

EFFECT OF SUPPLEMENTAL SAFFLOWER SEED AND VITAMIN E  
TO LATE GESTATING EWES ON LAMB GROWTH AND THERMOGENESIS

by

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## ABSTRACT

Fifty-one twin bearing Targhee ewes (Trial 1) and 1182 single and twin bearing white face range ewes (Trial 2) were used in a 2 x 2 factorial arrangement of treatments to determine the effect of supplemental energy source and level of vitamin E on lamb serum metabolites and thermogenesis (Trial 1), and lamb growth (Trial 2). During the last 30 d of gestation, ewes were individually (Trial 1) or group (Trial 2) fed a daily supplement. Supplements were: 226 g safflower seeds (SS) and either 350 (VE) or 0 (VC) IU vitamin E or 340 g of a grain-based supplement (GC) and either VE or VC. One h postpartum in Trial 1, twin born lambs were placed in a 0°C dry cold chamber for 30 min. Lamb rectal temperature was recorded every 60 s and blood samples were taken immediately before and after cold exposure. In Trial 2 lambs were weighed at birth, turnout and weaning. Ewes were weighed at turnout and weaning. During the cold exposure in Trial 1, lambs from SSVC ewes had the lowest ( $P = 0.01$ ) body temperature, a decrease ( $P < 0.08$ ) in NEFA concentration, and an increase ( $P = 0.06$ ) in serum glucose while lambs born to GCVC ewes had a decrease in serum glucose. The SS lambs tended to have lower ( $P < 0.11$ ) levels of BUN and  $T_4$  at 0 min than lambs born to GC ewes. After 30 min of cold exposure, SS lambs increased and GC lambs decreased in BUN,  $T_3$ , and  $T_3T_4$  concentration ( $P < 0.10$ ). At turnout in Trial 2, GC lambs weighed more and had higher survival rates than SS lambs ( $P < 0.07$ ). Based on lower body temperature in at birth of lambs born to ewes fed safflower seeds and vitamin control, the greater change in BUN during the cold exposure and the higher mortality rate for lambs born to ewes fed safflower seeds than lambs born to ewes fed grain control, it appears that safflower seed and vitamin control supplemented ewes gave birth to lambs with an apparent decrease in basal metabolic rate. This may compromise the newborn lamb's ability to adapt to extreme environmental conditions. This study was unable to demonstrate an increase in lamb production due to feeding supplemental safflower seeds during late gestation.

## CHAPTER 1

### INTRODUCTION

Hypothermia, starvation, scours, and pneumonia are the major causes of neonatal lamb mortality with 50% of lamb losses occurring within 24 hours of birth (Rowland et al., 1992). Alexander (1961) estimated that 50% of the heat generated by ruminant neonates comes from non-shivering thermogenesis which is fueled solely by brown adipose tissue (BAT). Linoleic and linolenic acid supplements, such as safflower seeds, increased the thermogenic capacity of BAT by 75% and doubled the content of uncoupling protein-1 (UCP-1) in rats (Nedergaard et al., 1983). Increased heat production from BAT increases the oxidation of BAT, which results in the formation of free radicals. Free radicals cause damage to cellular membranes; therefore creating a need for more antioxidants to maintain cell integrity.

Encinias et al. (2004) reported that lambs born to ewes fed a late gestation diet that included 4.6% safflower seeds had increased survivability and lowered pneumonia rates than lambs born to ewes fed a 1.9% fat, isocaloric prepartum diet. Lammoglia et al. (1999) found similar results in calves that were born to cows fed a high fat diet (5.1%) when compared to calves born to cows fed a low fat (2.2%) control diet.

Vitamin E, a potent antioxidant, protects cellular membranes by sequestering free radicals and sparing cell membranes from oxidative degradation (Horton et al., 1996). Ewes lambing early in the season and fed harvested forages may have low plasma

vitamin E concentrations because dry, stored feeds have lower vitamin E content than fresh, spring forage (Hatfield et al., 1999).

It is unknown if feeding supplemental safflower seeds to increase the thermogenic capacity of BAT will increase anti-oxidant requirements in sheep. Therefore, the objective of this study was to determine the effects of safflower seed and vitamin E, supplemented to late gestating ewes, on lamb growth, serum metabolites, and thermogenesis in lambs born to spring-lambing ewes.

## CHAPTER 2

### LITERATURE REVIEW

#### Lamb Survival

Neonatal lamb mortality rates of 10 to 35 % are considered normal in large sheep operations in the western U.S. (Rowland et al., 1992). Alexander (1984) reported that approximately 50% of lamb mortality occurs within the first 3 days of life, independent of the production system. In a review of mortality records from typical western range sheep operation, Rowland et al. (1992) reported that more than 50% of all lamb deaths occurred within 24 hours of birth. In Montana, Safford and Hoversland (1960), after examination of approximately 1000 lamb autopsies, noted pneumonia was the leading cause of death in neonatal lambs. Neonatal lamb mortality rates and causes reported by Safford and Hoversland (1960) are similar to those reported by more recent research (Rowland et al., 1992). Results from these studies, spanning more than 30 yr, suggest that little improvement has been made in preventing neonatal lamb mortality.

#### Brown Adipose Tissue

The unique structure and development of BAT contribute to its importance in heat regulation for neonates. In the differentiation of adipose tissue multipotent stem cells, with unknown origin, will differentiate into unipotent adipoblast that are committed to developing into adipose tissue. These adipoblasts will differentiate into preadipocytes,

which give rise to immature adipose cells and finally to mature adipose cells (Ailhaud et al., 1992). Brown adipose tissue proliferation and differentiation is  $\beta$ -adrenergic mediated, while mature adipose cells form in response to thyroid hormone  $T_3$  (Ailhaud et al., 1992).

Newborn ruminants are dependent on BAT fueled nonshivering thermogenesis to generate approximately 50% of their body heat (Alexander, 1961; Stott and Slee, 1985; Symonds et al., 1992). Due to the role BAT plays in fueling nonshivering thermogenesis, the development of BAT is important during fetal development. Neonatal ruminants are capable of generating heat from BAT for the first 2-3 wk of life because the enzyme uncoupling protein-1 (UCP-1) has the ability to separate the metabolic reactions oxidative phosphorylation and mitochondrial respiration (Carstens, 1994). When neonates lose BAT they also lose the ability to generate heat via nonshivering thermogenesis (Alexander, 1962; Alexander and Williams, 1968).

Brown adipose tissue appears in lambs at 70 d of gestation with rapid proliferation until 110 d of gestation (Alexander, 1978; Klein et al., 1983). Mitochondria begin to increase in number within BAT cells at 80-90 d gestation and continue until birth (Klein et al., 1983). Landis et al. (2002) described the development of BAT in calves; these authors reported that in calves at 96 d prepartum BAT had little accumulation of mitochondria, the mitochondria present were small, spherical and dense. By 24 d prepartum mitochondria were large with cristae (folds in the inner membrane of mitochondria) that were distinct but not extensive. At 14 d prepartum mitochondria were elongated with extensively differentiated cristae. The development of

cristae is an important event in the maturation of BAT because it indicates the growth of the surface area of the inner membrane of the mitochondria. Uncoupling protein-1 is found in mitochondrial inner membrane. An increase in this membrane as indicated by the development of cristae, increase the potential for UCP-1 within the mitochondria, which will then increase the thermogenic capacity of the cell. Uncoupling protein-1 abundance rises from 145 d gestation to birth and decreases soon after birth (Ailhaud et al., 1992; Clarke et al., 1997; Mostyn et al. 2003 ).

At about the same time as mitochondrion increase in number within BAT there is an increase in lipid deposition within the vacuoles resulting in increased cell size (Klein et al., 1983). The increase in lipid content within the cell is the primary determining factor of cell metabolism and thermogenesis (Klein et al., 1983) as lipids provide metabolites that fuel oxidative phosphorylation. By the time the fetus is born its BAT has a multivacuolar appearance due to stored fat reserves; however these fat reserves disappear with age (Thompson and Jenkinson, 1969; Gemmell et al., 1972). Thompson and Jenkinson (1969) reported that 96% of cells from fat tissue surrounding kidney of lambs had a multivacuolar appearance at birth but by 4 d postpartum only 60% of cells in adipose tissue had multivacuolar appearance. This indicates that neonates require stored fat reserves to sustain heat production and growth, once fat reserves stored in BAT are utilized the animal must rely on ingested milk for energy.

Uncoupling protein-1 and fat reserves are both present in the cell before the fetus is born. However, nonshivering thermogenesis does not occur prior to birth (Gunn et al., 1993). If nonshivering thermogenesis were to occur before birth, potential energy stored

within BAT cells would be exhausted. The inhibitory mechanism is unknown at this time. Gunn et al. (1993) speculated that oxygen available to fetus may be part of the regulation. Nonshivering thermogenesis is a process that requires large amounts of oxygen to oxidize fat reserves within the cell, if nonshivering thermogenesis occurred in utero oxygen demands would increase compromising lamb survival.

