Effect of ractopamine-hydrochloride and trenbolone acetate on longissimus muscle fiber area, diameter, and satellite cell numbers in cull beef cows

J. M. Gonzalez,* J. N. Carter,† D. D. Johnson,* S. E. Ouellette,* and S. E. Johnson*2

*Department of Animal Sciences, University of Florida, Gainesville 32611, and †North Florida Research and Education Center, Marianna 32446

ABSTRACT: The objective of this study was to evaluate the effects of coadministration of ractopamine-HCl (RAC) and trenbolone acetate plus estradiol (TBA) on LM fiber cross-sectional area (CSA), diameter, fiber-associated myonuclei, and satellite cell number. Culled crossbred beef cows (n = 98; 11 ± 1.8 yr old; BCS 4.3 ± 0.03) from a single ranch in south Florida were fed a concentrate diet for 92 d in a 2 × 2, randomized block design. Cows were blocked by BW on arrival into light (initial BW = 369.75 ± 2.68 kg and end BW = 501.96 ± 6.90 kg) and heavy (initial BW = 418.31 ± 2.75 kg and end BW = 522.15 ± 7.09 kg) groups before assignment to treatment. Factors included dietary treatment (0 or 15 ppm) and implant status (0 or 80 mg of trenbolone acetate + 16 mg of estradiol). Ractopamine was provided to 2 pens or half the treatments during the final 35 d of feeding. Cows were slaughtered on d 92. Forty-eight hours postmortem, the 6th-rib portions of the LM were obtained from 10 randomly selected carcasses from each treatment group (n = 40). Cryosections (12 μm) were immunostained for dystrophin and myosin heavy chain I or II for the measurement of fiber CSA and type, respectively. Fiber-associated nuclei and satellite cell numbers were measured in serial cryosections. There was a RAC × TBA interaction (P < 0.05). Type I fiber CSA and diameter were increased (P < 0.05) by TBA and RAC. Type I CSA and diameter were larger (P < 0.05) in TBA + RAC than RAC only. Type II fiber CSA and diameter were not affected by TBA (P = 0.48), RAC (P = 0.15), or TBA + RAC (P = 0.60). Satellite cell numbers and fiber-associated nuclei were not affected (P > 0.05) by implant status or ractopamine supplementation. These results indicate that TBA and RAC preferentially increase the size of type I fibers in cull cows.

Key words: beta-agonist, cull cow, muscle fiber, ractopamine, satellite cell

©2007 American Society of Animal Science. All rights reserved.

INTRODUCTION

Ractopamine HCl (RAC), a beta-agonist with established nutrient partitioning capabilities, promotes skeletal muscle accretion at the expense of fat deposition (for review see Mersmann, 1998). Many studies exist detailing the positive effects of RAC on swine performance and carcass composition (Dunshea et al., 1993, 1998; Sainz et al., 1993). At the cellular level in pigs, beta-agonists increase the size of type IIB muscle fibers. Cross-sectional area and fiber diameters were larger in pigs fed RAC (Aalhus et al., 1992) and an increase in the relative amount of myosin IIB was apparent (Depreux et al., 2002). A second effective means of altering feed efficiency and carcass composition is the use of steroid implants. In growing steers, treatment with Revalor-S (trenbolone acetate + estradiol; TBA) resulted in an increase in type IIB fibers without a change in the size or number of type I muscle cells (Fritsche et al., 2000).

Satellite cells, or muscle stem cells, are responsible for postnatal muscle fiber growth and repair (Mauro, 1961; Schultz et al., 1978). This typically quiescent population of cells lies adjacent to the muscle fiber under the basal lamina and is identified in vivo by their expression of Pax7. Pax7 is a member of the paired-box family of transcriptional mediators and is implicated in the establishment of the satellite cell lineage (Seale et al., 2000). Mice devoid of Pax7 exhibit severe muscle size and functional deficits that are due to an absence of satellite cells (Mansouri et al., 1996, Seale et al., 2000). The animals die within 3 to 4 wk of age. Pax7 does not alter proliferation rates but does inhibit satellite cell differentiation and apoptosis (Olguin and Olwin, 2004; Relaix et al., 2006; Zammit et al., 2006).

Due to limited information regarding aged bovine muscle fiber size, satellite cell numbers, and growth
capabilities, we examined LM fiber morphometrics and myonuclei numbers in cull cows fed RAC and TBA.

