# THERMOGENESIS, SERUM METABOLITES AND HORMONES, AND GROWTH IN LAMBS BORN TO EWES SUPPLEMENTED WITH DOCOSAHEXAENOIC ACID

by

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# ABSTRACT

Neonatal lamb mortality is a major factor effecting profitability in the sheep industry. Lamb thermogenesis and immunocompetence are key elements in neonatal lamb survival. Research has shown an increase in lamb vigor, when ewes were supplemented during late gestation with algae-derived docosahexaenoic acid (DHA). However, the impact of DHA on lamb thermogenesis and immunocompetence has not been investigated. Eighty twin-bearing Targhee ewes were assigned randomly to 1 of 2 supplemental treatments to determine the effects of feeding (DHA) to ewes during late gestation and early lactation on lamb thermogenesis, immunocompetence, serum metabolites and hormones, and lamb growth. Treatments within supplements were: 1) 12 g/ewe daily of the product DHA Gold in the form of algal biomass (ALGAE), and 2) no DHA (CONTROL). Treatment supplements were individually fed daily during the last 30 d of gestation and pen fed (6 pens/treatment, and 6 or 7 ewes/pen) during the first 38 d of lactation. One h after lambing and before nursing, twin-born lambs were weighed, bled via jugular puncture, and placed in a dry cold chamber for 30 min (0°C). Lamb rectal temperatures were recorded every 1 min. After 30 min, lambs were removed from the cold chamber, bled, warmed for 15 min, and returned to their dam. Ewes were bled and colostrum samples collected 1 h postpartum. Ewe and lamb sera were assayed for glucose, non-esterified fatty acids (NEFA), cortisol, leptin and anti-Parainfluenza Type 3 ($PI^3$) titers. Lamb rectal temperature, glucose, NEFA, cortisol, leptin, anti-$PI^3$ titers, and birth weights did not differ between treatments. Thirty-eight-d BW was greater ($P$ = 0.03) in lambs born to CONTROL-supplemented than lambs born to ALGAE-supplemented ewes; however, the colostrum of ALGAE-supplemented ewes had a greater specific gravity ($P$ = 0.05), indicating greater IgG concentrations, than colostrum of CONTROL-supplemented ewes. Supplementation of DHA during late gestation and early lactation had a negative impact on lamb BW and did not affect indices of lamb thermogenesis, but may have improved IgG concentrations in ewe colostrum.

# CHAPTER 1

# LITERATURE REVIEW

## Lamb Survival

Nearly 50% of pre-weaning lamb mortalities occur during the first 24 h of life (Dwyer, 2007), with lamb mortality rates of 10 to 35% occurring between birth and weaning in typical western range sheep operations (Rowland et al., 1992). Similar mortality rates were reported in Montana, where the annual lamb mortality rate averaged 23% from 1967 to 1984 (Kott and Thomas, 1987). Similar percentages of lamb mortaility have been reported for sheep in other countries including Australia (Hatcher et al., 2009) and New Zealand (Everett-Hincks and Dodds, 2008).

After an extensive review of lamb survival literature, Dwyer (2007) concluded that birth weight is the single most determining factor in neonatal survival. Larger lambs have a greater risk of death during the birthing process, while smaller lambs have a greater risk of starvation and pneumonia at, or soon after birth. In Australia, lamb survival records of 14,142 Merino lambs born between 1975 and 1983 were examined (Hatcher et al., 2009). Lambs with heavier birth weights (4.60 kg for singles and 4.13 kg for twins) were more likely to survive to weaning than lambs of average birth weights (4.00 for singles and 3.35 kg for twins). Likewise, in New Zealand, lambing records of 20 flocks with a total of 15, 821 white-faced lambs from 2003 and 2004 were examined (Everett-Hincks and Dodds, 2008). For singles, twins, and triplets, the optimum lamb birth weight was 5.5 to 6 kg. Survival data of Scottish Blackface lambs from 2

experiment stations in Scotland were examined (Sawalha et al., 2007). Although single (usually larger) born lambs had lower "hazard" rates from d 1 to 14 after birth, they had greater morality rates due to dystocia than twin-born (usually smaller) lambs. They concluded that selection should be based on lamb viability at birth rather than maximum birth weights.

Hypothermia, starvation, stillbirth, dystocia and pneumonia are leading causes of lamb mortality (Rook et al., 1997). Elsevier (2009) concluded that time from birth to standing is a significant contributor to lamb survival, and harsh environmental temperatures add to the difficulty of a lamb to stand and begin suckling (Falck et al., 2008). Hypothermia brought on by cold environmental conditions comprised over 50% of neonatal lamb deaths (Samson and Slee, 1981) due to physical weakness, depression, and poor nursing response (Olson et al., 1987). In a 3-yr study conducted by Falck et al. (2008), survival rates were significantly affected by the minimum temperature on the day of parturition. Survival rates began to decline at 4 C°, with a 5% decline at -1 C°, and 23% decline in lamb survival when temperatures decreased to -6 C°.

## Lamb Body Temperature Regulation

The lowest ambient temperature at which a homoeothermic animal can no longer maintain a constant core temperature is known as its 'critical temperature' (Li and Tokura, 1997). At this point of cold-stress, the body must generate extra heat to survive. If a newborn lamb remains in a cold-stressed environment and does not stand and suckle, body energy reserves will be depleted and the lamb will eventually lose its ability to

thermoregulate and die of hypothermia (Clarke et al, 1997b; Nowak and Poindron, 2006). In lambs, the active biological processes involved in generating heat include shivering and non-shivering thermogenesis (Alexander, 1979).

Shivering Thermogenesis

The primary motor center for shivering is anatomically located in dorsomedial portion of the hypothalamus (Lessard et al., 1988). Cold signals from the skin and spinal cord activate this center, causing contraction of the skeletal muscles throughout the body. Shivering begins once this skeletal structure meets a certain level of contraction (Lessard et al., 1988).

Although an important source of heat in adult mammals, shivering thermogenesis may be insignificant in new-borns (Taylor, 1960; Hull and Segall, 1965). Shivering thermogenesis only accounted for 10% of the total heat production in guinea pigs born in an environmental temperature of 8 C° (Brück and Wünnenberg, 1965). Hull and Segall (1965) concluded that shivering in the newborn rabbit did not occur until heat production was stressed to the maximum. Shivering and non-shivering thermogenesis were evaluated in newborn lambs by Alexander and Williams (1968). They reported that in the lamb, shivering is just as important as non-shivering thermogenesis; accounting for 50% of the heat production, with the other 50% derived from non-shivering thermogenesis.

Non-Shivering Thermogenesis and Brown Adipose Tissue (BAT)

Non-shivering thermogenesis greatly enhances the ability of a newborn mammal to maintain homeothermy under cold environmental conditions (Alexander and Williams, 1968) and accounts for up to 50% of the total heat production (Alexander, 1961; Stott and Slee, 1985). Non-shivering thermogenesis replaces shivering thermogenesis within the first hour of life, with energy derived from BAT (Foster and Frydman, 1979; Curtis, 1981; Symonds and Lomax, 1992; Clarke et al., 1997a). In cold environments, newborn lambs rely on BAT to produce heat and prevent hypothermia (Alexander and Williams, 1968).

Brown adipose tissue is found in the perirenal-abdominal region of lambs (Alexander, 1978), and accounts for 1 to 2% of the birth weight (Alexander and Bell, 1975). Lambs are unique in that their fat stores at birth are composed of almost 100% BAT; whereas other mammals possess both brown and white fat at birth (Encinias et al, 2004). Mitochondrial uncoupling protein (UCP1) is found in the many mitochondria of brown adypocytes. Heat is generated from BAT when UCP1 "uncouples" the electron transport chain, producing heat rather than adenosine triphosphate (ATP, Cannon and Nedergaard, 1985). The amount of heat that is produced depends upon the concentration of UCP1 in the mitochondria of the BAT and the activity of the pathway by which the electron transport chain is uncoupled (Clarke et al., 1997a).

Non-shivering thermogenesis decreases rapidly with age. Shivering thermogenesis replaces non-shivering thermogenesis, as brown fat dissipates (Alexander and Williams, 1968); thereafter, BAT is replaced with white adipose tissue (Symonds et

al., 1992). Lambs placed in a cold chamber at 6 C° had depleted all of the lipid droplets in their brown fat cells by 48 h (Smith et al., 2004) and had reached 'summit metabolism.' In mammals, summit metabolism is defined as the point at which heat loss exceeds the ability to produce heat, and is associated with the onset of clinical hypothermia (Eales and Small, 1980).

The main difference between brown and white adipose tissue is that UCP1 is only present in BAT (Clark et al., 1997a). Clarke et al. (1997a) evaluated the development of adipose tissue during the first 30 d of life in lambs and found that the activity of UCP1 decreased rapidly between 0 and 4 days of age, and again, between 7 and 14 days of age, then remained very low thereafter. Also, between 7 and 30 days of age, there was a large increase in brown adipose tissue weight and lipid content, supposedly due to decreased UCP1 activity. This observation is supported by previous studies that established that BAT functions in the manner of white adipose tissue over the first month of life (Thompson and Jenkinson, 1969; Gemmel et al., 1972) but is eventually depleted and replaced by white adipose tissue (Ailhuad et al., 1992).

## Dietary Supplementation

Newborn lambs derive energy from their tissues until they begin to suckle (Mellor and Cockburn, 1986). An optimal period for enhancing lamb thermogenic potential is during late gestation of the ewe (Encinias et al., 2004). In fact, a majority of the growth of BAT in unborn calves occurred during the last 28 d of gestation (Casteilla et al., 1987).

