TEXAS TECH UNIVERSITY
Libraries™

ADDITIVE EFFECTS OF A STEROIDAL IMPLANT AND ZILPATEROL HYDROCHLORIDE ON FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS, AND SKELETAL MUSCLE MESSENGER RIBONUCLEIC ACID ABUNDANCE IN FINISHING STEERS

Additive effects of a steroidal implant and zilpaterol hydrochloride on feedlot performance, carcass characteristics, and skeletal muscle messenger ribonucleic acid abundance in finishing steers[1]

T. J. Baxa,* J. P. Hutcheson,† M. F. Miller,‡ J. C. Brooks,‡ W. T. Nichols,† M. N. Streeter,† D. A. Yates,† and B. J. Johnson*[2,3]

*Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506;
†Intervet/Schering-Plough Animal Health, DeSoto, KS 66018;
and ‡Department of Animal and Food Sciences, Texas Tech University, Lubbock 79409

ABSTRACT: This experiment investigated the effects of zilpaterol hydrochloride (ZH) and the steroidal implant Revalor-S (RS; 120 mg of trenbolone acetate and 24 mg of estradiol-17β) on finishing steer performance and the mRNA concentration of β-adrenergic receptors (β-AR) types I and II, and types I, IIA, and IIX myosin heavy chain (MHC) isoforms. A total of 2,279 feedlot steers weighing 426 ± 6.4 kg were administered no implant or RS on d 0, and fed 0 or 8.3 mg of ZH/kg of diet DM during the last 30 d with a 3-d withdrawal. Treatments were randomly assigned to 24 pens (n = 6 pens/treatment). At slaughter, semimembranosus muscle tissue was excised for RNA isolation from 4 carcasses per pen. No interactions were detected for any of the variables measured in the experiment. Administration of ZH during the last 30 d of the feeding period increased ($P < 0.01$) ADG, G:F, HCW, and LM area; decreased ($P < 0.01$) 12th-rib fat depth and marbling; and improved ($P < 0.01$) yield grade. Treatment had no effect on β1-AR mRNA levels, but there was an increase ($P = 0.01$) in β_2-AR mRNA levels due to ZH inclusion. Myosin heavy chain-I (MHC-I) mRNA levels were unaffected by treatment. For MHC-IIA mRNA concentrations, administration of RS tended ($P = 0.08$) to increase mRNA levels, whereas ZH feeding the last 30 d tended ($P = 0.08$) to decrease mRNA levels for this isoform of myosin. Feeding ZH the last 30 d before slaughter increased ($P < 0.01$) mRNA concentrations of MHC-IIX in semimembranosus muscle of steers. These data indicate the combined use of ZH and RS additively contributes to BW and carcass gain in finishing feedlot steers and decreases marbling scores and USDA quality grades. The LM area increased and fat thickness decreased. In addition, ZH feeding changes the mRNA levels of MHC isoforms to a faster, more glycolytic fiber type in bovine skeletal muscle. These changes in mRNA concentrations of MHC isoforms, due to ZH feeding, could be affecting skeletal muscle hypertrophy.

Key words: β-adrenergic agonist, cattle, implant, myosin, zilpaterol hydrochloride

J. Anim. Sci. 2010. 88:330–337
doi:10.2527/jas.2009-1797

INTRODUCTION

Zilpaterol hydrochloride (**ZH**) is an orally active β-adrenergic agonist (**β-AA**) approved for use in finishing beef cattle in the United States. Inclusion of ZH in cattle feed the last 20 to 40 d improves ADG, feed efficiency, carcass yield grade, HCW, LM area, and dressing percentage in finishing steers (Avendaño-Reyes et al., 2006; Vasconcelos et al., 2008). These biological effects are a result of ZH binding to a β-adrenergic receptor (**β-AR**) located on the cell surface of tissues, including skeletal muscle and adipose tissue (Mersmann, 1998). There are 3 subtypes of β-AR (β_1, β_2, and β_3) on most mammalian cells with β_2-AR being the most abundant subtype in bovine skeletal muscle and adipose tissue (Sillence and Matthews, 1994). Zilpaterol hydrochloride can bind to the β_1-AR and β_2-AR, with a greater affinity for β_2-AR (Verhoeckx et al., 2005). The β-AA increase skeletal muscle hypertrophy. These improvements in skeletal muscle hypertrophy are a result of changes in protein synthesis and degradation rates, whereas in adipose tissue they promote lipolysis (Beermann, 2002; Birkelo, 2003; Verhoeckx et al., 2005).