MATERIALS AND METHODS

Animals and Diets

This experiment was approved by the University of Florida Institutional Animal Care and Use Committee. Ninety-two crossbred beef cows (11 yr ± 1.8) culled from a commercial cow-calf operation in south Florida (Lykes Bros., Okeechobee, FL) were shipped on the same day by 2 truckloads to a feeding facility near Gainesville, FL. On arrival, the cattle were weighed and their general health was evaluated. Cows were given individual ID tags, dewormed with a generic anthelmintic (Agri Laboratories, Ltd., St. Joseph, MO), and tail switches were trimmed. Cows were blocked on arrival by BW into 2 replicates (heavy and light) and randomly assigned to treatments according to a 2 × 2 factorial arrangement. At the beginning of the study, light cows weighed 369.75 ± 2.68 kg and heavy cows weighed 418.31 ± 2.75 kg.

Cows were fed in 4 pens, with implant status and dietary treatment as the main effects. All diets were fed ad libitum in self-feeders. The BCS of all cows on arrival was uniform (4.3 ± 0.03; Carter et al., 2006). One-half of the cows in each pen were implanted with Revalor-IS (80 mg of trenbolone acetate plus 16 mg of estradiol; Intervet, Millsboro, DE), whereas the remainder received no implant. The basal diet was fed to one-half of the cows (2 pens) for the duration of the 92 d on feed. The remaining one-half (2 pens) were fed the same basal diet from d 0 to 55. On d 56, a pelleted supplement containing RAC was added to the basal diet, delivered to the empty self-feeders, and fed ad libitum for the remaining 35 d on feed.

The basal diet consisted of (DM basis) soybean hulls (21.1%), citrus pulp (19.7%), cracked corn (14.4%), wheat middlings (14.2%), cottonseed hulls (12.7%), cottonseed meal (7.0%), liquid molasses (7.0%), vitamins and minerals (included sodium bicarbonate; 2.1%), talc (1.3%), and urea (0.4%). The diet provided 87.6% DM, 14% crude protein (DM basis), and 79.5% TDN prescribed rate. Analytical results indicated that the B premix contained on average 2.15 g/kg of RAC (as-fed basis) and would adequately provide the targeted level of RAC.

The basal diet also included an ionophore [Rumensin 80 (monensin, granulated), Elanco Animal Health] formulated at the rate of 22 mg/kg of feed. Feed samples were collected randomly over the feeding period and analyzed for monensin concentration, which averaged 22.22 mg/kg.

Slaughtering and Sample Collection

On d 92, cows were slaughtered under USDA inspection in a commercial slaughter facility located in Center Hill, FL. Preslaughter BW was 501.96 ± 6.90 kg for light cows and 522.15 ± 7.09 kg for the heavy cows. After a 48-h chill period and carcass data collection, 10 wholesale ribs were randomly selected from each treatment group (n = 40). The 6th-rib steak from each wholesale rib section was removed. Two 1 × 1 × 1-cm portions of the 6th-rib LM were suspended in OCT tissue freezing medium (Fisher Scientific, Hampton, NH), frozen by submersion in supercooled isopentane, and stored at −80°C.

Immunohistochemistry

Three serial cryosections (12 μm), one for each fiber isoform, were collected on frost-resistant slides (Fisher Scientific) for each LM sample. Two sets of serial cryosections were collected for each animal, and the protocol of Watson et al. (2003) was followed with modifications. Nonspecific antigen sites were blocked with 5% horse serum in PBS for 20 min at room temperature. Cryosections were incubated for 60 min at room temperature with the primary antibodies. Antibodies and dilutions were: α-dystrophin (Abcam, Cambridge, MA), 1:500; undiluted, supernatant, myosin heavy chain type 1 (BAD.5, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City); myosin heavy chain type 2A (SC.71, Developmental Studies Hybridoma Bank); myosin heavy chain type 2A (SC.71, Developmental Studies Hybridoma Bank); myosin heavy chain type 2B (BF.F3, Developmental Studies Hybridoma Bank); 1:5 diluted, supernatant Pax7 (Developmental Studies Hybridoma Bank). The myosin heavy chain antibodies were directed toward bovine skeletal muscle myosin (Schiavino et al., 1989).

