Effects of ractopamine and sex on serum metabolites and skeletal muscle gene expression in finishing steers and heifers

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ABSTRACT: We evaluated growth-related responses to ractopamine in steers and heifers. Sixteen Angus steers (512 kg) and 16 Angus heifers (473 kg) housed in individual pens were used in a complete block design. At 90 to 97 d before the experiment, steers were implanted with 120 mg of trenbolone acetate and 24 mg of estradiol-17β (Component TE-S) and heifers were implanted with 140 mg of trenbolone acetate and 14 mg of estradiol-17β (Component TE-H). Treatments were arranged as a 2 × 2 factorial and included sex (steer vs. heifer) and ractopamine-HCl (0 or 200 mg/d). Cattle were fed a diet based on steam-flaked corn once daily. Blood and LM and biceps femoris (BF) biopsy samples were collected on d 0 (before ractopamine feeding) and after 14 and 28 d of ractopamine feeding. Serum insulin concentrations were not affected by ractopamine or sex. Serum IGF-I concentrations were greater in steers than heifers (P < 0.001), and steers demonstrated greater IGF-I mRNA expression in BF than heifers (P = 0.05). Ractopamine decreased serum IGF-I concentrations in heifers on d 14, but increased serum IGF-I concentrations in steers on d 28 (sex × ractopamine × day interaction; P = 0.03). Ractopamine did not affect (P ≥ 0.19) mRNA expression of IGF-I, IGFBP-3, or calpastatin in BF or LM. However, ractopamine led to increases in LM expression of IGFBP-5 in heifers, but to decreases in expression in steers (ractopamine × sex interaction; P = 0.04). Ractopamine decreased myosin heavy chain IIA mRNA expression in BF (P = 0.04) but not in LM (P = 0.99). Ractopamine decreased β2-receptor mRNA expression in LM of steers on d 14, but not on d 28; in contrast, expression of β2-receptor mRNA in LM of heifers was not affected by ractopamine (sex × ractopamine × day interaction; P = 0.03). Although there were a few criteria for which ractopamine led to differences in response between steers and heifers, there were no striking disparities to suggest that the effectiveness of ractopamine would markedly differ between sexes.

Key words: cattle, gene expression, muscle, ractopamine, sex

INTRODUCTION

Ractopamine-HCl is a β-adrenergic agonist that is approved by the Food and Drug Administration for use in finishing cattle. Ractopamine administration elicits responses through β-adrenergic receptors (β1 and β2), with predominant affinity for β1 adrenergic receptors (Moody et al., 2000) and results in increased muscle mass with minimal effects on adipose tissue (Mersmann, 1998). Ractopamine-HCl fed at 200 mg/d for the final 28 d can improve ADG and G:F by 17 and 18% (Laudert et al., 2004) and by 15 and 17% (Gruber et al., 2007) in finishing steers and by 18 and 15% (Schroeder et al., 2005a) and 11 and 12% (Laudert et al., 2007) in finishing heifers. In contrast, Quinn et al. (2008) reported no change in carcass gain, DMI, or carcass efficiency when nonimplanted heifers were fed 200 mg/d of ractopamine-HCl for the final 28 d. Over the entire 178- to 187-d feeding period, Sissom et al. (2007) demonstrated 2.2 and 4.0% improvements in ADG and G:F with no effect on DMI in finishing heifers fed 200 mg/d of ractopamine-HCl for the final 28 d. Hot carcass weight has been increased by 8 (Winterholler et al., 2007), 6 (Laudert et al., 2004), and 5.5 kg (Gruber et al., 2007) in feedlot steers fed ractopamine. In feedlot heifers, HCW was increased by 4.6 (Laudert et al., 2007) and 2.9 kg (Schroeder et al., 2005a) in response to ractopamine.

Although improvements in growth and carcass performance are observed in feedlot steers and heifers, the responses in heifers seem more variable, suggesting that ractopamine may be acting somewhat differently in heif-
ers than in steers and that heifers are more variable as a research model compared with steers. Our objectives were to determine the effects of feeding ractopamine to finishing steers and heifers on skeletal muscle gene expression and to determine if responses differed between steers and heifers.

**MATERIALS AND METHODS**

Procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee.

**Animals**

Sixteen Angus steers and 16 Angus heifers were used in a randomized complete block design experiment to evaluate the responses of steers and heifers to ractopamine feeding. Cattle originated from 2 different sires and were fed similarly for 1 mo before arrival. Cattle were received 100 to 107 d before initiation of the experiment, grouped by sex in 2 pens, and transitioned from a 60% concentrate:40% roughage diet to a 90% concentrate:10% roughage diet within the first 30 d. Thereafter, all cattle were fed a common diet based on steam-flaked corn for ad libitum consumption for 70 to 77 d before initiation of the trial. Ten days after arrival, corresponding to 90 to 97 d before cattle starting on the trial, steers were implanted with 120 mg of trenbolone acetate (TBA) and 24 mg of estradiol (E2; Component TE-S, Vet Life, West Des Moines, IA) and heifers were implanted with 140 mg of TBA and 14 mg of E2 (Component TE-H, Vet Life). Steers and heifers averaged 15.6 and 15.3 mo of age, respectively, when started on the trial. As a group, the cattle graded 68% USDA Choice, 45% USDA Yield grade 2, and 50% USDA Yield grade 3 after the experiment.

