Effects of Treadmill Exercise on Stress Physiology and the Incidence of Dark-Cutting Longissimus Muscle in Holstein Steers

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Story in Brief

Holstein steers (n = 25) were used to evaluate the influence of treadmill exercise (TME) on meat quality and formation of dark-cutting beef. Calves were blocked by weight and assigned within blocks to one of five treatments of a 2 x 2 factorial design with an unexercised control (NS). Calves were exercised at either 2.5 or 5.0 mph for 10 or 15 min, then immediately harvested. Blood samples were collected via indwelling jugular catheters at 10 and 2 min pre-exercise, at initiation of exercise (0-min), and at 2.5-min intervals during exercise for quantification of serum cortisol and plasma glucose, lactate, and non-esterified fatty acids (NEFA). Upon completion of TME, calves were harvested, and longissimus muscle (LM) samples were removed from right sides at 0, 1.5, 3, 6, 12, 24, and 48 h post-exsanguination for pH determinations. After a 48-h chill at 34°F, subjective and objective color measurements of the LM were obtained, and samples of LM were used to measure bound and expressible moistures using the Carver press methodology. Serum cortisol levels increased (P < 0.01) during the movement of calves from home stanchions to the treadmill, and cortisol concentrations were higher (P < 0.01) during TME in calves exercised at 5.0 mph for 10 min compared to calves exercised at 2.5 mph, regardless of duration, and NS. Additionally, TME caused increases in plasma lactate and NEFA concentrations; however, TME did not affect (P = 0.30) postmortem pH decline, and had no (P ≥ 0.29) effect on LM color or moisture content. Even though TME elicited a noticeable stress response, this physical stressor failed to produce dark-cutting beef.

Introduction

Dark-cutting beef is a persistent quality defect characterized by an elevated muscle pH (≥ 6.0), high water-holding capacity, dry, firm, and “sticky” lean, and a dark-red to almost-black lean color, and is associated with depletion of muscle glycogen reserves prior to harvest. More importantly, the dark-cutting condition (DCC) costs the U.S. beef industry between $132 and $170 million annually (Smith et al., 1995). Glycogen degradation prior to harvest can occur during heavy exercise or in response to catecholamines in the absence of physical exertion. The aggressive activity associated with mixing unfamiliar bulls or heifers in estrus elicits pre-harvest muscle glycogen depletion and formation of dark-cutting beef (Kenny and Tarrant, 1988; Schaefer et al., 1990). Furthermore, chasing sheep to exhaustion also resulted in dark-cutting meat (Crystall et al., 1982). Because cattle are quite lethargic and normally expend little energy moving, it is reasonable to suspect that muscular activity could lead to depletion of muscle glycogen reserves and lead to the development of dark-cutting beef. Therefore, the objective of this experiment was to determine the effects of treadmill exercise on blood metabolites associated with energy metabolism and the stress response, and meat quality. The ultimate aim of this study was to evaluate treadmill exercise as a potential animal-model that could repeatedly produce dark-cutting meat.

Experimental Procedures

Twenty-five Holstein calves, weighing approximately 300 lb, were purchased from a local supplier and blocked by weight into five blocks. Within blocks, calves were assigned randomly to one of five treatments arranged in a 2 x 2 factorial design with an unexercised control, with two treadmill speeds (2.5 or 5.0 mph) and two exercise durations (10 or 15 min). Calves had ad libitum access to a high-concentrate diet and water for a minimum of 4 weeks before each replicate. Seven days before exercise treatments, each block of calves was moved from the University of Arkansas Beef Cattle Research Unit to the University of Arkansas Calf Research Facility, and subjected to a single, 10-min training/screening session where all calves were exercised at 1.25 mph for approximately 10 min. Afterwards, calves were stanchioned in individual metabolic crates, and individually fed the high-concentrate diet at a rate of 2.5% of their individual body weight. Calves had ad libitum access to water via automated bowl-waterers attached to each crate.

Calves were fitted with indwelling jugular catheters 24 h before stressor treatment to facilitate repeated blood sampling. On the morning of exercise treatment, a sample of blood was collected 10 min before moving the calf from its home stanchion to the treadmill. After a 4-min acclimation period on the treadmill, a blood sample was collected (designated –2 min) and the treadmill speed was gradually increased from 0 to the appropriate speed (2.5 or 5.0 mph) in a 2-min period of time. When the treatment speed was achieved, a sample of blood was collected (designated as the 0-min sample) and the calf was exercised for either 10 or 15 min (additional blood samples were collected at 2-min intervals until completion of exercise). Unexercised control calves were placed upon the treadmill, but were not subjected to exercise. Samples of blood were collected at 2-min intervals for the duration of each treatment, and assayed for serum cortisol (by direct RIA), and plasma glucose, lactate, and non-esterified fatty acids (NEFA; by commercially-available diagnostic kits). Upon completion of the specified exercise treatment, calves were moved approximately 100 yards to the University of Arkansas Red Meat Abattoir, and harvested according to industry-accepted procedures. Immediately following non-penetrating, captive bolt stunning and exsanguination, samples of the longissimus muscle (LM) were excised from the right side of each carcass. Subsequently, LM samples were removed at 45 min, 1.5, 3, 6, 12, and 24 h after stunning. Approximately 2 g of LM was used for pH determinations, whereas...
the remainder of the sample was frozen immediately in liquid nitrogen for determination of muscle glycogen and lactate concentrations at a later date.