It was initially thought that BAT reverted to white adipose tissue after birth (Gemmell and Alexander, 1978; Gemmell et al., 1972). Yet, continued research conducted by Ailhaud et al. (1992) concluded that BAT is a separate tissue from white adipose. White adipose tissue cells replace actually replace BAT cells rather than BAT cells differentiating into white adipose tissue cells. Ailhaud et al. (1992) came to the conclusion that BAT and white adipose tissue are separate tissues because proliferation of BAT cells is highest in preadipocytes suggesting that committed cells proliferate, but fully differentiated cells do not. Mostyn et al. (2003) agreed with this assessment and stated that there was a marked difference in the development of mitochondrial protein between brown adipose tissue and other tissues within the fetus as describe in the lung tissue.

Studies supporting the theory of BAT changing to white adipose tissue suggest that several factors affect the rate of change between the two. These factors include ambient temperature and amount of colostrum ingested by the neonate. Changes from BAT to white adipose tissue occur over a longer period of time in lambs that have been fed colostrum and kept in colder environment than lambs fed colostrum and kept in a warm environment (Gemmell et al., 1972), which is presumably due to an increased need

for heat production in cold lambs. Lambs that are kept in a warm environment and fed colostrum do not require as much heat to be produced from BAT deposits; the changes evident in BAT of lambs in a warm environment indicate a shift to white adipose tissue occur at an accelerated pace compared to lambs that were not fed colostrum and kept in the same warm environment (Gemmell et al., 1972). It appears that once a lamb's body temperature has been stabilized BAT is replaced by white adipose tissue. The variations in rate the BAT is replaced by white adipose tissue would indicate that cold stress is a better indicator than age of how long BAT remains functional.

### Lamb Temperature Regulation

Newborn lambs typically have well-developed thermogenic mechanisms, including both shivering and non-shivering responses (Alexander, 1979). Alexander (1961) estimated that 50% of the heat generated by ruminant neonates comes from non-shivering thermogenesis. Non-shivering thermogenesis is fueled in part by BAT (Stott and Slee, 1985; Symonds et al., 1992). Summit metabolism, the highest metabolic rate attainable at normal body temperature without voluntary muscular activity, is established within hour postpartum (Alexander, 1962) and cannot be induced days after birth (Alexander, 1962; Alexander and Williams, 1968) due to the loss of BAT.

Alexander (1961) reported newborn lambs are able to maintain body temperature in ambient temperature as low as  $-5^{\circ}\text{C}$ , by increasing heat production 2-3 times basal levels when exposed to cold environment. Decrease in lamb body temperature associated with cold stress is preceded by an initial rise in temperature without shivering, therefore,

Alexander (1961) concluded that this heat production could be attributed to nonshivering thermogenesis.

The lamb's ability to maintain its temperature is affected by several factors including ingestion of colostrum, breed, birth type, and birth weight. Ingestion of colostrum is an important part of the newborn's ability to produce heat. Newborn lamb ingestion of milk increased heat production, a phenomenon that could not be replicated in lambs 3 d old with 12 h fast (Alexander, 1961). Lambs that were 3 d old lambs had already reached summit metabolism shortly after birth and ingestion of warm milk did not provide enough calories to fuel increased metabolism throughout the body. Sampson and Slee (1981) reported that initial temperature was dependent on breed. Breed also had an effect on skin thickness, coat depth, birth weight and, litter size. Sampson and Slee (1981) concluded that certain breeds are more cold tolerant. However, when Hamadeh et al. (2000) studied Rambouillet and Targhee lambs, they reported that breed and sex were poor indicators of cold tolerance in lambs. In that study, single lambs had a higher body temperature than twin born lambs and lambs that were fed colostrum had higher body temperature than lambs that were not fed colostrum. Therefore, Hamadeh et al. (2000) concluded the lamb's ability to tolerate cold environments was influenced by birth type and colostrum intake.

### BAT and Lamb Survival

Alexander (1961) conducted research on starvation and hypothermia in lambs and determined that lambs weighing more than 3 kg had a higher rate of survival. Thompson and McEwan Jenkinson (1969) estimated that only 0.3% of a lamb's body weight at birth is BAT, while Alexander and Bell (1975) estimated that BAT accounts for 2% of body weight at birth. Carstens et al. (1997) determined that thermometabolic rate and peak metabolic rates are positively correlated with birth weight; heavier calves were shown to have higher temperature than light calves. Thermometabolic rate and peak metabolic rate result from BAT within the newborn. It is possible to conclude from Thompson et al. (1969), Alexander and Bell (1975) and Carstens et al. (1997) that heavier neonates have more BAT stores within the body which enable them to produce more heat. Due to the relationship BAT has with body temperature it can be concluded deposition of BAT dictates the ability of the newborn to adapt to the extra uterine environment.

Alexander (1962) illustrated the important role that ewe nutrition during gestation has on lamb BAT by testing starved lambs from ewes with 'high', 'medium,' and 'low' nutrition levels during gestation. It was concluded that lambs from 'low' nutrition ewes had a shorter survival period than lambs born to ewes with 'medium' or 'high' nutrition in any of the experimental environments. It appears that lambs from 'low' nutrition ewes not only had less BAT but may have also had less UCP-1 which decreased the lamb's cold tolerance. Additionally, lambs placed at 9°C survived on average shorter periods than lambs placed at 23°C. As lambs became hypothermic they utilized fat reserves

within the body to fuel heat production. Adipose tissue samples taken from starved lambs appeared to be completely oxidized, indicating that the lambs had exhausted virtually all fatty acids. Increasing the amount of BAT within the body would increase the lamb's ability to produce heat, increasing survival rates under severe conditions. Ewe nutrition level during gestation, coupled with birth type, will affect the amount of fat reserves available to lambs (Alexander 1962). Thus, Alexander et al. (1962) concluded that by increasing gestation nutrition levels it is possible to greatly increase lamb survival.

Twin born lambs are certainly at a disadvantage when compared to single born lambs. Budge et al. (2003) reported that adipose tissue was decreased in twin born lambs compared to single born lambs. Not only did twin born lambs have less BAT, but the same BAT was found to be less thermogenic when compared to BAT from single born lambs, possibly due to a decreased amount of UCP-1 in twin born lambs in comparison to single born lambs. These results agree with Hamadeh et al. (2000), who cold stressed lambs in a controlled environment for 30 min, stated that birth type is a useful indicator of the lamb's ability to withstand cold stress.

#### Dam Effects on Lamb BAT

The dam and her environment are extremely influential in the composition of the fetus. Lambs born to nutrient restricted ewes had shorter, lighter digestive tracts than lambs born to ewes fed adequate nutrients (Molle et al., 2004; Scholljegerdes et al. 2004). Heifers that were nutrient restricted had shorter gestation length and lighter calves than

non-nutrient restricted heifers (Warrington et al., 1988). Nutrient restricted dams lost more body condition or in the case of growing heifers had decreased growth rates compared to dams fed 100% recommended nutrients. In studies that provided at least 100% of recommended nutrients to all animals, calf and lamb birth weight were not different between treatments. This is expected due to the isocaloric nature of all diets in these experiments (Martin et al., 1997; Lammoglia et al., 1999; Bottger et al., 2002; Dietz et al., 2003; Encinias et al., 2004).

Chronic cold exposure resulting from shearing of ewes during late gestation can increase lamb birth weight. Symonds et al. (1992) reported that lambs born to ewes shorn four weeks prior to expected delivery were 15% heavier than lambs born to unshorn ewes with no difference in growth rates over the first month of lamb's life. Heavier lambs tend to have more BAT reserves, therefore shearing dams during late gestation could increase lamb survivability.

In addition to dictating the amount of BAT within a fetus, maternal nutrition can also impact the composition of BAT. Martin et al. (1997) fed heifers a protein restriction diet and concluded that there was no difference in composition of BAT. However, this was refuted by Budge et al. (2000) in which ewes were fed 150% of metabolic requirements. Lambs born to ewes fed 150% of metabolic requirements were not only heavier at birth, but the BAT had higher thermogenic activity, as measured by concentration of UCP-1.

### Safflower Effects on Animal Production

The oil content of safflower seeds, which will increase energy available to the animals, is part of the reason safflower supplementation has been used in all parts of animal production. There are two types of oil that safflower produces, the first is high in monounsaturated fatty acids (oleic acid) and the second is high in polyunsaturated fatty acids (linoleic acid; Wikipedia, 2006). When fed as part of a feedlot diet safflower supplementation resulted in a higher amount of total polyunsaturated fatty acids in muscle tissue extract than a control diet (Kott et al., 2003). This is important because dietary conjugated linoleic acids have been shown to have anti-carcinogenic activity on prostate and colon cancer, as well as mammary cancer. It is also important to note that the major dietary source of conjugated linoleic acids for humans is ruminant meats and dairy products (Wang and Jones, 2004).

As well, by including safflower oil in the diet few if any adverse effects were seen in feedlot performance. Kott et al. (2003) compared a group fed 6% safflower oil and a isocaloric, starch based control group, and reported improvement in ADG and improved lamb gain per 100 kg of feed. Van Wagoner et al. (2001) found that wethers fed safflower seeds had a greater average daily gain in comparison to those fed a control diet. Surprisingly, the concentration of oil in the diet did not impact the average daily gain or dry matter intake levels (Boles et al., 2005). Van Wagoner et al. (2001) found no differences in dry matter intake between safflower-fed lambs and non-safflower-fed lambs.