**Overall Design**

Treatments included sex (steer or heifer) and ractopamine-HCl (0 or 200 mg/d; Optaflexx; Elanco Animal Health, Greenfield, IN). Steers and heifers were blocked (8 blocks with each containing 2 steers and 2 heifers) based on BW and ADG during the 90 d before the start of the experiment. To accomplish blocking, cattle were sorted into the heaviest 8 and the lightest 8 animals within each sex. Within sex and BW group, cattle were sorted into groups of 2 animals based on ADG. Within each block of 4 cattle (2 steers and 2 heifers), sex was predetermined and the ractopamine treatment was randomized between the 2 cattle of the same sex with a flip of a coin. All cattle were fed and housed individually in 4.5- × 1.5-m pens for 7 to 8 d before initiation of the experiment and were given ad libitum access to water and feed (Table 1) for 28 d, which represents both the shortest feeding time for which ractopamine usage is labeled as well as the most typical feeding period for ractopamine by the feedlot industry. Cattle were fed once daily at 1600 h with bunk management designed to yield slick bunks at feeding.

Due to logistics of the procedures imposed, complete blocks of steers and heifers were started on trial staggered over time. Trial initiation for blocks 1 and 2 occurred 1 d before that for blocks 3 and 4, 7 d before that for blocks 5 and 6, and 8 d before that for blocks 7 and 8. Beginning at 0630 h on each sampling day, a portable chute was placed in front of each pen and cattle were placed into the chute. Blood samples were collected, and biopsy samples were collected from the biceps femoris (BF) and LM. Sample collection occurred on d 0 before initiation of ractopamine feeding, and on d 14 and 28 of ractopamine feeding. After sample collection on d 0 (before initiation of ractopamine feeding) and on d 28 of ractopamine feeding for each block, cattle were weighed at 1100 h. For 2 blocks, cattle assigned to the ractopamine treatment mistakenly received ractopamine on the day before the initial sampling; their d-0 data were not used for statistical analysis (see below), and samples collected after 15 and 29 d of ractopamine feeding were considered equivalent to those collected after 14 and 28 d of ractopamine feeding.

**Blood Samples**

Jugular blood samples (10 mL) were collected into vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, immediately placed on ice, and centrifuged for 20 min at 1,000 × g at 4°C to obtain plasma. Blood (10 mL) was also collected into vacuum tubes without additives, allowed to clot for 24 h at 4°C, and then centrifuged for 20 min at 1,000 × g at 4°C to obtain serum. Plasma samples were stored (−20°C) for later analysis of glucose (Gochman and Schmitz, 1972) and urea (Marsh et al., 1965). Sera were stored (−20°C)

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**Table 1. Composition of the diet fed to finishing steers and heifers**

<table>
<thead>
<tr>
<th>Item</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td></td>
</tr>
<tr>
<td>Steam-flaked corn</td>
<td>75.31</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>10.17</td>
</tr>
<tr>
<td>Steep liquor</td>
<td>5.77</td>
</tr>
<tr>
<td>Solvent soybean meal</td>
<td>4.02</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.37</td>
</tr>
<tr>
<td>Urea</td>
<td>0.57</td>
</tr>
<tr>
<td>Salt</td>
<td>0.30</td>
</tr>
<tr>
<td>Vitamin/mineral premix1</td>
<td>0.07</td>
</tr>
<tr>
<td>Monensin/tylosin premix2</td>
<td>2.22</td>
</tr>
<tr>
<td>Analyzed composition</td>
<td></td>
</tr>
<tr>
<td>OM</td>
<td>95.3</td>
</tr>
<tr>
<td>CP</td>
<td>14.5</td>
</tr>
</tbody>
</table>

1To provide per kilogram of diet DM: 60 mg of Mn, 60 mg of Zn, 10 mg of Cu, 0.6 mg of I, 0.25 mg of Se, 0.1 mg of Co, and 2,650 IU of vitamin A.

2Mixed with ground corn to provide per kilogram of diet DM: 30 mg of monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) and 9 mg of tylosin (Tylan 40, Elanco Animal Health). By analysis, provided per kilogram of diet DM: 36 mg of monensin and 9.1 mg of tylosin.
Muscle Biopsies

Biopsy samples were collected using a Bergstrom biopsy needle from the BF and LM (Dunn et al., 2003; Pampusch et al., 2003) for measuring gene expression. Biopsies on d 0 and 28 were collected from the left side and on d 14 from the right side. On each sampling day, each animal was biopsied one time in each muscle, and each insertion of the biopsy needle yielded approximately 2 g of tissue. The LM sampling sites initially were at the last rib and moved 5 cm anterior for the final sampling. The BF sampling sites initially were midway between the trochanter major of the femur and the tuber ischii and moved 5 cm ventral for the final sampling.

