Effects of supplemental safflower and vitamin E during late gestation on lamb growth, serum metabolites, and thermogenesis


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ABSTRACT: Twin-bearing Targhee ewes (Exp. 1, 1 yr, n = 42) and 1,182 single- and twin-bearing white-face range ewes (Exp. 2, n = 8 experimental units over 2 yr) were used in a 2 × 2 factorial arrangement of treatments to determine the effect of supplemental energy source and level of vitamin E supplement on lamb serum metabolites and thermogenesis (Exp. 1) and on lamb growth (Exp. 2). During late gestation, ewes were individually fed (Exp. 1) or group-fed (Exp. 2) a daily supplement. Supplements were 226 g/ewe of daily safflower seed (DM basis; SS) with either 350 IU/ewe daily (VE) or no added supplemental (VC) vitamin E; or 340 g/ewe daily of a barley-based grain supplement (DM basis; GC) and either VE or VC. One hour postpartum in Exp. 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 Exp. 2, lambs were weighed at birth, at turnout from confinement to spring range (32 d of age ± 7; turnout), and at weaning (120 d of age ± 7). Ewes were weighed at turnout and weaning. In Exp. 1, a level of vitamin E × energy source interaction was detected (P < 0.10) for body temperature and change in NEFA and glucose concentrations. Lambs from SSVC ewes had the lowest (P = 0.01) body temperature and had decreased (P = 0.08) NEFA concentration. The SS lambs tended to have decreased (P < 0.11) concentrations of blood urea N (BUN) and thyroxine at 0 min than did lambs born to GC ewes. After 30 min of cold exposure, SS lambs had increased and GC lambs had decreased BUN, triiodothyronine, and triiodothyronine:thyroxine concentrations (P<0.10). In Exp. 2, kilograms of lamb per ewe at turnout and weaning and lamb survival at weaning were greater (P < 0.07) for GC than SS lambs. Based on the decreased body temperature in SSVC lambs at birth, the greater change in BUN during the cold exposure for SS than GC lambs, and the decreased survival rate for SS than GC lambs, SSVC-supplemented ewes appeared to give birth to lambs with an apparently decreased energetic capacity. This may compromise the ability of the newborn lamb to adapt to extreme environmental conditions.

Key words: gestation, lamb survival, safflower seed, sheep, thermogenesis, vitamin E

INTRODUCTION

Hypothermia, starvation, scours, and pneumonia are the major causes of lamb mortality, with 50% of lamb losses occurring within 24 h of birth (Rowland et al., 1992). Alexander (1961) estimated that 50% of the heat generated by ruminant neonates comes from nonshivering thermogenesis, which is fueled solely by brown adipose tissue (BAT). Linoleic and linolenic acid supplements, such as safflower seed, increased the thermogenic capacity of BAT by 75% and doubled the content of uncoupling protein-1 in rats (Nedergaard et al., 1983). Encinias et al. (2004) reported that lambs born to ewes fed a late-gestation diet that included 4.6% safflower seed had increased survivability and reduced pneumonia rates compared with lambs born to ewes fed a 1.9% fat, isocaloric late-gestation diet. However, we speculate that increased heat production from BAT may increase the oxidation of BAT, which results in the formation of free radicals. Free radicals cause damage to cellular membranes, thereby creating a potential 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). Ewes lambing late in the winter or early in the spring and fed harvested forages may have reduced plasma vitamin E concentrations because dry, stored feeds have a decreased vitamin E content than fresh spring forage (Kivimae and Carpena, 1973). It is un-
known whether feeding supplemental safflower seed to increase the thermogenic capacity of BAT will increase the antioxidant 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 growth, serum metabolites, and thermogenesis in lambs born to spring-lambing ewes.

**MATERIALS AND METHODS**

All animal procedures were approved by the Montana State University Institutional Animal Care and Use Committee.

**Exp. 1**

Fifty-one twin-bearing Targhee ewes were stratified by age and assigned randomly to a 2 × 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 Bluff Research Ranch near Norris, Montana (45°47′ N, 111°9′ W). Ewes were assigned to treatments 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 Montana State University Fort Ellis facilities near Bozeman, Montana, where they were housed in a 3,721 m² pen with ad libitum access to long-stemmed alfalfa hay (Table 1) and water.