The addition of safflower to the diet was shown to cause no differences in measured carcass characteristics (Van Wagoner et al. 2001; Kott et al., 2003). The dressing percentage, longissimus muscle area, or kidney fat weight did not differ between safflower fed lambs and those receiving a control diet. Yet, fat thickness was greater in safflower supplemented animals compared to the control group. Kott et al. (2003) found no measurable differences in leg, loin, and shoulder primal wholesale cut weight, however safflower-supplemented lambs were found to have heavier racks. No differences existed when comparing dressing percentage, loin muscle area, or kidney fat weight between lambs fed safflower and those fed a control diet (Van Wagoner et al., 2001).

Encianan et al. (2004) reported that lambs born to ewes fed a late gestation diet including safflower seeds (4.6%) had increased survivability rates and lowered pneumonia rates when compared to lambs born to ewes fed an isocaloric and isonitrogenous low fat (2%) prepartum diet. Although ewes fed a low fat diet raised fewer lambs, the weaning weights of lambs that survived were comparable to lambs born to ewes receiving a high fat diet.

Maternal nutrition has long been recognized as an essential part of the calf or lamb's ability to withstand cold stress. Lammoglia et al. (1999) found calves born to cows fed a high fat (4.6%) diet had a higher peak body temperature, and maintained that temperature longer than calves born to cows fed a low fat (1.9%) diet. However, this was contradicted by Dietz et al. (2003) in which no difference in calf birth weight or vigor

score was found, thereby it was concluded that high fat prepartum diets did not improve newborn calves' response to cold stress.

When supplementing the ewe with safflower seeds ewe performance needs to be considered. When dam is in good condition, body condition score or weight of dam did not differ with level of safflower seed supplementation when control diets were formulated to be isocaloric (Encinias et al., 2004). Yet, Bottger et al. (2002) concluded that fat supplementation could be advantageous to any dam found to be in poor body condition. Bottger et al. (2002) found that dam in poor condition had increased milk fat and weight gains when supplemented with fat during late gestation.

There are differing results on impact the safflower seed supplementation has on reproductive performance. Scholljegerdes et al. (2004) suggested that increased IGF levels found in tissues essential for reproduction could have negative effects on reproductive performance and therefore productivity. Bottger et al. (2002) reported that linoleate and oleate safflower seeds did not effect postpartum reproductive performance when compared to grain based control diets.

#### Vitamin E Effects on Cellular Level

Vitamin E levels are important during late gestation because it is the cells's first line of defense against lipid peroxidation (Hatfield et al., 1999). Vitamin E protects cellular membranes by scavenging for free radicals and sparing the cell membranes from oxidation (Horton et al., 1996). Rapidly proliferating cells are prone to damage by free radicals, peroxides, and superoxides (Tengerdy, 1990) due to increased activity within the

cell. Young dams that are growing while gestating their first calf or lambs may be especially susceptible to cellular damage because the dam and fetus are both growing. Cell membranes are protected from free radicals, peroxides, and superoxides by vitamin E which is an integral part of lipid membranes (McDowell et al., 1996).

Pascoe et al. (1987) determined that cellular damage due to lipid peroxidation can be prevented if  $\alpha$ -tocopherol is maintained in cellular membranes. In addition to rapid cell division, nonshivering thermogenesis also strains  $\alpha$ -tocopherol levels within the cell. The generation of heat via non-shivering thermogenesis in BAT is due to the ability of UCP-1 to uncouple oxidative phosphorylation from ATP synthesis and of release energy as heat (Nicholls and Locke, 1984; Casteilla et al., 1987). The increased heat production in BAT caused by increased oxidation results in more free radicals being formed; therefore more antioxidants are needed to maintain cell integrity. By sparing the membranes from the damage of oxidation the ewe may be able to direct more energy towards fetal development possibly as BAT.

#### Vitamin E Effects on Animal Production

Vitamin E is an essential part of animal reproduction and production. Vitamin E is found in whole cereal grains, particularly in germ and byproducts that include the germ (McDowell et al., 1996). However, naturally occurring tocopherols are unstable and substantial loss of vitamin E activity occurs during processing and storing of feedstuff. Vitamin E content of feed is affected by season, stage of maturity, time of forage cutting, and the time from cutting to drying (McDowell et al., 1996). Stored feed does not have

adequate vitamin E levels for production especially during late gestation (Hatfield et al., 1999).

Due to the fact that natural tocopherols are unstable and are less available during times of productive stress, including late gestation and parturition, it is possible to add more stable, synthetic forms of tocopherols to feed stuffs. Free D- and DL- $\alpha$ -tocopherol have the highest bioavailability (McDowell et al., 1996). Naziroglu et al. (2002) determined that supplementing feedstuffs with vitamin E in the form of  $\alpha$ -tocopherol results in serum  $\alpha$ -tocopherol levels increase linearly with level of supplementation. It was also determined that serum  $\alpha$ -tocopherol concentrations can be reliably used to assess vitamin E status in adult animals. Vitamin E supplementation can be important during late gestation. Hatfield et al. (1999) reported that vitamin E levels in gestating ewes can decline by 50% seven days prepartum and levels do not return to normal until 20-30 d postpartum.

There are several routes of administration of vitamin E commercially available to producers, however the route of administration impacts the bioavailability. Intramuscular injection of vitamin E does increase serum  $\alpha$ -tocopherol (Njeru et al., 1992; Daniels et al., 2000), and Hidiroglou et al. (1990) determined that intraperitoneal injections also increase serum  $\alpha$ -tocopherol. Route of administration affects biological activity,  $\alpha$ -tocopherol administered intravenously is more active than that given intramuscularly, which is more active than when given orally or intraruminally (Hidiroglou and Karpinski, 1987). Route of administration also affects how quickly the  $\alpha$ -tocopherol is eliminated from the system. Alpha-tocopherol given intravenously is the most active but is also

eliminated from the system quickest, oral administration is eliminated more quickly than  $\alpha$ -tocopherol given intraruminally or intramuscularly (Hidroglou and Karpinski, 1987).

When pregnant ewes have been supplemented with mega doses of vitamin E little differences in production of the dam have been shown. Thomas et al. (1995) found a 330 IU injection of  $\alpha$ -tocopherol had no effect on ewe body weight or body condition score. Also, Kott et al. (1998) found no difference in ewe weight, body condition score, fertility or prolificity when ewes were given 330 IU/ewe/day orally three weeks prepartum. Capper et al. (2005) reported that vitamin E supplementation had no effect on milk yield.

Vitamin E has been reported to have an effect on lamb production (Lauzurica et al., 2005). Vitamin E supplementation has been used in feedlot to improve shelf life of meat. The short shelf life of packed lamb meat is one of the main problems in its commercialization (Lauzurica et al., 2005). It was concluded that feeding vitamin E may reduce early lamb mortality between birth and turnout to summer range. Kott et al. (1998) also reported lamb mortality rate reduced in lambs born to ewes supplemented with vitamin E. Thomas et al. (1995), Williamson et al. (1995), and Kott et al. (1998), reported no differences in lamb birth weights due to vitamin E supplementation, however Capper et al. (2005) found that ewes fed supplemental vitamin E during late gestation produced lambs with heavier birth weights than ewes without supplemental vitamin E. Thomas et al. (1995) reported that ewes fed vitamin E tended to wean more kg of lambs due to lower mortality rates of lambs born to ewes fed vitamin E than control ewes.

### Summary

Lambs are born with well developed thermogenic mechanisms including nonshivering thermogenesis that relies on BAT. Brown adipose tissue is a specialized organ that is only present in ruminants as neonates. The enzyme UCP-1 found in BAT is responsible for uncoupling oxidative phosphorylation from the electron transport chain, resulting in increased heat production. Due to the fact that BAT produces heat as lambs are adjusting to the extrauterine environment, lamb early survival can be directly tied to the amount of BAT available to the lamb. The dam and her environment play important roles in the amount and composition of BAT available to neonates. Supplementing with safflower seeds, which are high in PUFAs, can increase the amount of UCP-1 found in BAT therefore increasing thermogenic capacity of BAT and survival rates of newborns. While UCP-1 is essential for nonshivering thermogenesis it also increases stress within the cell in the form of free radicals. Vitamin E is a potent antioxidant that can spare cellular membranes from oxidation. Moreover, due to the high rate of oxidation associated with PUFAs it is especially important to maintain antioxidant levels within the cell to aid in preventing cellular damage caused by free radicals formed during oxidation.

## CHAPTER 3

### MATERIALS AND METHODS

#### Trial 1:

##### Objective & Hypotheses

The objective of trial 1 is to determine the effects of supplemental safflower seeds and vitamin E to late gestating ewes on lamb thermogenesis and serum metabolites.

Three hypotheses were used to test this objective. Hypothesis 1 ( $H_{01}$ ): lambs born to ewes supplemented during late gestation with safflower seeds or an isocaloric and isonitrogenous control will not differ in: body temperature or serum metabolites.