RNA Isolation

Muscle biopsy samples (2 g) were rapidly frozen in liquid N2 and stored at -80°C for subsequent RNA isolation. Total RNA was isolated from BF and LM samples as described by Dunn et al. (2003) and Pampusch et al. (2003). The concentration of RNA was determined by absorbance at 260 nm. To verify the integrity of the RNA, visualization of the 28S and 18S rRNA was accomplished with a 2100 Bioanalyzer (Agilent, Foster City, CA). One microgram of total RNA was reverse-transcribed to produce the first-strand cDNA using TaqMan reverse transcriptase (Applied Biosystems, Foster City, CA) following the protocol recommended by the manufacturer. Random hexamers were used as primers in cDNA synthesis.

Real-Time PCR

Real-time quantitative-PCR was used to measure the quantity of mRNA for β1-, β2-, and β3-adrenergic receptors, IGF-I (Class 1), IGFBP-3 and IGFBP-5, calpastatin, myosin heavy chain (MHC) IIA, and 18S rRNA in total RNA isolated from BF and LM. Measurement of the relative quantity of cDNA was conducted using TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM of the appropriate forward and reverse primers, 200 nM of the appropriate TaqMan detection probe, and 1 µL of the cDNA mixture. Sequences for primers and probes are presented in Table 2. The primers for IGF-I spanned exon 1 and exon 3 and were designed to measure class 1 IGF-I mRNA. Commercially available eukaryotic 18S rRNA primers and probes were used as an endogenous control (Applied Biosystems; GenBank Accession No. X03205). Assays were performed in an ABI Prism 7000 sequence detection system (Applied Biosystems) using thermal cycling parameters recommended by the manufacturer (40 cycles of 15 s at 95°C and 1 min at 60°C). Relative expressions of mRNA for β1-, β2-, and β3-adrenergic receptors, IGF-I (Class 1), IGFBP-3 and IGFBP-5, calpastatin, and MHC IIA were corrected for PCR efficiency and normalized to the 18S rRNA endogenous control using the equation: 

\[ \frac{(1 + \text{efficiency for gene of interest}) - \text{Ct for gene of interest}}{2^{\text{Ct for 18S rRNA}}} \]

where Ct = cycles to threshold and efficiencies for β1-, β2-, and β3-adrenergic receptors, IGF-I (Class 1), IGFBP-3 and IGFBP-5, calpastatin; and MHC IIA primer sets were 1.08, 0.88, 0.97, 0.90, 0.71, 0.87, 0.80, and 0.91, respectively. One sample from d 0 (LM from steer receiving no ractopamine) was misplaced, and therefore, the data were missing.

Statistical Analyses

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Performance data were analyzed as a randomized complete block design. The model contained the effects sex, ractopamine, and ractopamine × sex. Block was included as a random effect. All other data were analyzed as a randomized complete block design with repeated measures with d-0 values used as a covariate. The model contained the effects of the covariate, sex, ractopamine, ractopamine × sex, day, ractopamine × day, sex × day, and ractopamine × sex × day. Block was included as a random effect. The repeated statement included day as the repeated variable with animal as experimental unit, and the covariance structure was unstructured. To estimate missing values from d 0, values were predicted using the same model but without the covariate. Because the covariates (d-0 values) would remove most of the effects of sex, which was fixed before the d-0 measures, data were analyzed for the main effect of sex, using the model described above, without the use of covariates as well as without inclusion of predicted d-0 values. Treatment means were computed using the LSMEANS option, and pair-wise t-tests were used to separate means when interactions were significant.

RESULTS AND DISCUSSION

Effects of Sex and Ractopamine on Performance

Performance data for steers and heifers are presented in Table 3. Heifers fed ractopamine consumed 16% less DM than control heifers, but in steers there were no differences in DMI in response to ractopamine (sex × ractopamine interaction; P = 0.05). Dry matter intake in finishing heifers generally is not affected by 200 mg/d of ractopamine (Schroeder et al., 2005a; Walker et al., 2006; Sissom et al., 2007; Quinn et al., 2008). Gruber et al. (2007) observed no change in DMI in feedlot steers administered ractopamine. Heifers assigned to the rac-
topamine treatment consumed less feed relative to the other cattle for the week before the study (7.7, 6.3, 6.7, and 7.2 kg for control heifers, heifers fed ractopamine, control steers, and steers fed ractopamine), indicating that the decreased DMI for ractopamine-fed heifers probably does not reflect a response to ractopamine. The BW of heifers on d 0 and 28 were less than those of steers ($P < 0.001$). The final BW (d 28) for steers and heifers were not affected by ractopamine feeding ($P = 0.99$). Daily BW gains and G:F were not affected by ractopamine or sex, although ractopamine numerically increased ADG (16%) and G:F (21%) in steers. Our ability to detect performance responses to ractopamine was limited by the unexpectedly decreased DMI of heifers fed ractopamine. Efficiencies of gain above maintenance were calculated (Table 3) because they are