**Treatments**

The main effects tested in Exp. 1 were supplemental energy source and level of vitamin E. The isocaloric and isonitrogenous treatments were 226 g/ewe daily of whole safflower seed (*Carthamus tinctorius* L., variety Centennial; DM basis; SS, Table 1) and either 350 IU/ewe daily (VE) or no added supplemental (VC) vitamin E; or 340 g/ewe daily of a barley-based supplement (DM basis; GC) and either VE or VC (Table 1). An additional 114 g/ewe daily of GC was required to provide an equal amount of energy as the SS supplement. Ewes were placed in individual pens (1.5 m²) once daily and fed the appropriate supplemental treatments. Ewes remained in individual pens until all supplement had been consumed. Treatments were administered from March 7, 2005, to April 10, 2005. Ten days (±5) before lambing, ewes were returned to the flock at Red Bluff, where they were group-fed the appropriate supplement treatments in 1 of 4 groups and allowed ad libitum access to long-stemmed alfalfa hay (Table 1).

**Data Collection**

Ewes were observed 24 h/d during the lambing season. Forty-two (SSVE = 12, SSVC = 10, GCVE = 9, GCVC = 11) 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 parturition, lambs were not allowed to suckle; vigorous lambs were muzzled to prevent nursing. The ewe and her lambs were placed in a pen (1.5 m²) for 1 h to allow maternal bonding. At 1 h postpartum, lamb sex and birth BW were recorded and the umbilical cord was clipped and dipped in iodine. Jugular blood samples (10 mL) were taken from

### Table 1. Chemical analysis (DM basis) of long-stemmed alfalfa hay and supplements fed to ewes during late gestation

<table>
<thead>
<tr>
<th>Item</th>
<th>Alfalfa hay</th>
<th>Energy supplement</th>
<th>Vitamin E supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2005</td>
<td>2006</td>
<td>SS</td>
</tr>
<tr>
<td>DM, %</td>
<td>91.1</td>
<td>90.5</td>
<td>95.6</td>
</tr>
<tr>
<td>CP, %</td>
<td>15.2</td>
<td>15.8</td>
<td>19.6</td>
</tr>
<tr>
<td>ADF, %</td>
<td>37.2</td>
<td>35.3</td>
<td>13.4</td>
</tr>
<tr>
<td>TDN, %</td>
<td>58.6</td>
<td>60.2</td>
<td>107.2</td>
</tr>
<tr>
<td>Sulfur, %</td>
<td>0.18</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.24</td>
<td>0.26</td>
<td>0.82</td>
</tr>
<tr>
<td>Potassium, %</td>
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<td>2.01</td>
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</tr>
<tr>
<td>Magnesium, %</td>
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<td>0.28</td>
<td>0.38</td>
</tr>
<tr>
<td>Calcium, %</td>
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<td>1.56</td>
<td>0.26</td>
</tr>
<tr>
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<td>0.05</td>
<td>0.02</td>
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<tr>
<td>Iron, mg/kg</td>
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<td>212</td>
<td>125</td>
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<tr>
<td>Manganese, mg/kg</td>
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<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Copper, mg/kg</td>
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<td>7</td>
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<td>Zinc, mg/kg</td>
<td>55</td>
<td>49</td>
<td>62</td>
</tr>
<tr>
<td>Selenium, mg/kg</td>
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<td>&lt;0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>Vitamin E, IU/kg</td>
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<td>14.4</td>
<td>52.8</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>1.3</td>
<td>1.4</td>
<td>49.3</td>
</tr>
</tbody>
</table>

1Chemical analysis conducted by Midwest Laboratories Inc. (Omaha, NE).
2SS = 226 g/ewe⁻¹.d⁻¹ of safflower seed; GC = 350 g/ewe⁻¹.d⁻¹ of isocaloric and isonitrogenous grain-based control supplement.
3VE = 350 IU/ewe⁻¹.d⁻¹ of supplemental vitamin E; VC = no added supplemental vitamin E.
479.1% linoleic, 6.2% palmitic, 2.1% stearic, and 10.3% oleic fatty acids.
ewes within 1 h of parturition. Lambs were then bled (10 mL) via jugular puncture by using nonheparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Lambs were fitted with a rectal temperature sensor connected to a mini logger 2000 (Mini Mitter Company Inc., Bend, OR). After an initial temperature reading, both lamb twins were placed in crates (183 cm²) 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 (10 mL), warmed for 15 min in a 25°C warming box, and returned to their dam.