Hypothesis 2 ( $H_{02}$ ): lambs born to ewes supplemented during late gestation with 350 IU of vitamin E or an isocaloric and isonitrogenous control supplement with no added vitamin E will not differ in: body temperature or serum metabolites. Hypothesis 3 ( $H_{03}$ ): lambs born to ewes supplemented during late gestation with a combination of safflower seeds and/or vitamin E or an isocaloric and isonitrogenous control will not differ in: body temperature or serum metabolites.

##### Ewe Selection

All animal procedures were approved by the Montana State University Institutional Animal Care and Use Committee (protocol #AA-030). Fifty-one twin bearing Targhee ewes were assigned randomly to a 2 x 2 factorial arrangement of treatments. Real-time

ultrasound was used to identify pregnant ewes carrying twins conceived early in the breeding season from the Targhee flock managed at Montana State University's Red

Table 1. Analysis of feed fed to ewes during late gestation

|                   | Alfalfa | Safflower<br>Seed | Safflower<br>Control | Vitamin E | Vitamin E<br>Control |
|-------------------|---------|-------------------|----------------------|-----------|----------------------|
| CP (%)            | 15.2    | 19.6              | 16.2                 | 24.2      | 24.2                 |
| TDN (%)           | 58.6    | 78.5              | 71.4                 | 78.3      | 78.4                 |
| Ether Extract (%) | 1.28    | 49.29             | 2.76                 | 3.90      | 3.70                 |
| Vitamin E         | 7       | 24                | 11                   | 161       | 35                   |

Bluff Research Ranch near Norris, Montana. Ewes were assigned to treatment in such a way that the average age of each treatment group was 4.4 to 4.5 yr. Ewes were moved March 1 2005, from the range flock at Red Bluff to the Fort Ellis Sheep Facilities near Bozeman, Montana where they were housed in a 3721 m<sup>2</sup> pen with ad libitum access to long stemmed alfalfa hay (Table 1) and water.

### Treatment

Isocaloric and isonitrogenous treatments were: 226 g·ewe<sup>-1</sup>·d<sup>-1</sup> safflower seeds (SS) and either 350 (VE) or 0 (VC) IU·ewe<sup>-1</sup>·d<sup>-1</sup> vitamin E or 340 g·ewe<sup>-1</sup>·d<sup>-1</sup> of a grain-based supplement (SC) and either VE or VC (Table 1). An additional 115 g of SC was required to provide an equal amount of energy as the SS supplement. Ewes were placed in individual pens once daily to receive supplemental treatments. Ewes remained in individual pens until all trial supplements had been consumed. Treatments were administered March 7, 2005 to April 10, 2005. Immediately (10 d ± 5) before lambing, ewes were returned to the range flock at Red Bluff.

### Data Collection

Ewes were constantly observed 24 h/d during lambing season. Forty-two of the 51 ewes were identified at parturition and lambs born to these ewes were used to evaluate treatment effects on lamb body temperature and blood metabolites. When ewes were observed to be in labor they were monitored constantly until parturition. Immediately after birth, lambs were prevented from suckling; vigorous lambs were muzzled to prevent nursing. The ewe and her lambs were placed in a pen (1.5 m<sup>2</sup>) for 30 min to 1 h to allow maternal bonding. At 1 h postpartum, lamb sex and birth weight were recorded and the umbilical cord was clipped and dipped in iodine. Colostrum and blood samples were taken from ewes within 1 h of parturition, before lambs suckled. Lambs were then bled via jugular puncture using non-heparinized vacutainers. Lambs were fitted with a rectal temperature sensor connected to a mini-logger 2000 (Mini Mitter Company, Inc, Survivor, OR). After an initial temperature reading, both twin lambs were placed in crates (183 cm<sup>2</sup>) and put in a 0°C dry cold environmental chamber for 30 min; lamb rectal temperature was recorded automatically every 60 s. After cold exposure, lambs were removed from the cold chamber, bled via jugular puncture, warmed artificially and returned to their dam.

### Sample Analysis

Blood samples were centrifuged for 20 min at 1000 x g. Serum from ewes was then decanted into plastic tubes and stored at -20<sup>0</sup> C until assayed for blood urea nitrogen (BUN), NEFA, glucose, cholesterol, and total protein. Serum from lamb was assayed for

glucose, cholesterol, total protein, BUN, NEFA, cortisol, triiodothyronine ( $T_3$ ), thyroxine ( $T_4$ ), and  $T_3:T_4$  ratio. Non-etherified 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 (Catalog 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. Total protein concentrations were measured using a bichromatic (540, 700 nm) endpoint technique. Cortisol,  $T_3$ , and  $T_4$  concentrations were assayed by a solid-phase RIA kits (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA) (Berardinelli et al., 1992).

### Statistical Analysis

Temperature data were analyzed using the repeated measures procedure of SAS (SAS Inst., Inc., Cary, NC). Blood metabolite data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The model included the effects of ewe age, lambing date, energy source, level of supplemental vitamin E, and the interaction between energy source and vitamin E. Statistical significance level was set at alpha 0.10. Ewe was the experimental unit so lamb weights were summed to calculate kilograms of lamb born, turned out, and weaned per ewe.

Trial 2:Objective & Hypotheses:

The objective of trial 2 was to determine the effects of supplemental safflower seeds and vitamin E to late gestating ewes on lamb growth. Three hypotheses were used to test this objective. Hypothesis 1 ( $H_{01}$ ) lambs born to ewes supplemented during late gestation with safflower seeds or an isocaloric and isonitrogenous control will not differ in: body weight. Hypothesis 2 ( $H_{02}$ ) lambs born to ewes supplemented during late gestation with vitamin E or an isocaloric and isonitrogenous control supplement with no added vitamin E will not differ in: body weight. Hypothesis 3 ( $H_{03}$ ) is that lambs born to ewes supplemented during late gestation with a combination of safflower seeds and/or vitamin E or an isocaloric and isonitrogenous control will not differ in: body weight.

Ewes

Single and twin bearing white-faced ewes ( $n = 573$  in 2005,  $n = 609$  in 2006) were randomly assigned to one of the four treatment combinations described in Trial 1. Pregnant ewes were managed at Montana State University's Red Bluff Research Ranch near Norris, Montana. Ewes were randomly assigned within breed (Columbia, Rambouillet, and Targhee) and age (2 to 7 years old) so that each treatment group had a similar average age and number of each breed. Ewes were mass fed within treatment groups their assigned supplements the last 30 days of gestation. Ewes had ad libitum access to alfalfa hay (Table 1) and water. Treatments were administered March 10, 2005 to April 10, 2005 and March 13, 2006 to April 15, 2006. All animal procedures were

approved by the Montana State University Institutional Animal Care and Use Committee (Protocol #AA-030).

### Data Collection

Lambs were processed according to MSU protocol at birth with sex, birth type, birth weight, birthday, and breed information recorded. During lambing ewes were observed 24 h/d. Ewes and lambs were in individual pens for 24 h postpartum. Lamb body weights were recorded again at turnout and weaning with kilograms of lamb/ewe calculated. Lambs that died were included in the analysis as 0 kg body weight. Body condition score was assigned to all ewes prior to treatment (February, 2005), at shearing (April, 2005), at spring turnout (May 24, 2005), and at weaning (August 24, 2005).

### Statistical Analysis

Production data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The model included the effects of year, ewe breed, ewe age, number born, lambing date, energy source, level of vitamin E, and all two and three way interactions, and the four way interaction. Lamb survival was analyzed using the GLM procedure of SAS, the model included the effects of year, ewe age, lambing date, birth type, energy source, level of vitamin E. Significance level was set at alpha 0.10. Ewe was the experimental unit so lamb weights were summed to calculate kilograms of lamb born, turned out, and weaned per ewe; lamb survival was presented as lambs alive at spring turnout and weaning per lambs born per ewe.

## CHAPTER 4

## RESULTS AND DISCUSSION

Trial 1 Results:Lamb Serum Metabolites

There was an energy source by level of vitamin E supplementation interaction for glucose numeric change; T<sub>3</sub>:T<sub>4</sub> 30 min and percent change; and NEFA 0 min, numeric change and percent change ( $P \leq 0.10$ ). Lambs from SSVC ewes had an increase ( $P = 0.06$ ) in serum glucose during the cold exposure, while lambs born to GCVC ewes had a decrease in serum glucose (Table 3). Lambs born to SSVE ewes had greater ( $P = 0.10$ ) level of T<sub>3</sub>:T<sub>4</sub> at 30 min than all other lambs. Lambs born to GCVC ewes were the only group that had a decrease ( $P = 0.08$ ) in percent change of T<sub>3</sub>:T<sub>4</sub> over the cold exposure. Lambs born to SSVC ewes had the greatest ( $P = 0.03$ ) concentration of NEFA at 0 min and were the only group to have a decrease ( $P < 0.08$ ) in numeric and percent change in NEFA concentration over the cold exposure.

Lambs born to SS ewes tended to have lower ( $P = 0.11$ ) levels of BUN and lower T<sub>4</sub> ( $P = 0.07$ ) at 0 min than lambs born to GC ewes (Table 3). Percent change in BUN and numeric change in BUN, T<sub>3</sub>, and T<sub>3</sub>T<sub>4</sub> differed ( $P < 0.10$ ) between lambs born to SS and GC ewes. After 30 min of cold exposure, SS lambs increased and GC lambs decreased in concentration of these metabolites.

