Effects of Restraint and Isolation Stress on Stress Physiology and the Incidence of Dark-cutting Longissimus Muscle in Holstein steers


Story in Brief

Thirty-two Holstein steers, weighing approximately 300 lb, were used to test the effects of varying durations of restraint and isolation stress (RIS) on endocrine and blood metabolite status, and the incidence of dark-cutting longissimus muscle (LM). Calves were blocked by weight, and assigned randomly within blocks to one of four treatments: unstressed controls, or 2, 4, or 6 h of RIS. Serum cortisol, as well as plasma glucose and lactate, concentrations were elevated (P < 0.01) in RIS-calves, regardless of stressor duration, compared with their unstressed counterparts. Insulin concentrations were similar among treatment groups during the first 80 min after stressor application, but, from 100 to 340 min, calves subjected to RIS had greater (P < 0.01) plasma insulin levels than unstressed calves. In the LM, 24- and 48-h pH values were in excess of 6.0, and higher (P < 0.01) in calves subjected to 6 h RIS than unstressed controls and calves subjected to 2 or 4 h RIS. Dark-cutting meat is characterized by muscle pH in excess of 6.0, a high water-holding capacity, and a dark-red to almost-black lean color. Based on these criteria, 75% of carcasses from calves exposed to 6 h of RIS were deemed to be dark-cutters; whereas, only 25% of carcasses of calves subjected to 2 or 4 h RIS, and no controls, were classified as dark-cutters. Therefore, subjecting young, lightweight Holstein steers to 6 h RIS may be an effective, reliable animal-model to study the dark-cutting condition.

Introduction

Dark-cutting meat 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. More importantly, the dark-cutting condition (DCC) costs the U.S. beef industry between $132 and $170 million annually (Smith et al., 1995). It is widely accepted that reduced muscle glycogen reserves prior to harvest is the mechanism responsible for the formation of the DCC. Reductions in muscle glycogen reserves may arise from any change in the physical or psychological well-being of an animal, including handling, transportation, feed deprivation, and co-mingling cattle. However, the inability to consistently produce dark-cutting carcasses impedes replication of experimental results, and hinders the ability to test possible management practices and/or treatments to reduce/eliminate the DCC.

Attempting to develop an animal-model to study the DCC, Apple and co-workers used sheep and tested a physical stressor (Apple et al., 1994) and a psychological stressor (Apple et al., 1993). After some modifications, Apple et al. (1995) demonstrated that exposing sheep to a single 6-h bout of restraint and isolation stress effectively reduced antemortem glycogen reserves, and produced 100% dark-cutting carcasses. However, questions were raised concerning the applicability of this animal-model to cattle; therefore, the primary objective of this study was to develop a repeatable, reliable animal-model using lightweight Holstein steers.

Experimental Procedures

Thirty-two Holstein steers, weighing approximately 300 lb, were purchased from a local supplier and blocked by weight into four blocks. Within blocks, calves were assigned randomly to one of four treatments: 1) unstressed controls (Ctrl); 2) subjected to a single 2-h bout of restraint and isolation stress (RIS); 3) subjected to a single 4-h bout of RIS; or 4) subjected to a single 6-h bout of RIS. Calves had ad libitum access to a high-concentrate diet and water for a minimum of four weeks before each replicate. Seven days before stressor treatment, each block of calves was moved from the University of Arkansas Beef Cattle Research Unit to the University of Arkansas Calf Research Facility, individually stanchioned in 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 stressor treatment, samples of blood were collected at 40, 20, and 0 min before stressor application. After the 0-min blood sample, RIS-calves were moved from their home stanchions to another area where they were isolated from visual and tactile contact with other calves. Restraint was achieved by placing calves on their right sides, then binding both forelimbs and both hindlimbs together with non-adhesive tape. Finally, both sets of limbs were bound together with elastic tape. To minimize physical discomfort during stressor treatment, restrained calves were placed on 4 to 5 in of carpet padding. Control calves remained in their home stanchions, and, with the exception of blood sampling, were subjected to minimal handling and stress. Samples of blood were collected at 20-min intervals for the duration of each treatment, and assayed for serum cortisol and plasma glucose, lactate, and insulin.

