E44

DEVELOPING A RELIABLE ANIMAL MODEL TO STUDY THE DARK-CUTTING CONDITION IN SHEEP

JASON K. APPLE[1], MICHAEL E. DIKEMAN[2], AND J. ERNEST MINTON[2]
[1]Department of Veterinary and Animal Sciences, Paige Laboratory, University of Massachusetts, Amherst, MA 01003-6450
[2]Department of Animal Sciences and Industry, Weber Hall, Kansas State University, Manhattan, KS 66506-0201

Key Words: Sheep, Glycogen, Stress, Dark-Cutting Meat

BACKGROUND. Dark-cutting meat is a persistent quality defect characterized by an ultimate pH value in excess of 6.0; a high water-holding capacity; a dry, firm, "sticky" lean cut surface; and a dark-red to almost-black lean color. Research has indicated that the incidence of dark-cutting carcasses is approximately 5.1% of all cattle (Smith et al., 1992) and between 4 and 15% of all sheep beef industry approximately $132.5 million in 1991, or approximately $5 for every steer and heifer slaughtered (Smith et al., 1992).

Antemortem glycogenolysis has been generally accepted as the mechanism whereby the DCC is elicited. Experimentally, muscle glycogen reserves have been reduced in response to several different types of stressors. Tarrant (1981) indicated that the difficulty of consistently inducing the DCC under controlled experimental conditions was a significant drawback to eliciting the causes and mechanisms responsible for this quality defect, as well as researching possible treatments or alterations in animal welfare practices to reduce the incidence of dark-cutting carcasses.

Glycogenolysis in skeletal muscle is regulated by the activity of glycogen phosphorylase. Activation of glycogen phosphorylase may be triggered by either an adrenergic mechanism or a contractile mechanism, or by both mechanisms acting in concert (Tarrant, 1989). However, he implied that the predominant mechanism responsible for antemortem glycogenolysis and the subsequent formation of the DCC may be species dependent. It has been suggested that the contractile mechanism was the primary mechanism responsible for antemortem glycogenolysis in cattle, while the adrenergic mechanism was the predominant mechanism in sheep. Therefore, the primary objective of our study was to develop a reliable animal model that would consistently result in producing dark-cutting carcasses. A secondary objective was to determine the role that muscle contraction plays in antemortem glycogen degradation and subsequent formation of the DCC in sheep.

METHODS. In Experiment 1, 12 Suffolk wether lambs were used to evaluate the influence of a physical stressor (treadmill exercise; TME) on physiological changes and meat quality. Lambs underwent two screening sessions and were randomly assigned to 1 of 4 treatments (n=3/treatment): 1) exercise at 5.6 km/h on a 9°incline; 2) exercise at 7.2 km/h on a 9° incline; 3) exercise at 8.8 km/h on a 9° incline; and 4) nonexercised controls (C). In applying the treatments, each lamb was moved from its home stanchion to another room and placed on a variable-speed, variable-incline electric treadmill. After a 30-min acclimation period, each lamb was exercised at its assigned treatment speed for 10 min, followed by a 10-min postexercise walk at 4 km/h on the level. To reduce the potential of emotional stress caused by isolating a single lamb from its contemporaries, another lamb (not in the study) was walked with the lamb to the room and placed in a stanchion facing the lamb on the treadmill. Control lambs were placed on the treadmill, but not subjected to exercise. After the postexercise walk, each lamb was transported immediately less than 1 km to the Kansas State University Meats Laboratory and slaughtered.

Lambs were rendered unconscious by a concussion stunning method. Five minutes after stunning, a 20 g sample of longissimus muscle (LM) was removed and frozen in liquid-N_2 for glycogen analysis (McVeigh, 1981; Tarrant and McVeigh, 1979). Carcasses were chilled conventionally at 2°C. At 48-h postexsanguination, an additional muscle sample from the LM was removed for pH determination (Bendall, 1973). After sampling, visual color of the LM was scored using an 8-point scale for fresh meat color (1=bleached red and 8= very dark red). Carcasses then were fabricated into primal cuts, and CIE L*a*b* values for the LM were measured with a Minolta CR-200 Chroma Meter on cross-sections after a 30-min bloom period.

Table 1. Least squares means for carcass quality traits of exercised lambs.