Dwyer (2007) concluded that undernourished, pregnant ewes gave birth to lighter lambs resulting in increased mortality rates. Light lambs, reduced mammary weight and development, delayed onset of lactation, reduced colostrum, and reduced total milk production are associated with poor nutrition of ewes during gestation (Dwyer, 2007). The effects of cold exposure during gestation (shorn ewes vs unshorn ewes, all well-fed at 100% ME requirements) on BAT (thermogenic activity) of lambs born to these ewes were evaluated by Gate et al. (1999). They reported that maternal cold exposure had no effect on birth weight or perirenal adipose tissue thermogenic activity (based on measures of guanosine diphosphate, GDP, binding to mitochondria and catecholamine content) of lambs. This most likely was due to the fact that ewes in this study were well-fed.

Hess et al. (2008) reviewed a decade of research in the area of dietary fat supplementation in ruminants. They concluded that altering maternal nutrition to incorporate increased levels of fatty acids in the unborn lamb could benefit neonates born under harsh environmental conditions. This assertion is supported by an experiment by Budge et al. (2000) who used 15 Welsh Mountain ewes supplemented with barley-based (primarily starch) diets containing either 100% ME requirements or 150% ME requirements during the last 64 d of gestation (Budge et al., 2000). Diets were either 1 kg of hay and 150 to 200 g of a barley-based concentrate (100% ME) or 1.2 kg of hay and 225 to 300 g of the barley-based concentrate (150% ME). Ewes fed increased levels of energy during the second half of gestation not only produced larger lambs, but these lambs were born with increased quantities of UCP1 , as well as more thermogenic activity (i.e. less guanosine 5'-diphosphate binding) in their BAT.

Levels and Types of Fat

If the goal is to prevent the substitution of forage intake with the consumption of a supplemental fat, fat should be fed at a level of 2% or less of the DMI (Hess et al., 2008). However, on a high concentrate diet, fat can be fed at levels up to 6% of the DMI without negatively impacting starch digestion (Hess et al., 2008). Encinias et al. (2004) evaluated two different levels of dietary fat (2.8% in the form of safflower meal and corn, and 5.7% in the form of safflower seed) fed during the last 42 d of gestation in ewes. Ewes lambed either outdoors or in an unheated barn at North Dakota State University. They discovered that ewes fed the high-fat diet had greater lamb survival rates than ewes fed the low-fat diet. Furthermore, more lambs died that were born to ewes fed the low-fat diet than lambs born to ewes fed the high-fat diet. Both fat levels were higher than those suggested by Hess et al. (2008), so forage intake was likely reduced, although total energy was likely higher due to the high percentage of fat in the study by Encicias et al. (2004),

Likewise, in cattle, Lammoglia et al. (1999a) fed heifers low and high levels of dietary fat (2.2 or 5.1%) during the last 55 d of gestation. At 5 h of age, after being fed 30 mL/kg BW of pooled dairy cow colostrum, calves were placed in a 30°C cold chamber for 140 min. Rectal temperatures and blood samples of calves were collected at 10- and 20-min intervals. They concluding that survival, probably caused by increased cold tolerance in newborn calves, could be improved by feeding high-fat diets to pregnant heifers. In a similar study, Dietz et al. (2003) supplemented pregnant cows during the last 47 d of gestation with three (1.5, 4, and 5%) levels of dietary fat in a hay-based diet.

At 6.5 h of age, calves were placed in a 5°C cold chamber for 90 min. Rectal temperatures of calves born to cows fed 5% fat decreased to a greater degree in response to cold exposure than calves born to cows fed 1.5% fat. Calf BW, vigor, shivering, dystocia score, time to stand, time to nurse, serum glucose concentrations, and serum IgG were not affected by level of fat supplement. One interpretation of these results is that supplementing pregnant cows with fat altered activity of BAT in newborn calves. However, Chen et al. (2007) reported that neonatal BAT stores (determined by UCP1 activity) were not affected by level (2, 4, or 8%) or type (saturated versus monounsaturated) of supplemental fat fed to ewes during late gestation. Nevertheless, supplementing pregnant ewes late in gestation with 2 or 4% of either fat type improved cold tolerance in newborn lambs (rectal temperatures were higher at birth and increased more in the 0°C cold chamber); whereas, rectal temperatures were depressed in lambs born to ewes fed 8% saturated or monounsaturated fatty acids (Chen et al., 2007).

Linoleic acid is a polyunsaturated fatty acid (PUFA) thought to be a major precursor for heat production in BAT of lambs. Pups born to female rats fed high-fat diets rich in linoleic acid during pregnancy had increased BAT activity, as indicated by the increase in adenyl cyclase activity (Cresteil, 1977). In a second trial by Encinicas et al. (2004), ewes fed a late-gestation diet high in linoleic acid (4.6% fat from safflower seed) produced lambs that had increased survivability compared to lambs born to ewes fed low fat diets (1.9% fat from safflower meal and corn). In a study by Dafoe et al. (2008), safflower seed supplement was compared to a barley-based supplement (with or without vitamin E) in ewes late in gestation. Lambs born to ewes fed safflower seed

without vitamin E had a decrease in cold tolerance. Turnout BW (32 d of age) and weaning BW (124 d of age) were lower in lambs born to ewes fed safflower seed (with or without vitamin E) than lambs born to ewes fed the barley-based supplement. Vitamin E is an antioxidant, important in the processing of fatty acids. Therefore, diets high in fat, but without vitamin E were not likely broken down and utilized to their full potential by the ewe.

Docosahexaenoic Acid (DHA)

Docosahexaenoic acid is a 22-carbon chain PUFA and is the most abundant omega-3 fatty acid in the mammalian brain (Innis, 2007). Found in fish and algae oils, DHA has been shown to be a major contributor to brain growth and development of the human neonates, and maintenance of brain function in adult humans (Horrocks and Yeo, 1999). Maturation of the retina and visual cortex are also dependent on DHA, with elevated levels of DHA improving both mental and visual functions in humans (Uauy and Dangour, 2006).

Infants receive DHA through human breast milk; the level of which in milk is highly depending on the dietary intake of DHA by women during lactation. Women who consumed fish and diets high in omega-3 PUFAs during lactation had increased levels of milk DHA than women who consumed diets low in these fatty acids (Yuhas et al., 2006). Docosahexaenoic acid can also be synthesized from other omega-3 PUFAs (Williard et al., 2001) via the omega-3 PUFA elongation-desaturation pathway (Williams and Burdge, 2006).

Lambs born to ewes supplemented with fish oil had similar proportions of all fatty acids in their blood profiles as their dams (Capper et al., 2006). Pickard et al. (2008) studied the effects of DHA supplementation to ewes on lamb vigor. During the last 63 d of gestation, control ewes received no DHA while supplemented ewes received 12 g DHA/ewe daily for either 3, 6, or 9 wk. Concentrations of eicosapentaenoic acid (a PUFA and precursor of DHA) and DHA (both omega-3 fatty acids) in ewe plasma throughout gestation and at lambing were elevated in proportion to the length of time that the DHA diet was fed. Concentrations of DHA in colostrum of ewes increased linearly with the amount of time that DHA was fed. They concluded that lamb vigor was improved by feeding DHA for longer periods during late gestation since lambs from ewes fed DHA for a longer duration (9 and 6 wk) stood sooner than lambs from ewes fed no DHA or lambs from ewes fed DHA for a 3-wk period. Also, growth rates of lambs at 5 wk of age did not differ between ewes supplemented or not supplemented with DHA.

The effects of DHA (herring and salmon oil) supplementation to ewes during late gestation on lamb output and colostrum production were evaluated by Annett et al. (2009). Supplementation with 20 g of fish oil daily increased number of lambs born per ewe and BW of lambs weaned per ewe compared to lambs born to ewes that did not receive DHA. However, increasing the level of fish oil to 40 g daily led to a linear decline in colostrum yield at 10 h, as well as a decline in total colostrum output. Colostrum of ewes supplemented with fish oil also had lower IgG content at 10 h after lambing. Annett et al. (2009) concluded that supplementing late gestating ewes with fish oil (up to 20 g/ewe daily) reduces lamb mortality, and increases lamb output and BW at

weaning. However, supplementation with greater concentrations of fish oil (40 g/ewe daily) has a negative impact on survival and characteristics of colostrum in ewes.

## Lamb Survival Factors

### Immunocompetence

A healthy immune system is necessary for an animal to prevent or combat infection. The ability to mount an immune response after exposure to an antigen is termed immunocompetence (Norris and Evans, 2000).

### Colostrum IgG

Colostrum is the first source of energy that a lamb receives after birth and it supplies passive immunity via transfer of maternal immunoglobulins to the neonatal lamb primarily during the first, and perhaps, the second day of life (McCarthy and Mc-Dougall, 1953; Halliday, 1971). Concentration and quantity of IgG in colostrum is proportional to the level of passive immunity transmitted to newborn lambs (Al-Sabbagh, 2009). Immunoglobulin G (IgG) accounts for 92% of the total immunoglobulin in colostrum of ewes, while 6% is IgA, and 2% is IgM (Smith et al., 1975).

Hunter et al. (1977) investigated factors affecting IgG concentrations in the serum of 1 d-old lambs, and reported that litter size had a large affect. Single lambs absorbed an average of 28% more IgG than twins at 24 h of age; the difference between singles and triplets was even greater (Halliday, 1974; Hunter et al., 1977). Gender did not make a difference when all lambs were averaged as a group; however, in same-sex twins (either

two males or two females), males absorbed higher concentrations of IgG than females (Halliday, 1974; Hunter et al., 1977). In the study by Hunter et al. (1977), ewes were classified as "small," "medium," or "large" colostrum suppliers according to the size and feel of the udder and ease at which colostrum could be milked from the udder. Ewes classified as "medium" had lambs with the most IgG absorption, due to the higher concentrations of IgG in the colostrum compared to "large" ewes with diluted colostrum. "Large" ewes would require their lambs to consume a larger volume to receive the same levels of IgG as lambs from ewes classified as "medium" suppliers. Lambs that nursed "small" suppliers had less serum IgG than lambs nursing "medium" and "large" colostrum suppliers.

