Effects of Dietary Copper and Molybdenum Supplementation on Meat Characteristics of Texel and Finnsheep Rams[1]

T.L. Wheeler[2,3], M. Koohmaraie[2,3] and E.T. Littledike[2,3]

Summary

Eight purebred Texel and eight purebred Finnsheep rams, eight months of age, were fed a growing diet containing either of two levels of copper/molybdenum (Normal = 9.6 ppm Cu + 1.9 ppm Mo; High = 19.2 ppm Cu + 3.8 ppm Mo) for 13 weeks in a 2 × 2 factorial arrangement to estimate effects on muscle traits and meat palatability. Live and carcass weights and fat thickness were not different ($P > 0.05$) between breeds or diets. Average daily gain (ADG) in Texels fed the high Cu/Mo diet was lower ($P < 0.05$) than in the other groups and longissimus muscle area was greater ($P < 0.05$) in Texels than in Finnsheep regardless of diet. Calpastatin activity was not affected ($P > 0.05$) by breed, but was higher ($P < 0.05$) in animals fed the high Cu/Mo diet, measured both with a crude assay at 0 hours and three days postmortem and partially purified at 0 hours. Calpain activity (µ- and m-calpain) tended to be higher ($P < -0.10$) in the high Cu/Mo diet. Warner-Bratzler shear force at three days postmortem was higher ($P < 0.05$) and Myofibril Fragmentation Index (MFI) at day 1 and day 3 was lower ($P < 0.05$) in meat from rams fed the high Cu/Mo diet. Meat from Texels had higher ($P < 0.05$) MFI at day 14 postmortem than meat from Finnsheep. Metal ion concentrations in longissimus muscle were affected by treatments, but no definitive association with tenderness was detected. Finnsheep had larger ($P < 0.05$) βR muscle fibers regardless of diet. These data indicate the Texel breed was similar in meat palatability traits to the Finnsheep breed, and that increased dietary copper and molybdenum was detrimental to meat tenderness due to increased calpastatin activity in the muscle.

Key words: calpastatin, copper, molybdenum, sheep, tenderness, Texel.

Introduction

Tatum et al. (1988) reported from a 1987 national survey of lamb cutability that 39% of all lamb carcasses were yield grade 4 or 5 and the average fat thickness at the 12th rib was 0.74 cm. In addition, the average fatness of retail lamb cuts was 0.35 cm and 53.0% of the separable fat from retail cuts was seam fat (Harris et al., 1990). These reports indicate that the lamb industry needs to pursue methods for producing leaner meat.

The Texel breed of sheep was imported into the U.S. by USDA-ARS, at the Roman L. Hruska U.S. Meat Animal Research Center (MARC) to compare it to Suffolk as a terminal sire breed. Texels were thought to have potential to improve muscularity and leanness of U.S. lambs. Research to date comparing Texels to Suffolks confirmed the lower rate of gain of Texel-sired lambs, but sire breed did not affect carcass composition (Leymaster and Jenkins, 1991).

Texels have been made available to the commercial sheep industry in the U.S., but no data are available evaluating eating quality of meat from Texel sheep. In addition, Texels appear to be particularly susceptible to copper toxicity and are reported to develop symptoms (high liver concentration of copper, periportal cirrhosis and jaundice) after 13 weeks of feeding on a diet containing 12 mg copper/kg DM (Woolliams et al., 1982). Thus, toxic effects of dietary

[1] Name brands are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2] U.S. Department of Agriculture, Clay Center, NE 68933. Phone: (402) 762-4229, fax: (402) 762-4148. USDA-ARS, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933.

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copper on various tissues may occur at lower dietary copper levels in Texel sheep than in most other breeds of sheep. Copper is a potent inhibitor of the muscle protease calpain. The calpain system is thought to be involved in myofibrillar protein turnover (Goll et al., 1992). Thus, different copper metabolism by Texel sheep may be associated with their improved muscularity. In addition, calpain is primarily responsible for postmortem myofibrillar proteolysis that results in increased meat tenderness (Koohmaraie, 1991, 1992).