Upon completion of the specified duration of RIS, calves were transported approximately 300 yd 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 0.75, 1.5, 3, 6, 12, and 24 h after stunning. Approximately 2 g of LM were used for pH determinations, while the

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removal 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 six-point, Japanese color standards for pork (Nakai et al., 1975) by a three-person panel after a 30-min bloom period at 38°F. Objective color of LM chops steaks was measured with a Hunter Lab MiniScan XE. Simultaneously, L*, a*, and b* values were determined from a mean of four random readings on each steak using illuminant C and a 10° standard observer. After color measurements were collected, both 1.0-in thick steaks were paper-wrapped and stored at -20°F for further analyses.

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 (Bendall, 1973). 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). In addition, moisture content of the LM was determined according to the freeze-drying procedure of Apple et al. (2001), and LM water-holding capacity was measured using the Carver press/compressing planar planimeter method of Urbin et al. (1962).

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 stressor 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

Serum cortisol concentrations were elevated (P < 0.01) in RIS-calves, regardless of stressor duration, compared with their unstressed counterparts (Figure 1). Typically, an animal responds to a stressor by releasing ACTH from the anterior pituitary gland, which propagates the release of glucocorticoids (cortisol and corticosterone) from the adrenal cortex. The glucocorticoids serve to adapt the body to stressors by affecting cardiovascular, energy-producing, and immune systems. Therefore, the treatment imposed on the calves effectively elicited a stress response.

Plasma glucose (Figure 2) and lactate (Figure 3) levels were higher (P < 0.01) in calves subjected to RIS than in unstressed controls. Plasma glucose levels began to rise after 40 to 60 min after stressor application, but it wasn’t until after 80 min of RIS that circulating concentrations were different between stressed-calves and controls (Figure 2). On the other hand, plasma lactate levels began to rise immediately after stressor application, and after 40 min of RIS, calves subjected to 2 h of RIS had higher plasma lactate levels than calves subjected to either 4 or 6 h of RIS (Figure 3). Insulin concentrations were similar among treatment groups during the first 80 min after stressor application, but, from 100 to 340 min, calves subjected to RIS had greater (P < 0.01) plasma insulin levels than unstressed calves (Figure 4).

The hyperglycemia observed in this study is a typical response observed during stress, and is induced by increased adrenal secretion of epinephrine and cortisol. These hormones accelerate glucose production by stimulating glycogenolysis and gluconeogenesis, as well as reducing glucose clearance from circulation; therefore, the net result is elevated plasma glucose concentrations. In the present study, concomitant increases in both glucose and insulin by RIS was expected; however, plasma glucose levels did not decrease in response to the elevated levels of plasma insulin – a classical example of insulin resistance.