<table>
<thead>
<tr>
<th>Primer and probe</th>
<th>Sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I (Accession # X15726)</td>
<td>TGTGATTTCTTGAAGCAGGTGAA</td>
</tr>
<tr>
<td>Forward</td>
<td>AGCACAGGGCCAGATAGAAGAG</td>
</tr>
<tr>
<td>Reverse</td>
<td>6FAM-GCCCATCACATCCTCTCGCA-TAMRA</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>CATTCCCAACTGCAGCAAGAAG</td>
</tr>
<tr>
<td>IGFBP-3 (Accession # M76478)</td>
<td>GCAAGAACCAGGCTTCTCT</td>
</tr>
<tr>
<td>Forward</td>
<td>6FAM-AGAAAAACGATGCTGCCCTTCAA-TAMRA</td>
</tr>
<tr>
<td>Reverse</td>
<td>TCCTCCGCGCCAAACACA</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>TCTTTCGCGGTCTTCTTCAC</td>
</tr>
<tr>
<td>IGFBP-5 (Accession # M62782)</td>
<td>6FAM-CGCACTCTCAGCTGAAGGCTGAA-TAMRA</td>
</tr>
<tr>
<td>Forward</td>
<td>CAGCTCCAGAGATCAGACAAATC</td>
</tr>
<tr>
<td>Reverse</td>
<td>CTGCTTACATTGACGTTGATAG</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>6FAM-AGGGCGGCTTCCATGCCC-TAMRA</td>
</tr>
<tr>
<td>β1-Adrenergic receptor (Accession # AF188187)</td>
<td>GTGGGACCGCTGGGAGATGAT</td>
</tr>
<tr>
<td>Forward</td>
<td>TGCACACAGGTCTCAATGCG</td>
</tr>
<tr>
<td>Reverse</td>
<td>6FAM-CTCCTTCGCGGTCTGAGGACCTC-TAMRA</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>CAGCTCCAGAGATCAGACAAATC</td>
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<tr>
<td>IGFBP-3 (Accession # NM_174231)</td>
<td>CTGCTTACATTGACGTTGATAG</td>
</tr>
<tr>
<td>Forward</td>
<td>6FAM-AGGGCGGCTTCCATGCCC-TAMRA</td>
</tr>
<tr>
<td>Reverse</td>
<td>CAGCTCCAGAGATCAGACAAATC</td>
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<tr>
<td>TaqMan probe</td>
<td>6FAM-AGGGCGGCTTCCATGCCC-TAMRA</td>
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<td>β2-Adrenergic receptor (Accession # X85961)</td>
<td>AGGCAACCTGCTGGTATACG</td>
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<td>GCCCTGAGAAGGCTGTTGATAG</td>
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<td>Reverse</td>
<td>6FAM-CCCGGAGCCCAGACTCAG-TAMRA</td>
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<tr>
<td>TaqMan probe</td>
<td>CAGCTCCAGAGATCAGACAAATC</td>
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<tr>
<td>Calpastatin (Accession # X67333)</td>
<td>CCCCTGATCAACTTTGCTGACG</td>
</tr>
<tr>
<td>Forward</td>
<td>TGACCTTTATCCGCTGCTGCTGCTG</td>
</tr>
<tr>
<td>Reverse</td>
<td>6FAM-TCCGGGCAAGACAGGCTGATC-TAMRA</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>CCCCTGAGAAGGCTGTTGATAG</td>
</tr>
<tr>
<td>Myosin heavy chain IIA (Accession # AB059398)</td>
<td>TCTTCCGCTGTAGCTGACG</td>
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<tr>
<td>Forward</td>
<td>CCCCGCCCCACATCTT</td>
</tr>
<tr>
<td>Reverse</td>
<td>6FAM-TCTCTGACAGGCTTACGTT</td>
</tr>
<tr>
<td>TaqMan probe</td>
<td>CCCCGCCCCACATCTT</td>
</tr>
</tbody>
</table>

Table 3. Effect of ractopamine (RAC) on growth characteristics of finishing steers and heifers