Thus, changes in the activity of the calpain system would likely affect meat tenderness. The objectives of this research were: 1) to characterize muscle traits related to palatability of meat from Texel sheep relative to the Finnsheep breed; 2) to determine the basis for any differences in meat palatability; and 3) to determine the effect of dietary supplementation of copper and molybdenum on muscle traits.

Materials and Methods

Animals

The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of animals in this study. Eight purebred Texel and eight purebred Finnsheep rams (each eight months of age) were given ad libitum access for 13 weeks to a diet formulated to meet NRC requirements for growing sheep (NRC, 1985) containing one of two levels of copper/molybdenum (Table 1). Although both levels are within recommendations, the higher level (19.2 ppm copper) may be inappropriate for Texel sheep. At the end of the feeding period, the sheep were humanely slaughtered at the MARC abattoir.

Calpain Proteolytic System Quantification

Fifty grams of longissimus muscle were removed from the left carcass sides (first to fourth lumbar vertebrae) and processed within 20 minutes postmortem for quantification of the calpain proteolytic system (μ-calpain, m-calpain and calpastatin) after partial purification using ion-exchange chromatography according to Koohmaraie (1990). Crude (without ion-exchange chromatography purification) calpastatin (specific calpain inhibitor) also was quantified in the supernatant after heating and centrifugation of a muscle homogenate from 10 g of longissimus muscle according to Shackelford et al. (1993).

Temperature and pH Measurement

Temperature and pH were measured in the longissimus muscle of the left carcass sides at 0, 3, 6, 9 and 24 hours postmortem. The pH was determined by homogenizing 2.5 g of muscle in 25 mL 5 mM iodoacetate in 150 mM KCl according to Bendall (1973).

Carcass and Muscle Traits

At 24 hours postmortem, fat thickness and longissimus muscle area were measured at the 12th rib. The right sides (6th thoracic to 4th lumbar vertebra) then were cut into 2.54 cm thick chops and assigned to Warner-Bratzler shear force (day 1, 3 and 14), Myofibril Fragmentation Index (MFI) (day 1, 3 and 14) crude calpastatin determination (day 1 and 3) or fiber-typing (day 1). Chops assigned to 3- or 14-day aging were vacuum packaged and stored at 2 °C for the appropriate time period.

Shear Force and MFI Determination

At the end of each aging period, the loin chops were broiled on electric broilers[4] to 70 °C internal temperature. The chops were turned after reaching 40 °C. Temperature was monitored with iron constantan thermocouple wires inserted into the geometric center of a chop and attached to a potentiometer multipoint recorder.[5] Cooked chops were chilled 24 hours at 3 °C, then 1.27 cm diameter cores were removed parallel to the muscle fiber orientation and sheared once each on an Instron Universal testing instrument[6] with a Warner-Bratzler shear attachment. The crosshead speed was 5 cm/minute. Myofibril Fragmentation Index (MFI) was determined on 5 g of fresh muscle according to Culler et al. (1978).

Muscle Fiber Histochemistry

Longissimus muscle was obtained at 24 hours postmortem. Several 0.7 cm^3 samples were frozen on cork in liquid nitrogen and stored at -70 °C. Transverse cryostat sections, 10 μm thick, were cut and allowed to air dry. Sections were stained according to the procedures for simultaneous staining of bovine muscle fiber-types (pre-incubation pH = 4.30) described by Solomon and Dunn (1988).

A minimum of 200 fibers per animal were classified as βR, αR or αW according to Ashmore and Doerr (1971). Fiber areas were measured by Microcomp PM[7] interactive image analysis for planar morphometry.

Metal Ion Concentration

Longissimus muscle (50 g) was obtained at 30 minutes postmortem and stored on ice for 3 hours. Following wet ashing, calcium, magnesium and zinc concentrations were determined by flame atomic absorption spectrophotometry, and copper and molybdenum concentrations were determined by graphite

Table 1. Experimental diets.