Capper et al. (2006) investigated the effect of PUFA and palm oils in twin and triplet-bearing ewes during late gestation on time to standing and suckling and colostral concentrations of IgGs. They found that fat and protein levels were lower in the colostrum from ewes supplemented with fish oil (PUFAs) than colostrum from ewes supplemented with palm oil (high in saturated fats). However, lambs born to ewes fed fish oil were faster to stand and suckle, i.e., more vigorous. Furthermore, colostrum from ewes fed PUFAs had lower levels of IgG potentially reducing the ability of lambs to mount an effective immune response to antigens.

Immune Response and Parainfluenza Type 3 Vaccination

Reffett et al. (1988) conducted a study to determine effects of dietary selenium and vitamin E on immune responses of lambs challenged with Parainfluenza type 3 ($PI_3$) virus. After the primary inoculation, anti- $PI_3$ titers increased in lambs given selenium

but not vitamin E. This relationship reversed after the second vaccination: anti- $PI_3$ titers increased in lambs given vitamin E but not selenium. Daniels et al. (2000) evaluated effects of vitamin E on the immune response of lambs born from ewes supplemented with or without vitamin E and challenged with or without $PI_3$ during late gestation. They found that serum anti-$PI_3$ titers and IgG concentrations in the serum of newborn lambs were not affected by vitamin E supplementation of ewes.

Rittacco et al. (1986) conducted an experiment to investigate effects of supplemental vitamins A and E on ovine immune response using a different vaccination. To evoke an immune response and determine if treatments would have an effect on immune response, lambs were injected with 1 ml *Brucella ovis* bacterin. They reported that lambs treated with 3000 mg oral vitamin E had increased anti-Brucella ovis titers than those not treated with vitamin E; however, vitamin A had no effect on anti-*Brucella ovis* titers.

Cold Exposure and Rectal Temperatures

Cold temperatures play an important role in depletion of BAT in ruminant neonates. Dietary energy generates body heat rather than productive functions in extreme cold environmental conditions (Young, 1983). Lambs placed in a cold chamber at 4 C° for 48 h reach summit metabolism; associated with this is a depletion all of lipid droplets in brown fat cells (Smith et al., 2004).

Measurements of thermal balance in livestock have been based on measurements of body temperature changes in animals exposed for short durations in controlled

temperature chambers (Young, 1983). The rise in rectal temperatures during initial exposure to cold environmental temperature (as the body attempts to maintain its temperature) is consistent with heat provided by BAT (Alexander and Williams, 1968). An increase in lamb rectal temperatures at 4 and 22 h of age was found in lambs from ewes supplemented with 2, 4, or 8% fat during late gestation (Chen et al., 2007). Temperatures leveled off at 15 min; however, higher rectal temperatures were maintained in lambs at both 4 and 22 h of age born to ewes supplemented with 2 or 4% fat than in lambs born to ewes fed 8% fat in high-concentrate diets. These results indicated that of fat supplementation in ewes late in gestation appears to provide endogenous protection against cold stress in newborn lams. However, feeding fat at greater than 6% will begin to decrease starch digestion, even on a high-concentrate diet and reduce the benefit of fat supplementation (Hess et al., 2008). Consistent with these results are those of Lammoglia et al. (1999a) who reported calves born to cows fed high fat diets (5.1%) during late gestation had a larger increase in rectal temperature from 0 to 40 min once placed under cold stress (0.50°C increase vs. 0.36°C), and temperatures decreased less rapidly over the 140-min cold exposure period (0.19°C decrease vs. 0.34°C) than calves born to cows fed a lower fat (2.2%) diet. Taken together these results illustrate that moderately high fat diets fed to ruminants during late gestation may be beneficial to the response of offspring to cold environmental conditions.

## Blood Serum Metabolites and Metabolic Hormones

### Glucose

Glucose is a natural form of sugar and the primary precursor for glycogenesis (Guyton and Hall, 2000). Once glucose is ingested (or passed from the ewe to the lamb through the placenta), it is transported via the bloodstream to be utilized by the body or stored as glycogen. Glucocorticoids, adrenal steroids involved with glucose homeostasis, support thermogenesis through lipid and glycogen mobilization to provide energy precursors needed for thermogenesis (Himms-Hagen, 1990). This mobilization mechanism elevates glucose and non-esterified fatty acid (NEFA) levels in hypothermic calves and is associated with an increase in temperature in calves (Godfrey et al., 1991).

Lammoglia et al. (1999a) investigated factors (length of time in cold exposure, breed, and diet) that effect glucose concentrations. Heifers were supplemented during the last 55 d of gestation with low (2.2%) or high (5.1%) fat diets. Plasma glucose concentration was affected by duration of neonate exposure to cold (glucose concentrations peaked at 50 min of cold exposure). This supported results of another study by Lammoglia et al. (1999b) in which crossbred heifers were supplemented with low (1.7%) or high (4.7%) fat diets, both high in linoleic acid, during the last 8 wk of gestation. Glucose concentrations were greater in calves born to dams consuming the high fat diets than in calves born to dams consuming low fat diets. These results indicate that increased levels of energy reserves at birth in calves can be reflected in dietary fat content of cows during late gestation. Furthermore, Lammoglia et al. (1999b) found that

glucose concentrations were affected by the interaction of fat percentage in the diet and duration of cold exposure. Supplementation of diets of cows with fat during late gestation appeared to improve calf survival by improving cold tolerance.

During gestation, cold exposure caused increased plasma glucose in ewes (Symonds et al., 1992; Clarke et al., 1997b) and fetuses (Thompson et al., 1982), suggesting that cold exposure may alter the manner by which nutrients are processed and metabolized by pregnant ewes. Ewes adapt to cold stress by storing more nutrients for thermoregulation.

A decrease in body temperature along with an increase in plasma glucose concentration occurred during a 2-h cold exposure of newborn calves (Godfrey et al., 1991), indicating a mobilization of energy due to cold stress. This same relationship was found in lambs during summit metabolism; although no increase in glucose concentration occurred while lambs were under basal (unstressed) conditions (Alexander and Williams, 1968; Alexander et al., 1968). This illustrates the relationship of glucose to thermogenesis; glucose increased in newborn lambs only when cold-stressed to produce heat for the neonate (summit metabolism). This increase in glucose comes at the price of depleting glycogen stores.

Non-Esterified Fatty Acids (NEFA)

Non-esterified fatty acids are free fatty acids in the blood which increase in concentration during fasting due to release of fatty acids from adipose tissue and can be used for metabolic energy (Bender, 2005). Changes in NEFAs are a measure of the degree of mobilization of lipid stores (Encinias et al., 2004). Elevated NEFA

concentrations are a sign of limited dietary or body energy reserves in ruminants, enabling fat mobilization in response to energy scarcity (Peters, 1986). Godfrey et al. (1991) reported an increased in NEFA concentrations in cold-exposed calves. Interestingly, Encinias et al. (2004) reported plasma NEFA concentrations were lower at parturition in ewes fed high fat diets (5.7%) than in ewes fed low fat (2.8%) diets during gestation, indicating that high fat diets limit utilization of fat stores at parturition in ewes.

Cortisol

Cortisol is a glucocorticoid produced by the adrenal cortex. It mediates numerous metabolic processes, including gluconeogenesis, glycogenolysis, lipolysis, lipogenesis, and amino acid utilization, and is a primary mediator of physical or psychological stress (Guyton and Hall, 2000). Greater concentrations of cortisol have been reported in cold-stressed than in non-stressed lambs. Cortisol under these conditions would increase lipolysis, glycogenolysis, and utilization of BAT for energy metabolism and heat production (Bassett and Alexander, 1971; Bell and Thompson, 1979).

In a study conducted by Godfrey et al. (1991), Brahman and Brahman crossbred calves were removed from their dams at 20 min of age and assigned either a warm (31°C) or cold (4°C) environment. Blood samples were collected every 20 min for 180 min. At 100 to 120 min, calves were given 1.2 L of colostrum from their dams, and all calves were placed in the warm chamber. Calves responded to the cold environment with elevated cortisol concentrations to mobilize energetic precursors and increase metabolic rate. A peak in cortisol concentrations in newborn calves after 20 to 50 min of cold exposure was reported by Lammoglia et al. (1999a). This was determined to be a result

of thermoreceptor activation in the skin that is transmitted to the hypothalamus via the spinal cord to stimulate processes of thermogenesis.

Leptin

Leptin is an adipokine peptide hormone produced by fat cells; predominantly white adipose tissue (Ehrhardt et al., 2003). It interacts with the hypothalamus to regulate appetite and suppress lipolysis in adipose tissue (Guyton and Hall, 2000). Factors that regulate leptin concentration in the circulation of newborn lambs were evaluated by Ehrhardt et al. (2003). Within 8 h of birth, they randomly assigned 8 large (4.9 $\pm$ 0.02 kg) and small (2.2 $\pm$ 0.03 kg) newborn lambs to either high (ad libitum milk replacer, 265 g CP and 272 g fat) or low (enough of the same milk replacer to sustain a growth rate of 150 g/d) planes of nutrition. Blood samples were collected every 2 d, and 2 lambs from each birth size by nutrition category were slaughtered at approximately 7.5, 10, 15, and 20 kg (live weight) to analyze body fat content. Blood was also collected from 6 large and 4 small lambs at birth, and these lambs were immediately sacrificed for analysis of fat content. Although large lambs were twice as heavy as small lambs, all lambs had similar body fat and leptin concentrations at birth. Birth weight did not affect leptin concentrations at birth or at any other time in early postnatal life. However, they did report that leptin concentration increased rapidly after birth in lambs on a high nutrition plane, but did not change from birth until about 120 d of age in lambs on the low nutrition plane. Furthermore, lambs on the high nutrition plane had approximately 30% more fat than lambs on the low nutritional plane at every slaughter weight increment. Ehrhart et al. (2003) concluded that maternal nutrition did not have an effect

on leptin concentrations of the newborn lamb but did exert stimulatory effects within days following birth. Also, they concluded that nutrition and fat level were the main contributors to leptin regulation in early postnatal life; whereas, nutrition plays a more dominant role in adult sheep.