Anabolic steroidal implants containing a combination of trenbolone acetate (**TBA**) and estradiol-17β (**E_2**)

[1]Contribution number 09-011-J of the Kansas Agric. Exp. Stn., Manhattan 66506.
[2]Corresponding author: bradley.johnson@ttu.edu
[3]Present address: Department of Animal and Food Sciences, Texas Tech University, Lubbock 79409.
Received January 14, 2009.
Accepted September 21, 2009.

have been reported to improve feedlot performance and stimulate carcass protein accretion in feedlot steers (Johnson et al., 1996; Pampusch et al., 2003). Data also indicated that TBA + E_2 implantation increased proliferation, and fusion of muscle satellite cells, which may be an important mechanism by which anabolic steroids enhance muscle hypertrophy (Johnson et al., 1998; Johnson and Chung, 2007).

Currently, there are no data on the comparative efficacy of these 2 distinct types of growth promotants in feedlot steers. The purpose of this study was to investigate the effects of ZH administration in combination with a steroidal implant containing TBA and E_2 on steer performance and the mRNA abundance for β_1-AR; β_2-AR; calpastatin; and myosin heavy chain (**MHC**) types I, IIA, and IIX.

MATERIALS AND METHODS

The following experiments were a collaboration of Intervet Inc. (Millsboro, DE), Texas Tech University, and Kansas State University. Experimental procedures with cattle were in compliance with the guidelines stated in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and were approved by the Kansas State University Institutional Animal Care and Use Committee.

Animals

English × Continental yearling steers (n = 2,279), with initial BW of 426 ± 6.4 kg, were utilized in this study. All animals were housed at a commercial research facility in Texas in soil-surface pens. At approximately d −61, all animals were administered a Component TE-IS (16 mg of estradiol and 80 mg of TBA; Vetlife Inc., West Des Moines, IA) implant. Before allotment, animals were weighed and ultrasound was used to estimate empty body fat (**EBF**), so that pens could be stratified by EBF. Extreme animals in terms of BW or EBF were removed from the trial. Furthermore, animals with missing electronic identification tags or with visual performance or health problems were also eliminated from the trial.

Experimental Design, Treatment, and Pen Assignment

Four treatments were arranged in a 2 × 2 factorial design with the main effects of feeding ZH (0 or 8.3 mg/kg of diet; Zilmax, Intervet Inc., Millsboro, DE) for the last 30 d on feed with a 3-d withdrawal (in accordance with Food and Drug Administration regulations), and a terminal implant of Revalor-S [**RS**; 120 mg of TBA and 24 mg of estradiol (**E_2**); Intervet Inc.] 91 d before slaughter or no implant. Treatments were randomly assigned to each pen (n = 24) with approximately 100 steers per pen, as follows: 1) no RS or ZH (**CON**), 2) only ZH (ZH), 3) only RS (RS), or 4) RS and ZH (ZH+RS). The different treatment groups were designed to be reflective of the typical commercial finishing period and to evaluate the impact of inclusion of ZH in the diet. The ZH was included by means of water-based slurry through a commercial micro-ingredient machine (Table 1). At the time of the study, ZH was not approved by the FDA to be fed in combination with monensin (Rumensin, Elanco Animal Health, Indianapolis, IN) or tylosin (Tylan, Elanco Animal Health); therefore, during the ZH feeding period, these feed additives were removed from the diets of treatments receiving ZH for 30 d, then administered again during the 3-d withdrawal to all treatments. The diet ingredient and chemical compositions are provided in Table 1. The diet was formulated to meet or exceed NRC (1996) requirements for nutrients. Throughout this study, a clean bunk method was implemented so that all feed was consumed each day without limiting feed available. Feed allocation (increase or decrease in daily amount) was based on the time bunks were empty and visual appraisal of cattle appetite aggression. Cattle were fed 3 times daily with equal distribution of the daily feed across the 3 feedings. Pens were fed in the same order each day. At the end of the feeding period BW was measured, and an industry standard of 4% pencil shrink was used to calculate final BW. All animals were transported approximately 193 km to a commercial abattoir to be slaughtered.