Component	As fed	
Corn, %	63.4	
Alfalfa, %	20.0	
Soybean meal, %	8.0	
Liquid molasses, %	3.0	
Durabond, %	2.5	
Limestone, %	1.5	
Ammonium chloride, %	0.5	
Steamed bonemeal, %	0.5	
NaCl, %	0.5	
Vitamin ADE, ppm	500.0	
Aureomycin, ppm	500.0	
Vitamin E, ppm	300.0	
Rumensin 60, ppm	80.0	
	Cu/Mo level	
	Normal	High
Cu, ppm	9.6	19.2
Mo, ppm	1.9	3.8

[4] Farberware Company, Bronx, NY, 10461.

[5] Honeywell, Incorporated, Fort Washington, PA 19034.

[6] Instron Corporation, Canton, MA 02021.

[7] Southern Micro Instruments, Atlanta, GA 30339.

furnace atomic absorption spectrophotometry.

Statistical Analyses

Data were analyzed with General Linear Models (GLM) of SAS (1989) for a 2 × 2 (breed × diet) factorial arrangement of a completely randomized design for shear force, MFI, carcass traits, calpain system, fiber-type and metal ion concentration data. Temperature and pH were analyzed as a repeated measures design with breed/diet treatment as the whole plot and postmortem time as the split-plot. Treatment effects were tested with treatment × replication as the error term and split-plot effects were tested with the residual error.

Results and Discussion

Beginning and ending live weight, carcass weight and 12th rib fat thickness were not affected ($P > 0.05$) by breed or diet (Table 2). The animals used in this study were ram lambs that were slightly older and heavier than normal slaughter lambs. ADG was decreased ($P < 0.05$) in the Texel lambs on the high Cu/Mo diet. This may have been due to the toxicity of this level of copper to Texel lambs (Woolliams et al., 1982). Texel carcasses had greater ($P < 0.05$) longissimus muscle area than Finnsheep carcasses, regardless of diet. However, the Texels were not leaner than the Finnsheep, possibly because they were fed longer than normal for slaughter lambs.

There was no difference ($P > 0.05$) in the temperature decline of the longissimus muscle during postmortem chilling between breeds or diets (Figure 1). In addition, sire-breed and diet did not interact ($P > 0.05$) to affect temperature or pH decline in the longissimus muscle and all treatments reached the same ultimate pH. These results would be expected because carcass weight and fatness were similar.

The calpain proteolytic system is highly related to meat tenderness (for review see Koohmaraie, 1992) and thus, was measured in order to explain possible differences in tenderness due to breed or diet treatment. Breed did not affect ($P > 0.05$) calpain or calpastatin activity (Table 3). However, higher Cu/Mo in the diet increased ($P < 0.05$) calpastatin activity in the longissimus muscle measured with two procedures and at different times postmortem. Partially purified calpastatin activity at 0 hours (within 20 minutes postmortem) was higher ($P < 0.05$) in the muscle of lambs fed the high Cu/Mo diet compared to those fed the normal Cu/Mo diet, regardless of sire-breed. Crude calpastatin activity, measured at 0 hours and at 3 days postmortem, also was greater ($P < 0.05$) in the higher Cu/Mo diet and tended to differ ($P < 0.07$) between diets at 1 day postmortem. The *in vivo* regulation of calpastatin has not been elucidated; thus it is impossible to speculate on the physiological reasons for the increased calpastatin activity with high dietary Cu/Mo. There was a tendency for increased ($P < 0.10$) μ- and m-calpain activity in muscle from animals fed the higher Cu/Mo diet. It has previously been shown that higher calpastatin activity remaining in muscle at 1 to 7 days postmortem is associated with tougher meat (Whipple et al., 1990; Koohmaraie et al., 1991; Shackelford et al., 1991; Wheeler and Koohmaraie, 1992; Morgan et al., 1993a), thus implicating the factor(s) responsible for the decline in calpastatin activity as the determinant of meat tenderness. It also has been shown that initial calpastatin activity (0 hours postmortem) was higher in β-agonist-fed steers (Wheeler and Koohmaraie, 1992) and lambs (Koohmaraie et al., 1991) and in bulls compared to steers (Morgan et al., 1993b) and the higher calpastatin activity was associated with decreased tenderness.

