INTRODUCTION

Copper (Cu) is an essential element required by lambs and other animals for a number of biochemical functions (Davis and Mertz, 1987). Dietary Cu, when fed at pharmacological concentrations, has been shown to alter lipid metabolism in calves (Jenkins and Kramer, 1989) and goats (Solaiman et al., 2006). Some research suggests that dietary Cu at physiological concentrations may also affect lipid metabolism in ruminants, but the results are inconsistent. Sinnett-Smith and Woolliams (1987) reported that supplementing Cu (from Cu oxide needles) to Cu-deficient sheep led to increased adipose cell volume and in vitro lipolytic rates of adipose tissue. In addition, several studies in finishing steers found that Cu supplementation decreased backfat depth (Engle et al., 2000a; Engle and Spears, 2000a) and increased unsaturated fatty acid concentrations of longissimus muscle (Engle et al., 1999, 2000a). However, 10 or 40 mg Cu/kg DM given to Simmental steers fed a corn silage-soybean meal-based diet had no effect on lipid metabolism (Engle and Spears, 2000b; 2001; Solaiman et al., 2001). The reasons for conflicting performance and lipid metabolism by Cu supplementation are not clear, and no research had been done on the mechanism of reducing backfat and altering lipid metabolism by copper treatment.

It was documented that tumor necrosis factor (TNF)
could be produced in adipose tissues (Hotamisligil and Spiegelman, 1994) and that it could affect lipid metabolism (Hauner et al., 1995; Kushibiki et al., 2000) and caused an elevated rate of lipolysis in adipocytes (Kawakami et al., 1987; Green et al., 1994). Furthermore, Cu has been shown to affect TNF metabolism in humans (Nasulewicz et al., 2004) and calves (Gengelbach et al., 1997). Therefore, it is speculated that alteration of lipid metabolism by Cu supplementation may be due to altering TNF metabolism.

Recent research has shown that significant differences exist in mineral metabolism between species (Arnold et al., 1993), even between ruminants (Haenlein, 2004). NRC (1980) indicated that sheep are more sensitive to high Cu supplementation than cattle and goats. Limited research had been done on Cu supplementation in sheep. Therefore, the objective of this study was to determine the effects of dietary Cu source and level on performance, carcass characteristics and lipid metabolism in terms of plasma TNF-α concentration and other plasma parameters in Dorper×Mongolia sheep.

### Materials and Methods

#### Animals and diets

Fifty Dorper×Mongolia wether lambs (approximately 3 month of age; 23.8±0.6 kg of body weight) were housed in individual wooden pens with slatted floors in an open-sided barn and fed a maize-soybean meal-based diet (Table 1: basal diet contained on dry matter basis 6.74 mg Cu/kg, 33 mg Zn/kg, 171.5 mg Fe/kg, 1.2 mg Mo/kg, 0.26 mg S/kg). The basal diet was formulated to meet or exceed all nutrient requirements for lambs with the exception of Cu (NRC, 1985).

Feeds were offered daily at 07:00 and 17:00 h in two equal portions. The forage and concentrate were prepared for each lamb before the morning meal; oat hay and alfalfa hay were offered first and the concentrate was offered 30 min later. All animals had free access to water containing undetectable concentrations of Cu (analyzed less than 0.01 mg/kg).

After 3 weeks adjustment to the experimental feeding system, lambs were weighed for two consecutive days, stratified by body weight and assigned randomly to one of five experimental treatments (n = 10 lambs per treatment): i) control (no supplemental Cu), ii) 10 mg Cu/kg DM from Cu-lysine (JH BIOTECH, INC.), iii) 20 mg Cu/kg DM from Cu-lysine, iv) 10 mg Cu/kg DM from TBCC (Cu2(OH)3Cl; HERITAGE, INC.), v) 20 mg Cu/kg DM from TBCC. Cu was added (as Cu-lysine or TBCC) to the premix using finely ground maize flour as a carrier and mixed with concentrate. Feed offered and refusals were recorded daily prior to the morning feeding and feed intake was adjusted weekly. The experiment lasted for 60 days.

#### Sample collection

Blood samples were collected from each lamb before morning feeding via jugular venipuncture into heparinized and nonheparinized vacutainer tubes on day 60, then centrifuged at 1,100×g for 10 min at 4°C to obtain plasma and serum and stored at -30°C for plasma TNF-α and serum lipid profile determinations.