Sample Preparation and RNA Isolation

Within 10 min of slaughter at the abattoir, a muscle sample was collected from the semimembranosus muscle of 4 randomly selected steers per pen. The samples were rapidly frozen in liquid N_2 and shipped to Kansas State University for analysis. Total RNA was isolated from muscle samples with Tri Reagent (Sigma, St. Louis, MO). Briefly, the semimembranosus muscle tissue (200 mg) was transferred to a steel mortar bowl cooled by liquid N_2. The samples were homogenized by using a sterile pestle in liquid N_2, and Tri Reagent (2 mL) was added to the ground tissue sample. Muscle tissue (1 mL) in Tri Reagent was incubated at room temperature for 5 min. After incubation, chloroform (Sigma) was added, and the samples were centrifuged for 15 min at 12,000 × *g* at room temperature. After centrifugation, the upper aqueous phase was removed and transferred to a new microcentrifuge tube. Isopropanol (Sigma) was added and incubated at room temperature for 5 min. Then the samples were centrifuged for 10 min at 12,000 × *g* to isolate the RNA pellet. The isopropanol was then removed and the RNA pellet was suspended in 70% ethyl alcohol (1 mL) and stored at −80°C.

The RNA pellet was treated to remove any contaminating genomic DNA by using the DNA-free kit (Ambion, Austin, TX). The RNA concentration was determined by absorbance at 260 nm. The integrity of the RNA was determined by gel electrophoresis. Total

RNA with ethidium bromide was loaded onto a 1% agarose gel to separate and visualize the 28S and 18S rRNA. Total RNA (1 μg) was then reverse-transcribed to produce the first-strand cDNA using TaqMan Reverse Transcription Reagents and Multi-Scribe 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 Quantitative PCR

Real-time quantitative PCR was used to measure β_1-AR, β_2-AR, MHC-I, MHC-IIA, and MHC-IIX quantitative gene expression relative to the quantity of ribosomal protein S9 (**RPS9**) in total RNA isolated from muscle tissue using the delta CT method. Measurements of the relative quantity of cDNA were performed by using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 900 n*M* of the appropriate forward and reverse primers, 200 n*M* of the appropriate TaqMan detection probe, and 1 μL of the cDNA mixture. The bovine-specific β_1-AR, β_2-AR, MHC-I, MHC-IIA, and MHC-IIX forward and reverse primers and TaqMan detection probes (Table 2) were synthesized by using published GenBank sequences. CustomRPS9 (GenBank Accession No. DT860044) rRNA primers and probes were used as an endogenous control. The ABI Prism 7000 detection system (Applied Biosystems) was used to perform the assay by using the recommended thermal cycling variables from the manufacturer (50 cycles of 15 s at 95°C and 1 min at 60°C). The RPS9 rRNA endogenous control was used to normalize the expression of β_1-AR, β_2-AR, MHC-I, MHC-IIA, and MHC-IIX using the delta CT method. The data were expressed as relative units.

Statistical Analysis

Data were analyzed as a 2 × 2 factorial in a randomized complete block design with PROC MIXED (SAS Inst. Inc., Cary, NC). Pen served as experimental unit for all feedlot, carcass characteristics, and gene expression data. Treatment and interaction means were analyzed and separated ($P < 0.05$) with the least significant difference procedure of SAS and Fisher's exact test.

RESULTS AND DISCUSSION

Effect of ZH and RS on Performance and Carcass Characteristics

Performance data represent the entire 91-d period from RS implant until slaughter. All performance and carcass data are shown in Table 3. The RS treatment increased ($P < 0.01$) ADG and G:F and increased ($P = 0.02$) DMI by 2.2%. These results are similar to previous studies on steroidal implants showing improvements on finishing steer performance and feed efficiency (Bartle et al., 1992; Johnson et al., 1996; Guiroy et al., 2002; Pampusch et al., 2003). The increase in DMI was in agreement with previous studies utilizing estrogen implants that reported an increase in DMI (Rumsey et al., 1992).