Sample Analysis

Blood samples were centrifuged for 20 min at 1,000 x g. Serum was then decanted into plastic tubes and stored at −20°C. Ewe serum was assayed for blood urea nitrogen (BUN), NEFA, glucose, cholesterol, and total protein. Serum from each lamb was assayed for glucose, cholesterol, total protein, BUN, NEFA, cortisol, triiodothyronine (T₃), thyroxine (T₄), and T₃:T₄ ratio. Nonesterified fatty acids were assayed by 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 by 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 by using a bichromatic (340- and 383-nm) rate technique. Cholesterol concentrations were determined in serum samples by using a polychromatic (540-, 452-, and 700-nm) end-point technique. Total protein concentrations were measured by using a bichromatic (540- and 700-nm) end-point technique. Cortisol, T₃, and T₄ concentrations were assayed by using solid-phase RIA kits (Coat-A-Count; Siemens Medical Solutions Diagnostics, Los Angeles, CA; Berardinelli et al., 1992). Intra- and interassay CV for the T₃ serum pool, which contained 8.3 ng/mL, and the T₄ serum pool, which contained 55.1 µg/mL, were 3.9 and 16.9%, and 5.5 and 7.7%, respectively. Intra- and interassay CV for the high (38.0 ng/mL) and low (2.7 ng/mL) cortisol concentration pools were 5.6 and 9.5%, and 4.5 and 11.0%, respectively.

Statistical Analysis

Data were analyzed as a completely randomized design, with treatments arranged factorially, by using the GLM procedure (SAS Inst. Inc., Cary, NC). Temperature data were analyzed by using the repeated measures procedure of SAS. Ewe and lamb production data and blood metabolite data, which included values for 0 min, 30 min, the numeric change, and the percentage change, were analyzed by using the GLM procedure. The model included effects of energy source (SS vs. SC), level of supplemental vitamin E (VE vs. VC), and the interaction between energy source and level of vitamin E. Ewe age and lambing date were included as covariates. Ewe was the experimental unit, so lamb BW were summed to calculate kilograms of lamb born, turnout, and weaned per ewe. Means were separated by the LSD procedure.

Exp. 2

In a 2-yr study, single- and twin-bearing whiteface ewes (n = 8) were stratified by age and breed and assigned randomly to a 2 x 2 factorial arrangement of treatments as described in Exp. 1. Pregnant ewes were managed at Montana State University’s Red Bluff Research Ranch near Norris, Montana. Ewes were assigned randomly within breed (Columbia, Rambouillet, and Targhee) and age (2 to 7 yr old) so that each treatment group had a similar average age (3.6 ± 1.4) and number of each breed. Within treatment groups, ewes were group-fed their assigned supplements the last 40 ± 12 d of gestation. Ewes had ad libitum access to alfalfa hay (Table 1) and water.

Data Collection

Lambs were processed according to the Montana State University protocol at birth (April 22 ± 7), with sex, birth type, birth BW, birthday, and breed information recorded. During lambing, ewes were observed 24 h/d. Ewes and lambs were individually penned for 24 h postpartum. Lamb BW was recorded again at turnout from confinement to spring range (May 24, 2005, 32 d of age ± 7; and May 27, 2006, 34 d of age ± 7) and weaning (August 24, 2005, 124 d of age ± 7; and August 26, 2006, 126 d of age ± 7). Lambs that died were included in the analysis as 0 kg of BW. Body condition scores (Russel, 1991) were assigned to all ewes before treatment (February 15, 2005; and February 17, 2006), at shearing (April 15, 2005; and April 20, 2006), at turnout, and at weaning.

Statistical Analysis

Production data were analyzed as a completely randomized design, with treatments arranged factorially, by using the GLM procedure. The model included effects of energy source, level of vitamin E, and the 2-way interaction. Pen was the experimental unit, thus n = 8, with 4 treatments and 2 yr of data collection. The number of ewes in SSVE, SSVC, GCVE, and GCVC in 2005 and 2006 was 143, 149, 136, 142, 155, 153, 150, and 150, respectively. Lamb BW were summed to calculate kilograms of lamb born, turned out, and weaned per ewe; lamb survival was presented as the percentage of lambs alive at spring turnout and weaning. Means were separated by the LSD procedure.
RESULTS

Exp. 1

There was an energy source × level of vitamin E supplementation interaction for glucose numeric change, \( T_3/T_4 \) 30 min and percentage change, and NEFA 0 min, numeric change, and percentage change (\( P \leq 0.10 \); Table 2). Serum glucose in lambs from SSVC ewes increased during cold exposure, whereas it decreased during cold exposure in lambs from GCVC ewes (\( P = 0.06 \); Table 2). Lambs born to SSVE ewes had greater (\( P = 0.10 \)) \( T_3/T_4 \) at 30 min than did all other lambs. Lambs born to GCVC ewes were the only group that had a decreased (\( P = 0.08 \)) change in \( T_3/T_4 \) (%) 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 decreased (\( P < 0.08 \)) change in NEFA concentration (both numeric and percentage) over the cold exposure.