Consistent with the above discussion, shear force was higher ($P < 0.05$) at 3 days postmortem and MFI (a measure of muscle proteolysis and thus, of tenderness) was lower ($P < 0.05$) at 1 and 3 days postmortem in muscle from animals fed the higher Cu/Mo diet (Table 4). Shear force at 1 day postmortem was higher ($P < 0.05$) in muscle from Finnsheep compared to muscle from Texels. At 14 days postmortem, shear force was higher ($P < 0.05$) in muscle from Texels fed the higher Cu/Mo diet compared to the other three treatment combinations and MFI was lower ($P < 0.05$) in muscle from Finnsheep compared to Texels, regardless of diet. These data are consistent with the involvement of calpastatin activity in determining meat tenderness. In addition, it appears that dietary copper/molybdenum influences calpastatin activity in muscle.

Molybdenum and magnesium concentration in the longissimus muscle were not affected ($P > 0.05$)

Table 2. Effect of sire-breed and diet on live and carcass traits[a].

	Texel		Finnsheep			Probability		
	Normal	High	Normal	High	SEM	Breed	Diet	B × D
Initial weight, kg	52.6	49.0	53.4	51.8	2.5	0.31	0.15	0.76
Slaughter weight, kg	66.2	55.5	68.0	68.2	3.5	0.07	0.17	0.16
ADG, kg	0.15	0.07	0.16	0.18	0.02	0.02	0.44	0.04
Hot carcass weight, kg	37.4	31.6	34.2	34.7	2.0	0.97	0.20	0.14
Fat thickness, cm	0.54	0.47	0.32	0.38	0.1	0.16	0.98	0.53
Longissimus muscle area, cm^2	21.9	25.6	15.3	17.1	1.0	0.01	0.02	0.37

[a] Normal = 9.6 ppm Cu and 1.9 ppm Mo; High = 19.2 ppm Cu and 3.8 ppm Mo in the diet.

by breed or diet (Table 5). Calcium concentration was higher ($P < 0.05$) and zinc concentration lower in longissimus muscle from Texels compared to Finnsheep, regardless of diet. Copper was lower ($P < 0.05$) in muscle from Texels fed the normal Cu/Mo diet. Because calcium activates calpains and zinc and copper both inhibit calpains, theoretically, these data indicate that muscle from the Texels on normal Cu/Mo diet could have greater ***in vivo*** calpain activity. In fact, shear force and MFI data would support that possibility (Table 4). However, due to the complex (and as yet undetermined) regulation of the calpain system and the rigid control of metal ions that affect calpains, it is difficult to determine the net effect of these changes on calpain activity. It does appear that changes in metal ion concentration in longissimus muscle were partially associated with changes in shear force and MFI. Thus, the increased calpastatin activity and corresponding decreased tenderness indicated by day 3 shear force and MFI and day 1 MFI may be partially explained by changes in metal ion concentrations.

Percentage fiber type distribution was not affected ($P > 0.05$) by breed or diet (Table 4). However, Finnsheep lambs, regardless of dietary treatment, had larger ($P < 0.05$) βR fibers than Texels, particularly in those rams fed the normal Cu/Mo diet. This difference in βR fiber areas was not translated into greater longissimus muscle area, probably due to the low percentage of βR fibers. The area of αR and αW fibers was not affected ($P < 0.05$) by treatment. The greater longissimus muscle area of Texels (Table 2) cannot be explained by percentage fiber type distribution or relative fiber areas.