After a 48-h chilling period at 34°F, left sides were ribbed between the 12th and 13th ribs, and the wholesale rib was fabricated from the forequarter. Three 1.0-in thick LM steaks were removed from each rib, and subjective and objective color measurements were collected on two steaks; whereas, the third LM steak was used to measure moisture content and water-holding capacity. Longissimus muscle color was subjectively evaluated using the Japanese color standards for pork (Nakai et al., 1975) by a three-person panel after a 30-min bloom period at 38°F. The Japanese color scoring system, consisting of six plastic disks with meat-like texture, is widely used as the standards for visual appraisal ofveal color (E. D. Mills, personal communication). Additionally, L* (a measure of darkness to lightness; a larger number indicates a lighter color), a* (a measure of redness; a larger number indicates a redder color), and b* (a measure of yellowness; a larger number indicates a more yellow color) values of LM steaks were determined from a mean of four random readings with a Hunter MiniScan XE (Hunter Associates Laboratory, Reston, VA) using illuminant C. After color measurements were collected, both 1.0-in thick steaks were paper-wrapped and stored at -20°F for determination of cooking loss percentage and Warner-Bratzler shear force (WBSF) analyses.

**Muscle pH and Water-Holding Capacity.** Approximately 1 g of excised muscle at each sampling time was homogenized with 10 mL of 5 mM sodium iodoacetate in 150 mM of potassium chloride. The pH of the homogenate was measured with a temperature-compensating, combination electrode attached to a pH/Ion/FET-meter (Denver Instrument Co., Arvada, CO). Moisture content of the LM was determined according to the freeze-drying procedure of Apple et al. (2001). Additionally, the amount of free and bound moisture was measured using the Carver press/compensating planar planimeter method of Urbin et al. (1962).

**Warner-Bratzler Shear Force Determination.** Rib steaks were thawed for 16 h at 36°F, deboned, weighed, and then cooked to an internal temperature of 174°F in a commercial convection oven (Zephaira E; Blodgett Oven Co., Burlington, VT) preheated to 365°F. Internal temperature was monitored with Teflon-coated thermocouple wires (Type T; Omega Engineering, Inc., Stamford, CT) placed in the geometric center of each steak and attached to a multichannel data logger (model 245A; VAS Engineering, Inc., San Diego, CA). Immediately after removal from the oven, steaks were blotted dry, weighed, and the difference between pre- and post-cooked weights was used to calculate cooking loss percentage. Then, steaks were chilled overnight at 38°F, and at least five 0.5-in diameter cores were removed parallel with the muscle fiber orientation, and each core was sheared once through the center with a Warner-Bratzler shear force device attached to an Instron Universal Testing Machine (model 4466; Instron Corp., Canton, MA) equipped with a 110-lb tension/compression load cell and a crosshead speed setting of 10 in/min.

**Statistical Analyses.** All data were analyzed as a randomized complete block design with calf as the experimental unit. Blood data, as well as postmortem pH decline data, were analyzed as a repeated measure using PROC MIXED (SAS Inst., Inc., Cary, NC) with sampling time as the repeated variable, calf as the subject, and stressor treatment and treatment x sampling time included in the model as fixed effects (random effects included block and block x treatment x calf). PROC MIXED was also used to generate the analysis of variance for all beef quality data. The lone fixed effect was exercise treatment, whereas block and block x treatment were the random effects included in the model. Least squares means were computed for all main and interactive effects, and separated statistically using pair-wise t-tests (PDFF option of SAS).

### Results and Discussion

**Physiological Responses.** Serum cortisol concentrations were similar (P > 0.10) among treatments before exercise (-10 and -2 min), and during the first 2 min of exercise (Fig. 1). However, after 4 min of exercise, serum cortisol levels were higher (P < 0.01) in calves exercised for 10 min at 5.0 mph than unstressed controls (treatment x time interaction; P < 0.0001). Additionally, after 6 and 8 min of exercise, calves exercised for 10 min at 5.0 mph had higher (P < 0.01) serum cortisol levels than unexercised calves and calves exercised for 10 min at 2.5 mph, and, after 10 min of exercise, calves exercised at 5.0 mph, regardless of the duration of exercise, had greater (P < 0.01) circulating cortisol levels than control calves. Thus, exercise at 5.0 mph appeared to have effectively elicited a stress response.