After extensive washing with PBS, tissues were incubated for 40 min with goat anti-mouse Alexa Flour 568 (1:500; Invitrogen, San Diego, CA) for α-dystrophin, or goat anti-mouse biotin (1:100; Vector Laboratories, Burlingame, CA), followed by streptavidin Alexa Flour 488 (1:500; Invitrogen) for Pax7 and myosin heavy chain isoform detection. After Pax7 immunostaining, Hoechst 33245 (1 μg/mL in PBS) was used to identify
Feeding ractopamine-hydrochloride to cull beef cows

Figure 1. Representative photomicrographs of LM fibers immunostained for type I and type II fibers from control diet-fed cows, control diet + ractopamine-HCl-fed cows (RAC), control diet + implant-fed cows (TBA), and control diet + RAC + implant-fed cows (RAC/TBA). Scale bar equals 100 μm.

Figure 2. Number of Pax7-positive nuclei counted per field during a 72-h period postmortem. Samples were taken at 0, 24, 48, and 72 h postmortem and subjected to the Pax7 staining protocol used in the current study. Area of field equals 41.5 mm².

Statistics

The study was designed as a randomized complete block design, with individual carcasses of the 4 feeding regimens as the experimental unit (Matulis et al., 1987; Cranwell et al., 1996a; Schnell et al., 1997). Fiber frequencies were tabulated and compared by χ² analysis using PROC FREQ (SAS Inst. Inc., Cary, NC). Treatment group frequencies within a fiber type were compared with one another by a 2-sample t-test for proportions. Data for fiber area and diameter were sorted and analyzed by individual fiber type, whereas FAN and Pax7 nuclei were not sorted. Data were analyzed with PROC MIXED of SAS, where implant status, dietary treatment, and their interaction were the fixed effects. Random effects included BW replicate, truckload, and animal within treatment. Each combination of BW replicate and truckload were grouped and used in the random statement. Pairwise comparisons between the least squares means of the factor levels were computed by using the PDIFF option of the LSMEANS statement.
RAC, or TBA + RAC. No differences (diameters of type II fibers occurred in response to TBA, fit for type II muscle fiber histograms do not differ (with larger CSA than control (Figure 3). Curve of best TBA + RAC caused a shift in numbers of type I fibers type I and type II LM fibers. Trenbolone acetate and RAC/TBA.

Type II fibers in the LM of cull cows treated with 1). There was an increase (across the control, RAC, and TBA treatments (Table 1). Feeding RAC to TBA implanted cull cows did not

The numbers of nuclei contained within the dystrophin boundary were measured as an index of hypertrophy. Fiber-associated nuclei were not affected (P > 0.38) by any of the treatments given (Table 2). Satellite cell number, as measured by antiPax7 at 48 h postmor-

tem, did not change (P > 0.89) in response to any treatment (Table 2).

The UNIVARIATE procedure of SAS was used to generate histograms and to analyze the distributions of fiber diameter and area within each treatment group for each fiber type.

RESULTS

Muscle fiber types were measured using antibodies specific to myosin heavy chain type I, IIA, and IIB isoforms. No type IIB immunoreactivity was observed, suggesting that LM was composed solely of type I and IIA fibers (Figure 1). Muscle fiber diameters were measured following colocalization of myosin and dystrophin. There was a significant (P < 0.01) RAC × TBA interaction for type I fibers. The main effects of TBA and RAC increased (P < 0.05) the diameter and computed cross-sectional area (CSA) of type I fibers (Table 1). Feeding RAC to TBA implanted cull cows did not further increase the LM fiber diameter (P = 0.88) or CSA (P = 0.77). No change (P > 0.05) in the CSA or diameters of type II fibers occurred in response to TBA, RAC, or TBA + RAC. No differences (P > 0.05) in the percentage of type I and type II fibers were observed across the control, RAC, and TBA treatments (Table 1). There was an increase (P < 0.001) in the percentage of type II fibers in the LM of cull cows treated with RAC/TBA.

Size-frequency histograms of CSA were generated for type I and type II LM fibers. Trenbolone acetate and TBA + RAC caused a shift in numbers of type I fibers with larger CSA than control (Figure 3). Curve of best fit for type II muscle fiber histograms do not differ (P = 0.18) between the groups (Figure 4).