Table 1. Composition and analyzed nutrient content (DM basis) of the finishing diet

	Treatment[1]	
Item	Control[2]	ZH[3]
Flaked corn	34.60	34.60
High-moisture corn	21.20	21.20
Wet corn gluten feed	24.00	24.00
Tallow	4.67	4.67
Corn silage	10.03	10.03
Finisher supplement[4]	5.49	5.49
Control microingredients[5]	0.02	—
ZH microingredients[6]	—	0.02
Nutrient level, DM basis		
DM, %	62.46	62.46
CP, %	12.87	12.87
NPN, %	1.52	1.52
ME, Mcal/kg	3.37	3.37
NDF, %	18.99	18.99
Calcium, %	0.72	0.72
Phosphorus, %	0.46	0.46

[1]Diets represent feed for the last 33 d on feed; from the start of the zilpaterol hydrochloride (ZH; Zilmax, Intervet Inc., Millsboro, DE) administration until slaughter.
[2]Treatments not receiving ZH inclusion in the diet.
[3]Treatment receiving ZH inclusion in the diet.
[4]The finisher supplements contained (DM basis) 58.43% wheat middlings; 7.25% urea; 5.35% salt; 27.20% limestone; 0.50% choice white grease; 0.07% vitamin premix; and 1.20% trace mineral premix.
[5]Added at the time of diet preparation to provide monensin (Rumensin, Elanco Animal Health, Indianapolis, IN) at 28.00 mg/kg of diet DM and tylosin (Tylan, Elanco Animal Health) at 8.57 mg/kg of diet DM.
[6]Added at the time of diet preparation to provide ZH at 8.30 mg/kg of diet DM.

Carcass yield improved with increases ($P < 0.01$) in HCW and LM area as well as an increase ($P < 0.05$) in dressing percentage as a result of RS treatment (Table 3). There was a change in quality grade, with marbling scores decreasing ($P < 0.01$) with RS treatment; however, no differences were observed for 12th-rib fat depth. Our results are similar to a previous study that found improvements in performance with implant use; however, quality grade also decreased with increased implant use in steers (Platter et al., 2003). These results show that RS implant improves finishing steer performance through increased efficiency of nutrient conversion to skeletal protein; however, it does have a negative impact on carcass quality grade due to decreases in final adipose amounts.

The ZH treatment increased ($P < 0.01$) ADG, G:F, HCW, dressing percentage, and LM area and decreased ($P < 0.01$) 12th-rib fat depth and marbling scores. With the ZH treatment, there was no effect on DMI. These results show that ZH improves performance and enhances carcass protein accumulation without stimu-

Table 2. Sequences for bovine-specific PCR primers and TaqMan probes for β_1- and β_2-adrenergic receptors; types I, IIA, and IIX myosin heavy chain (MHC) mRNA; and ribosomal protein S9 (RPS9)

Item	Sequence (5′ to 3′)
β_1-Adrenergic receptor (accession No. AF188187)	
Forward	GTGGGACCGCTGGGAGTAT
Reverse	TGACACACAGGGTCTCAATGC
TaqMan probe	6FAM-CTCCTTCTTCTGCGAGCTCTGGACCTC-TAMRA
β_2-Adrenergic receptor (accession No. NM_174231)	
Forward	CAGCTCCAGAAGATCGACAAATC
Reverse	CTGCTCCACTTGACTGACGTTT
TaqMan probe	6FAM-AGGGCCGCTTCCATGCCC-TAMRA
MHC I (accession No. AB059400)	
Forward	CCCACTTCTCCCTGATCCACTAC
Reverse	TTGAGCGGGTCTTTGTTTTTCT
TaqMan probe	6FAM-CCGGCACGGTGGACTACAACATCATAG-TAMRA
MHC IIA (accession No. AB059398)	
Forward	CCCCGCCCCACATCTT
Reverse	TCTCCGGTGATCAGGATTGAC
TaqMan probe	6FAM-TCTCTGACAACGCCTATCAGTTCAT-TAMRA
MHC IIX (accession No. AB059399)	
Forward	GGCCCACTTCTCCCTCATTC
Reverse	CCGACCACCGTCTCATTCA
TaqMan probe	6FAM-CGGGCACTGTGGACTACAACATTACT-TAMRA
RPS9 (accession No. DT860044)	
Forward	GAGCTGGGTTTGTCGCAAAA
Reverse	GGTCGAGGCGGGACTTCT
TaqMan probe	6FAM-ATGTGACCCCGCGGAGACCCTTC-TAMRA