The only blood metabolite influenced by vitamin E supplementation was  $T_3:T_4$  ratio at 0 min (Table 3). Lambs born to ewes that received supplemental vitamin E had higher ( $P < 0.10$ )  $T_3:T_4$  levels than lambs born to ewes that did not receive supplemental vitamin E.

Table 2. Least squares means for initial (0 min) and final (30 min) serum metabolites for cold stressed lambs (exposed to 0°C dry cold for 30 min) born to ewes fed 226 g whole safflower seeds or 340 g safflower control and 350 IU vitamin E or 0 IU vitamin E control supplement for the last 30 d gestation

| n=42                           | Treatment <sup>1</sup> |                    |                    |                    |       | S x. V <sup>2</sup> | SS vs. GC | VE vs. VC |
|--------------------------------|------------------------|--------------------|--------------------|--------------------|-------|---------------------|-----------|-----------|
| Metabolite                     | SSVE                   | SSVC               | GCVE               | GCVC               | SEM   | P-value             | P-value   | P-value   |
| Glucose, mg/dl                 |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 44.2                   | 48.6               | 43.0               | 49.2               | 8.05  | 0.90                | 0.97      | 0.48      |
| 30 min                         | 41.8                   | 57.0               | 44.2               | 41.8               | 7.22  | 0.20                | 0.35      | 0.35      |
| Change                         | -2.4 <sup>ab</sup>     | 8.4 <sup>a</sup>   | 1.2 <sup>ab</sup>  | -7.4 <sup>b</sup>  | 5.46  | 0.06                |           |           |
| % Change                       | -2.7                   | 28.9               | 9.4                | 0.6                | 14.53 | 0.14                | 0.55      | 0.41      |
| Cholesterol, mg/dl             |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 21.3                   | 22.7               | 22.7               | 24.3               | 1.54  | 0.96                | 0.32      | 0.31      |
| 30 min                         | 23.1                   | 23.0               | 23.1               | 23.9               | 1.29  | 0.71                | 0.71      | 0.78      |
| Change                         | 1.8                    | 0.2                | 0.4                | -0.4               | 0.93  | 0.67                | 0.26      | 0.20      |
| % Change                       | 9.3                    | 1.2                | 2.5                | 1.1                | 3.51  | 0.32                | 0.30      | 0.15      |
| Total Protein, mg/dl           |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 4.3                    | 4.4                | 4.3                | 4.2                | 0.09  | 0.23                | 0.72      | 0.82      |
| 30 min                         | 4.2                    | 4.2                | 4.2                | 4.2                | 0.06  | 0.26                | 0.99      | 0.61      |
| Change                         | -0.1                   | -0.1               | -0.1               | -0.1               | 0.06  | 0.56                | 0.61      | 0.85      |
| % Change                       | -2.1                   | -3.1               | -2.2               | -1.6               | 1.45  | 0.56                | 0.62      | 0.88      |
| BUN, mg/dl                     |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 22.5                   | 21.3               | 23.2               | 24.7               | 1.38  | 0.30                | 0.11      | 0.91      |
| 30 min                         | 22.8                   | 22.0               | 22.3               | 24.3               | 1.38  | 0.28                | 0.48      | 0.66      |
| Change                         | 0.4                    | 0.8                | -0.9               | -0.4               | 0.44  | 0.90                | 0.01      | 0.30      |
| % Change                       | 2.2                    | 3.9                | -4.2               | -1.9               | 2.08  | 0.87                | 0.01      | 0.30      |
| T <sub>3</sub> , ng/ml         |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 27.4                   | 23.0               | 29.9               | 27.6               | 2.91  | 0.66                | 0.14      | 0.16      |
| 30 min                         | 28.6                   | 24.6               | 25.4               | 25.2               | 3.62  | 0.52                | 0.66      | 0.49      |
| Change                         | 1.1                    | 1.6                | -4.6               | -2.4               | 3.28  | 0.75                | 0.07      | 0.63      |
| % Change                       | 10.6                   | 15.2               | -14.7              | 11.9               | 14.24 | 0.35                | 0.23      | 0.19      |
| T <sub>4</sub> , ug/ml         |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 0.97                   | 0.97               | 1.06               | 1.09               | 0.061 | 0.75                | 0.07      | 0.74      |
| 30 min                         | 0.94                   | 1.03               | 1.06               | 1.06               | 0.077 | 0.54                | 0.32      | 0.57      |
| Change                         | -0.02                  | 0.06               | 0.01               | -0.04              | 0.063 | 0.29                | 0.54      | 0.71      |
| % Change                       | -1.41                  | 8.48               | 0.91               | -2.75              | 6.236 | 0.25                | 0.45      | 0.60      |
| T <sub>3</sub> :T <sub>4</sub> |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 29.1                   | 23.8               | 28.4               | 26.3               | 2.65  | 0.47                | 0.67      | 0.09      |
| 30 min                         | 31.4 <sup>a</sup>      | 24.9 <sup>b</sup>  | 24.3 <sup>b</sup>  | 25.2 <sup>b</sup>  | 2.77  | 0.10                |           |           |
| Change                         | 2.3                    | 1.1                | -4.2               | -1.1               | 3.02  | 0.38                | 0.08      | 0.69      |
| % Change                       | 16.1 <sup>a</sup>      | 9.1 <sup>ab</sup>  | -13.7 <sup>b</sup> | 16.3 <sup>a</sup>  | 12.96 | 0.08                |           |           |
| Cortisol, ng/ml                |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 124.5                  | 101.6              | 111.5              | 98.4               | 14.58 | 0.72                | 0.55      | 0.19      |
| 30 min                         | 82.5                   | 83.9               | 74.2               | 70.2               | 11.53 | 0.81                | 0.31      | 0.91      |
| Change                         | -42.0                  | -17.7              | -37.3              | -28.2              | 11.13 | 0.47                | 0.78      | 0.12      |
| % Change                       | -33.5                  | 17.7               | -30.3              | -11.4              | 11.37 | 0.90                | 0.70      | 0.17      |
| NEFA, mEq/L                    |                        |                    |                    |                    |       |                     |           |           |
| 0 min                          | 1.16 <sup>a</sup>      | 1.52 <sup>b</sup>  | 1.35 <sup>ab</sup> | 1.18 <sup>a</sup>  | 0.124 | 0.03                |           |           |
| 30 min                         | 1.31                   | 1.42               | 1.41               | 1.27               | 0.097 | 0.18                | 0.79      | 0.84      |
| Change                         | 0.15 <sup>a</sup>      | -0.12 <sup>b</sup> | 0.06 <sup>ab</sup> | 0.11 <sup>a</sup>  | 0.098 | 0.08                |           |           |
| % Change                       | 16.13 <sup>a</sup>     | -7.08 <sup>b</sup> | 8.63 <sup>ab</sup> | 15.86 <sup>a</sup> | 8.322 | 0.06                |           |           |

<sup>a,b</sup> Within row, means without a common superscript letter differ,  $P < 0.10$

<sup>1</sup> SSVE = 226 g whole safflower seeds (SS) and 350 IU vitamin E (VE); SSVC = SS and 0 IU vitamin E (VC); GCVE = 350 g whole safflower seeds and 350 IU vitamin E (VE); GCVC = GC and VC

<sup>2</sup> S x. V = Interaction between safflower treatments and vitamin E treatments

### Ewe Serum Metabolite

No source of energy by level of vitamin E supplementation interaction was detected ( $P \geq 0.46$ ) for ewe serum metabolites (Table 4). Ewes fed SS had a greater ( $P = 0.01$ ) cholesterol and NEFA concentration than ewes that were fed the grain control. There were no other differences ( $P > 0.16$ ) in ewe serum metabolites when comparing SS to GC. There was no difference ( $P \geq 0.18$ ) in any of the serum metabolites from ewes due to level of supplemental vitamin E.

Table 3. Least squares means for serum metabolites for ewes (one hour postpartum) fed 226 g whole safflower seeds or 340 g safflower control and 350 IU vitamin E or 0 IU vitamin E control supplement for the last 30 d gestation.

| n = 42        | Treatment <sup>1</sup> |       |       |      | S x V <sup>2</sup> |         | SS vs.GC | VEvs.VC |
|---------------|------------------------|-------|-------|------|--------------------|---------|----------|---------|
| Metabolite    | SSVE                   | SSVC  | GCVE  | GCVC | SEM                | P-value | P-value  | P-value |
| Glucose       | 106.9                  | 111.9 | 104.8 | 90.8 | 14.13              | 0.46    | 0.37     | 0.72    |
| Cholesterol   | 68.5                   | 59.9  | 48.9  | 47.2 | 3.23               | 0.46    | 0.01     | 0.18    |
| BUN           | 20.8                   | 20.6  | 22.5  | 23.2 | 1.65               | 0.75    | 0.16     | 0.90    |
| NEFA          | 1.3                    | 1.2   | 0.8   | 0.9  | 0.13               | 0.49    | 0.01     | 0.88    |
| Total Protein | 6.5                    | 6.7   | 6.4   | 6.4  | 0.19               | 0.60    | 0.20     | 0.51    |

<sup>1</sup> SSVE = 226 g ewe<sup>-1</sup>d<sup>-1</sup> Safflower Seeds (SS) and 350 IUewe<sup>-1</sup>d<sup>-1</sup> supplemental vitamin E (VE); SSVC = SS and 0 IUewe<sup>-1</sup>d<sup>-1</sup> supplemental Vitamin E (VC); GCVE= 350 g ewe<sup>-1</sup>d<sup>-1</sup> Isocaloric and Isonitrogenous Grain Control to Safflower Seeds and VE; GCVC = GC and VC

<sup>2</sup> S x. V = Interaction between safflower treatments and vitamin E treatments

### Temperature

All lambs had a higher ( $P < 0.02$ ) rectal temperature after 30 min of cold exposure relative to 0 min (Figure 1). An energy source by level of supplemental vitamin E interaction was detected ( $P < 0.01$ ). The SSVC lambs had a lower body temperature throughout the cold exposure ( $P < 0.03$ ) when compared to all other treatment lambs.