The type IIA antibody used in this study reportedly

DISCUSSION

Growing pigs fed RAC demonstrate an increase in loin eye area that is a result of increased type II fiber size. Coincident with the larger fiber diameter and CSA is a shift from the fast oxidative (type IIA) to the fast glycolytic (type IIB) metabolic phenotype (Aalhus et al., 1992). A 2-fold increase in the numbers of type IIB fibers, at the expense of the type IIA fibers, was recorded in RAC-supplemented hogs (Depreux et al., 2002). In the current study, neither type IIB nor type IIX muscle fibers were detected by immunocytochemistry. The population of type IIA muscle fibers remained constant at approximately 68% of the total fibers in cattle fed RAC or implanted with TBA. A greater percentage of type IIA fibers at the expense of type I fibers was observed in TBA implanted cows fed RAC. This result is intriguing in light of our inability to detect a change in the proportion of type II fibers in cows fed RAC only. In cattle and mice, it is well established that beta agonists increase the percentage of type IIA fibers at the expense of type I fibers (Vestergaard et al., 1994; Rajab et al., 2000; Bricout et al., 2004). By contrast, anabolic agents have no effect on the distribution of type I or type IIA fibers (Ono et al., 1996; Fritsche et al., 2000). The underlying mechanism of action of RAC/TBA that elicits a change in myosin isoforms remains unknown and warrants further investigation.

The type IIA antibody used in this study reportedly reacts with myosin IIX and IIB in cattle (Duris et al., 2000) and type IIX fibers in swine (Depreux et al., 2000). Based on the assumption of that all type II fibers are immunoreactive, 60% the LM fibers in cull cows are classified as fast, a value in agreement with prior publications. Using enzymatic techniques, Brand-

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>RAC</th>
<th>TBA</th>
<th>RAC/TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I fiber</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td>32.35a</td>
<td>31.54a</td>
<td>32.35a</td>
<td>28.78b</td>
</tr>
<tr>
<td>Area, μm²</td>
<td>2,432.97 ± 73.56a</td>
<td>3,191.89 ± 99.18b</td>
<td>3,678.72 ± 190.40c</td>
<td>3,615.42 ± 96.36c</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>54.61 ± 0.73a</td>
<td>62.62 ± 0.98b</td>
<td>66.89 ± 1.78c</td>
<td>66.58 ± 0.93c</td>
</tr>
<tr>
<td>Type II fiber</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td>67.65a</td>
<td>68.46a</td>
<td>67.65a</td>
<td>71.22b</td>
</tr>
<tr>
<td>Area, μm²</td>
<td>5,087.12 ± 261.30</td>
<td>3,626.91 ± 253.48</td>
<td>4,690.68 ± 446.62</td>
<td>4,830.49 ± 241.12</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>79.70 ± 1.56</td>
<td>66.55 ± 1.52</td>
<td>75.23 ± 3.60</td>
<td>76.13 ± 1.50</td>
</tr>
</tbody>
</table>

* = Means within a row with a different letter are significantly different (P < 0.05).

1Control = Cull cows fed the control diet (n = 10); RAC = Cull cows fed the control diet plus Optaflexx (15 ppm ractopamine-HCl) supplement during the last 35 d on feed (n = 10); TBA = Cull cows implanted with Revalor-IS (80 mg of trenbolone acetate plus 16 mg of estradiol) and fed the control diet (n = 10); and RAC/TBA = Cull cows implanted with Revalor-IS, fed the control diet plus Optaflexx supplement during the last 35 d on feed (n = 10).

2Percentage fiber type detected by monoclonal antibody immunohistochemistry.

Table 1. LM type I and type II fiber percentage and least squares means of fiber cross-sectional area and diameter of cull cows fed 4 feeding regimens

--Gonzalez et al.1896--
Feeding ractopamine-hydrochloride to cull beef cows

stetter et al. (1998) identified the percentage of type I, type IIA, and type IIB fibers as 25, 25, and 50%, respectively, in the LM. Fritsche et al. (2000) reported a similar percentage with 20 to 30% of fibers classified as oxidative and approximately 55% of the total fibers present as fast glycolytic fibers. In a similar manner, the inability to detect type IIB fibers may be a limitation of the immunodetection method. Watson et al. (2003) failed to detect type IIB fibers in harbor seals using the same antibody. Duris et al. (2000) reported this antibody demonstrates a low specificity for type II myosin heavy chain in bovine tissues and indicated that antimyosin IIB (N3.36) is a suitable alternative. However, monoclonal N3.36 failed to detect type IIB fibers in the LM of cull cows fed RAC. Our inability to detect myosin type IIB fibers agrees with Tanabe et al. (1998) and Toniolo et al. (2005), who were unable to establish the presence of myosin IIB in bovine LM by semiquantitative reverse transcription-PCR. Chikuni et al. (2004) also reported a lack of myosin IIB mRNA as measured by real-time PCR, and hypothesized the absence of this isoform may explain the differences between beef and pork. Thus, the inability of RAC to shift myosin expression to the fastest isoform may be unique to cattle. Alternatively, fiber type shifts in response to RAC may occur only in muscle that normally expresses limited amounts of myosin IIB.