lating DMI, leading to improved efficiency with which dietary nutrients are converted to BW gain. However, there is a reduction in adipose tissue. These results are similar to other studies that have detected increases in performance and carcass lean tissue accumulation in cattle and sheep (Plascencia et al., 1999; Salinas-Chavira et al., 2004). Specifically, we observed approximately a 20-kg increase in HCW due to ZH feeding for 30 d with a 3-d withdrawal. Our findings are very similar to Elam et al. (2009) and Vasconcelos et al. (2008) in which 30-d ZH feeding resulted in approximately 17 and 16 kg heavier HCW in steers, respectively, compared with steers receiving the control diet. Similar to previous work, the HCW advantage was greater in magnitude than BW advantage between ZH and no ZH treatment. These findings indicate that ZH is preferentially mobilizing tissue from noncarcass components and directing these nutrients to the carcass during the ZH feeding period.

The cattle receiving the combination ZH+RS treatment had the greatest increase in ADG and G:F and appeared to be additive when compared with the individ-

Table 3. Effects of implanting with Revalor-S (RS) and feeding zilpaterol hydrochloride (ZH) for the final 30 d on feed plus a 3-d withdrawal on performance during the final 91 d on feed by finishing steers

	No ZH		ZH[1]			*P*-value		
Item	No RS	RS[2]	No RS	RS[2]	SEM[3]	RS	ZH	RS × ZH
Initial BW,[4] kg	426.1	426.5	426.0	426.2	6.37	0.72	0.84	0.91
Final BW, kg	589.6	603.6	600.2	614.0	9.23	<0.01	<0.01	0.96
ADG, kg	1.81	1.96	1.92	2.07	0.083	<0.01	<0.01	1.00
DMI, kg/d	10.31	10.54	10.21	10.44	0.269	0.02	0.27	0.99
G:F	0.176	0.186	0.188	0.198	0.0036	<0.01	<0.01	0.63
HCW, kg	371.1	382.2	392.6	402.6	5.97	<0.01	<0.01	0.60
Dressing percentage	62.94	63.31	65.42	65.57	0.139	0.05	<0.01	0.38
LM area, cm^2	90.1	94.8	101.7	107.0	1.28	<0.01	<0.01	0.66
12th-rib fat depth, cm	1.52	1.50	1.40	1.35	0.033	0.31	<0.01	0.78
Marbling score[5]	369.5	353.4	339.9	323.5	6.53	<0.01	<0.01	0.96
KPH, %	2.16	2.09	2.02	1.90	0.066	<0.01	<0.01	0.15

[1]Zilpaterol hydrochloride (Zilmax, Intervet Inc., Millsboro, DE) inclusion in the diet (8.30 mg/kg of feed on a DM basis) for the last 30 d of feed with a 3-d withdrawal.

[2]Revalor-S (Intervet Inc.) implantation (120 mg of trenbolone acetate and 24 mg of estradiol-17β) 91 d before slaughter.

[3]Pooled SEM of simple-effect means n = 6 pens/treatment with 90 to 100 steers/pen initially and 89 to 100 steers/pen at slaughter.

[4]A 4% shrink was applied to initial and final BW; dead or removed animals did not contribute to initial or final BW.