<table>
<thead>
<tr>
<th>Item</th>
<th>No ractopamine</th>
<th>Ractopamine, 200 mg/d</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heifers</td>
<td>Steers</td>
<td>Heifers</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>476</td>
<td>509</td>
<td>471</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>516</td>
<td>548</td>
<td>503</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>8.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>1.42</td>
<td>1.41</td>
<td>1.17</td>
</tr>
<tr>
<td>G:F</td>
<td>0.169</td>
<td>0.159</td>
<td>0.164</td>
</tr>
<tr>
<td>G:F above maintenance&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.302</td>
<td>0.287</td>
<td>0.337</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means in rows not bearing a common superscript differ, $P < 0.05$.
<sup>1</sup>Calculated with feed (2.14 Mcal of NE<sub>m</sub>/kg) necessary to meet NE<sub>m</sub> requirements subtracted from DMI; NE<sub>m</sub> requirements = 0.077 Mcal × (BW × 0.96)<sup>0.75</sup>.
independent of DMI; ractopamine tended ($P = 0.07$) to increase G:F above maintenance by 20%, which is similar to improvements in G:F observed in other trials. Schroeder et al. (2005b) demonstrated 20 and 21% increases in ADG and G:F in feedlot steers fed 20 mg/kg of ractopamine for the final 28 to 42 d. Gruber et al. (2007) reported 15 and 17% improvements in ADG and G:F in implanted finishing steers when fed 200 mg/d of ractopamine for the final 28 d. Schroeder et al. (2005a) observed increases of 18 and 15% in ADG and G:F in finishing heifers fed 20 mg/kg of ractopamine for the final 28 to 42 d. Laudert et al. (2007) demonstrated 11 and 14% increases in ADG and G:F in implanted finishing heifers fed 200 mg/d of ractopamine for the final 28 d. Implanted finishing heifers in response to feeding 200 mg/d of ractopamine for the final 28 d demonstrated 18% increases in ADG and G:F (Walker et al., 2006).

**Effect of Sex**

Data for blood metabolites are presented in Table 4. Steers tended to have greater plasma glucose concentrations than heifers ($P = 0.08$). Plasma urea concentrations in steers were less ($P = 0.003$) than those in heifers. Serum insulin concentrations were not affected ($P = 0.32$) by sex. Serum IGF-I concentrations were greater ($P < 0.001$) in steers than heifers. Plouzek and Trenkle (1991) using nonimplanted cattle demonstrated 20 and 43% greater serum IGF-I concentrations in steers than in heifers at 12 and 15 mo of age. Because steers deposit more lean tissue than heifers at the end of the finishing period, decreased plasma urea and greater serum IGF-I concentrations might be expected in steers compared with heifers.

**Effects of Ractopamine and Interaction of Ractopamine with Sex**

Data for blood metabolites are presented in Table 5. Plasma glucose concentrations were not affected by ractopamine ($P = 0.34$). Ractopamine tended to decrease

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### Table 4. Effect of sex\(^1\) on blood metabolites and gene expression

<table>
<thead>
<tr>
<th>Item</th>
<th>Heifers</th>
<th>Steers</th>
<th>SEM(^2)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>488</td>
<td>695</td>
<td>29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.02</td>
<td>1.32</td>
<td>0.21</td>
<td>0.32</td>
</tr>
<tr>
<td>Plasma, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea-N</td>
<td>5.26</td>
<td>4.59</td>
<td>0.16</td>
<td>0.003</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.5</td>
<td>6.5</td>
<td>0.39</td>
<td>0.08</td>
</tr>
<tr>
<td>Gene expression, arbitrary units, $10^{-6}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biceps femoris</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IGF-I</td>
<td>4.0</td>
<td>5.3</td>
<td>0.44</td>
<td>0.05</td>
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<tr>
<td>IGFBP-3</td>
<td>172</td>
<td>193</td>
<td>22</td>
<td>0.32</td>
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<tr>
<td>IGFBP-5</td>
<td>702</td>
<td>846</td>
<td>89</td>
<td>0.26</td>
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<tr>
<td>Calpastatin</td>
<td>201</td>
<td>209</td>
<td>16</td>
<td>0.59</td>
</tr>
<tr>
<td>Myosin heavy chain IIA</td>
<td>1,355</td>
<td>1,455</td>
<td>149</td>
<td>0.56</td>
</tr>
<tr>
<td>$\beta_1$-Adrenergic receptor</td>
<td>0.71</td>
<td>0.98</td>
<td>0.21</td>
<td>0.37</td>
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<tr>
<td>$\beta_2$-Adrenergic receptor</td>
<td>113</td>
<td>128</td>
<td>16</td>
<td>0.38</td>
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<tr>
<td>$\beta_3$-Adrenergic receptor</td>
<td>3.4</td>
<td>5.5</td>
<td>0.94</td>
<td>0.08</td>
</tr>
<tr>
<td>LM</td>
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</tr>
<tr>
<td>IGF-I</td>
<td>4.2</td>
<td>3.8</td>
<td>0.40</td>
<td>0.33</td>
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<tr>
<td>IGFBP-3</td>
<td>169</td>
<td>217</td>
<td>25</td>
<td>0.10</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>640</td>
<td>801</td>
<td>83</td>
<td>0.17</td>
</tr>
<tr>
<td>Calpastatin</td>
<td>179</td>
<td>207</td>
<td>16</td>
<td>0.22</td>
</tr>
<tr>
<td>Myosin heavy chain IIA</td>
<td>1,694</td>
<td>1,594</td>
<td>130</td>
<td>0.59</td>
</tr>
<tr>
<td>$\beta_1$-Adrenergic receptor</td>
<td>0.56</td>
<td>0.54</td>
<td>0.15</td>
<td>0.92</td>
</tr>
<tr>
<td>$\beta_2$-Adrenergic receptor</td>
<td>86</td>
<td>108</td>
<td>15</td>
<td>0.11</td>
</tr>
<tr>
<td>$\beta_3$-Adrenergic receptor</td>
<td>3.1</td>
<td>3.4</td>
<td>1.0</td>
<td>0.79</td>
</tr>
</tbody>
</table>