Quartile analysis of all type II fibers demonstrates no apparent difference in the percentage of fibers present with a larger diameter in RAC cull cows by comparison

Table 2. Least squares means of LM, fiber-associated nuclei per fiber, and satellite cells per hundred fibers of cull cows fed 4 feeding regimens

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>RAC</th>
<th>TBA</th>
<th>RAC/TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber-associated nuclei</td>
<td>4.69 ± 0.75</td>
<td>4.62 ± 0.77</td>
<td>5.16 ± 0.71</td>
<td>4.97 ± 0.76</td>
</tr>
<tr>
<td>Satellite cells</td>
<td>4.63 ± 0.91</td>
<td>3.20 ± 0.88</td>
<td>5.89 ± 0.70</td>
<td>4.24 ± 0.64</td>
</tr>
</tbody>
</table>

1Total nuclei counted in a field divided by number of fibers counted per field (field equals 381 mm²).
2Total satellite cells counted in a field divided by number of fibers counted per field multiplied by 100 (field equals 381 mm²).
3Control = Cull cows fed the control diet (n = 10); RAC = Cull cows fed the control diet plus Optaflexx (15 ppm ractopamine-HCl) supplement during the last 35 d on feed (n = 10); TBA = Cull cows implanted with Revalor-IS (80 mg of trenbolone acetate plus 16 mg of estradiol) and fed the control diet (n = 10); and RAC/TBA = Cull cows implanted with Revalor-IS, fed the control diet plus Optaflexx supplement during the last 35 d on feed (n = 10).
Figure 4. Histograms of LM fiber, cross-sectional areas of all type IIA fibers sampled from control diet-fed cows, control diet + ractopamine-HCl-fed cows (RAC), control diet + implant-fed cows (TBA), and control diet + RAC + implant-fed cows (RAC/TBA).

with control suggesting that the response of IIA fibers was minimal. Indeed, control animals appear to have the greatest percentage of large diameter fibers that may represent type IIB. The unresponsive nature of type II fibers of cull cows to RAC in the current study may reflect the muscle environment. Finishing hogs fed normal or supraphysiological levels of RAC contained an equivalent amount of myosin IIA in the LM as control animals. By contrast, the semintendinosus muscle contained less myosin IIA in response to RAC (Depreux et al., 2002). Alternatively, the resident type II population may be refractile to growth enhancing agents due to the advanced age of the animal. The differential response of bovine and porcine type II muscle fibers to the beta agonist is intriguing and warrants further investigation.

A substantial increase in the size of type I fibers was evident in cows treated with RAC or TBA. Administration of TBA to cull cows increased LM area and carcass fat-free lean (Cranwell et al., 1996b). In a similar manner, implantation of feedlot steers with TBA increased type I and IIA CSA in the LM (Hughes et al., 1998). Thus, the larger diameter type I fibers in cull cows receiving TBA reflects previous reports. Conversely, an increase in LM type I CSA by RAC supplementation is novel. No change in the size of type I fibers is apparent in finishing hogs supplemented with RAC (Aalhus et al., 1992). The LM type I fibers from lambs fed cimaterol showed no change to a modest 15% increase in fiber CSA (Beermann et al., 1987; Kim et al., 1987). The 30% increase in type I CSA found in RAC-supplemented cows suggests that this population of cells is 2 to 3 times more responsive in cattle than other species. Further support for species differences is reflected by a 35% larger type I CSA in bulls fed cimaterol (Vestergaard et al., 1994). However, cull cows are unique in that their type II fiber population is completely refractile to RAC induced hypertrophy, whereas cimaterol stimulated hypertrophy in bulls (Vestergaard et al., 1994). The mechanism behind the differential response in cattle may be a reflection of the age of the animal. During aging in humans, the numbers of type IIA/X muscle fibers and size are reduced (Lee et al., 2006; Verdijk et al., 2006). Type I fiber CSA remains unchanged largely, but the percentage of type I fibers are increased. In addition, the ability of the muscle to respond to hypertrophic events is altered in extreme age (for review see Carmeli et al., 2002). Advanced age in rodents, birds, and humans demonstrates an impaired ability to increase in size that is associated with reduced type II muscle fiber numbers (Carson et al., 1995; Blough and Linderman, 2000; Short et al., 2005; Lee et al., 2006). The percentage of type I and II fibers is established by 24 mo of age in cattle, irrespective of breed, and the LM is composed predominantly of type I and IIB (Wegner et al., 2000; Kirchofer, et al., 2002). In the event that type II fibers are limited in their protein synthetic capacity and are intolerant to hypertrophic stimuli, the nutrients supplied by diet coupled with the partitioning agents lead to an excess of available substrate for type I fiber growth.
One of the primary reasons for supplementing livestock with RAC or TBA is to improve carcass value. Schroeder et al. (2005a,b) reported an increase in ribeye area and fat-free lean in steers and heifers fed RAC. Therefore, based on the findings above we predicted that cull cows receiving 15 ppm RAC daily for 35 d would possess a larger REA. However, no improvement in carcass characteristics, including REA, due to RAC or TBA supplementation was detected (Carter et al., 2006). This may be a reflection of an unresponsive type II fiber population. Approximately 30% of the total number of fibers was present as type I. Based on the 30% increase in type I size found in young bulls (Vestergaard et al., 1994), we would predict a minimal 9% increase in REA. This small change may require more animals to reach statistical significance.