McFadin et al. (2002) conducted a study to investigate whether BCS and weight affect leptin concentration in sheep. One wk prior to expected lambing date, mixed-parity ewes were bled, weighed, and assigned an initial BCS. Within 2 h of parturition, blood samples were collected from each ewe and lamb, and colostrum samples were collected from each ewe. Blood samples were collected daily for 5 d, and then weekly until d 47; when ewes and lambs were weighed, bled, assigned a final BCS, and a milk sample was collected. They found that ewe BCS, rather than BW, was highly correlated with leptin concentrations; indicating that leptin is directly associated with the extent of body fat tissue. Also, they found that colostrum samples had increased concentrations of leptin compared to milk samples collected later in lactation. They concluded that these higher concentrations of leptin could be used by the neonate for energy, as a mechanism of thermogenesis.

# CHAPTER 2

# MATERIALS AND METHODS

## Objective and Hypothesis

The objective of this study was to determine the effects of feeding DHA to ewes during late gestation and early lactation on lamb thermogenesis, passive immunity, serum metabolites and hormones, and body weight at 38 d. The null hypothesis was that supplementing ewes during late gestation and early lactation with or without algae-derived DHA in the diet does not alter body temperature of cold-exposed lambs, birth weight, growth (from birth to 38 d), passive immunity, or serum metabolites and hormones.

## Ewe Selection and Management

Eighty twin-bearing Targhee ewes (ages 2-5; 68.5 $\pm$ 3 kg) were stratified by age and assigned randomly to 1 of 2 treatment diets. Ewes were selected from a band of approximately 2,000 ewes from the Targhee flock owned by the Bair Ranch Foundation and managed at the Bair Ranch in Martinsdale, Montana (Meagher County). Ewes were selected based on stage of pregnancy and number of fetus (determined by real-time ultrasound at 65-75 d after breeding); twin-bearing ewes which conceived early in the breeding season (early November) and were expected to lamb in early April were chosen for the study. Ewes were transported from the Bair Ranch to Montana State University's

Fort Ellis facilities near Bozeman on February 2, 2009 where they were confined in a dry lot with access to shelter. Ewes had ad libitum access to water and grass hay (10% CP; Table 1), trace mineral (Westfeeds, Inc., Billings, MT) and salt (mixed with the trace mineral) during late gestation. Beginning on February 7, ewes were fed 80% alfalfa/20% barley pellets (16.8% CP) and whole barley (~ 454 g of each/ewe daily) to supplement the grass hay and acclimate ewes to the area in which they were to be individually fed experimental diets. On February 18, 2009, ewes were shorn, vaccinated with Covexin 8 (clostridial perfringens type C and D; Schering-Plough Animal Health Corporation, Omaha, NE), and treated with Ultra Boss, an internal and external parasite pour-on (Schering-Plough Animal Health Corporation, Summit, NJ).

## Lambing Management

Ewes were observed 24 h/d during lambing season. Ewes observed during the act of parturition and lambs born to these ewes were used to evaluate effects of DHA on lamb thermogenesis, growth (from birth to 38 d), immunocompetence, and serum metabolites and hormones. When ewes were observed in labor, they were visually monitored constantly until parturition. Immediately after parturition, lambs were not allowed to suckle; vigorous lambs were separated from their ewe to prevent nursing. After parturition, a ewe and her lambs were placed in a pen (1.5 $m^2$) for 30 min to allow maternal bonding. At 30 min after lambing, lamb sex and birth weight were recorded, and umbilical cords were clipped and dipped in iodine. After 24 h, ewes and lambs were moved to mixing pens, and at 48 to 60 h to 65 $m^2$ pens in groups of 5 or 6 ewes/pen for

the duration of the study which was 38 ( ± 7 d; SD). All lambs were vaccinated with Covexin 8 and Ovine Ecthyma (sore mouth; Colorado Serum Company, Denver, CO) vaccines. All animal procedures were approved by the Montana State University Agricultural Animal Care and Use Committee (Protocol #AA-030)

Treatments

Supplements were formulated by Westfeeds (Billings, MT) to be isocaloric and isonitrogenous, and to be individually fed daily at levels of 0.9 kg/ewe. Treatments within diets were: 1) 12 g/ewe daily of the product DHA Gold (Advanced Bionutrition Corporation, Columbia, MD), in the form of algal biomass which contained 2.2 g of actual DHA, and 2) no DHA (CONTROL). The amount of DHA (2.2 g/daily) fed contained 10 times more than the recommended level of DHA for pregnant women, which is 0.22 g daily (Simopoulos et al., 1999). Diets were individually fed (40 ewes/treatment) daily during the last 30 d of gestation (beginning March 6, 2009) and pen fed (6 pens/treatment, and 6 or 7 ewes/pen) during the first 38 d of lactation (beginning April 15, 2009). Ewes were placed in individual pens (0.75 $m^2$**)** once daily and fed the appropriate supplement. Ewes remained in individual pens until all supplement had been consumed. During late gestation, ewe was the experimental unit. After lambing, pen was the experimental unit.

Table 1. Chemical analysis (DM basis) of trace mineral, treatment supplements (Algae and Control), grass hay, and alfalfa/barley pellets[3], fed to ewes during late gestation and early lactation

| Item | Trace Mineral[1] | Algae[2,4] Supplement | Control[2,5] Supplement | Grass Hay[3] | Alfalfa/Barley Pellet[6] |
|---|---|---|---|---|---|
| CP, % | | 27.2 | 27.2 | 10.0 | 16.8 |
| TDN, % | | 70.0 | 70.0 | 58.1 | 68.3 |
| Ether Extract, % | | 2.0 | 2.0 | 1.0 | 1.3 |
| ADF, % | | 8.2 | 8.3 | 40.5 | 37.6 |
| NDF, % | | 21.7 | 21.8 | 58.8 | 48.2 |
| Calcium, % | 12.0 | 1.1 | 1.1 | | |
| Phosphorus, % | 8.0 | 0.8 | 0.8 | | |
| Salt, % | 10.0 | 1.6 | 1.6 | | |
| Potassium, % | 1.0 | 1.4 | 1.4 | | |
| Magnesium, % | 2.0 | 0.3 | 0.3 | | |
| Sulfur, % | 1.0 | 0.3 | 0.3 | | |
| Zinc, ppm | 3800 | 176.0 | 176.4 | | |
| Manganese, ppm | 3200 | 155.6 | 156.0 | | |
| Selenium, ppm | 36 | 0.6 | 0.6 | | |
| Vitamin A, IU/kg | 200,000 | 22,000 | 22,000 | | |
| Vitamin D, IU/kg | 20,000 | 2,200 | 2,200 | | |
| Vitamin E, IU/kg | 500 | 330 | 330 | | |
| DHA, g | | 2.2 | | | |
| Decoquinate, g/kg | 1.2 | | | | |

[1] Chemical analysis provided by Westfeeds, Inc., Billings, MT.
[2] Chemical analysis provided by CHS Nutrition, Great Falls, MT.
[3] Chemical analysis conducted at the Oscar Thomas Nutrition Center, Montana State University, Bozeman, MT.
[4] Algae = 0.9 kg/(ewe·d) supplement containing 12 g of the product DHA Gold (Advanced Bionutrition Corporation, Columbia, MD). Each 12 g of DHA Gold contains 2.2 g of actual DHA.
[5] Control = 0.9 kg/(ewe·d) isocaloric and isonitrogenous control supplement containing no DHA .
[6] 80% alfalfa, 20% barley pellet.

## Data Collection and Sample Analyses

### Blood Collection and Thermogenesis

At 1 h after lambing, lambs and ewes were bled via jugular puncture using non-hepranized vacutainers, and lamb thermogenesis was measured in the manner described by Dafoe et al. (2008). Each lamb was fitted with a rectal temperature sensor connected to a data-logger (Omega Engineering, Inc., HH506RA). After an initial temperature

recording, both twin lambs were placed into crates (183 $cm^2$) in a dry cold environmental chamber for 30 min; rectal temperatures of each lamb were recorded automatically every 1 min. Lambs were removed from the cold chamber and a blood sample was collected via jugular puncture. Lambs were warmed for 15 min and returned to their dam. A final blood sample was collected from each ewe and lamb on the last day of the study.

The first 7 d of lambing, lambs were placed into a -15°C cold chamber (Appendix A; Figure 2); thereafter, lambs were placed into a 0°C cold chamber (Appendix A; Figure 3). This occurred as a result of an incorrect adjustment of the temperature regulator on the chamber. Temperature of chamber (defined by early and late period) by supplement treatment interaction for rectal temperature of lambs was not significant ($P = 0.22$) and the data for temperature of chamber were pooled for statistical evaluation. Thus, the results reflect the effect of DHA supplementation on lamb body temperature.

Blood Metabolites and Hormones

Blood samples were centrifuged for 30 min at 1,000 x *g*. Serum was decanted into plastic tubes and stored at -20°C. Ewe and lamb sera were assayed for glucose, NEFA, cortisol, leptin, and anti-$PI_3$ titers.

Glucose concentrations were assayed in duplicate (10 uL) using the Infinity Glucose Hexokinase Reagent (Thermo Fisher Scientific, Inc., Waltham, MA) to create a reagent to sample volume of 150:1. Standards and high and low quality controls were also prepared with each assay. The culture tubes were then covered and vortexed for 30 s, and incubated at 37°C in a hot water bath for 30 min. Samples were then placed in a cold water bath for 10 min, and poured into plastic micro cuvettes, which were placed in

a UV 1201 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) read at an absorbance of 340 nm. This assay was validated for sheep serum and intra- and inter-assay CVs for pooled serum that contained 26.8 and 101.4 mg/dL glucose were < 10%, respectively.