\(^1\)Averages from d 0, 14, and 28 for cattle receiving 0 or 200 mg/d of ractopamine.

\(^2\)Largest SEM among treatments.
plasma urea concentrations (4.68 vs. 5.03 mM; \( P = 0.06 \)). Growing Holstein steers demonstrated no change in plasma urea concentrations in response to ractopamine (Walker et al., 2007). In contrast, Dunshea and King (1994) reported a 5% decrease in plasma urea-N in gilts fed 20 mg/kg of ractopamine for 22 d. Ractopamine increases lean tissue deposition and increases N retention (Walker et al., 2007) and, therefore, would be expected to decrease urea production. Serum insulin concentrations were not affected by ractopamine. Similarly, growing Holstein steers demonstrated no change in insulin concentrations when fed ractopamine for 28 d (Walker et al., 2007).

In response to ractopamine feeding, serum IGF-I concentrations (Table 5) were increased by ractopamine feeding in steers on d 28 (sex \( \times \) ractopamine \( \times \) day interaction; \( P = 0.03 \)). The serum IGF-I response in steers fed ractopamine might allow an extended growth curve enabling them to deposit more lean tissue compared with heifers. Heifers fed ractopamine had less DMI, and it is plausible that the reduced DMI, independent of ractopamine, resulted in decreases in circulating IGF-I concentrations. Ellenberger et al. (1989) demonstrated decreases in serum IGF-I concentrations when steers were feed restricted. The majority of IGF-I in circulation is derived from hepatic production and, therefore, changes in serum IGF-I concentrations in steers and heifers fed ractopamine could be attributed to ractopamine affecting hepatic IGF-I synthesis or release, or ractopamine might alter the IGFBP such that IGF-I clearance from the blood is affected. Beermann et al. (1987) reported 46.5 and 21.5% decreases in serum IGF-I concentrations in lambs fed cimaterol for 6 and 12 wk compared with lambs not fed cimaterol. Serum IGF-I concentrations tended to decrease by 13% in Holstein steers in response to feeding 200 mg/d of ractopamine for 28 d (Walker et al., 2007). In contrast, Winterholler et al. (2008) demonstrated no change in circulating concentrations of IGF-I in yearling steers fed 200 mg/d of ractopamine for 28 d.

Data for mRNA expression of IGF-I, IGFBP-3 and IGFBP-5, MHC IIA, calpastatin, and \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \) receptors in BF and LM are presented in Table 5. Ractopamine did not significantly alter mRNA expression of IGF-I in BF (\( P = 0.21 \)) or in LM (\( P = 0.18 \)). In LM, mRNA expression of IGF-I decreased in growing Holstein steers fed 200 mg/d of ractopamine for 14 or 28 d (Walker et al., 2007). Sissom et al. (2007) demonstrated a reduction of IGF-I mRNA expression in response to ractopamine in semimembranosus of heifers initially implanted with 80 mg of TBA/8 mg of E\(_2\) and reimplanted with 200 mg of TBA 96 d before feeding 200 mg/d of ractopamine for the final 28 d. However, in the same study, heifers implanted with 200 mg of TBA/20 mg of E\(_2\) 154 d before ractopamine feeding demonstrated a numerical increase in IGF-I mRNA expression in the semimembranosus, suggesting that the intensity of the implant program may affect the responsiveness of heifers to ractopamine. In contrast, Winterholler et al. (2008) reported no change in IGF-I mRNA expression in the LM of yearling steers fed 200 mg/d of ractopamine for 28 d. Yimlamai et al. (2005) reported that clenbuterol fed to rats for 2 wk decreased protein expression of IGF-I in the plantaris muscle (fast twitch), but did not affect protein expression of IGF-I in the tibialis anterior muscle. In contrast, Awede et al. (2002) demonstrated a 5-fold increase in IGF-I mRNA expression in the soleus muscle (slow twitch) of 3-mo-old rats administered 10 mg/kg of clenbuterol in the drinking water for 3 d. Fiber composition of the muscle might determine responsiveness to \( \beta \)-agonists, but in our study, IGF-I mRNA expression numerically increased in BF and LM due to the ractopamine. Clearly, the response of local IGF-I expression to \( \beta \)-agonists is still equivocal.

