P > 0.10$) and thus ewe and lamb treatment comparisons were made across years and ewe and lamb treatments (Tables 4 and 5 for blood metabolites prior to and after cold exposure, respectively).

Table 4. Least square means for pre-cold exposure (0 min) serum metabolites for lambs born to ewes receiving either safflower or barley supplements.

	Ewe Treatment			
Metabolite	Control	Safflower	SE	*P*-value
Glucose, mg/dl	53.95	43.93	3.45	0.05
Cholesterol, mg/dl	17.98	16.17	0.88	0.88
Total Protein, mg/dl	4.26	4.10	0.05	0.05
BUN, mg/dl	28.94	31.08	0.67	0.03
T_3, ng/ml	32.88	32.60	1.31	0.89
T_4, ug/ml	1.13	1.23	0.04	0.08
Cortisol, ng/ml	39.52	36.00	2.84	0.40
NEFA, mEq,L	1.60	1.37	0.05	0.003

Table 5. Least square means for post-cold exposure (30 min) serum metabolites for lambs born to ewes receiving either safflower or barley supplements.

	Ewe Treatment			
Metabolite	Control	Safflower	SE	*P*-value
Glucose, mg/dl	73.98	65.06	4.17	0.15
Cholesterol, mg/dl	19.49	19.04	0.90	0.74
BUN, mg/dl	30.25	31.85	0.73	0.14
T_3, ng/ml	32.68	33.27	1.36	0.77
T_4, ug/ml	1.19	1.24	0.04	0.35
Cortisol, ng/ml	32.09	29.72	2.55	1.53
NEFA, mEq,L	1.62	1.31	0.08	0.01

Table 6. Least square means for post-cold exposure (30 min) serum total protein for lambs born to ewes receiving either safflower or barley supplements by year.

	Ewe Treatment			
Total Protein, mg/dl	Control	Safflower	SE	*P*-value
Year 1	3.89	3.88	0.079	0.96
Year 2	4.59	4.11	0.083	<0.01
P-value	<0.01	0.04		

Table 7. Least square means for pre (0 min) and post (30 min) cold exposure alpha-1-acid glycoprotein (αGP) serum concentrations for lambs born to ewes receiving either safflower or barley supplements by year.

	Ewe Treatment			
αGP, mg/ml	Control	Safflower	SE	*P*-value
0 minute				
Year 1	246.25	491.50	49.40	<0.01
Year 2	268.89	228.33	52.07	0.56
P-value	0.75	<0.01		
30 minute				
Year 1	235.75	497.00	49.29	<0.01
Year 2	252.88	220.83	52.27	0.64
P-value	0.81	<0.01		

At 0 minutes lambs born to SS supplemented ewes (Table 4) had lower glucose ($P = 0.05$), total protein ($P = 0.05$) and NEFA ($P = 0.003$) and higher BUN ($P = 0.03$) than those lambs from control supplemented ewes. Lambs from SS supplemented ewes continued to have lower NEFA ($P = 0.01$; Table 5) after cold exposure (30 minutes). Total protein concentration was similar in year 1 but lower ($P < 0.01$) in year 2 (Table 6).

Blood concentrations of NEFA, glucose and cholesterol are considered to be indicators of energy balance. Increased NEFA levels are the result of adipose tissue breakdown (Steinberg, 1964), but may also reflect fat addition to the diet (Gummer and Carroll, 1991). Circulating NEFA's are absorbed and metabolized for energy by the liver and other tissues. Soares (1986) reported that in goats there is a substantial transfer of lipids across the placenta and suggest that fatty acid status of newborn kids can be raised by manipulation of the maternal diet. He found that the feeding of a diet containing protected polyunsaturated fatty acids (PUFA) supplemented during the last month of pregnancy increased maternal plasma NEFA and subsequent newborn kid NEFA levels. Additional maternal sources of NEFA could come from increased maternal mobilization of body fat reserves because of increased energy demands during late gestation. In our study, feeding supplements high in linoleic oil prepartum actually decreased NEFA blood levels in lambs, suggesting limited to no dietary fatty acids were available to the fetus. Increased NEFA concentrations in lambs born to control supplemented ewes could be a result of increased mobilization of body fat in these ewes. These results are supported by North Dakota work (Encinias et al., 2004), which indicated that supplementation with high linoleic safflower seeds to ewes prepartum did not affect blood NEFA

concentrations of ewes or their lambs. Results in our study would agree with Freetly and Ferrell (2000), who reported that liver use of NEFA increased as parturition approached. In contrast, MSU research by Dafoe et al. (2008) reported that safflower supplementation increased blood NEFA concentrations in ewes and subsequently in lambs prior to cold exposure.