At the end of the study, final body weights were measured on two consecutive days, and all lambs were slaughtered after an overnight period of feed withdrawal. Hot carcass weight (HCW) was determined on the day of slaughter and was used to determine dressing percent (HCW/live BW). Fat depth over the longissimus muscle (between the 12th and 13th ribs), percentage of kidney fat (expressed as a percentage of live BW), and longissimus muscle area (LMA) were determined by a certified USDA grader 48 h after slaughter. After carcass grading, a longissimus muscle sample was sliced from the 9th to 11th rib interface (approximately weight, 50 g) of the right side of the carcass. The samples were placed in sealed whirlpack bags and immediately chilled on ice. Upon arrival at the laboratory, the samples were frozen at -80°C until they were analyzed for fatty acid composition.

#### Analytical procedures

Plasma TNF-α concentrations were determined by enzyme-linked immunosorbent assays using

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**Table 1. Ingredients and chemical composition of the basal diet**

<table>
<thead>
<tr>
<th>Item</th>
<th>Ingredients (%) as fed basis</th>
<th>Chemical composition (%) as dry matter basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat hay</td>
<td>16</td>
<td>Dry matter: 88.7</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>20</td>
<td>Metabolizable energy (MJ/kg): 11.32</td>
</tr>
<tr>
<td>Maize flour</td>
<td>46.8</td>
<td>Crude protein: 14.71</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8</td>
<td>Neutral detergent fiber: 29.92</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>6</td>
<td>Acid detergent fiber: 18.22</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.6</td>
<td>Calcium: 0.61</td>
</tr>
<tr>
<td>Di-calcium phosphates</td>
<td>0.1</td>
<td>Phosphorus: 0.39</td>
</tr>
<tr>
<td>Salt</td>
<td>0.5</td>
<td>Cu (mg/kg): 6.74</td>
</tr>
</tbody>
</table>

* Provided per kilogram of the diet: 30 mg of Zn as ZnSO4·7H2O; 20 mg of Mn as MnSO4·H2O; 0.5 mg of I as KI; 0.1 mg of Co as CoCl2; 0.1 mg of Se as Na2SeO3; 1,500 IU of vitamin A; 250 IU of vitamin D and 16 IU of vitamin E.

* Analyzed values except metabolizable energy. Metabolizable energy was calculated by metabolizable energy in ingredient of the basal diet.
commercially available kits (R&D Systems, Minneapolis, MN). Serum samples were analyzed for total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and triglyceride (TG) concentrations by automatic analyzer (HITACHI 7600-020, Japan) using commercial kits (Sigma Chemical Co., St. Louis, Mo). Serum NEFA concentrations were measured with a commercially available enzymatic colorimetric kit (Wako Chemicals USA, Inc., Richmond, VA).

Lipids of longissimus muscle samples were extracted according to the procedure described by Folch et al. (1957). The longissimus muscle samples (0.2 g) were transferred into a test tube, and 8 ml chloroform-methanol (2:1, v/v) solution and 1 ml heptadecanoic acid (C 17:0, 2 mg/ml in solution and 1 ml heptadecanoic acid (C 17:0, 2 mg/ml in chloroform-methanol solution, internal standard, Sigma-Aldrich) were added. The homogenate was filtered, and the filtrate was collected. The crude extract was washed with 4 ml of solution (chloroform: methanol: 0.04% MgCl2 = 3:48:47, v/v/v). The upper phase was removed and the lower phase was dried under the flow of nitrogen. Fatty acid methyl esters (FAME) were prepared by incubating the lipids extracted at 50°C for 30 min in the presence of 1.2 M sodium methoxide and, after cooling, the solution was incubated at 80°C for 60 min with methanolic hydrochloric acid, which was prepared by slow addition of 10 ml acetyl chloride to 10 ml methanol. The FAME were analyzed using a gas chromatograph HP6890 (Hewlett-Packard, Avondale, PA, USA) equipped with an automatic sampler HP15896C (Hewlett-Packard, Avondale, PA, USA). Separations were accomplished using a 100m CP-Sil 88 (Varian, Walnut Creek, CA, USA) capillary column (0.25 mm i.d. and 0.2 μm film thickness). The carrier gas was helium. The conditions of gas chromatography were: 250°C injector temperature; 250°C detector temperature; the oven temperature was programmed from an initial temperature of 180°C for 45 min to a final temperature of 215°C at the rate of 10°C/min, and the final temperature was maintained for 17 min. Standard FAME mixture (Supelco, Bellefonte, PA, USA) were used to identify FAME of samples. Identification of the fatty acid was made by comparing the relative retention times of fatty acid methyl ester peaks from samples with those of known standards. These were calculated as normalized area percentages of fatty acids.