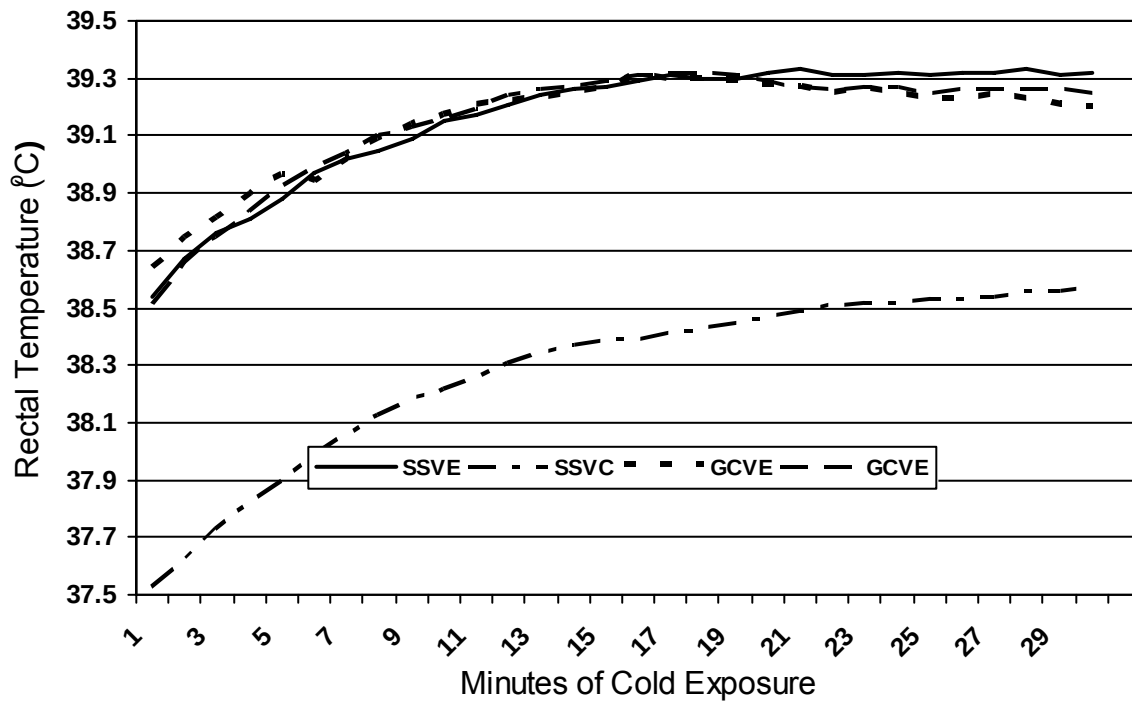


Figure 1. Least squares means of rectal temperature for twin lambs exposed to 0°C for 30 min 1 h after parturition. Treatments were: SSVE = 226 g·ewe<sup>-1</sup>·d<sup>-1</sup> safflower seeds (SS) and 350 IU·ewe<sup>-1</sup>·d<sup>-1</sup> supplemental vitamin E (VE); SSVC = SS and 0 IU·ewe<sup>-1</sup>·d<sup>-1</sup> supplemental vitamin E (VC); GCVE = 340 g·ewe<sup>-1</sup>·d<sup>-1</sup> isocaloric and isonitrogenous grain based control (GC) and VE; GCVV = GC and VC. The SEM ranged from 0.189 at 30 min to 0.333 at 1 min. Repeated measures evaluations of the effects of time, and energy source vs. level of vitamin E interaction were detected ( $P < 0.02$ ).

### Lamb Production

Birth wt did not differ ( $P = 0.58$ ) between SS and GC lambs (Table 5). Lambs born to SS ewes had a lower weaning weight ( $P = 0.10$ ) and tended ( $P = 0.16$ ) to have a lower turnout weight than lambs born to GC ewes. Lamb body weights did not differ ( $P < 0.46$ ) between VE and VC lambs.

Table 4. Least squared means of lamb body weight at birth, turnout, and weaning of lambs born to ewes fed 226 g whole safflower seeds or 340 g safflower control and 350 IU vitamin E or 0 IU vitamin E control supplement for the last 30 d gestation.

| n = 42         | Treatments <sup>1</sup> |      |      |      | SEM  | S x. E <sup>2</sup> |         | SSvsGC | VE vs.VC |
|----------------|-------------------------|------|------|------|------|---------------------|---------|--------|----------|
|                | SSVE                    | SSVC | GCVE | GCVC |      | P-value             | P-value |        |          |
| Birth Weight   | 9.2                     | 9.0  | 9.1  | 8.7  | 0.41 | 0.78                | 0.58    |        | 0.47     |
| Turnout Weight | 22.9                    | 23.4 | 25.2 | 26.2 | 1.86 | 0.89                | 0.16    |        | 0.65     |
| Weaning Weight | 53.3                    | 54.3 | 59.7 | 60.5 | 4.06 | 0.99                | 0.10    |        | 0.81     |

<sup>1</sup> SSVE = 226 g ewe<sup>-1</sup>d<sup>-1</sup> Safflower Seeds (SS) and 350 IUewe<sup>-1</sup>d<sup>-1</sup> supplemental vitamin E (VE); SSVC = SS and 0 IUewe<sup>-1</sup>d<sup>-1</sup> supplemental Vitamin E (VC); GCVE= 350 g ewe<sup>-1</sup>d<sup>-1</sup> Isocaloric and Isonitrogenous Grain Control to Safflower Seeds and VE; GCVC = GC and VC

<sup>2</sup> S x V = Interaction between safflower treatments and vitamin E treatments

### Ewe Production:

Interactions were detected for ewe body weight at turnout and weaning (Table 6). Ewes supplemented with SSVC weighed more ( $P < 0.06$ ) at turnout and weaning than GCVC ewes. There was no difference in BCS at turnout between SS and GC ewes ( $P > 0.37$ ) or VE and VC ewes ( $P > 0.71$ ). The VE ewes had a greater ( $P = 0.10$ ) BCS at weaning than VC ewes.

Table 5. Least squared means of body weight and body condition score for ewes fed 226 g whole safflower seeds or 340 g grain control and 350 IU vitamin E or 0 IU vitamin E control supplement for the last 30 d gestation.

| n = 42         | Treatments <sup>1</sup> |                   |                    |                   | SEM  | S x E <sup>2</sup> |         | SS vs.GC | VEvs.VC |
|----------------|-------------------------|-------------------|--------------------|-------------------|------|--------------------|---------|----------|---------|
|                | SSVE                    | SSVC              | GCVE               | GCVC              |      | P-value            | P-value | P-value  | P-value |
| Turnout Weight | 63.0 <sup>ab</sup>      | 67.8 <sup>b</sup> | 65.2 <sup>ab</sup> | 61.0 <sup>a</sup> | 2.26 | 0.05               |         |          |         |
| Weaning Weight | 71.5 <sup>a</sup>       | 76.8 <sup>b</sup> | 74.0 <sup>ab</sup> | 70.8 <sup>a</sup> | 2.37 | 0.04               |         |          |         |
| Turnout BCS    | 2.9                     | 3.0               | 2.9                | 2.8               | 0.14 | 0.38               | 0.38    |          | 0.71    |
| Weaning BCS    | 3.1                     | 2.9               | 3.0                | 2.8               | 0.12 | 0.78               | 0.44    |          | 0.10    |

<sup>a, b</sup> Within row, means without a common superscript letter differ,  $P < 0.10$

<sup>1</sup> SSVE = 226 gwe<sup>-1</sup>d<sup>-1</sup> Safflower Seeds (SS) and 350 IUewe<sup>-1</sup>d<sup>-1</sup> supplemental vitamin E (VE); SSVC = SS and 0 IUewe<sup>-1</sup>d<sup>-1</sup> supplemental Vitamin E (VC); GCVE= 350 gwe<sup>-1</sup>d<sup>-1</sup> Isocaloric and Isonitrogenous Grain Control to Safflower Seeds and VE; GCVC = GC and VC

<sup>2</sup> S x V = Interaction between safflower treatments and vitamin E treatments