Lambs born to SS ewes tended to have decreased (\( P = 0.11 \)) concentrations of BUN and less \( T_4 \) (\( P = 0.07 \)) at 0 min than did lambs born to GC ewes (Table 2). The percentage change in BUN and numeric change in BUN, \( T_3 \), and \( T_3/T_4 \) increased in SS and decreased in GC lambs after 30 min of cold exposure (\( P < 0.10 \)).

The only blood metabolite influenced by vitamin E supplementation was \( T_3/T_4 \) ratio at 0 min (Table 2). Lambs born to ewes that received supplemental vitamin E had a greater (\( P = 0.09 \)) \( T_3/T_4 \) ratio than lambs born to ewes that did not receive supplemental vitamin E.

Ewe Serum Metabolites

No source of energy × level of vitamin E supplementation interaction was detected (\( P \geq 0.46 \)) for ewe serum metabolites (Table 3). Ewes fed SS had greater (\( P = 0.01 \)) cholesterol and NEFA concentrations than ewes fed GC. There were no other differences (\( P > 0.16 \)) in ewe serum metabolites when SS was compared with GC. There was no difference (\( P \geq 0.18 \)) in any of the serum metabolites from ewes attributable to level of supplemental vitamin E.

Temperature Data

All lambs had a greater (\( P < 0.02 \)) rectal temperature after 30 min of cold exposure relative to 0 min (Figure 1). An energy source × level of supplemental vitamin E interaction was detected (\( P = 0.01 \)). The SSVC lambs had a decreased body temperature throughout the cold exposure (\( P < 0.02 \)) when compared with lambs in all other treatments.

Lamb BW

Birth BW did not differ (\( P = 0.58 \)) between SS and GC lambs (Table 4). Lambs born to SS ewes had decreased weaning weights (\( P = 0.10 \)) than lambs born to GC ewes. Body weights did not differ (\( P = 0.46 \)) between VE and VC lambs.

Ewe Production

Interactions were detected for ewe BW at turnout and weaning (Table 5). Ewes supplemented with SSVC weighed more (\( P = 0.06 \)) at turnout and weaning than did GCVC ewes and weighed more (\( P = 0.05 \)) at weaning than did SSVE ewes. There was no difference in BCS at turnout between SS and GC ewes (\( P > 0.37 \)) or between VE and VC ewes (\( P > 0.71 \)). The VE ewes had a greater (\( P = 0.10 \)) BCS at weaning than did the VC ewes.

Exp. 2

No interactions were detected (\( P > 0.18 \)) in Exp. 2. Lamb birth BW (kg/ewe) and lambs born (%) did not differ (\( P > 0.42 \)) between energy sources or levels of supplemental vitamin E (Table 6). However, lambs born to SS ewes had decreased (\( P < 0.06 \)) turnout and weaning weights (kg/ewe), had decreased (\( P = 0.06 \)) survival at weaning, and tended (\( P = 0.12 \)) to have a decreased percentage of survival at turnout than did lambs born to GC ewes. Kilograms of lamb per ewe and percentage of lamb survival did not differ (\( P > 0.28 \)) between levels of vitamin E supplementation (Table 6). Ewe BW and ewe BCS did not differ (\( P > 0.17 \)) between SS and GC ewes or between VE and VC ewes at turnout or weaning (Table 7).

DISCUSSION

Hypothermia, starvation, scours, and pneumonia are the major causes of neonatal lamb mortality, with 50% of lamb losses occurring within 24 h of birth (Rowland et al., 1992). Alexander (1961) estimated that 50% of the heat generated by ruminant neonates comes from nonshivering thermogenesis, which is fueled solely by BAT. Nedergaard et al. (1983) reported that linoleic and linolenic acid supplements, such as safflower seed, increased the thermogenic capacity. Activation of BAT causes large increases in oxygen consumption and consequently causes increases in the generation of oxygen radicals (Barja de Quiroga, 1992). Free radicals cause damage to cellular membranes, thereby creating a need for more antioxidants to maintain cell integrity. Vitamin E is an integral component of lipid membranes. The use of BAT in the lamb would suggest a need for ample amounts of antioxidants to reduce the amount of free radical buildup. Barja de Quiroga (1992) stated that with the relatively low activities of antioxidants in BAT and increased generation of free radicals, BAT activation could lead to a physiological oxidative stress on the body. Newborns are susceptible to vitamin E deficiency, and because of the negligible amount of vitamin E crossing to the fetus in utero, it
becomes important for colostrum to supply the lamb with sufficient amounts of vitamin E (Scott, 1980; McDowell et al., 1996).