Postnatal skeletal muscle growth is accomplished through the satellite cell population. These normally quiescent muscle stem cells become mitotically active, proliferate, and fuse into existing muscle fibers (for review see Collins, 2006). The number of satellite cells declines with age, and the activation potential of these cells is reduced in older individuals (Collins and Partridge, 2005). In aged rats and elderly humans, satellite cells represent 1 to 2% of the total myonuclei (Gallegly et al., 2004; Sajko et al., 2004; Brack et al., 2005). The number of Pax7 immunopositive satellite cells in cull cows represents approximately 1% of the total number of myonuclei, in close agreement with rodent data. Satellite cells isolated from TBA implanted steers exit the dormant state sooner than their contemporaries, suggesting that the anabolic steroid affects self-renewal and subsequent proliferation. In addition, these cells fused into larger muscle fibers in vitro (Johnson et al., 1998). The enhanced myogenic capabilities may account for the larger fiber sizes found in TBA-implanted cull cows. Alternatively, TBA increased circulating levels of IGF-I and autocrine synthesis of the growth factor (Thompson et al., 1989; White et al., 2003; Kamangapot, 2005). The lack of effect on FAN and constant satellite cell numbers suggests that any hypertrophy occurred due to changes in protein synthesis, degradation rates, or both. It is hypothesized that because protein synthesis is limited in older animals, this prevented the response to TBA and RAC normally seen in younger animals.

Satellite cells proliferate, differentiate, and fuse with the muscle fibers to provide FAN for increased contractile gene expression and maintenance of the myonuclear domain (Aberle et al., 2001). The lack of an increase of FAN found in all the supplemented cows indicates that the 2 growth promotants may act through a similar pathway of altered protein synthesis, degradation rates, or both. It was reported that RAC stimulates protein synthesis without an apparent effect on satellite cell cycle kinetics or fusion (Shappell et al., 2000). Beta-agonists may also augment nutrient supply to the muscle cell by increased blood flow. Muscle accretion is supported and possibly bolstered by the enhanced delivery of substrates and energy needed for protein synthesis (Mersmann, 1998). In several swine and cattle studies, the mechanism of muscle growth due to ractopamine supplementation was attributed to enhanced protein synthesis (Smith et al., 1987; Dunshea et al., 1993, 1998; Williams et al., 1994). Beermann et al. (1987) and Kim et al. (1987) reported that DNA concentration per gram of protein was less in beta-agonist-supplemented lambs, and both groups concluded that muscle growth was due to a reduction in protein degradation and independent of satellite cell activity. A similar observation was reported for rats (Maltin et al., 1986) and lambs (Bohorov et al., 1987) fed clenbuterol. The discrepancies between increased protein synthesis or reduced degradation rates may be a reflection of the beta-agonist fed and the beta-receptor isoform activated.

Conclusion

Supplementing cull cows with RAC or TBA alone or the combination increased LM fiber CSA and diameter for type I fibers, although had no effect on type II fibers. There were no fiber type shifts between the different myosin heavy chain isoforms due to the presence of TBA or RAC. Fiber-associated or satellite cell numbers were not affected by the RAC or TBA treatments. The lack of effect on FAN and constant satellite cell numbers suggests that any hypertrophy occurred due to changes in protein synthesis, degradation rates, or both. It is hypothesized that because protein synthesis is limited in older animals, this prevented the response to TBA and RAC normally seen in younger animals.

LITERATURE CITED