Non-esterified fatty acid concentrations were assayed in duplicate by the Diagnostic Center for Population and Animal Health at Michigan State University (Lansing, MI) on an Olympus chemical analyzer (Olympus America, Inc., Melville, NY) using NEFA-C kits (Wako Chemicals USA, Inc., Richmond, VA), as described by Carr et al. (1995). Intra- and inter- assay CVs for pooled serum at 0.08 and 0.80 mEq/L NEFA were < 10%, respectively.

Cortisol concentrations were assayed in duplicate using solid-phase RIA kits (Seimens Medical Diagnostics, Inc., Los Angeles, CA), as described by Berardinelli et al. (1992). Briefly, 25 µl of each sample, in duplicate, were placed in antibody-coated tubes (from the Coat-A-Count kit), followed by 1 ml of a buffer that contained $^{125}$I cortisol. Standards and high and low quality controls were also prepared with each assay. Tubes were vortexed for 30 sec and incubated for 12 h at 37ºC in a dry incubator. Tubes were thoroughly decanted, removing all moisture, and placed in a Packard Cobra II Gamma Scintillation Counter (Packard Instrument Company, Meridan, CT) for estimating percentage bound to antibody. Intra- and inter-assay CVs for pooled serum that contained 12.9 ng/mL were 8.49 and 11.7%, respectively. Intra- and inter-assay CVs for pooled serum that contained 45.5 ng/mL were 5.7 and 10.4%, respectively.

Leptin concentrations were assayed in triplicate by Dr. Duane Keisler at the Animal Science Research Laboratory of the University of Missouri using a liquid-liquid phase, double-antibody leptin RIA procedure described by Delavaud et al. (2000). Intra- and inter- assay CVs for pooled sera were all <10%, respectively.

Colostrum, Milk, and Lamb Growth

During the first h after lambing, 60-mL colostrum samples were hand-milked from each ewe for assay of fat, protein, lactose, total solids, and solids, non-fat. Samples were poured into covered plastic containers and stored at -20°C. On the final day of the experiment (38 d after lambing), 50-mL milk samples were hand-milked from each ewe and assayed for fat, protein, lactose, total solids, and solids, non-fat. Constituents of milk and colostrum samples were analyzed by infrared analyses (MilkoScan FT120; Foss America, Eden Prairie, MN) at the Montana Veterinary Diagnostic Laboratory in Bozeman.

At birth, lambs were weighed with a spring balance hand scale and sling (Premier 1 Supplies, Washington, IA). Lambs were weighed again (un-shrunk, live weight) on the last day of the esperiment (38 d after lambing) with a digital livestock scale, and growth was determined as kg gained from birth to 38 d of age.

Lamb Immunocompetence

Parainfluenza type 3 ($PI_3$) vaccination was administered to each ewe on February 18 and March 14; approximately 42 and 16 d, respectively, before expected lambing to evaluate the effect of DHA supplementation to ewes on immuno–response of lambs, as

well as the degree of passive immunity from a ewe to her lambs (Daniels et al., 2000). Ewe and lamb sera collected at parturition were used for this assay. Anti-$PI_3$ titer concentrations of ewe and lamb sera were indicative of the strength of an immune response, i.e., how much immunity was passed from ewe to lamb). Anti-$PI_3$ titers were assayed at the Montana Veterinary Diagnostic Laboratory by the hema-absorption method using an end-point titer assay described by Daniels et al. (2000) and Redden et al. (2010).

Colostrum samples were assayed for specific gravity using a colostrometer (JorVet, Jorgensen Laboratories, Loveland, CO). In this procedure, 15 mL of colostrum was poured into the glass colostrometer. The colostrometer was then placed in a plastic graduated cylinder containing distilled water. The colostrometer floated or sank to a certain level, at which point reading was taken to assess the weight of the colostrum. Specific gravity is indicative of the concentration of IgG within a colostrum sample. The quantity of IgG in the colostrum is proportional to the level of passive immunity that reaches the newborn lambs (Tareq Al-Sabbagh, 2009).

Statistical Analyses

Data were analyzed as a completely randomized design using the Proc GLM and Proc Mixed procedures of SAS (SAS Inst., Inc., Cary, NC). Temperature data were analyzed using the Proc Mixed repeated measures procedure of SAS (SAS Inst., Inc., Cary, NC). Temperature data were also analyzed using the Proc GLM repeated measures of SAS (Appendix B; Figures 3,4, and 5). However, Proc Mixed analysis was the more appropriate model as it addressed the covariance structure of repeated measures, whereas

Proc GLM does not. Colostrum and milk data were analyzed using the Proc GLM procedure of SAS. Ewe blood metabolite data included values for 0 d, 38 d, and numeric change from 0 to 38 d, and were also analyzed using the GLM procedure of SAS. Similarly, lamb blood metabolite data included values for 0 min, 30 min, 38 d, numeric change from 0 min to 30 min, and numeric change from 0 min (0 d) to 38 d. The models included the effects of supplement treatment, lambing date, lamb gender, and birth weight. Ewe was the experimental unit for blood metabolite, colostrum, and anti-$PI_3$ data, so lamb weights, temperatures, and blood metabolite data were averaged to calculate lamb values per ewe. Pen was the experimental unit for milk and lamb growth data. Means were separated using the LSD procedure.

# CHAPTER 3

# RESULTS

## Lamb Body Temperature

Although lambs born to ALGAE-supplemented ewes had consistently higher rectal temperatures over the 30-min cold exposure period, rectal temperature did not differ between ALGAE and CONTROL lambs (Figure 1). When evaluated within time, lambs born to ALGAE-supplemented ewes had higher rectal temperatures at 0 min ($P$ = 0.07) and at 1 min ($P$ = 0.08); however, rectal temperatures did not differ at any other time during cold exposure.

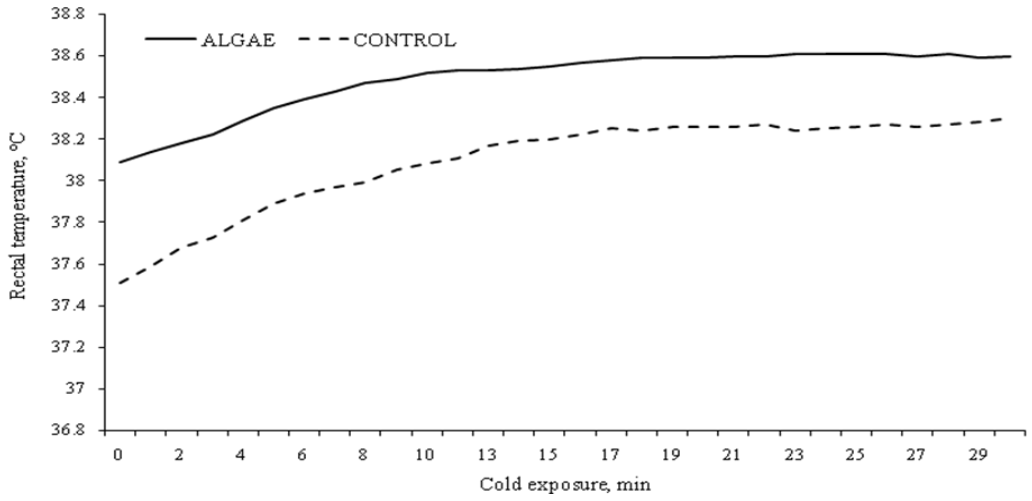


Figure 1: Least squared means of rectal temperature for 44 twin lamb pairs exposed to 0 or -15°C for 30 min 1 h after birth. Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA (CONTROL). The SEM for differences = 0.22°C. Rectal temperatures of lambs from ewes supplemented with ALGAE were greater at time 0 ($P$ = 0.07) and time 1 ($P$ = 0.08), but did not differ at any other time ($P \geq 0.11$). Proc Mixed repeated measures evaluated the effects of time, treatment (trt), trt x time interaction. None of these factors differed ($P \geq 0.11$), with the exception of time ($P < 0.0001$).

## Lamb and Ewe Serum Metabolites/$PI_3$

Glucose, NEFA, cortisol, and leptin concentrations of lambs did not differ ($P > 0.19$) between supplement treatments before (0 min), after (30 min), or when comparing the change in metabolite or hormone concentrations over the 30-min cold exposure period (Table 2). There were no ($P > 0.13$) ewe supplement effects on glucose, NEFA, cortisol, or leptin of lambs at birth (0 d), turnout (38 d), or in the change of these over the 38 d post-lambing period (Table 3).

Table 2. Least squared means for initial (0 min) and final (30 min) serum metabolites for cold-stressed lambs (0 of -15 ºC) born to ewes individually fed 0.9 kg of Algae or Control supplement daily during the last 30 d gestation

| | Treatment[1] | | | |
|---|---|---|---|---|
| Variable | Algae | Control | SEM[2] | *P*-Value |
| Glucose, mg/dl | | | | |
| 0 min | 90.30 | 87.69 | 5.45 | 0.74 |
| 30 min | 73.44 | 79.61 | 5.25 | 0.41 |
| Change | -16.86 | -8.08 | 5.12 | 0.23 |
| Cortisol, ng/ml | | | | |
| 0 min | 126.90 | 131.48 | 11.11 | 0.77 |
| 30 min | 80.79 | 83.01 | 7.97 | 0.84 |
| Change | -46.11 | -48.47 | 7.80 | 0.83 |
| NEFA, mEq/L | | | | |
| 0 min | 0.86 | 0.87 | 0.10 | 0.95 |
| 30 min | 1.27 | 1.12 | 0.08 | 0.19 |
| Change | 0.41 | 0.27 | 0.10 | 0.33 |
| Leptin, ng/ml | | | | |
| 0 min | 1.27 | 1.26 | 0.26 | 0.96 |
| 30 min | 0.84 | 0.83 | 0.07 | 0.94 |
| Change | 0.47 | 0.42 | 0.28 | 0.91 |

[1] Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA **(**CONTROL).