### Trial 1 Discussion

Levels of glucose, NEFA and BUN indicate that SSVC lambs were unable to metabolize their fat reserves as efficiently as other lambs. The SSVC lambs experienced an increase in glucose over the cold exposure which indicates that they utilized energy reserves from the body to increase blood glucose levels as none of the lambs were fed colostrum before cold exposure. The SSVC lambs were the only group to have a decrease in NEFA over the cold exposure indicating they were unable to metabolize fat reserves as quickly as lambs in the other treatment groups. The SS lambs also had lower BUN at 0 min than GC lambs; however SS lambs increased BUN over the cold exposure

while GC lambs decreased. This indicates that the SS lambs may have mobilized protein reserves within the muscle to fuel metabolism and possibly non-shivering thermogenesis. Contrary to our results, Encinias et al. (2004) reported NEFA and glucose concentrations were not different between lambs born to ewes on a 5.7% fat or 2.8% fat prepartum diet, indicating that metabolism of energy reserves, specifically lipid metabolism, of lambs in that study were occurring at a similar rate. Furthermore, SSVC lambs had the highest level of NEFA before cold exposure; it is possible that during the 60 min between parturition and cold exposure SSVC lambs had already utilized blood glucose and free fatty acids in a warm environment. When SSVC lambs were metabolically challenged by the cold environment they may have had to utilize more protein reserves as they were not able to utilize fat reserves as efficiently as lambs in other treatments groups.

Data from lamb temperature during cold exposure indicates that SSVC lambs had a decreased cold tolerance. An interaction between energy source and level of vitamin E supplementation on lamb temperature supports the conclusion SSVC lambs were unable to mobilize fat reserves as efficiently as lambs in other treatment groups. Conflicting results have been reported for the effect of safflower seed supplementation on body temperature. There have been no literature on the effect of both supplemental safflower seeds and vitamin E on neonatal ruminant temperature. Lammoglia et al. (1999) reported that calves born to cows receiving a 5.1% fat diet prepartum had a higher initial rectal temperature and maintained that temperature longer than calves born to cows receiving a 2.2% fat diet prepartum. Similarly, Encinias et al. (2004) reported that lambs born to

ewes receiving 2.8% fat diet prepartum had a higher rectal temperature than lambs born to ewes receiving 5.7% fat diet prepartum.

In Lammoglia et al. (1999) and Encinias et al. (2004) safflower supplementation increased temperature, this was not the case in our study. Our findings are that safflower seed supplementation without vitamin E will result in lambs with lower body temperature; there was no difference between SSVE, GCVE and GCVC lamb's temperature indicating that safflower seed supplementation did not increase cold tolerance in lambs. This is supported by Dietz et al. (2003) who reported no difference in response to cold stress between calves born to heifers receiving 1.5% fat control diet, 4.0% fat safflower diet, and 5.0% fat cottonseed diet. The calves in this study were not in a maintained cold environment, the ambient air was considered to be sufficiently different from the uterine environment to induce cold stress. If the calves had been born during severe weather, or been exposed to a regulated cold environment, the results may have been different. Dietz et al. (2003) concluded that high fat diets may not be beneficial when calving during mild spring conditions.

The effects of SSVC supplementation on serum metabolites and cold tolerance did not result in decreased lamb weights at birth or turnout. Our study is supported by Dietz et al., 2003, Bottger et al., 2002, Encinas et al. 2004 who reported no differences in birth weights of calves and lambs in studies comparing high and low fat diets using safflower seed supplementation during late gestation.

Our results conflict with Bottger et al. (2002) who reported no difference in calf weaning weights when comparing safflower seed and grain based diets. In our study,

lambs that died or were missing were assigned 0.0 kg body weight. It is unclear if the differences in weaning weight and turnout weight in this study are due to differences in survival or weight gain. Numerically SS ewes lost more lambs than GC ewes (7 lambs vs. 1 lamb); however the sample size was not large enough for statistical analysis. Daniels et al. (2000) reported no difference in lamb survival rates at 30 d postpartum; Encinias et al. (2004) reported lambs born to ewes on a high fat diet had increased survival compared to lambs born to ewes on a low fat diet. Our results however were not what we expected as SS lambs had lower survival than GC lambs.

Lamb body weights did not differ between VC and VE lambs. This is in agreement with research done by Kott et al. (1998) and Williamson et al. (1995) which conclude that weaning weights did not differ due to vitamin E, however Thomas et al. (1995) reported that ewes supplemented with vitamin E weaned more kg of lambs due to a decrease in mortality rates in lambs born to ewes supplemented with vitamin E. In the current study there was no numerical difference in mortality rates. Thomas et al. (1995), Kott et al. (1998) and Williamson et al. (1995) all reported that birth weight did not differ with vitamin E supplementation which supports our study. However, Capper et al. (2005) reported that vitamin E supplementation increased lamb birth weights.

The SSVC ewes had heavier body weight at turnout and weaning than ewes in other treatment groups. Our study contradicts, Thomas et al. (1995), Kott et al. (1998), Daniels et al. (2000), and Encinias et al. (2004) all of whom reported no differences in ewe body weight when supplemented with vitamin E. Capper et al. (2005) reported no differences in ewe production as measured by milk production. While there may be a

statistical difference in BCS between VE and VC ewes, the difference in condition is minimal and probably not a biological significance. Interestingly the SSVC ewes had the heaviest body weight and yet their lambs were the least tolerant of cold exposure. The SS also numerically lost more lambs than GC ewes.

The SS ewes had the greater concentration of NEFA than GC ewes; Encinias et al. (2004) also reported ewes fed 5.7% fat prepartum diet had higher NEFA concentration at parturition than ewes fed a 2.8% fat diet. Interestingly in that same study glucose concentration were higher for ewes fed 2.8% fat than ewes fed 5.7% fat at parturition while there were no differences in ewe glucose in our study.

Trial 2 Results:

There were no two-way interactions ( $P > 0.29$ ) between energy source and level of vitamin E supplementation, year and energy source, year and level of vitamin E, ewe breed and energy source, or ewe breed and level of vitamin E for lamb body weight, lamb survival, ewe body weight or ewe BCS. There were no 3-way interactions ( $P > 0.25$ ) between energy source, level of vitamin E supplementation, and year for lamb body weight, lamb survival, ewe body weight or ewe BCS. There was no 4-way interaction ( $P > 0.14$ ) between energy source, level of vitamin E supplementation, year, and ewe breed for lamb body weight, lamb survival, ewe body weight or ewe BCS.

Lamb birth weight did not differ ( $P > 0.42$ ) between energy sources (Table 7). The SS lambs weighed less ( $P = 0.07$ ) at turnout than GC lambs. Although there was no difference ( $P = 0.26$ ) in percent lambs born, SS ewes had a lower ( $P = 0.02$ ) percent survival at turnout than GC ewes (Table 7). Lamb birth weight, body weight and percent lamb survival at turnout and weaning did not differ ( $P > 0.36$ ) between levels of vitamin E treatments (Table 8).

Table 6. Least squared means of body weight and percent survival for lambs born to ewes fed 226 g whole safflower seeds or 340 g grain control for the last 30 d gestation.

| n = 1166                          | Treatments <sup>1</sup> |       | SS vs. GC |         |
|-----------------------------------|-------------------------|-------|-----------|---------|
|                                   | SS                      | GC    | SEM       | P-value |
| Birth weight (kg)                 | 6.8                     | 6.9   | 0.16      | 0.42    |
| Turnout Weight (kg) <sup>2</sup>  | 14.5                    | 15.1  | 0.45      | 0.07    |
| Weaning Weight (kg) <sup>3</sup>  | 38.0                    | 41.4  | 0.98      | 0.01    |
| Lambs Born (%)                    | 143.6                   | 140.4 | 3.66      | 0.26    |
| Turnout Survival <sup>2</sup> (%) | 122.3                   | 127.8 | 3.13      | 0.02    |
| Weaning Survival <sup>3</sup> (%) | 123.0                   | 126.4 | 0.03      | 0.21    |

<sup>1</sup> SS = 226 gwe<sup>-1</sup>d<sup>-1</sup> Safflower Seeds; GC= 350 gwe<sup>-1</sup>d<sup>-1</sup> Isocaloric and Isonitrogenous Grain Control

<sup>2</sup> measured at spring turnout May 25, 2005 and May 18, 2006

<sup>3</sup> measured at weaning Aug. 25, 2005 and Aug. 23, 2006

Table 7. Least squared means of body weight and percent survival for lambs born to ewes fed 350 IU vitamin E or 0 IU vitamin E control supplement for the last 30 d gestation.

| n = 1166                          | Treatments <sup>1</sup> |       | VE vs. VC |         |
|-----------------------------------|-------------------------|-------|-----------|---------|
|                                   | VE                      | VC    | SEM       | P-value |
| Birth weight                      | 6.8                     | 6.8   | 0.22      | 0.93    |
| Turnout Weight <sup>2</sup>       | 14.7                    | 14.9  | 0.44      | 0.66    |
| Weaning Weight <sup>3</sup>       | 39.3                    | 40.1  | 0.79      | 0.36    |
| Lambs Born (%)                    | 143.6                   | 140.4 | 3.67      | 0.26    |
| Turnout Survival <sup>2</sup> (%) | 124.8                   | 125.2 | 3.11      | 0.88    |
| Weaning Survival <sup>3</sup> (%) | 123.7                   | 125.7 | 0.03      | 0.45    |

<sup>1</sup> VE = 350 IUewe<sup>-1</sup>d<sup>-1</sup> supplemental vitamin E; VC = 0 IUewe<sup>-1</sup>d<sup>-1</sup> supplemental Vitamin E

<sup>2</sup> measured at spring turnout May 25, 2005 and May 18, 2006

<sup>3</sup> measured at weaning Aug. 25, 2005 and Aug. 23, 2006

Ewe body weight at turnout did not differ ( $P = 0.17$ ) between energy sources (Table 9). The GC ewes had a higher BCS ( $P = 0.03$ ) than SS ewes. At weaning, SS ewes had lighter ( $P = 0.04$ ) body weight and greater ( $P = 0.06$ ) BCS than GC ewes. Although ewe body weight at turnout and weaning did not differ ( $P \geq 0.18$ ) between levels of vitamin E (Table 10), VE ewes had greater ( $P = 0.09$ ) BCS at turnout and weaning than VC ewes.