Total protein and BUN are indicators of protein metabolism. Lower total protein (4.10 vs. 4.26; $P = 0.05$) and higher BUN (31.08 vs. 28.94; $P = 0.03$) in blood samples collected prior to cold exposure (0 min) in lambs from safflower vs. control supplemented ewes, respectively, suggests that there was increased protein catabolism in lambs from safflower supplemented ewes at and just prior to birth. Lower total protein and higher BUN blood concentrations could also be a result of slight increases in gestation length or time of parturition. This could not be evaluated in the present study. These results are in disagreement with previous MSU work (Dafoe et al., 2008) that found no difference in BUN or total protein values in lambs born to safflower supplemented ewes but decreased lamb body temperatures during cold exposure.

An interaction ($P < 0.01$) was detected between ewe supplement and year for αGP concentration in lambs both prior to (0 min) and post (30 min) cold stress and thus, ewe supplement comparisons were conducted within year (Table 7). Concentrations of αGP ($P < 0.01$) were higher in lambs from safflower supplemented ewes at both 0 and 30 min in year 1. Lambs from ewes supplemented with safflower had higher ($P < 0.01$) blood αGP levels at both 0 and 30 min in year 1 than in year 2. Serum αGP is an acute phase protein manufactured in the liver and is an indicator of stress (Stull et al. 1999). Level of

αGP found in lambs from safflower supplemented ewes during year 1 (491 ± 52.1 and 497 ± 52.3 at 0 and 30 min, respectively) are above the value of 450 mg/ml established by Bezkorovainy (1985) for calves that showed ailments. Iooh et al., (1989) suggested that in pigs, when used in conjunction with herd history, αGP could be used as an early detection tool of stress and morbidity.

No differences ($P > 0.30$) were detected between lambs from ewes supplemented with safflower vs. barley for changes in any of the blood metabolites measured during the cold stress period (Table 8). This data is supported by cold stress temperature data where ewe supplement had no affect on lamb temperature response to cold stress.

Table 8. Least square means for change in serum blood metabolites during cold exposure for lambs born to ewes receiving either safflower or barley supplements by year.

	Ewe Treatment			
Metabolite	Control	Safflower	SE	*P*-value
Glucose, mg/dl	19.93	21.14	3.25	0.80
Cholesterol, mg/dl	1.49	0.86	0.51	0.42
Total Protein, mg/dl	-0.02	-0.11	0.05	0.30
BUN, mg/dl	1.30	0.78	0.41	0.39
T_3, ng/ml	-0.28	0.67	1.03	0.53
T_4, ug/ml	0.05	0.01	0.03	0.31
Cortisol, ng/ml	-7.51	-6.28	3.77	0.82
αGP, mg/ml	-14.63	-1.00	18.23	0.61
NEFA, mEq,L	0.02	-0.07	0.07	0.42

Blood metabolites did not differ post cold exposure ($P < 0.15$; Table 9) or for changes during cold exposure ($P < 0.50$; Table 10) between lambs receiving colostrum from safflower vs. barley supplemented ewes. The lack of differences between lambs fed

colostrum from safflower vs. barley supplemented ewes combined with no difference in the lambs temperature response to cold stress strongly suggest that any positive effects on lambs that may be found form oil supplements to dams are not due to differences in colostrum composition.

Table 9. Least square means for post-cold (30 min) serum metabolites for lambs receiving pooled colostrum from ewes fed either safflower or barley supplements.

	Lamb Treatment			
Metabolite	Control	Safflower	SE	*P*-value
Glucose, mg/dl	69.86	69.18	4.27	0.91
Cholesterol, mg/dl	18.87	19.66	0.92	0.55
Total Protein, mg/dl	4.09	4.14	0.05	0.58
BUN, mg/dl	30.83	31.28	0.74	0.68
T_3, ng/ml	32.91	33.04	1.39	0.94
T_4, ug/ml	1.20	1.23	0.04	0.65
Cortisol, ng/ml	29.46	32.35	2.60	0.44
αGP, mg/ml	293.96	309.27	34.12	0.76
NEFA, mEq,L	1.50	1.42	0.08	0.54

Table 10. Least square means for change in serum blood metabolites during cold exposure for lambs receiving pooled colostrum from ewes fed either safflower or barley supplements.