[2] n = 22 observations/treatment (blood metabolites); ewe was the experimental unit (values from twin lambs were averaged per ewe).

Table 3. Least squared means for birth (d 0) and turnout (d 38) serum metabolites for cold-stressed lambs (0 of -15 ºC) born to ewes individually fed (last 30 d gestation) and group fed (first 38 d lactation; 5 to 7 ewes/pen; 6 pens/treatment) 0.9 kg of Algae or Control supplement daily

| | Treatment[1] | | | |
|---|---|---|---|---|
| Metabolite | Algae | Control | SEM[2] | *P*-Value |
| Glucose, mg/dL | | | | |
| 0 day | 90.30 | 87.69 | 5.45 | 0.74 |
| 38 day | 79.82 | 82.27 | 2.79 | 0.54 |
| Change | -10.88 | -0.77 | 7.65 | 0.29 |
| Cortisol, ng/mL | | | | |
| 0 day | 126.90 | 131.48 | 11.11 | 0.77 |
| 38 day | 9.82 | 10.80 | 1.06 | 0.52 |
| Change | -118.61 | -121.45 | 11.73 | 0.86 |
| NEFA, mEq/L | | | | |
| 0 day | 0.86 | 0.87 | 0.10 | 0.95 |
| 38 day | 0.42 | 0.46 | 0.02 | 0.13 |
| Change | -0.46 | -0.40 | 0.10 | 0.68 |
| Leptin, ng/mL | | | | |
| 0 day | 1.27 | 1.26 | 0.26 | 0.96 |
| 38 day | 1.15 | 1.14 | 0.08 | 0.96 |
| Change | -0.13 | -0.13 | 0.28 | 0.98 |

[1] Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA **(**CONTROL).

[2] n = 23 samples/treatment (d 0, change in d), n = 33 samples/treatment (d 38); ewe was the experimental unit (values for twin lambs were averaged per ewe).

Glucose, NEFA, cortisol, and leptin concentrations of ewes did not differ (*P* = 0.20) between supplements at 1 h postpartum (0 d), at turnout (38 d), or in the change over the 38 d period (Table 4). Ewe and lamb anti-$PI_3$ titers did not differ (*P* = 0.32) between supplements (Table 5).

Table 4. Least squared means for initial (1 h after lambing) and final (38 d after lambing) serum metabolites from ewes individually supplemented (last 30 d gestation) and group supplemented (first 38 d lactation; 5 to 7 ewes/pen; 6 pens/treatment) 0.9 kg of Algae or Control supplement daily

| | Treatment[1] | | | |
|---|---|---|---|---|
| Metabolite | Algae | Control | SEM[2] | *P*-Value |
| Glucose, mg/dL | | | | |
| 0 day | 132.94 | 140.42 | 8.80 | 0.55 |
| 38 day | 63.04 | 70.31 | 4.85 | 0.29 |
| Change | -69.90 | -70.11 | 10.26 | 0.96 |
| Cortisol, ng/mL | | | | |
| 0 day | 22.60 | 26.37 | 2.47 | 0.29 |
| 38 day | 6.61 | 15.52 | 4.85 | 0.20 |
| Change | - 15.99 | -10.85 | 0.11 | 0.55 |
| NEFA, mEq/L | | | | |
| 0 day | 0.98 | 1.06 | 0.10 | 0.56 |
| 38 day | 0.43 | 0.42 | 0.05 | 0.93 |
| Change | -0.55 | -0.64 | 0.11 | 0.46 |
| Leptin, ng/mL | | | | |
| 0 day | 2.45 | 2.34 | <0.01 | 0.71 |
| 38 day | 1.93 | 1.89 | <0.01 | 0.90 |
| Change | -0.45 | -0.50 | 0.03 | 0.85 |

[1] Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA **(**CONTROL).

[2] n = 29 samples/treatment (d 0, change in d), n = 33 samples/treatment (d 38); ewe was the experimental unit.

Table 6. Least squared means of specific gravity, percent fat, protein, lactose, total solids, and solids non-fat levels in colostrum samples (1 h after lambing) from ewes individually supplemented (last 30 d gestation) 0.9 kg of Algae or Control supplement daily

| | Treatment[2] | | | |
|---|---|---|---|---|
| Item | Algae | Control | SEM[3] | *P*-value |
| Ewe serum anti-$PI_3$ titer | 1.35 | 1.22 | 0.10 | 0.34 |
| Lamb serum anti-$PI_3$ titer | 1.20 | 1.10 | 0.07 | 0.32 |

[1] Jugular blood samples were taken from ewes at 1 h postpartum, and from lambs at 90 min postpartum.
[2] Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA **(**CONTROL).
[3] n = 29 samples/treatment; experimental unit for all items was ewe (values for twin lambs were averaged per ewe).

## Colostrum and Milk

Percent fat and total solids were greater ($P < 0.05$) in colostrum of CONTROL-supplemented ewes than in ALGAE-supplemented ewes; however, colostrum of ALGAE-supplemented ewes contained a greater percentage of lactose ($P = 0.03$) and had a higher specific gravity ($P = 0.05$) than that of CONTROL-supplemented ewes. No supplement effects were detected in percent protein or solids, non-fat (Table 6). Milk samples of ewes at 38 d did not differ ($P = 0.27$) between supplements (Table 7).

Table 6. Least squared means of specific gravity, percent fat, protein, lactose, total solids, and solids non-fat levels in colostrum samples (1 h after lambing) from ewes individually supplemented (last 30 d gestation) 0.9 kg of Algae or Control supplement daily

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Algae | Control | SEM[2] | *P*-value |
| Specific gravity | 1.08 | 1.07 | 0.01 | 0.05 |
| Fat, % | 12.9 | 16.1 | 0.90 | 0.01 |
| Protein, % | 20.1 | 21.0 | 0.50 | 0.17 |
| Lactose, % | 2.7 | 2.3 | 0.12 | 0.03 |
| Total solids, % | 38.6 | 42.3 | 1.13 | 0.02 |
| Solids non-fat, % | 25.7 | 26.2 | 0.41 | 0.40 |

[1] Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA **(**CONTROL).
[2] n = 28 samples/treatment; experimental unit for all items was ewe.

Table 7. Least squared means of percent fat, protein, lactose, total solids, and solids non-fat in milk samples taken 38d after lambing from ewes individually supplemented (last 30 d gestation) and group supplemented (first 38 d lactation; 5 to 7 ewes/pen; 4 pens/treatment) 0.9 kg of Algae or Control supplement daily

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Algae | Control | SEM[2] | *P*-value |
| Fat, % | 6.7 | 7.5 | 0.53 | 0.31 |
| Protein, % | 4.4 | 4.5 | 0.09 | 0.81 |
| Lactose, % | 5.4 | 5.2 | 0.11 | 0.27 |
| Total solids, % | 18.3 | 19.0 | 0.52 | 0.38 |
| Solids Non-Fat, % | 11.7 | 11.5 | 0.12 | 0.42 |

[1] Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA **(**CONTROL).
[2] n = 4 samples/treatment; experimental unit for all items was pen (5 to 7 ewes/pen; 4 pens/treatment).

## Production Characteristics

Lamb BW at 38 d was greater ($P = 0.03$) in lambs born to CONTROL-supplemented ewes than in lambs born to ALGAE-supplemented ewes. However, ewe BCS were higher in ALGAE-supplemented ewes than in CONTROL-supplemented ewes ($P = 0.05$) by 38 d after lambing.

Table 8. Least squared means of ewe BW, ewe BCS, and lamb BW from ewes individually supplemented (last 30 d gestation) and group supplemented (first 38 d lactation; 5 to 7 ewes/pen; 4 pens/treatment) 0.9 kg of Algae or Control supplement daily

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | Algae | Control | SEM[2] | *P*-value |
| Initial Ewe BW, kg | 89.7 | 89.1 | 1.61 | 0.80 |
| Final Ewe BW, kg | 77.6 | 76.8 | 1.41 | 0.68 |
| Initial Ewe BCS | 3.0 | 3.1 | 0.04 | 0.21 |
| Final Ewe BCS | 2.8 | 2.7 | 0.05 | 0.05 |
| Lamb birth BW, kg | 5.4 | 5.2 | 0.11 | 0.26 |
| Lamb 38 d BW, kg | 30.4 | 32.3 | 0.53 | 0.03 |

[1] Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA **(**CONTROL).

[2] n = 6 samples/treatment; experimental unit for all items was pen (5 to 7 ewes/pen; 6 pens/treatment); values for lambs were averaged per ewe in her respective pen.

# CHAPTER 4

# DISCUSSION

Polyunsaturated fatty acids, such as linoleic acid, have been shown to be major contributors to the energy fueling heat production in the BAT of the lamb (Lammoglia et al., 1999b). Pups born to rats consuming high-fat diets rich in linoleic acid during pregnancy had increased BAT activity, as indicated by the increase in adenyl cyclase activity (Cresteil et al., 1977). Docosahexaenoic acid is a PUFA found in algae and fish oils. Pickard et al. (2008) studied the effects of DHA supplementation to ewes during the last 63 d of gestation on lamb vigor. In this study control ewes received no DHA, while treated ewes received 12 g DHA/ewe daily. They found that concentrations of eicosapentaenoic acid and DHA (both omega-3 fatty acids) in ewes throughout gestation and at lambing were elevated in proportion to the length of time that the DHA diet had been fed. In the present study, NEFA was measured but concentrations in ewes did not differ between treatments. Our supplements were isocaloric, isonitrogenous, and had 2% fat, so ewes were provided equal energy, thus, the fact that NEFA did not differ is not surprising.