Table 8. Least squared means of body weight and body condition score for ewes fed 226 g whole safflower seeds or 340 g grain control for the last 30 d gestation.

| n = 1166                    | Treatments <sup>1</sup> |      | SS vs. GC |         |
|-----------------------------|-------------------------|------|-----------|---------|
|                             | SS                      | GC   | SEM       | P-value |
| Turnout Weight <sup>2</sup> | 57.5                    | 58.1 | 0.51      | 0.17    |
| Turnout BCS <sup>2</sup>    | 3.1                     | 3.2  | 0.03      | 0.03    |
| Weaning Weight <sup>3</sup> | 60.7                    | 61.5 | 0.40      | 0.04    |
| Weaning BCS <sup>3</sup>    | 3.1                     | 3.0  | 0.03      | 0.06    |

<sup>1</sup> SS = 226 g ewe<sup>-1</sup>d<sup>-1</sup> Safflower Seeds; GC = 340 g ewe<sup>-1</sup>d<sup>-1</sup> Isocaloric and Isonitrogenous Grain Control

<sup>2</sup> measured at spring turnout May 25, 2005 and May 18, 2006

<sup>3</sup> measured at weaning Aug. 25, 2005 and Aug. 23, 2006

Table 9. Least squared means of body weight and body condition score for ewes fed 350 IU vitamin E or 0 IU vitamin E control supplement for the last 30 d gestation.

| n = 1166                    | Treatments <sup>1</sup> |      | VE vs. VC |         |
|-----------------------------|-------------------------|------|-----------|---------|
|                             | VE                      | VC   | SEM       | P-value |
| Turnout Weight <sup>1</sup> | 57.9                    | 57.7 | 0.51      | 0.74    |
| Turnout BCS <sup>1</sup>    | 3.2                     | 3.1  | 0.03      | 0.09    |
| Weaning Weight <sup>2</sup> | 61.4                    | 60.8 | 0.40      | 0.18    |
| Weaning BCS <sup>2</sup>    | 3.1                     | 3.0  | 0.03      | 0.09    |

<sup>1</sup> VE = 350 IU ewe<sup>-1</sup>d<sup>-1</sup> supplemental vitamin E; VC = 0 IU ewe<sup>-1</sup>d<sup>-1</sup> supplemental Vitamin E

<sup>2</sup> measured at spring turnout May 25, 2005 and May 18, 2006

<sup>3</sup> measured at weaning Aug. 25, 2005 and Aug. 23, 2006

### Trial 2 Discussion

In our study, there was no difference in lamb birth weight between lambs born to SS and GC ewes. These results are supported by Encinias et al. (2004) who reported no difference in birth weights between lambs born to ewes supplemented with either 2.8% fat or 5.7% fat diets during late gestation. Lammoglia et al (1999) also reported no differences in birth weights with calves born to heifers fed 2.2% fat or 5.1% fat diets during late gestation. Dietz et al. (2003) reported no difference in birth weights of calves born to heifers fed 1.5% fat control diet, 4.0% fat diet that included safflower seeds and 5% fat diet that included cotton seed. Encinias et al. (2004) reported no difference in weaning weights between lambs born to ewes fed 2.8% fat or 5.7% fat diets during late gestation diet.

The GC lambs in our study had higher survival rates at turnout than SS lambs (Table 6). In contrast to our study, Encinias et al. (2004) reported lambs born to ewes fed a 5.7% fat diet during late gestations had lambs with greater survivability than lambs born to ewes fed 2.8% fat prepartum diet. Supplementing with safflower seeds could have a negative effect on colostrum. Although colostrum was not analyzed in our study, Dietz et al. (2003) reported heifers fed safflower seeds had greater colostral solids than heifers fed control diet. Dietz et al. (2003) supports personal observations of colostrum in our study. The colostral solids may have put SS lambs at a disadvantage, as lambs born to

safflower supplemented ewe may not be able to consume adequate levels of colostrum. If these lambs were already metabolically stressed the added energy required to nurse and lower energy intake may have resulted in greater neonatal loss.

Our study indicates no difference in birth weight and turnout weights between levels of vitamin E supplementation (Table 9). Williamson et al. (1995) also reported no difference in birth weights or weaning weights when ewes were injected 2 weeks prior to lambing with 2400 IU vitamin E and lambs were injected with 1200 IU vitamin E. Thomas et al. (1995) and Kott et al. (1998) which also fed ewes vitamin E at the same amount and research facility as our study reported no difference in birth weight between vitamin E supplemented ewes and a non-supplement control. However in contrast with our study, Capper et al. (2005) who used 2x2 factorial arrangements of treatments to compare the effects of feeding PUFA vs. saturated fat and 500mg/kg vitamin E vs. 50 mg/kg vitamin E/ewe for the last 6 weeks of gestation indicated feeding 500 mg/kg ewe of vitamin E resulted in heavier birth weight than lambs born to ewes fed 50 mg/kg ewe. Interestingly lamb birth weight did not differ with type of fat supplementation the ewe received.

There was no difference between level of vitamin E supplementation for lamb survival at turnout. Our results are supported by Williamson et al (1995) but contradicted by Thomas et al. (1995) and Kott et al. (1998). Thomas et al. (1995) and Kott et al. (1998) fed ewes at Red Bluff Research ranch, the site of our study.

Both Thomas et al. (1995) and Kott et al. (1998) reported that supplementing with vitamin E can decrease lamb mortality of lambs born in the early part of the lambing season. Our study includes all ewes, not those ones lambing in the early part of the lambing season.

There was no difference in ewe body weight at turnout between SS and GC ewes, this could be due to the isocaloric nature of the diets. There was a statistical difference in BCS with GC ewes having greater BCS than SS ewes. However due to the subjective nature of assigning BCS and lack of variation in BCS this probably isn't biologically significant. Encinias et al (2004) also reported no difference in ewe body weight or condition with safflower seed supplementation.

There was no difference in ewe body weight at turnout between VE and VC ewes. Kott et al. (1998), however, reported ewes fed vitamin E weighted less at turnout than ewes fed control diets, however in that study there was no difference in BCS at turnout. Our study indicated that VE ewes had more body condition than VC ewes, but again, this probably isn't biologically significant.

## CHAPTER 5

### CONCLUSION

Hypothermia, starvation, scours, and pneumonia are the major causes of neonatal lamb mortality with 50% of lamb losses occurring within 24 hours of birth (Rowland et al., 1992). Alexander (1961) estimated that 50% of the heat generated by ruminant neonates comes from non-shivering thermogenesis which is fueled solely by BAT. Linoleic and linolenic acid supplements, such as safflower seeds, increased the thermogenic capacity of BAT (Nedergaard et al., 1983). Increased heat production from BAT increases the oxidation of BAT, which results in the formation of free radicals. Free radicals cause damage to cellular membranes; therefore creating a need for more antioxidants to maintain cell integrity. Vitamin E, a potent antioxidant, protects cellular membranes by sequestering free radicals and sparing cell membranes from oxidative degradation (Horton et al., 1996). Furthermore, the importance of antioxidants such as vitamin E can clearly be seen when dealing with high levels of PUFAs, such as safflower seed supplementation, which are known to increase rate of lipid oxidation and formation of free radicals.

In Trial 1, the effects of energy source and level of vitamin E on lamb body temperature and serum metabolites were examined, while Trial 2 examined the effect of energy source and level of vitamin E on lamb growth and survival. In Trial 1, based on lower body temperature at birth of lambs born to ewes fed safflower seeds and vitamin control and levels of glucose, NEFA, and BUN during the cold exposure, it appears that

these lambs were born with an apparent decrease in basal metabolic rate. This indicates that supplementing ewes with safflower seed but not vitamin E may compromise the newborn lamb's ability to adapt to extreme environmental conditions. In Trail 2, lambs born to ewes supplemented with safflower seeds also endured decreased weight gain and survival rates compared to lambs born to ewes supplemented with grain control. Supplementing ewes with safflower seeds during late gestation may result in a decrease in lamb production potential.

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