Postmortem pH decline in the LM is presented in Figure 5. In the LM, pH values were similar among treatment groups during the first 12 h after death; however, pH values were greater (P < 0.01) in the LM from calves subjected to 6 h RIS at 24 and 48 h postmortem than unstressed controls and calves subjected to 2 or 4 h RIS (Figure 5). More importantly, ultimate (24- and 48-h) pH values for the LM of calves stressed for 6 h were in excess of 6.0, the threshold level associated with the DCC. Normal postmortem muscle pH declines in a curvilinear fashion from its initial value of approximate 7.2, until it reaches a normal ultimate pH value between 5.5 and 5.7. The decline in pH is a response to the increased accumulation of lactic acid in the muscle as a result of anaerobic metabolism of muscle glycogen. Therefore, the high ultimate pH value for the LM of calves subjected to 6 h of RIS would indicate that muscle glycogen reserves were almost completely depleted at the conclusion of the stressor treatment. The LM from RIS-calves received darker (P < 0.01) color scores and lower L* (P < 0.01) values than the LM from unstressed calves (Table 1). Finally, even though LM moisture content was similar (P > 0.10) among the treatment groups, the LM from calves subjected to 6 h of RIS had a greater (P < 0.01) percentage of free, water than the other treatment groups. Dark-cutting meat is characterized by an elevated muscle pH in excess of 6.0, a high water-holding capacity, a dry, firm, and “sticky” lean, and a dark-red to almost-black lean color. Based on these criteria, 75% (6 of 8) of the carcasses from calves exposed to 6 h of RIS were deemed to be dark-cutters; only 25% of the carcasses of calves subjected to 2 or 4 h RIS were classified as dark-cutters; and no (0 of 8) controls were remotely close to being dark-cutters. Therefore, subjecting young, lightweight Holstein calves to 6 h of RIS may be an effective animal-model to study the DCC condition.

Implications

Subjecting calves to restraint and isolation stress effectively elicited dramatic elevations in cortisol and circulating energy reserves, and curtailed normal postmortem pH decline. Results from this study suggest that exposing calves to a single six-hour bout of restraint and isolation stress is an effective, reliable animal-model to study the dark-cutting condition in a controlled laboratory setting.

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**Literature Cited**


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**Fig. 1.** Effect of duration of restraint and isolation stress (RIS) on serum cortisol concentrations (treatment x time interaction; P < 0.0001).

**Fig. 2.** Effect of duration of restraint and isolation stress (RIS) on plasma glucose concentrations (treatment x time interaction; P < 0.0001).
Fig. 3. Effect of duration of restraint and isolation stress (RIS) on plasma lactate concentrations (treatment x time interaction; P < 0.0001).

Fig. 4. Effect of duration of restraint and isolation stress (RIS) on plasma insulin concentrations (treatment x time interaction; P < 0.0001).

Fig. 5. Postmortem pH decline in the longissimus muscle as affected by duration of restraint and isolation stress (RIS; treatment x time interaction; P < 0.0005).
Table 1. Effect of restraint and isolation stress on beef quality attributes of Holstein calves

<table>
<thead>
<tr>
<th>Quality trait</th>
<th>Control</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color score</td>
<td>3.9a</td>
<td>4.8d</td>
<td>4.9d</td>
<td>5.6c</td>
<td>0.29</td>
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<tr>
<td>CIE L&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.28c</td>
<td>40.59d</td>
<td>40.85d</td>
<td>38.28d</td>
<td>1.201</td>
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<tr>
<td>CIE a&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.40</td>
<td>10.19</td>
<td>9.69</td>
<td>10.09</td>
<td>0.392</td>
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<tr>
<td>CIE b&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.09x</td>
<td>12.46vy</td>
<td>12.08y</td>
<td>11.92y</td>
<td>0.692</td>
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<tr>
<td>Moisture content, %</td>
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<td>77.2</td>
<td>78.2</td>
<td>77.3</td>
<td>0.46</td>
</tr>
<tr>
<td>Bound water, %</td>
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<td>63.9d</td>
<td>64.4d</td>
<td>72.5c</td>
<td>2.52</td>
</tr>
<tr>
<td>Free water, %</td>
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<td>36.1c</td>
<td>35.6c</td>
<td>27.5d</td>
<td>2.52</td>
</tr>
</tbody>
</table>

*a Color score: 1 = pale gray to 6 = dark purple (Nakai et al., 1975).
*b L* values are a measure of lightness to darkness (lower number indicates a darker color); a* values are a measure of redness (larger number indicates redder color); and b* values are a measure of yellowness (larger number indicates more yellow color).
*c,d,e Within a row, least squares means lacking a common superscript letter differ (P < 0.01).
*x,y Within a row, least squares means lacking a common superscript letter differ (P < 0.06).