Vitamin E 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 be seen when dealing with high levels of PUFA (such as via supplementation with safflower seed), which are known to increase the rate of lipid oxidation and the formation of free radicals. These factors may help explain the interaction noted in our study between the level of vitamin E and the source of energy for lamb body temperature, in which lambs born to SS ewes sup-

Table 2. Least squares means for initial (0 min) and final (30 min) serum metabolites for cold-stressed lambs in Exp. 1 (exposed to 0°C dry cold for 30 min) born to ewes fed 226 g of whole safflower seed or 340 g of grain control and 350 IU of vitamin E or 0 IU of vitamin E control supplement for the last 30 d of gestation

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Treatment²</th>
<th>SSVE</th>
<th>SSVC</th>
<th>GCVE</th>
<th>GCVC</th>
<th>SEM</th>
<th>S × V³</th>
<th>SS vs. GC</th>
<th>VE vs. VC</th>
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<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>44.2</td>
<td>48.6</td>
<td>43.0</td>
<td>49.2</td>
<td>8.05</td>
<td>0.90</td>
<td>0.97</td>
<td>0.48</td>
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<tr>
<td>30 min</td>
<td>41.8</td>
<td>57.0</td>
<td>44.2</td>
<td>41.8</td>
<td>7.22</td>
<td>0.20</td>
<td>0.35</td>
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</tr>
<tr>
<td>Change</td>
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<td>8.4</td>
<td>1.2</td>
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<td>5.46</td>
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<td>22.7</td>
<td>22.7</td>
<td>24.3</td>
<td>1.54</td>
<td>0.96</td>
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<tr>
<td>30 min</td>
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<td>23.0</td>
<td>23.1</td>
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<td>0.71</td>
<td>0.71</td>
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<td>Change</td>
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<tr>
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<td>30 min</td>
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<td>Blood urea N, mg/dL</td>
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<td>22.5</td>
<td>21.3</td>
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<td>0.11</td>
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<tr>
<td>30 min</td>
<td>22.8</td>
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<tr>
<td>% Change</td>
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<td>−3.1</td>
<td>−2.2</td>
<td>−1.6</td>
<td>1.45</td>
<td>0.56</td>
<td>0.62</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>T₃,T₄ μg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>27.4</td>
<td>23.0</td>
<td>29.9</td>
<td>27.6</td>
<td>2.91</td>
<td>0.66</td>
<td>0.14</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>28.6</td>
<td>24.6</td>
<td>25.4</td>
<td>25.2</td>
<td>3.62</td>
<td>0.52</td>
<td>0.66</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>1.1</td>
<td>1.6</td>
<td>−4.6</td>
<td>−2.4</td>
<td>3.28</td>
<td>0.78</td>
<td>0.07</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>% Change</td>
<td>10.6</td>
<td>15.2</td>
<td>−14.7</td>
<td>11.9</td>
<td>14.24</td>
<td>0.35</td>
<td>0.23</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>0.97</td>
<td>0.97</td>
<td>1.06</td>
<td>1.09</td>
<td>0.061</td>
<td>0.75</td>
<td>0.07</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>0.94</td>
<td>1.03</td>
<td>1.06</td>
<td>1.06</td>
<td>0.077</td>
<td>0.54</td>
<td>0.32</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>−0.02</td>
<td>0.06</td>
<td>0.01</td>
<td>−0.04</td>
<td>0.063</td>
<td>0.29</td>
<td>0.54</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>% Change</td>
<td>−1.4</td>
<td>8.4</td>
<td>0.91</td>
<td>−2.75</td>
<td>6.236</td>
<td>0.25</td>
<td>0.45</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>NEFA, mEq/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>1.16</td>
<td>1.52</td>
<td>1.35</td>
<td>1.18</td>
<td>1.014</td>
<td>0.124</td>
<td>0.03</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>1.31</td>
<td>1.42</td>
<td>1.41</td>
<td>1.27</td>
<td>0.097</td>
<td>0.18</td>
<td>0.79</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>0.15</td>
<td>−0.12</td>
<td>0.06</td>
<td>0.11</td>
<td>0.098</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Change</td>
<td>16.3</td>
<td>−7.08</td>
<td>8.63</td>
<td>15.86</td>
<td>8.322</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

аБWithin a row, means without a common superscript letter differ, P < 0.10.