[5]Scores: 300 = Slight; 400 = Small; 500 = Modest.

ual treatments with ZH or RS. The ZH+RS treatment also had an additive increase in HCW, LM, and dressing percentage when compared with individual treatments of ZH or RS. Marbling scores, 12th-rib fat depth, and KPH were additively decreased for the ZH+RS treatment when compared with individual treatments of ZH or RS. There is little published research available that compares the effects of steroidal implants and ZH in beef cattle; however, these results are supported by similar studies that found increases in ADG, G:F, HCW, dressing percentage, and LM area and decreases in yield grade, 12th-rib fat depth, and marbling scores in finishing steers that were administered RS (120 mg of TBA and 24 mg of E_2) and received feed inclusion of ZH (8.33 mg/kg) for 0, 20, 30, or 40 d (Vasconcelos et al., 2008). Similarly, Avendaño-Reyes et al. (2006) reported increased ADG, G:F, HCW, and LM area; decreased 12th-rib fat depth; and no difference in DMI by steers that received Synovex Plus (200 mg of TBA and 28 mg of estradiol benzoate) 60 d before ZH administration (Avendaño-Reyes et al., 2006).

These results support our findings that steroid implantation and inclusion of ZH in typical finishing steer rations improve feed efficiency and animal performance without affecting DMI. Furthermore, the largest improvements in performance and efficiency are achieved and appear to be completely additive when steers are administered a steroidal implant before inclusion of ZH in the ration; however, carcass quality decreases to a similar extent. The additive nature of the data implies that these 2 growth promotants may be working through distinct mechanisms to enhance lean tissue deposition in beef cattle.

Real-Time PCR

In performing the real-time PCR, we initially determined quantitative values of each gene of interest relative to the quantity of the housekeeping gene (**HKG**) 18S rRNA to normalize the data. From our analysis, we determined there was a treatment effect ($P = 0.05$) of ZH on 18S rRNA concentrations. Total RNA isolated from muscle samples of ZH cattle had greater levels of 18S rRNA. Therefore, we used RPS9 as our HKG. In a previous study, various HKG were tested for variation with bovine hepatic tissue from animals of varying physiological and dietary experimental conditions. It was determined that of those tested, HKG RPS9 was the most stable relative to the various experimental types of hepatic tissue (Janovick-Guretzky et al., 2007). After analysis of our study, it was determined that no effect ($P = 0.43$) was detected with the HKG RPS9 for any of the treatment groups. From this discovery, the question arises about ZH possibly having an effect on ribosomal RNA gene expression. This would be another indicator that protein synthesis may be altered with ZH administration.

Effect of ZH and RS on Semimembranosus Muscle β_1-AR and β_2-AR mRNA Concentrations

The mRNA concentrations of β-AR are shown in Figures 1 and 2. There was no effect from any treatments on the expression of β_1-AR mRNA. The ZH treatment increased ($P = 0.01$) mRNA expression of the β_2-AR; however, there were no other treatment effects on the β_2-AR. These findings are similar to a study by Sissom et al. (2007) in heifers initially implanted with 80 mg of TBA and 8 mg of E_2 and reimplanted with 200 mg of TBA 96 d before feeding the β-AA, ractopamine hydrochloride (**RH**), for 28 d. These authors reported no change in β_1-AR mRNA levels, and a tendency to increase β_2-AR mRNA levels, due to β-AA administration (Sissom et al., 2007). In a similar study by Winterholler et al. (2007), with steers implanted with RS and administered RH, there was no β-AA treatment effect on the expression of β_1-AR mRNA levels. However, there was a tendency to increase β_2-AR mRNA levels (Winterholler et al., 2007). They further determined in vitro that primary bovine muscle cultures, in response to RH, showed an increase in the β_2-AR mRNA level during differentiation, but no effect on β_1-AR mRNA levels (Winterholler et al., 2007). These studies support our current findings that administration of a β-AA in steers can alter expression of skeletal muscle β_2-AR mRNA. The differences in the amount of change between these studies could be due to the differences in the type and duration of β-AA administered. Additionally, our study determined the expression of β_2-AR was nearly 1,000 times greater than the expression of β_1-AR, which suggests that the β_2-AR is the most abundant β-AR subtype in the semimembranosus muscle of steers. This corresponds with previous research that determined the β_2-AR mRNA is the most abundant mRNA subtype in the semimembranosus of heifers and steers (Sissom et al., 2007; Winterholler et al., 2007). No detectable changes of β_1-AR could also be attributed to its generally low expression making differences hidden by sample-to-sample variation.