	Treadmill speed, km/h			
Item	0	5.6	7.2	8.8
LM ultimate pH	5.56	5.54	5.57	5.61
LM glycogen[a]	43.6[c]	31.1[d]	42.9[c]	43.2[c]
LM color score[b]	6.75	6.75	6.75	6.75
CIE L*	34.9	34.2	34.8	33.9
CIE a*	20.9	20.0	20.7	19.9
CIE b*	8.5	8.6	8.7	8.2

[a]Muscle glycogen is reported in μmol/g of tissue wet weight.
[b]1=bleached red; ...; 6=moderately dark red; 7=dark red; 8=very dark red.
[c,d]Means in the same row with different superscript letters differ (P<.05).

In Experiment 2, 30 crossbred lambs were used to study the influence of an emotional stressor (restraint and isolation stress; RIS) on antemortem glycogenolysis and the formation of the DCC in the LM, and to determine the role of muscle contractions in the formation of dark-cutting meat. Lambs were assigned randomly to 1 of 3 treatments: 1) unstressed controls; 2) subjected to a single 6-h bout of RIS (RIS); and 3) subjected to a single 6-h bout of RIS and hindsaddle epidural-blockade (RISEB).

Stress treatment consisted of moving lambs from their home stanchions to a separate area, isolated from visual and tactile contact with other lambs, and placed in right lateral recumbency with their legs bound with nonadhesive wrap for restraint. Control lambs remained in their home stanchions and were subjected to minimal handling and stress. Fifteen hours before RIS, epidural-catheters were inserted into the epidural space between the last lumbar and first sacral vertebrae in RISEB lambs, and the tip of the catheter was advanced to the area of the first lumbar vertebra. After stressor treatment, 2% lidocaine (.2 ml./5 kg BW) was injected into the epidural catheter of RISEB lambs. Additional doses of lidocaine were given as needed to keep the level of contractile inhibition in all muscle groups of the hindsaddle, extending posterior of approximately the 10th thoracic vertebra, for 6 h. Upon completion of the 6-h period of RIS, lambs were transported less than 1 km and slaughtered.

Immediately after stunning and at .75, 3, 6, 12, and 24 h postmortem, samples were removed from the LM in the hindsaddle and foresaddle for pH determinations (Bendall, 1973), muscle glycogen (McVeigh, 1981; Tarrant and McVeigh, 1979), and muscle lactate (McGinnis et al., 1989) concentrations.

RESULTS. Carcass data for lambs in Experiment 1 are reported in Table 1. The ultimate pH, and subjective and objective measures of color from lambs subjected to TME were similar ($P > .05$) to those of LM from C, with values falling within ranges considered normal. Lambs exercised at 5.6 km/h had lower muscle glycogen concentrations than lambs exercised at 7.2 and 8.8 km/h and C. However, this reduction in muscle glycogen was not associated with higher pH or darker lean color.

When sheep were chased to exhaustion by dogs or humans, ultimate muscle pH values exceeded the characteristic 6.0 (Forrest et al., 1964; Chrystall et al., 1982). Our inability to detect these changes indicates that TME conditions of our study were not similar to stressor conditions described by Forrest et al.(1964) and Chrystall et al.(1982). Clearly, other physiological measures indicated that the untrained sheep in our study were challenged with significant physical stress (exercised at calculated values of 73% or greater VO_{2MAX}). However, it could be argued that, in addition to the physical stressors imposed in the aforementioned studies, significant emotional components also were associated with being chased, and that the combination of excitement and exercised resulted in the increased muscle pH.

Table 2. Least squares means for postmortem pH decline, glycogen degradation and lactate accumulation in the LM of RIS and RISEB lambs.

	Treatments		
Item	C	RIS	RISEB
Foresaddle pH, 0-h	6.92[c]	7.08[d]	7.02[cd]
Foresdaddle pH, 24-h	5.74[c]	6.32[d]	6.31[d]
Hindsaddle pH, 0-h	6.95[c]	7.08[c]	6.99[c]
Hindsaddle pH, 24-h	5.72[c]	6.33[d]	6.29[d]
Foresaddle glycogen[a], 0-h	37.95[c]	17.26[d]	20.99[d]
Foresaddle glycogen[a], 24-h	25.55[c]	6.85[d]	7.08[d]
Hindsaddle glycogen[a], 0-h	42.78[c]	7.12[d]	10.33[d]
Hindsaddle glycogen[a], 24-h	18.08[c]	2.16[d]	3.08[d]
Foresaddle lactate[b], 0-h	7.81[c]	5.86[d]	6.28[d]
Foresaddle lactate[b], 24-h	12.26[c]	6.33[d]	9.63[e]
Hindsaddle lactate[b], 0-h	6.01[c]	5.52[d]	4.70[d]
Hindsaddle lactate[b], 24-h	10.78[c]	5.22[d]	7.30[e]

[a]Muscle glycogen is reported in μmol/g of tissue wet weight.
[b]Muscle lactate is reported in mM/g of tissue wet weight.
[c,d,e]Means in the same row with different superscript letters differ ($P < .01$).