¹n = 42.
²SSVE = 226 g · ewe⁻¹ · d⁻¹ of safflower seed (SS) and 350 IU · ewe⁻¹ · d⁻¹ of supplemental vitamin E (VE); SSVC = SS and no added supplemental vitamin E (VC); GCVE = 340 g · ewe⁻¹ · d⁻¹ of isocaloric and isonitrogenous grain-based control (GC) and VE; GCVC = GC and VC.
³S × V = interaction between type of energy supplement and level of supplemental vitamin E.
⁴T₃ = triiodothyronine.
⁵T₄ = thyroxine.
supplemented with vitamin E maintained a greater body temperature than did lambs born to SS ewes and not supplemented with additional vitamin E.

Energy restriction often has been characterized by elevated NEFA concentrations in ruminants (DiMarco et al., 1981; Peters, 1986). In our study, in which lambs had not been allowed to consume colostrum before the 30-min cold period, the reduced concentrations of NEFA in SSVC lambs may be indicative of decreased energy reserves or a reduced ability to mobilize energy reserves compared with lambs in the other treatments. Lambs born to SS ewes had increased BUN, T3, and T3:T4 ratio during cold exposure, whereas lambs born to GC ewes had decreased levels of these metabolites. Leibholz (1970) reported that starvation can increase BUN levels as body protein is catabolized. In addition, during normal growth, thyroid hormones are involved in both the synthesis and degradation of protein (Goldberg et al., 1980). In a period of limited nutrient availability, increased thyroid function or greater circulating concentrations of thyroid hormones, or both could be indicative of greater levels of body protein degradation. It is possible that lambs born to SS ewes had to mobilize body protein stores to fuel metabolism and possibly nonshivering thermogenesis. We conclude from these observations that lambs born to SS ewes, and particularly those born to SSVC ewes did not have, or were unable to metabolize, the fat reserves as efficiently as

Table 3. Least squares means for serum metabolites for ewes in Exp. 1 (1 h postpartum) fed 226 g of whole safflower seed or 340 g of grain control and 350 IU of vitamin E or 0 IU of vitamin E control supplement for the last 30 d of gestation

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Treatment2</th>
<th>SEM</th>
<th>P-value</th>
<th>S × V3</th>
<th>SS vs. GC</th>
<th>VE vs. VC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>SSVE</td>
<td>106.9</td>
<td>0.46</td>
<td>0.37</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>SSVC</td>
<td>111.9</td>
<td>0.46</td>
<td>0.01</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>GCVE</td>
<td>104.8</td>
<td>0.46</td>
<td>0.01</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>NEFA, mEq/L</td>
<td>GCVC</td>
<td>90.8</td>
<td>0.13</td>
<td>0.49</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Total protein, mg/dL</td>
<td>SSVE</td>
<td>14.13</td>
<td>0.69</td>
<td>0.20</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSVC</td>
<td>3.23</td>
<td>0.60</td>
<td>0.20</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GCVE</td>
<td>1.65</td>
<td>0.19</td>
<td>0.60</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GCVC</td>
<td>1.3</td>
<td>0.13</td>
<td>0.49</td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>

1n = 42.  
2SSVE = 226 g·ewe⁻¹·d⁻¹ of safflower seed (SS) and 350 IU·ewe⁻¹·d⁻¹ of supplemental vitamin E (VE); SSVC = SS and no added supplemental vitamin E (VC); GCVE = 340 g·ewe⁻¹·d⁻¹ of isocaloric and isonitrogenous grain-based control (GC) and VE; GCVC = GC and VC.  
3S × V = interaction between type of energy supplement and level of supplemental vitamin E.  
4BUN = blood urea N.