### Statistical analysis

Statistical analysis of data was performed by least squares analysis of variance using the GLM procedure of SAS (2001). When treatment was significant (p<0.05), differences among means were determined using single degree of freedom orthogonal contrasts. Comparisons made were i) control versus Cu supplemented treatments, ii) 10 mg Cu/kg DM versus 20 mg Cu/kg DM, iii) 10 mg Cu/kg DM from Cu-lysine versus 10 mg Cu/kg DM from TBCC, iv) 20 mg Cu/kg DM from Cu-lysine versus 20 mg Cu/kg DM from TBCC.

### RESULTS AND DISCUSSION

#### Daily gain and daily feed intake

Average daily gain (ADG), average daily feed intake (ADFI) and gain:feed (G/F) were not affected by Cu level or source (Table 2).

It was shown that Cu level (10 or 20 mg Cu/kg DM) and source had no effects on ADG and ADFI in lambs. In agreement with the present study, Cu supplementation at 10 to 40 mg Cu/kg DM did not affect ADG, ADFI, and G/F compared with controls in steers (Engle and Spears, 2000a, b, 2001). Luginbuhl et al. (2000) found that ADG, ADFI and feed efficiency were not affected by supplemental Cu at levels of 0, 10, and 30 mg Cu/kg DM to growing meat goats. Moreover, Mullis et al. (2003) reported that Cu supplementation at 7 or 14 mg Cu/kg DM did not affect daily gain, feed intake, or G/F in Angus and Simmental heifers. In contrast, Mondal and Biswas (2007) reported that supplementation of Cu may improve ADG in Black Bengal kids. In addition, Engle and Spears (2000b) indicated that Cu supplementation to finishing steers at 20 or 40 mg Cu/kg DM decreased ADG, ADFI, and G/F relative to controls and the decreased steer performance may have been due to impaired ruminal fermentation by high dietary Cu. Furthermore, Zhang et al. (2007) found that ADG and feed efficiency were increased when 10 mg

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**Table 2. Effects of dietary copper source and level on performance of lambs**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control 0 ppm</th>
<th>Cu-lysine 10 ppm</th>
<th>TBCC 10 ppm</th>
<th>SEM</th>
<th>Control vs. Cu</th>
<th>Cu-lysine vs. TBCC at 10 ppm</th>
<th>Cu-lysine vs. TBCC at 20 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW (kg)</td>
<td>28.03</td>
<td>27.90</td>
<td>28.10</td>
<td>0.683</td>
<td>0.993</td>
<td>0.954</td>
<td>0.952</td>
</tr>
<tr>
<td>Final BW (kg)</td>
<td>43.87</td>
<td>44.25</td>
<td>43.63</td>
<td>0.845</td>
<td>0.979</td>
<td>0.961</td>
<td>0.954</td>
</tr>
<tr>
<td>ADG (kg)</td>
<td>0.264</td>
<td>0.273</td>
<td>0.259</td>
<td>0.004</td>
<td>0.721</td>
<td>0.112</td>
<td>0.822</td>
</tr>
<tr>
<td>ADFI (kg)</td>
<td>1.400</td>
<td>1.415</td>
<td>1.386</td>
<td>0.013</td>
<td>0.982</td>
<td>0.511</td>
<td>0.896</td>
</tr>
<tr>
<td>G/F (g/g)</td>
<td>0.189</td>
<td>0.193</td>
<td>0.187</td>
<td>0.002</td>
<td>0.627</td>
<td>0.080</td>
<td>0.847</td>
</tr>
</tbody>
</table>

*SEM = Standard error of mean.
Cu/kg DM was supplemented to a basal diet containing 7.38 mg Cu/kg DM in Inner Mongolian White Cashmere goats, but supplementation of 30 mg Cu/kg DM decreased the ADG of goats. It is evident that conflicting performance results exist in cattle, goats and sheep consuming diets supplemented with Cu. It may be attributed to the differences in breed (Mullins et al., 2003b), Cu supplemental level and the experimental diet (Arthington and Pate, 2002). Results in the current study suggested that Cu-lysine and TBCC are of similar availability in performance of lambs. Supplementation of 10 or 20 mg Cu/kg DM showed the similar effects on growth and feed efficiency in lambs.