Effect of ZH and RS on Semimembranosus Muscle Types I, IIA, and IIX MHC mRNA Concentrations

Administration of ZH and RS increases lean tissue deposition. From these findings we hypothesized that the expression of myosin isoforms had the potential to be altered as a result of the changes in lean tissue deposition, due to ZH and RS administration. The gene expression results for MHC are shown in Figures 3, 4, and 5. There was no effect on the expression of MHC-I mRNA for all treatments. The ZH treatment had a tendency to decrease ($P = 0.08$) MHC-IIA mRNA, and the RS treatment had a tendency to increase ($P = 0.08$) the expression of MHC-IIA mRNA. We observed

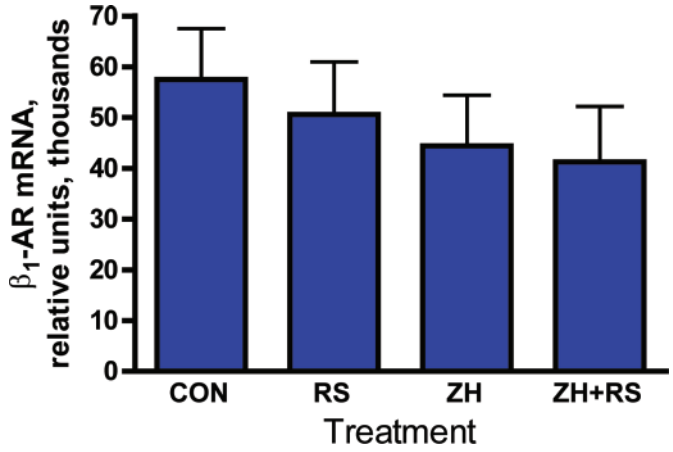

Figure 1. β_1-Adrenergic receptor (β_1-AR) mRNA abundance in bovine semimembranosus muscle collected from steers 10 min postslaughter. Four animals per pen were used in the analysis (6 pens/treatment). Treatments consisted of 1) control (CON), 2) zilpaterol hydrochloride (ZH; Zilmax, Intervet Inc., Millsboro, DE; 8.30 mg/kg), 3) Revalor-S (RS, Intervet Inc.; 120 mg of trenbolone acetate and 24 mg of estradiol-17β), or 4) ZH and RS (ZH+RS). There was no main effect or interaction of treatments on the expression of β_1-AR mRNA. Error bars are SEM.

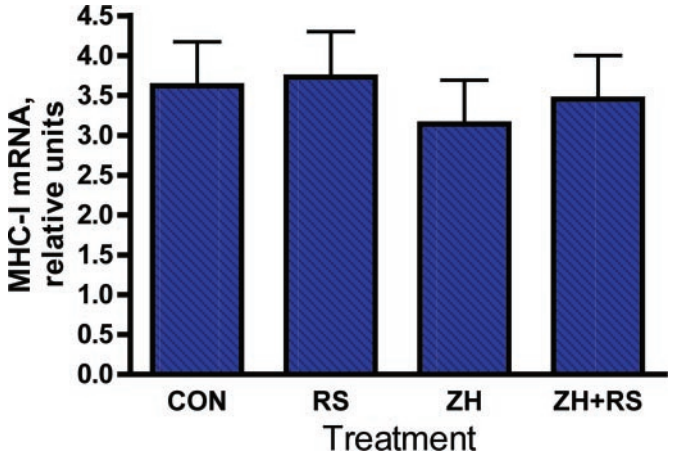

Figure 3. Type I myosin heavy chain (MHC-I) mRNA abundance in bovine semimembranosus muscle collected from feedlot steers 10 min postslaughter. Four animals per pen were used in the analysis (6 pens/treatment). Treatments consisted of 1) control (CON), 2) zilpaterol hydrochloride (ZH; Zilmax, Intervet Inc., Millsboro, DE; 8.30 mg/kg), 3) Revalor-S (RS, Intervet Inc.; 120 mg of trenbolone acetate and 24 mg of estradiol-17β), or 4) ZH and RS (ZH+RS). There was no main effect or interaction of treatments on the expression of MHC-I mRNA. Error bars are SEM.

an increase ($P < 0.01$) in the expression of MHC-IIX mRNA as a result of the ZH feeding with no apparent effect caused by the steroidal implant.