Conclusions

High dietary copper/molybdenum was associated with higher muscle calpastatin activity, decreased MFI and increased shear force of longissimus muscle, regardless of breed. Meat from Texels had lower MFI at 14 days postmortem that could be related to lower zinc and copper and higher calcium concentrations in the longissimus muscle compared with Finnsheep.

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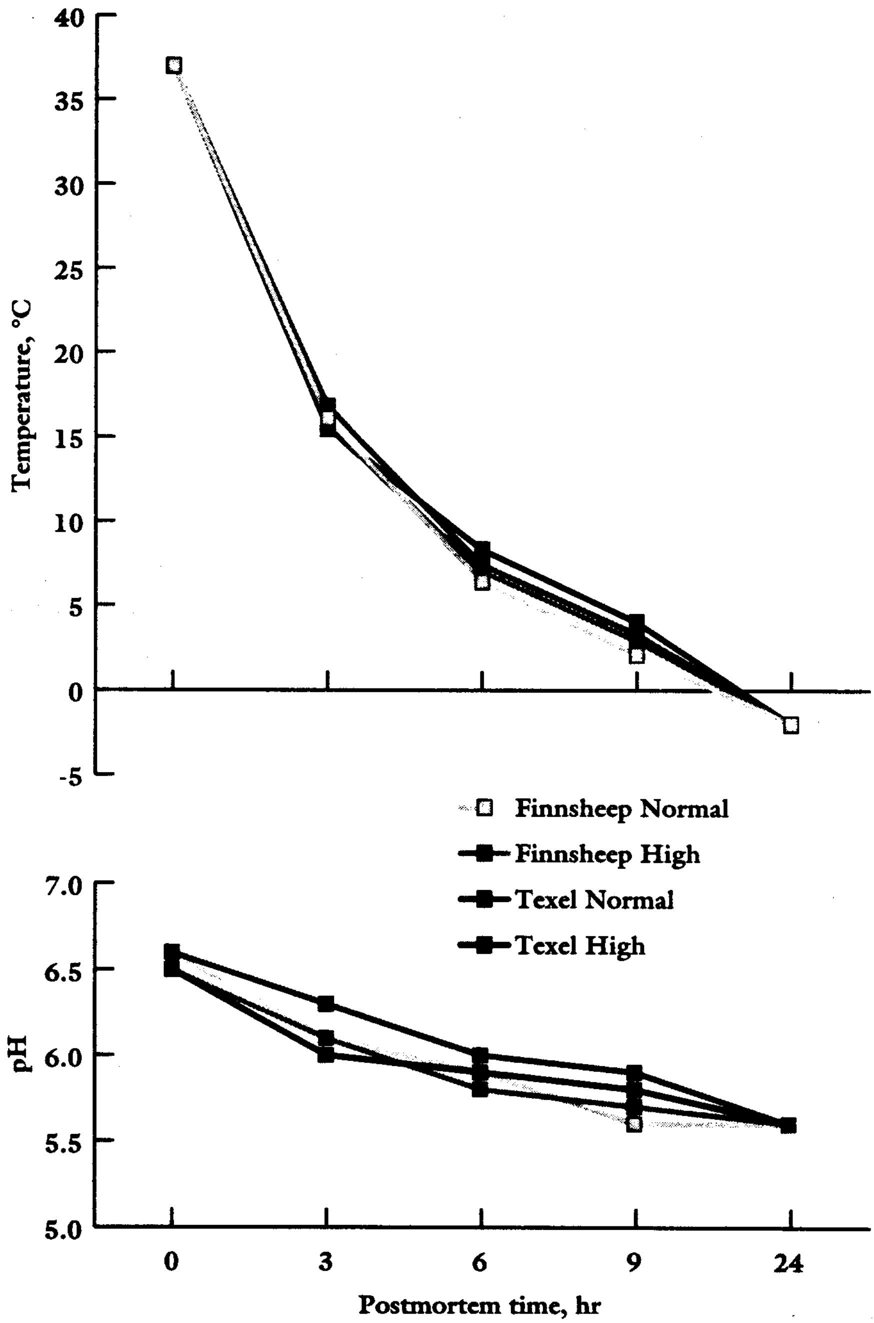
Figure 1. Effect of breed/dietary treatment on temperature and pH decline during carcass chilling.