In Experiment 2, LM pH values from both the foresaddle and hindsaddle were higher ($P < .01$) for RIS and RISEB lambs than C lambs at every sampling time (Table 2). Furthermore, there was no difference ($P > .05$) in LM pH from either the blocked hindsaddle or unblocked foresaddle of RISEB lambs. In addition, not only was the 24-h LM pH higher ($P < .01$) in stressed lambs, but it was elevated in excess of 6.0, the threshold level associated with the DCC. At slaughter, LM glycogen concentrations (Table 2) were unmistakenly lower ($P < .01$) in samples from stressed lambs than from C lambs, regardless of sampling location. It is important to note that epidural-blockage had no appreciable effect ($P > .05$) on antemortem or postmortem glycogen metabolism in stressed lambs.

The depletion of muscle glycogen reserves before slaughter and the subsequent pH decline curves of sheep in our study are a textbook example of the formation of the DCC, and are supported by numerous research studies. We employed epidural anesthesia to prevent muscle contraction without altering catecholamine secretion. At slaughter, LM glycogen concentrations were depleted almost completely, not only in our RIS lambs, but also in our RISEB lambs. Therefore, our data support Tarrant's conclusions and suggest that antemortem glycogenolysis in sheep likely was initiated and maintained by the adrenergic mechanism, and not by the contractile mechanism, at least in the hindsaddle LM.

At slaughter, LM lactate concentrations (Table 2) were higher ($P < .01$) in both the foresaddle and hindsaddle of C lambs than either RIS or RISEB lambs. However, at 24 h postmortem, LM lactate concentrations were higher ($P < .01$) in the foresaddle of RISEB lambs than that of RIS lambs. Moreover, with the exception of the 0-h sample, LM lactate levels were higher ($P < .01$) in the hindsaddle from RISEB lambs than from RIS lambs. Although postmortem glycogen degradation and pH decline were virtually identical in the LM from RIS and RISEB lambs, postmortem lactate formation and accumulation were not the same. The exact reason for this observation is unknown; however, differences in LM lactate could be interpreted as an indication that some factor(s), in addition to lactate, is responsible for pH decline in postmortem muscle.

CONCLUSIONS. Subjection of sheep to a single 6-h bout of RIS is an effective animal model to study the DCC. In addition, the failure of either TME to activate or epidural-blockade to inhibit antemortem glycogenolysis indicates that adrenergic stimulation and not muscle contraction is the primary mechanism responsible for the development of the DCC in sheep

PERTINENT LITERATURE

Bendall, J. R. (1973). In: *Structure and Function of Muscle*, Vol. 2, ed. G. H. Bourne Academic Press, NY, p. 244.

Chrystall, B. B., Devine, C. E., Snodgrass, M. & Ellery, S. (1982). *N. Z. J. Agric. Res.* 25:331.

Forrest, J. C., Merkel, R. A. & Mackintosh, D. L. (1964). *J. Anim. Sci.* 23:551.

Johnston, B. D. & Gahan, R. J. (1988). In: *Dark-Cutting in Cattle and Sheep, Proc. of an Australian Workshop*, ed., S. U. Fabiansson, W. R. Shorthose, and R. D. Warner, Australian Meat Live-Stock Research Development Corp., Sydney South, NSW, p. 84.

McGinnis, J. P., Fletcher, D. L., Papa, C. M. & Buhr, R. J. (1989). *Poultry Sci.* 68:386.

McVeigh, J. M. (1981). *Ph.D. Dissertation*, Natl. Univ. of Ireland, Bublin 2, Ireland.

Smith, G. C., Savell, J. W., Clayton, R. P., Field, T. G., Griffin, D. B., Hale, D. S., Miller, M. F., Montgomery, T. H., Morgan, J. B., Tatum, J. D. & Wise, J. W. (1992). *Improving the Consistency and Competitiveness of Beef — A Blueprint for Total Quality Management in the Fed-Beef Industry. The Final Report of the National Beef Quality Audit — 1991.* National Cattlemen's Assoc., Englewood, CO.

Tarrant, P. V. (1981). In: *The Problem of Dark-Cutting in Beef*, ed., G. E. Hood and P. V. Tarrant, Martinus Nijhoff Publishers, London, UK, p. 3.

Tarrant, P. V. (1989). *Ir. J. Food Sci. Tech.* 13:1.

Tarrant, P. V. & McVeigh, J. M. (1979). *Res. Vet. Sci.* 27:325.