The IGFBP regulate activity and clearance of IGF-I. Averaged across sex, mRNA expression of IGFBP-3 and IGFBP-5 were not affected by ractopamine in LM or BF (\( P \geq 0.19 \)). However, for IGFBP-5 in LM, there was an interaction between ractopamine and sex (\( P = 0.04 \)); ractopamine led to increases in expression of IGFBP-5 in heifers (from 459 to 610) but to decreases in expression in steers (from 1,046 to 731). For BF, the response of IGFBP-5 followed a pattern similar to that observed in LM, but there was no interaction between ractopamine and sex (\( P = 0.17 \)). Awede et al. (2002) demonstrated a 5-fold increase in IGFBP-5 mRNA expression in the soleus muscle of 3-mo-old rats administered clenbuterol for 3 d. The biological activity of IGF-I is regulated by 1 of 6 IGFBP (Duan, 2002).

Most IGF-I in circulation is bound to IGFBP, which can either inhibit or facilitate IGF-I binding to the IGF-I receptor (Duan, 2002). The most abundant IGFBP in circulation is IGFBP-3, and IGFBP-3 and IGFBP-5 have been implicated in inhibition of cell proliferation via IGF-I independent actions that negatively affect muscle growth (Yang et al., 1999; Kamanga-Sollo et al., 2005). Some of the difference between sexes in responsiveness to ractopamine could be linked to differences in expression of IGFBP-5 in the LM.

Ractopamine decreased MHC IIA mRNA expression in BF (991 vs. 1,373; \( P = 0.02 \)), but not in LM (\( P = 0.72 \)). Vestergaard et al. (1994) reported a decrease in frequency of type IIA fibers from 24.2 to 8.6% in LM and from 24.3 to 6.7% in semitendinosus of Friesian bulls fed 0.06 mg/kg of cimaterol for 90 d, whereas frequency of type IIB increased from 51.8 to 71.1% in LM and from 61.8 to 81.6% in the semitendinosus. Miller et al. (1988) demonstrated a 19% increase in type II fiber diameter with no change in type I diameter and frequency of type I and II in the LM of heifers fed 10 mg/d of clenbuterol for 50 d. Cull cows demonstrated decreases in type I fiber frequency in LM (34.4 to 32.2), semimembranosus (34.0 to 23.6), and vastus lateralis (35.3 to 24.6) and increases in type II fiber frequency in LM (65.6 to 67.8), semimembranosus (66.0 to 76.4), and vastus lateralis (64.7 to 75.4) when fed 200 mg/d of ractopamine for 28 d (Gonzalez et al., 2008). In the
Table 5. The effect of ractopamine (RAC) and sex¹ on blood metabolites and gene expression in finishing steers and heifers

<table>
<thead>
<tr>
<th>Item</th>
<th>Day 14</th>
<th>Day 28</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heifers 8</td>
<td>Steers 8</td>
<td>Heifers 8</td>
</tr>
<tr>
<td>Serum, ng/mL</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IGF-I</td>
<td>669c</td>
<td>691</td>
<td>607bc</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.39</td>
<td>1.39</td>
<td>1.10</td>
</tr>
<tr>
<td>Urea-N</td>
<td>4.45</td>
<td>4.60</td>
<td>4.02</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.0</td>
<td>6.6</td>
<td>5.1</td>
</tr>
<tr>
<td>Gene expression, arbitrary units, 10⁻⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biceps femoris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>3.0</td>
<td>5.6</td>
<td>5.2</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>121</td>
<td>179</td>
<td>128</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>559</td>
<td>889</td>
<td>737</td>
</tr>
<tr>
<td>Calpastatin</td>
<td>105</td>
<td>184</td>
<td>141</td>
</tr>
<tr>
<td>Myosin heavy chain IIA</td>
<td>1,320</td>
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<td>942</td>
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<td>β₁-Adrenergic receptor</td>
<td>0.24</td>
<td>1.85</td>
<td>0.18</td>
</tr>
<tr>
<td>β₂-Adrenergic receptor</td>
<td>72</td>
<td>161</td>
<td>56</td>
</tr>
<tr>
<td>β₃-Adrenergic receptor</td>
<td>1.4</td>
<td>8.4</td>
<td>1.1</td>
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<tr>
<td>LM</td>
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<td>3.3</td>
<td>6.1</td>
</tr>
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<td>IGFBP-3</td>
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<td>197</td>
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<tr>
<td>IGFBP-5</td>
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<td>1,242</td>
<td>696</td>
</tr>
<tr>
<td>Calpastatin</td>
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<td>228</td>
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<tr>
<td>Myosin heavy chain IIA</td>
<td>966</td>
<td>1,392</td>
<td>1,310</td>
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<td>β₁-Adrenergic receptor</td>
<td>0.22</td>
<td>0.93</td>
<td>0.32</td>
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<tr>
<td>β₂-Adrenergic receptor</td>
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<td>192</td>
<td>61b</td>
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<td>β₃-Adrenergic receptor</td>
<td>1.8</td>
<td>7.3</td>
<td>2.5</td>
</tr>
</tbody>
</table>

1 a–c Means in rows not bearing a common superscript differ, *P* < 0.05.
1 The use of d-0 data in covariate analysis removed much of the effect of sex.
2 Largest SEM among treatments.