All postmortem times differed ($P < 0.01$) from each other. The effect of breed/diet was not significant ($P > 0.05$). The interaction of breed/diet with postmortem time was not significant ($P > 0.05$).

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Table 3. Effect of sire-breed, diet treatment and postmortem time on crude calpastatin and 0-hour calpain system.

	Crude calpastatin[a]			0-hour calpain system[b]		
	0-hour	1-day	3-day	μ-Calpain	m-Calpain	Calpastatin
Texel:						
Normal	2.20	1.47	1.18	1.38	1.22	2.55
High	2.87	1.59	1.61	1.48	1.44	4.41
Finnsheep:						
Normal	2.05	1.44	1.14	1.33	1.24	2.65
High	2.59	1.72	1.57	1.72	1.37	3.67
SEM	0.11	0.09	0.09	0.10	0.11	0.20
Probability:						
Breed	0.36	0.66	0.69	0.43	0.82	0.17
Diet	0.02	0.07	0.01	0.08	0.10	0.01
B × D	0.78	0.46	0.99	0.27	0.61	0.09

[a] Measured in supernatant.
[b] Measured after partial purification by ion-exchange chromatography.

Table 4. Effect of sire-breed and diet treatment on Warner-Bratzler shear force and Myofibril Fragmentation Index (MFI) during postmortem storage.

	Texel		Finnsheep			Probability		
	Normal	High	Normal	High	SEM	Breed	Diet	B × D
Shear force, kg:								
day 1	6.20	6.61	7.02	7.47	0.37	0.05	0.30	0.96
day 3	4.16	5.69	4.35	5.87	0.51	0.76	0.03	0.99
day 14	2.81	3.79	2.91	2.83	0.20	0.08	0.07	0.04
MFI:								
day 1	54.3	42.7	52.3	43.5	3.8	0.89	0.02	0.73
day 3	71.8	58.7	70.3	56.5	2.7	0.57	0.01	0.92
day 14	73.8	70.7	62.2	62.5	1.6	0.01	0.44	0.37

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Table 5. Effect of sire-breed and diet on concentration of selected metal ions in longissimus muscle.

	Texel		Finnsheep			Probability		
	Normal	High	Normal	High	SEM	Breed	Diet	B × D
Copper, μg/g	0.61	0.83	0.83	0.86	0.07	0.04	0.11	0.21
Molybdenum, ng/g	8.6	8.6	7.6	6.2	0.8	0.08	0.46	0.44
Zinc, μg/g	19.4	21.6	25.8	22.8	0.8	0.01	0.63	0.01
Calcium, μg/g	12.2	14.0	11.0	11.1	0.8	0.04	0.32	0.36
Magnesium, μg/g	237.5	246.7	239.2	239.6	5.6	0.65	0.43	0.46

Table 6. Effect of sire-breed and diet on percentage muscle fiber type and muscle fiber area.

	Texel		Finnsheep			Probability		
	Normal	High	Normal	High	SEM	Breed	Diet	B × D
Percentage:								
βR	13.3	12.0	10.0	11.7	3.5	0.62	0.95	0.69
αR	28.5	35.3	40.0	35.7	4.0	0.18	0.76	0.20
αW	58.5	52.7	50.0	52.7	5.2	0.44	0.77	0.44
Area, μm^2								
βR	1520.0	1690.1	3049.1	2237.1	87.9	0.01	0.23	0.30
αR	1597.7	1685.4	1705.1	1836.6	54.1	0.11	0.65	0.79
αW	2281.5	2352.9	2218.7	2383.6	43.3	0.79	0.74	0.85