Birth weight of lambs did not differ between DHA and no DHA (to ewes) groups in the study by Pickard et al. (2008) which agrees with the results of the present study. However, lamb BW at 5 wk of age did not differ between treatments. This result differs from that of the present study, in that, lambs from CONTROL-supplemented ewes were

heavier than lambs of ALGAE-supplemented ewes at the end of the study (38 ± 7 d of age). The effects of PUFAs on lamb BW were studied by Dafoe et al. (2008). Late gestating ewes were assigned one of four supplemental diets that included: whole safflower seed (high in linoleic acid) with and without supplemental vitamin E or an isocaloric barley-based supplement with or without supplemental vitamin E. At 32 d of age, lambs born to ewes fed the barley-based supplement had higher BW than lambs born to ewes fed the linoleic acid-rich safflower seed supplement. These results agree with those of the present study; however, in both studies, supplemental PUFA did not affect lamb birth weight.

Lamb thermogenesis was evaluated by Dafoe et al. (2008). Lambs born to ewes supplemented with safflower but no supplemental vitamin E had lower rectal temperatures than all other treatment groups. In the present study, rectal temperature did not differ between treatments but was consistently greater in lambs born to ALGAE-supplemented ewes than lambs born to CONTROL-supplemented ewes. One possible explanation for the difference between these two studies is the level ofether extract in the treatment diets. In the present study the treatment diet was 2% ether extract. In the study by Dafoe et al., (2008) ether extract of the high-PUFA supplement was 49%.

Procedures for evaluating thermogenesis used by Dafoe et al. (2008) and in the present study were based on findings of Hamadeh et al. (2000), who concluded that 30 min in a 0°C dry, cold chamber was sufficient for determining thermogenic differences in factors such as birth type (twin vs single) and colostrum intake (none versus 20 mL) in newborn lambs. Thermogeneic changes of lambs in response to late gestational

supplementation of DHA have not been measured previously. However, Chen et al. (2007) supplemented late gestating ewes with 2, 4, or 8% saturated or monounsaturated fatty acids (SMFA) or polyunsaturated fatty acid (PUFA) and reported that rectal temperatures were higher at birth and increased to a higher level in the 0°C cold chamber (for 2 h) with both fat types when fed at levels of 2 or 4% fat; whereas, rectal temperatures were depressed in lambs born to ewes fed 8% SMFA. In the present study, supplementing diets of ewes with DHA did not have an effect on response of lambs to cold exposure, although ALGAE lambs tended to have higher rectal temperatures throughout the cold exposure period. As stated previously, level of fat in the treatment diets of the present study was 2%. The differences between our study and that of Chen et al. (2006) could be due to fat percentage. Perhaps if we had fed different fat levels (with DHA vs no DHA), we also would have seen a difference in rectal temperatures of lambs during cold exposure.

Heifers were supplemented during the last 55 d of gestation with low (2.2%) or high (5.1%) fat diets (Lammoglia et al., 1999a). Plasma glucose concentration was affected by two interactions: the interaction of diet (calves born to cows fed high fat diets had greater concentrations) and the duration of cold exposure (glucose levels peaked at 50 minutes of cold exposure). This supported the findings of another study by Lammoglia et al. (1999b), in which crossbred heifers were supplemented with low (1.7%) or high (4.7%) fat diets, high in linoleic acid, during the last 8 wk of gestation. Greater glucose concentrations were found in calves born to dams consuming the high fat diets during late gestation, suggesting increased levels of energy reserves at birth. The

present study showed no difference in blood metabolites, but this, again, could be due to the constant level of 2% fat in both supplements. Also, it may be that DHA supplementation does not influence energy homeostasis in lambs or ewes.

In conclusion, the results of this experiment indicate that supplementing ewes during late gestation and early lactation with algae-derived DHA in the diet does not affect birth weight, immunocompetence as indicated by anti-$PI^3$ titers, serum metabolites and hormones, or body temperature of lambs in response to cold exposure. However, supplementation of diets of ewes during late gestation and early lactation adversely affect growth and development of lambs early in lactation.

# CHAPTER 5

# IMPLICATIONS

Supplemental DHA fed to ewes during late gestation and early lactation did not improve indices of lamb thermogenesis and may result in decreased lamb weight gain. However, DHA is an important fatty acid in neural and visual development in infants (Cunnane et al., 1999; Horrocks and Yeo, 1999), and has been shown to increase lamb vigor (Capper et al., 2006; Pickard et al., 2008). It may be that DHA has more of a neurological benefit, rather than an energetic benefit. Although energy status (thermogenesis), serum metabolites and hormones, and BW at 38 d of age were measured in the present study, factors associated with brain and nervous system developments were not. These factors should be assessed in regards to supplementation of late gestating ewes with DHA in light of the fact that DHA is an intergral component of nervous system development in humans.

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# APPENDICES

# APPENDIX A

## RECTAL TEMPERUATURES OF COLD-STRESSED LAMBS (-15°C and 0°C) OVER A 30 MINUTE PERIOD ANALYZED WITH THE PROC MIXED REPEATED MEASURES OF SAS

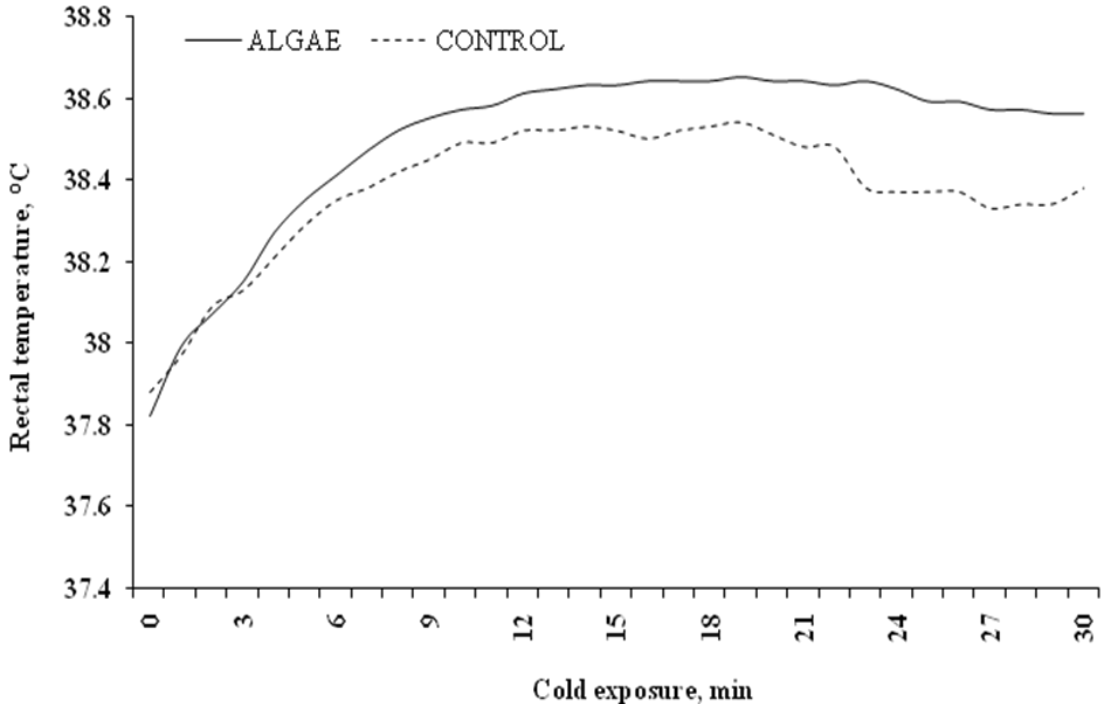


Figure 2: Least squared means of rectal temperature for 44 twin lamb pairs exposed to -15°C (early period) for 30 min 1 h after birth. Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA (CONTROL). The SEM for differences = 0.33°C. Rectal temperatures did not differ ($P > 0.10$) between treatments. Proc Mixed repeated measures evaluated the effects of time, treatment (trt), trt x time interaction. None of these factors differed ($P \geq 0.10$), with the exception of time ($P < 0.0001$).

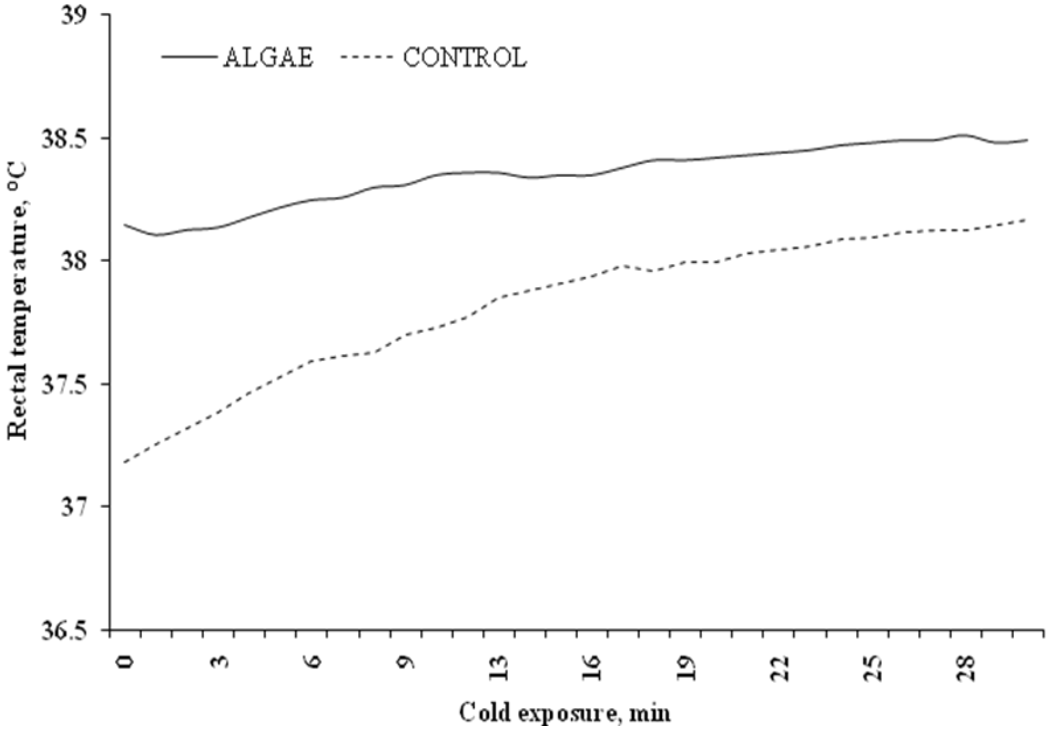


Figure 3: Least squared means of rectal temperature for 44 twin lamb pairs exposed to 0°C (late period) for 30 min 1 h after birth. Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA (CONTROL). The SEM for differences = 0.33°C. Rectal temperatures did not differ ($P > 0.10$) between treatments. Proc Mixed repeated measures evaluated the effects of time, treatment (trt), trt x time interaction. Treatment did not differ ($P \geq 0.10$), however time and trt x time were significant ($P < 0.0001$).