Ractopamine for finishing cattle
same study, frequency of type I fibers increased from 51.1 to 54.3% and frequency of type II fibers decreased from 48.9 to 45.7% in infraspinatus. Depreux et al. (2002) demonstrated a decrease in MHC IIA mRNA expression in LM and semimembranosus of pigs fed 20 mg/kg of ractopamine for 28 d. Muscle fibers, when subject to change, follow a well-documented transition pathway (type I to type IIA to type IIX), and these transitions are reversible (Pette and Staron, 1997). If type I fibers shift to type II fibers (no differentiation between type IIA and IIX) and gene expression data demonstrate a depression in type IIA MHC, then it could be concluded that ractopamine is transitioning the fiber type to type IIX; in our study this occurred in BF but not in LM.

Ractopamine did not affect calpastatin mRNA expression in BF or LM. Calpastatin regulates the activity of m-calpain and μ-calpain, which are responsible for initiating myofibrillar protein disassembly, and therefore, changes in mRNA expression of calpastatin could reflect changes in protein degradation. Parr et al. (1992) observed increases in activity of m-calpain and calpastatin by 27 and 76% in LM of steers fed 1.5 mg/kg of cimaterol for 16 wk. In addition, mRNA levels of m-calpain and calpastatin were increased by 30 and 96% due to cimaterol treatment. The authors were the first to report simultaneous upregulation of m-calpain and calpastatin due to β-agonist feeding and suggested that this may be an important physiological response to β-agonists. Koohmaraie et al. (1991) demonstrated a 63% increase in calpastatin activity, whereas activities of μ-calpain and m-calpain were not affected in the BF of lambs fed 4 mg/kg L_{641,609} for 6 wk. We did not observe significant changes in expression of calpastatin mRNA, and the numeric response in LM represented only a 20% increase. Benson et al. (1991) observed a reduction in 3-methylhistidine concentration in the medium at 0 and 2 h of incubation of soleus muscle removed from fasted rats treated with 2 mg/kg of clenbuterol in the water for 2 d compared with control fasted rats, demonstrating a reduction in degradation of myofibrillar protein.

Expressions of β_1- and β_2-receptor mRNA in the BF were not affected by treatment. Ractopamine tended to decrease β_1-receptor mRNA abundance in LM in steers on d 14 and in heifers on d 28 (sex × ractopamine × day interaction; P = 0.11). Ractopamine decreased β_2-receptor mRNA expression in LM of steers on d 14, but tended to increase it in steers on d 28, whereas β_2-receptor mRNA expression was unaffected by ractopamine in heifers (sex × ractopamine × day interaction; P = 0.03). Sisson et al. (2007) reported that, in the semimembranosus of implanted heifers, ractopamine did not affect β_1-receptor mRNA abundance and tended to increase β_2-receptor mRNA expression. Implanted yearling steers had increased β-receptor mRNA expression in semimembranosus when fed ractopamine for 28 d (Winterholler et al., 2007). Growing Holstein steers fed ractopamine for 28 d demonstrated decreases in β_1- and β_2-receptor mRNA expression in LM (Walker et al., 2007). Winterholler et al. (2008) observed a tendency for β_2-receptor mRNA to increase in LM of yearling steers in response to 200 mg/d of ractopamine fed for the final 28 d, but no change in β_2- and β_3-receptor mRNA was detected. In BF, ractopamine did not alter mRNA expression of β_2-receptor in steers or heifers (P = 0.19). Ractopamine did not affect β_3-receptor mRNA expression in growing Holstein steers (Walker et al., 2007). Chronic exposure to β-agonists is suggested to lead to desensitization of the β-receptors (Hausdorff et al., 1990) resulting in loss of β-receptors. We observed only numeric decreases in mRNA for β-receptors, although these numeric decreases were present for all 3 receptors in both muscles. It should be emphasized, however, that gene expressions of the β-receptors may not correlate with the functional β-receptors located on the cell membrane. Moreover, partial loss of β-receptor numbers might not greatly affect the responsiveness of the tissue to a β-agonist.

**Conclusions**

Feeding ractopamine seems to change the muscle fiber composition in BF as shown by decreases in MHC IIA mRNA expression. The role of IGF-I, if any, in mediating the action of ractopamine warrants further research. Although there were a few criteria for which ractopamine led to differences in response between steers and heifers, there were no striking disparities to suggest that the effectiveness of ractopamine would markedly differ between sexes.

**LITERATURE CITED**


Ractopamine for finishing cattle