Even though there was no exercise treatment x time interaction (P = 0.35) for plasma glucose (Fig. 2), plasma lactate (Fig. 3) levels increased sharply during the 2-min period where treadmill speed was gradually increased to the appropriate treatment speed (treatment x time interaction; P < 0.0001). In 0-min samples, calves exercised for 10 min at 5.0 mph had higher (P < 0.01) plasma lactate concentrations than calves exercised at 2.5 mph, regardless of length of exercise, and unexercised controls. Moreover, calves exercised at a treadmill speed of 5.0 mph had higher (P < 0.01) circulating lactate levels than either unstressed controls or calves exercised at a speed of 2.5 mph after 2, 4, 6, 8, and 10 min of exercise.

Plasma NEFA levels were similar (P > 0.10) before exercise and before treatment speeds had been achieved (Fig. 4); however, after 2 and 4 min of exercise, unexercised calves had higher (P < 0.01) plasma NEFA concentrations than calves exercised for 10 min at either 2.5 or 5.0 mph (treatment x time interaction; P = 0.008). Moreover, control calves continued to have higher circulating NEFA levels than exercised calves after 6 min of exercise. Plasma NEFA levels began to rebound in exercised calves after 8 min of exercise, when NEFA levels of controls were only higher (P < 0.01) than calves exercised at 5.0 mph (regardless of exercise duration); thereafter, there was no effect (P > 0.10) of exercise on plasma NEFA concentrations.

Even though plasma glucose levels were not affected by exercise, the increase in plasma lactate would indicate increased metabolism of muscle and liver glycogen, as well as available glucose. However, similar to the results of Apple et al. (1994) with sheep, circulating lactate levels declined as duration of exercise was extended which implies that calves never reached the anaerobic threshold where muscle metabolism shifts from aerobic metabolism and beta-oxidation of fatty (supported by the plasma NEFA results) acids to anaerobic metabolism of muscle glycogen.

**pH decline and LM Quality Attributes.** Postmortem pH decline in the LM is presented in Fig. 5. In the LM, pH values were similar among treatment groups at all sample times, and mean ultimate (48-h) pH values ranged from 5.67 to 5.73 (which is within the pH range of normal meat). There was no difference (P > 0.10) in visual color scores or instrumental measures of lightness (L*), redness (a*), and yellowness (b*) among treatments. Moreover, exercise did not (P > 0.10) impact the moisture content and water-holding capacity of the LM, or WBSF values of cooked steaks.
Implications

Treadmill exercise resulted in elevations in circulating cortisol, lactate, and non-esterified fatty acids, but failed to alter postmortem metabolism or produce dark-cutting carcasses. Therefore, subjecting lightweight calves to treadmill exercise, under these experimental conditions, would not be a suitable animal-model to study the dark-cutting condition in a controlled laboratory setting.

Table 1. Effect of treadmill exercise on beef quality attributes of Holstein calves.

<table>
<thead>
<tr>
<th>Quality trait</th>
<th>Control</th>
<th>2.5 mph</th>
<th>5.0 mph</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>10 min</td>
<td>15 min</td>
</tr>
<tr>
<td>Color score&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9</td>
<td>4.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Lightness (L&lt;sup&gt;+&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.04</td>
<td>40.40</td>
<td>41.71</td>
</tr>
<tr>
<td>Redness (a&lt;sup&gt;+&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.80</td>
<td>11.78</td>
<td>11.93</td>
</tr>
<tr>
<td>Yellowness (b&lt;sup&gt;+&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.50</td>
<td>13.72</td>
<td>14.03</td>
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<tr>
<td>Moisture content, %</td>
<td>76.6</td>
<td>76.3</td>
<td>77.1</td>
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<tr>
<td>Bound water, %</td>
<td>56.7</td>
<td>57.0</td>
<td>54.3</td>
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<tr>
<td>Free water, %</td>
<td>43.3</td>
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<td>Cooking loss, %</td>
<td>29.2</td>
<td>32.3</td>
<td>33.2</td>
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<tr>
<td>Shear force, lb</td>
<td>24.2</td>
<td>24.2</td>
<td>26.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Color score: 1 = pale gray to 6 = dark purple (Nakai et al., 1975).

<sup>b</sup> L<sup>+</sup> values are a measure of lightness to darkness (lower number indicates a darker color); a<sup>+</sup> values area measure of redness (larger number indicates redder color); and b<sup>+</sup> values are a measure of yellowness (larger number indicates more yellow color).

Fig. 1. Effect of treadmill exercise speed and duration on serum cortisol concentrations (treatment x time interaction; P < 0.0001).
Fig. 2. Effect of treadmill exercise speed and duration on plasma glucose concentrations (treatment x time interaction; P = 0.35).

Fig. 3. Effect of treadmill exercise speed and duration on plasma lactate concentrations (treatment x time interaction; P < 0.0001).
Fig. 4. Effect of treadmill exercise speed and duration on plasma NEFA concentrations (treatment x time interaction; \( P = 0.008 \)).

Fig. 5. Postmortem pH decline in the longissimus muscle (LM) as affected by speed and duration of treadmill exercise (treatment x time interaction; \( P = 0.92 \)).