Carcass characteristics

Hot carcass weight, dressing percentage and longissimus muscle area were not affected (p>0.05) by dietary treatments (Table 3). Cu supplementation, regardless of source and level, reduced (p<0.01) 12th rib backfat and kidney fat in lambs. The results showed that Cu-lysine and TBCC at the levels of 10 or 20 mg Cu/kg DM exercised a similar influence on carcass characteristics in lambs.

This study is the first to investigate the effects of dietary Cu on carcass characteristics in lambs. Several experiments in steers, however, have indicated that Cu supplementation had no effect on hot carcass weight, dressing percentage and longissimus muscle area (Engle and Spears, 2000a; 2001). In agreement with the current experiment, the decrease in backfat depth was also observed in previous studies in Angus and Angus×Hereford steers (Engle and Spears, 2000a; Engle et al., 2000a, b) and Boer×Spanish wether goat kids (Solaiman, 2006). It is speculated that the decreased backfat depth and kidney fat in the present study might have resulted from the increased in vitro lipolytic rate of adipose tissue by Cu supplementation reported previously (Sinnett-Smith and Woolliams, 1987; Johnson and Engle, 2003). In addition, it may be related to an influence of Cu on plasma TNF-α as discussed below.

Blood measurement

The effects of Cu treatment on TNF-α and plasma metabolites are shown in Table 4. Plasma TNF-α and serum triglyceride concentrations were increased (p<0.05), total cholesterol concentrations were decreased (p<0.05) and NEFA concentrations tended to be increased (p<0.07) by Cu supplementation. However, serum HDL-cholesterol and LDL-cholesterol concentrations were not affected (p>0.05) by dietary treatments. The results suggested that Cu-lysine and TBCC at the levels of 10 or 20 mg Cu/kg DM showed similar effects on TNF-α and plasma metabolites in lambs.

It has been documented that TNF is primarily secreted from macrophages and has a major role in mediating inflammatory responses. However, TNF-α is also produced in adipose tissues (Hotamisligil and Spiegelman, 1994) and has been shown to have important effects on lipid metabolism in the adipocyte (Lopez-Soriano et al., 1998). TNF-α may act as a local signal regulating fat accumulation directly by means of modulating the expression and synthesis of key enzymes in lipid accretion such as lipoprotein lipase (LPL) (Semb et al., 1987) or hormone-
sensitive lipase (HSL) (Pekala et al., 1983). In this study, Cu supplementation increased plasma TNF-α in lambs, which is similar to the results reported by Gengelbach et al. (1997) for calves. The increased serum triglycerides in the present study might be explained by the increased TNF-α which inhibited the hydrolysis of circulating triglycerides by LPL since studies in vitro (Hauner et al., 1995), and in vivo (Semb et al., 1987) have demonstrated that TNF-α inhibited LPL activity and down-regulated its protein expression. In addition, the higher plasma TNF-α concentration in Cu-supplemented lambs may increase lipolysis of adipose tissue, resulting in the observed reduction in backfat and kidney fat and the tendency for increasing plasma NEFA. The NEFA and triglyceride results in the current study are supported by Kushibiki et al. (2000) who found that administration of recombinant bovine TNF-α to dairy heifers induced an increase in plasma NEFA and triglyceride concentrations.

Serum total cholesterol concentrations were reduced on day 60 by Cu supplementation. This is in agreement with findings in goats receiving 20 or 40 mg Cu/kg DM (Datta et al., 2007) and in steers receiving 10 to 20 mg Cu/kg DM (Engle et al., 2000a; Engle and Spears, 2001). It is speculated that Cu supplementation decreases the cellular concentration of the reduced form of glutathione, then the activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, which is the rate-limiting enzyme in cholesterol synthesis, could potentially be reduced, thereby decreasing cholesterol synthesis (Roitelman and Schechter, 1984; Freedman et al., 1989; Bakalli et al., 1995).