# APPENDIX B

## RECTAL TEMPERUATURES OF COLD-STRESSED LAMBS OVER A 30 MINUTE PERIOD ANALYZED WITH THE PROC GLM REPEATED MEASURES OF SAS

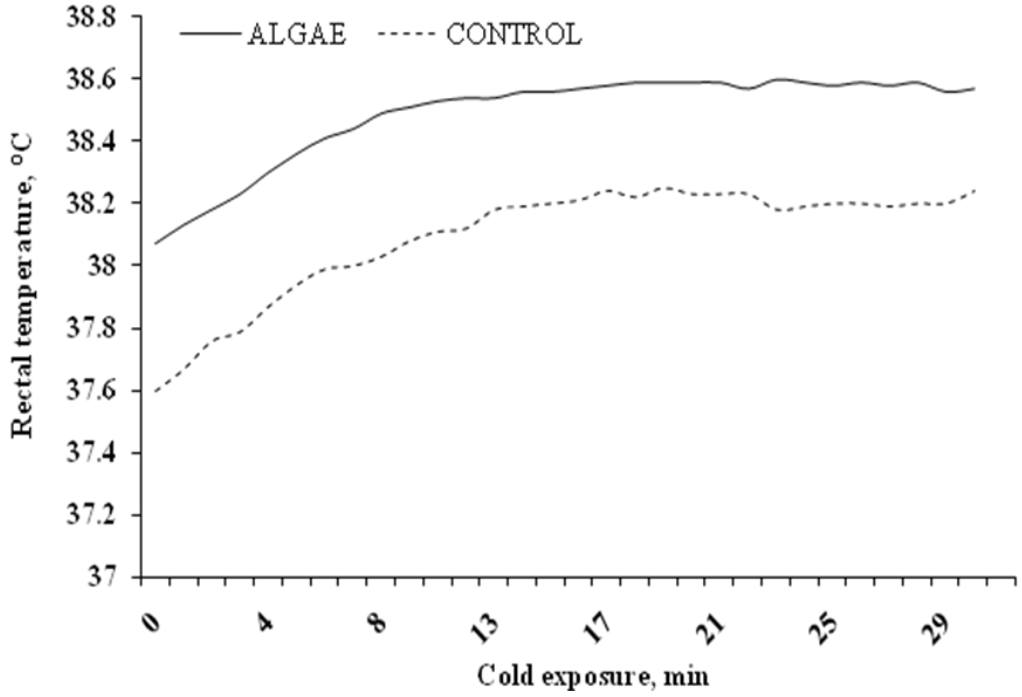


Figure 4: Least squared means of rectal temperature for 44 twin lamb pairs exposed to 0 or -15°C (Proc GLM early and late periods) for 30 min 1 h after birth. Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA (CONTROL). The SEM for Algae was 0.27°C; Control was 0.31°C. Proc GLM repeated measures evaluated the effects of treatment x period ($P$ = 0.2382), but because there was no interaction, values were averaged over period.

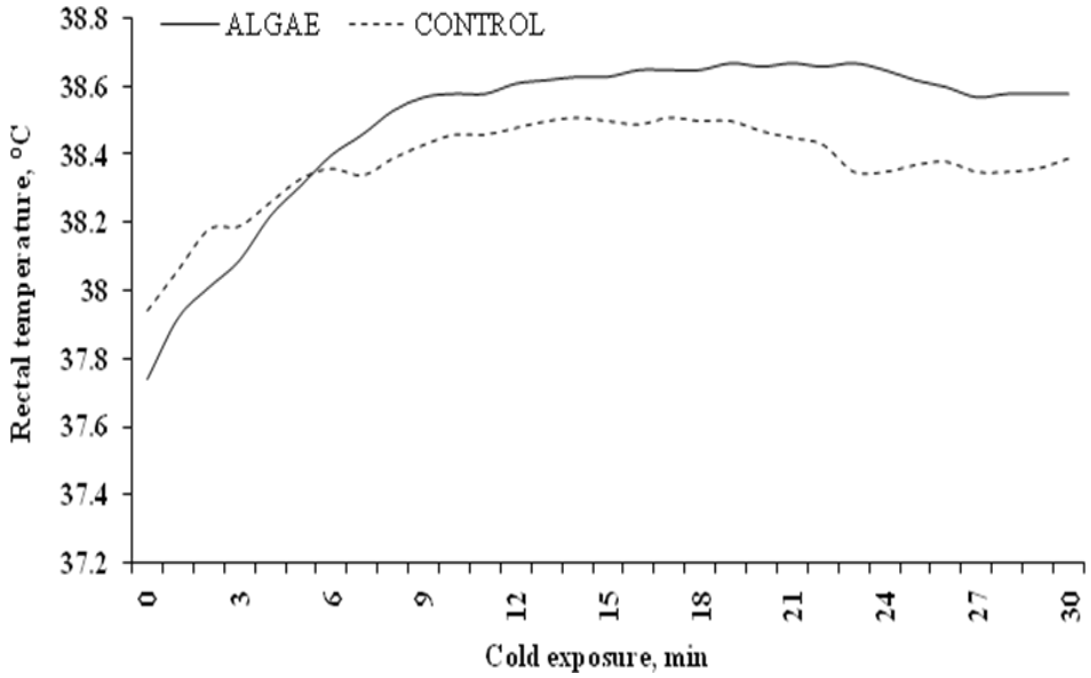


Figure 5: Least squared means of rectal temperature for 44 twin lamb pairs exposed to -15°C (Proc GLM early period) for 30 min 1 h after birth. Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA (CONTROL). The SEM for Algae was 0.38°C; Control was 0.45°C.

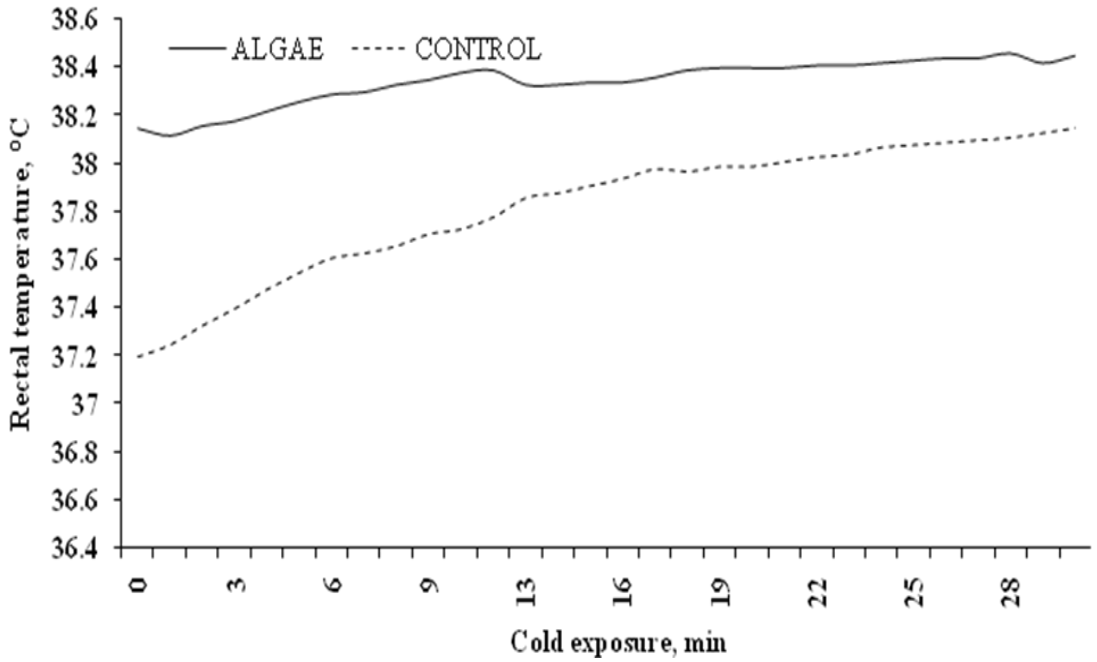


Figure 6: Least squared means of rectal temperature for 44 twin lamb pairs exposed to 0°C (Proc GLM late period) for 30 min 1 h after birth. Treatments within isocaloric and isonitrogenous supplements were: 1) 12 g/ewe daily DHA Gold (Advanced Bionutrition Corporation, Columbia, MD) containing 2.2 g of DHA, in the form of algal biomass (ALGAE**)**, and 2) no DHA (CONTROL). The SEM for Algae was 0.40°C; Control was 0.43°C.