Figure 1. Least squares means of rectal temperature for 42 twin lamb pairs in Exp. 1 exposed to 0°C for 30 min 1 h after parturition. Treatments were as follows: SSVE = 226 g·ewe⁻¹·d⁻¹ of safflower seed (SS) and 350 IU·ewe⁻¹·d⁻¹ of supplemental vitamin E (VE); SSVC = SS and no added supplemental vitamin E (VC); GCVE = 340 g·ewe⁻¹·d⁻¹ of isocaloric and isonitrogenous grain-based control (GC) and VE; GCVC = 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, energy source × level of vitamin E interaction, and SSVC vs. all other treatments were detected (P < 0.02).
or to the same extent as lambs in the other treatments. Contrary to our results, Encinias et al. (2004) reported that NEFA did not differ 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 occurred at a similar rate.

Lammoglia et al. (1999) and Encinias et al. (2004) reported that supplementation with safflower seed increased body temperature; this was not the case in our study. In our study, a source of energy × level of supplemental vitamin E interaction was detected. Data from lamb temperature during cold exposure indicating that SSVC lambs had a decreased cold tolerance supports the conclusion that SSVC lambs were unable to mobilize fat reserves as efficiently as lambs in the other treatment groups. Conflicting results have been reported for the effect of safflower seed supplementation on body temperature. There is no literature on the effect of both supplemental safflower seed and vitamin E on neonatal ruminant temperature. Lammoglia et al. (1999) reported that calves born to cows receiving a 4.7% fat diet had a greater initial rectal temperature and that they maintained that temperature longer than calves born to cows receiving a 1.7% fat diet. Similarly, Encinias et al. (2004) reported that lambs born to ewes receiving a 4.9% fat diet prepartum had a greater rectal temperature than lambs born to ewes receiving a 1.9% fat diet prepartum.

Ewes that received SSVC gave birth to lambs with lower body temperature compared with lambs in all other treatment combinations. In addition, no differences in body temperature between SSVE, GCVE, and GCVC indicated that safflower seed supplementation did not increase cold tolerance in lambs. This is supported by the report of Dietz et al. (2003), who found no difference in response to cold stress between calves born to heifers receiving a 1.5% fat control diet, a 4.0% fat safflower diet, and a 5.0% fat cottonseed diet. The calves in that study were not maintained in a 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 had 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 cows are calving during mild spring conditions.

Encinias et al. (2004) reported that lambs born to ewes fed a high-fat diet during late gestation had lambs with greater survivability than lambs born to ewes fed

<table>
<thead>
<tr>
<th>Item</th>
<th>SSVE</th>
<th>SSVC</th>
<th>GCVE</th>
<th>GCVC</th>
<th>SEM</th>
<th>S × V</th>
<th>SS vs. GC</th>
<th>VE vs. VC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth BW, kg/ewe</td>
<td>9.2</td>
<td>9.0</td>
<td>9.1</td>
<td>8.7</td>
<td>0.41</td>
<td>0.78</td>
<td>0.58</td>
<td>0.47</td>
</tr>
<tr>
<td>Turnout BW, kg/ewe</td>
<td>22.9</td>
<td>23.4</td>
<td>25.2</td>
<td>26.2</td>
<td>1.86</td>
<td>0.89</td>
<td>0.16</td>
<td>0.65</td>
</tr>
<tr>
<td>Weaning BW, kg/ewe</td>
<td>53.3</td>
<td>54.3</td>
<td>59.7</td>
<td>60.5</td>
<td>4.06</td>
<td>0.99</td>
<td>0.10</td>
<td>0.81</td>
</tr>
</tbody>
</table>

1n = 42.

2SSVE = 226 g-ewe-1d-1 of safflower seed (SS) and 350 IU-ewe-1d-1 of supplemental vitamin E (VE); SSVC = SS and no added supplemental vitamin E (VC); GCVE = 340 g-ewe-1d-1 of isocaloric and isonitrogenous grain-based control (GC) and VE; GCVC = GC and VC.

3S × V = interaction between type of energy supplement and level of supplemental vitamin E.

4Turnout = moved from confinement to spring range (May 24, 2005, 32 ± 7 d of age).

5Weaning = August 24, 2005, 124 ± 7 d of age.
a low-fat prepartum diet. This was not the case in our study. The effects of SS, and particularly SSVC, supplementation on serum metabolites and cold tolerance were reflected in decreased lamb BW and percentages of lambs born in both Exp. 1 and 2. In Exp. 1, GC-supplemented ewes weaned 6.2 kg more lamb/ewe than SS-supplemented ewes. The same relationship was noted in Exp. 2, in which kilograms of lamb weaned per ewe and lamb survival were both greater for GC ewes than SS ewes, with lambs born to SSVC ewes having the lowest survival and least BW per ewe.