Table 5. Effects of copper source and level on fatty acid composition (g/100 g total fatty acid) of longissimus muscle of lambs

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Treatment</th>
<th>SEM</th>
<th>Control vs. Cu</th>
<th>Cu-lysine vs. TBCC 10 ppm</th>
<th>Cu-lysine vs. TBCC 20 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>Control 0</td>
<td>0.10</td>
<td>0.09</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>C14:1</td>
<td>2.12</td>
<td>1.11</td>
<td>1.21</td>
<td>1.08</td>
<td>1.09</td>
</tr>
<tr>
<td>C16:0</td>
<td>22.77</td>
<td>23.43</td>
<td>24.33</td>
<td>24.01</td>
<td>24.51</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.41</td>
<td>1.45</td>
<td>1.71</td>
<td>1.39</td>
<td>1.52</td>
</tr>
<tr>
<td>C18:0</td>
<td>16.18</td>
<td>16.62</td>
<td>15.35</td>
<td>16.37</td>
<td>15.26</td>
</tr>
<tr>
<td>C18:1</td>
<td>40.07</td>
<td>41.36</td>
<td>41.82</td>
<td>40.69</td>
<td>40.75</td>
</tr>
<tr>
<td>C18:2</td>
<td>6.00</td>
<td>5.73</td>
<td>5.71</td>
<td>5.90</td>
<td>5.91</td>
</tr>
<tr>
<td>CLA</td>
<td>1.15</td>
<td>1.13</td>
<td>1.06</td>
<td>1.04</td>
<td>0.99</td>
</tr>
<tr>
<td>SFA</td>
<td>41.07</td>
<td>42.17</td>
<td>42.04</td>
<td>42.38</td>
<td>41.79</td>
</tr>
<tr>
<td>MUFA</td>
<td>41.58</td>
<td>42.90</td>
<td>43.64</td>
<td>42.16</td>
<td>42.36</td>
</tr>
<tr>
<td>PUFA</td>
<td>6.63</td>
<td>6.34</td>
<td>6.30</td>
<td>6.50</td>
<td>6.50</td>
</tr>
<tr>
<td>USFA</td>
<td>48.21</td>
<td>49.23</td>
<td>49.94</td>
<td>48.66</td>
<td>48.86</td>
</tr>
<tr>
<td>USFA:SFA</td>
<td>1.17</td>
<td>1.17</td>
<td>1.19</td>
<td>1.15</td>
<td>1.17</td>
</tr>
</tbody>
</table>

The longissimus muscle fatty acid profile is presented in Table 5. The composition of longissimus muscle fatty acid was not affected by Cu supplementation. There were no differences in longissimus muscle fatty acid between Cu-lysine and TBCC at the levels of 10 or 20 mg Cu/kg DM showed similar effects on lipid metabolism in lambs.

Composition of longissimus muscle fatty acids

The longissimus muscle fatty acid composition is limited in lambs, and direct comparisons could not be made. Several studies have been conducted in steers, but the results were not consistent. Results in this study were in accord with those observed in Simmental steers fed 10 or 40 mg Cu/kg DM (Engle and Spears, 2001) or in Angus steers fed 10 or 20 mg Cu/kg DM (Johnson and Engle, 2003), but contrary to those reported by Engle et al. (2000a, b) and Engle and Spears (2000a). This inconsistency may be explained partially by the difference in breed, Cu levels or sources, diet fed and the duration of supplementation. The unchanged composition of muscle fatty acid in the present study might have resulted from the unchanged stearoyl-CoA desaturase (SCD) gene expression caused by Cu supplementation, based on previous results (Lee et al., 2002).
IMPLICATIONS

The results of this study indicate that Cu-lysine and TBCC are of similar availability based on performance, carcass characteristics and fat metabolism in lambs. The addition of 10 or 20 mg Cu/kg DM to the basal diet containing 6.74 mg Cu/kg DM had no effect on performance and muscle fatty acid composition, but the backfat and kidney fat were reduced in Dorper-Mongolia wether lambs. Cu supplementation altered lipid metabolism, which is associated with improved plasma TNF-α. Supplementation of 10 or 20 mg Cu/kg DM showed similar effects on lipid metabolism in lambs. Further research is needed to determine the possible role of TNF-α as a mediator of the effects of copper on adipose tissue metabolism.

REFERENCES


Mondal, M. K. and P. Biswas. 2007. Different sources and levels