	Lamb Treatment			
Metabolite	Control	Safflower	SE	*P*-value
Glucose, mg/dl	23.12	17.94	3.33	0.28
Cholesterol, mg/dl	0.73	1.64	0.53	0.23
Total Protein, mg/dl	-0.11	-0.02	0.06	0.25
BUN, mg/dl	0.99	1.08	0.42	0.88
T_3, ng/ml	1.26	-0.87	1.05	0.16
T_4, ug/ml	0.003	0.05	0.03	0.25
Cortisol, ng/ml	-9.63	-4.17	3.85	0.33
αGP, mg/ml	-6.96	-8.67	18.64	0.95
NEFA, mEq,L	-0.01	-0.04	0.07	0.76

Lamb Survivability and Growth Trial

Production results from the lamb survivability and growth trial are presented in Table 11. No differences ($P > 0.12$) were detected for average date of birth, number of lambs born, number of lambs present at turnout (approximately 33 d of age) and weaning (approximately 124 d of age) or for weight of lambs at turnout or weaning for ewes receiving either the safflower or control supplements. These results suggest that safflower supplementation had no affect on ewe lamb production or survivability.

Table 11. Least square means of lamb survival at birth, turnout or weaning and lamb weights at birth, turnout and weaning of lambs born to ewes fed safflower or barley supplements.

	Ewe Treatment			
	Control	Safflower	SE	*P*-value
Birthdates	22-Oct	22-Oct	0.27	0.31
Number Lambs Born	1.41	1.37	0.03	0.12
Number Lambs at Turnout[1]	1.25	1.25	0.03	0.92
Number Lambs at Weaning[2]	1.18	1.15	0.03	0.37
Turnout Weight[1], kg	15.48	15.41	0.31	0.86
Weaning Weight[2], kg	33.65	33.37	0.74	0.75

[1]Measured at spring turnout, May 26, 2001 and May 26, 2002
[2]Measured at weaning Aug. 21, 2001 and Aug. 27, 2002

CHAPTER 5

CONCLUSION

Lamb mortality is one of the leading causes of lowered productivity in sheep operations. Rowland et al. (1992) found that lamb losses during the lambing period ranged from 8.2 to 12.2% with 50% of the lamb losses occurring within the first 24 hr after parturition. In most instances, cold and starvation interact together to be the major cause of death (Slee, 1987). Survival of lambs during the first 24 hr after birth depends on energy supply from lamb reserves and colostrum intake (Eales and Small, 1981). The neonatal lamb has well developed thermogenic mechanisms, including both shivering thermogenesis in muscle tissue and non-shivering thermogenesis (Alexander and Williams, 1968). The primary organ used for the production of thermoregulatory heat by non-shivering thermogenesis in mammals is brown adipose tissue (BAT) or brown fat (Nicholls and Locke, 1984; Stott and Slee, 1985). In newborn lambs non-shivering thermogenesis fueled by BAT is responsible for up to half the total heat production induced by cold conditions (Alexander, 1979). Linoleic and linolenic acid supplements, such as safflower seeds, increased the thermogenic capacity of BAT (Nedergaard et al., 1983).

Lamb tolerance to cold stress was tested and measured in lambs born to ewes receiving either a safflower seed or barley supplement late in gestation and lambs given a pooled colostrum from ewes receiving the safflower seed or barley supplement. There was no difference in body temperatures between lambs born to ewes supplemented with

safflower seeds vs. control or between lambs given pooled colostrum form safflower seed or control supplemented ewes. This indicates that supplementing ewes late in gestation with the high linoleic safflower seed had no apparent affect on the lamb's tolerance to cold stress through either increased BAT thermogenesis or colostrum intake. Feeding supplements high in linoleic oil prepartum decreased NEFA blood levels in lambs, suggesting limited to no dietary fatty acids were available to the fetus. There was an increase in NEFA concentrations in lambs born to control supplemented ewes, which could be a result of increased mobilization of body fat in these ewes. Lower total protein and higher BUN in blood samples collected prior to cold exposure in lambs from safflower vs. control supplemented ewes, suggests that there was increased protein catabolism in lambs from safflower supplemented ewes at and just prior to birth. Lower total protein and higher BUN blood concentrations could also be a result of slight increases in gestation length or time of parturition. The lack of differences in blood metabolites between lambs fed colostrum from safflower vs. barley supplemented ewes combined with no difference in the lambs temperature response to cold stress strongly suggest that any positive effects on lambs that may be found form oil supplements to dams are not due to differences in colostrum.

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