Our study indicated no difference in lamb BW or survival between levels of vitamin E supplementation. Williamson et al. (1995) also reported no difference in birth BW or weaning weights when ewes were injected 2 wk before lambing with 2,400 IU of vitamin E and lambs were injected with 1,200 IU of vitamin E. Kott et al. (1998), who fed ewes vitamin E at the same amount and in the same research facility as in our study, also reported no difference in birth BW between vitamin E-supplemented ewes and nonsupplemented control ewes. However, in contrast to our study, Capper et al. (2005), who used a 2 × 2 factorial arrangement of treatments to compare the effects of feeding PUFA versus saturated fat and a supplement that contained either 50 or 500 mg of vitamin E/kg for the last 6 wk of gestation, reported that feeding 500 mg of vitamin E/kg resulted in heavier birth BW compared with lambs born to ewes fed 50 mg of vitamin E/kg.

There were no consistent differences in ewe BW or BCS among treatments in either experiment. However, it is interesting to note that in Exp. 1, SSVC ewes had a tendency to weigh more than ewes fed the other treatments. Although this trend was not noted in Exp. 2, it does raise the question of how these different treatments affected nutrient partitioning between the ewe, her fetus, and milk and colostrum production.

In summary, based on the decreased body temperature in SSVC lambs at birth and the concentrations of thyroid hormones, NEFA, and BUN during cold exposure, SS-supplemented ewes, and particularly SSVC-supplemented ewes, appeared to give birth to lambs with a decreased basal metabolic rate. The interaction of type of energy × level of vitamin may also indicate

### Table 6. Least squares means of lamb BW and percentage survival for lambs in Exp. 2 born to ewes fed 226 g of whole safflower seed or 340 g of grain control and 350 IU of vitamin E or no added supplemental vitamin E for the last 30 d of gestation

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>S × V</th>
<th>SS vs. GC</th>
<th>VE vs. VC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth BW, kg/ewe</td>
<td>SSVE</td>
<td>7.1</td>
<td>0.36</td>
<td>0.71</td>
<td>0.96</td>
</tr>
<tr>
<td>Turnout BW, kg/ewe</td>
<td>SSVC</td>
<td>6.9</td>
<td>0.27</td>
<td>0.20</td>
<td>0.28</td>
</tr>
<tr>
<td>Weaning BW, kg/ewe</td>
<td>GCVE</td>
<td>7.1</td>
<td>0.67</td>
<td>0.48</td>
<td>0.46</td>
</tr>
<tr>
<td>Lambs born, %</td>
<td>GCVC</td>
<td>7.2</td>
<td>4.77</td>
<td>0.63</td>
<td>0.40</td>
</tr>
<tr>
<td>Turnout survival, %</td>
<td>SSVC</td>
<td>148.5</td>
<td>4.14</td>
<td>0.69</td>
<td>0.36</td>
</tr>
<tr>
<td>Weaning survival, %</td>
<td>SSVC</td>
<td>127.5</td>
<td>4.14</td>
<td>0.69</td>
<td>0.36</td>
</tr>
</tbody>
</table>

### Table 7. Least squares means of ewe BW and BCS in Exp. 2 fed 226 g of whole safflower seed or 340 g of safflower control and 350 IU of vitamin E or no added supplemental vitamin E for the last 30 d of gestation

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>S × V</th>
<th>SS vs. GC</th>
<th>VE vs. VC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turnout BW, kg</td>
<td>SSVE</td>
<td>58.6</td>
<td>0.95</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>Turnout BCS</td>
<td>SSVE</td>
<td>59.2</td>
<td>0.95</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>Weaning BW, kg</td>
<td>GCVE</td>
<td>59.0</td>
<td>0.95</td>
<td>0.95</td>
<td>0.98</td>
</tr>
<tr>
<td>Weaning BCS</td>
<td>GCVC</td>
<td>59.0</td>
<td>0.95</td>
<td>0.95</td>
<td>0.98</td>
</tr>
</tbody>
</table>
that supplementing ewes with safflower seed without additional vitamin E may compromise the ability of newborn lambs to adapt to cold environmental conditions. In both experiments, SS lambs had less BW and survival at weaning. We were unable to demonstrate any positive effects of safflower seed supplementation; in fact, supplementing ewes with safflower seed during late gestation may result in a decreased lamb production potential.

LITERATURE CITED