Companion data of this study including fabrication yield, measures of tenderness, and muscle fiber diameter are published in Kellermeier et al. (2009). These authors reported that the LM of the cattle fed ZH in this trial had greater fiber diameters compared with no ZH. Likewise, RS also caused a significant increase in LM fiber diameter. Similar to many other traits in this study, the combination of the 2 growth promotants was additive for LM fiber diameter. Our current finding describing a shift toward greater mRNA concentrations of MHC-IIX is consistent with the Kellermeier et al. (2009) report of increased fiber diameter caused by ZH feeding.

Within the bovine species, one study attempted to detect the fiber type IIB MHC mRNA, which is the fastest, most glycolytic fiber type, but none was detected in bovine skeletal muscle (Chikuni et al., 2004). This implied that the type IIX MHC mRNA may be the

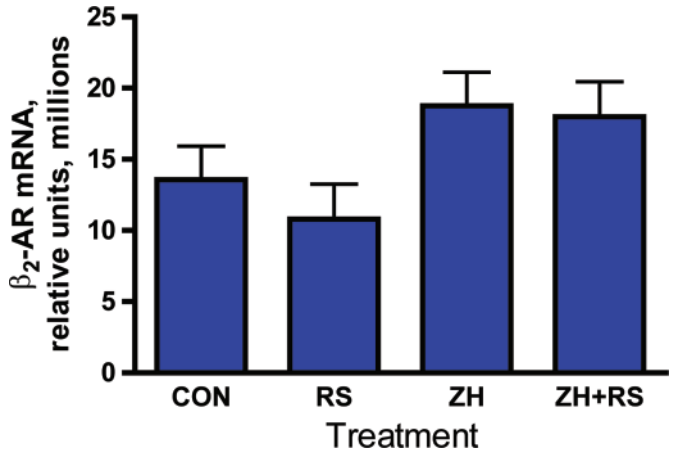

Figure 2. β_2-Adrenergic receptor (β_2-AR) mRNA abundance in bovine semimembranosus muscle collected from feedlot steers 10 min postslaughter. Four animals per pen were used in the analysis (6 pens/treatment). Treatments consisted of 1) control (CON), 2) zilpaterol hydrochloride (ZH; Zilmax, Intervet Inc., Millsboro, DE; 8.30 mg/kg), 3) Revalor-S (RS, Intervet Inc.; 120 mg of trenbolone acetate and 24 mg of estradiol-17β), or 4) ZH and RS (ZH+RS). There was no interaction of treatments on the expression of β_2-AR mRNA. The main effect of ZH administration increased ($P = 0.01$) the expression of β_2-AR mRNA, but there was no effect due to RS administration. Error bars are SEM.

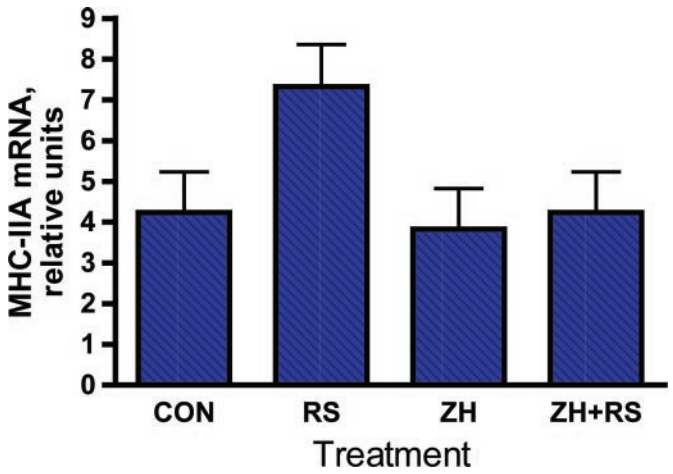

Figure 4. Type IIA myosin heavy chain (MHC-IIA) mRNA abundance in bovine semimembranosus muscle collected from feedlot steers 10 min postslaughter. Four animals per pen were used in the analysis (6 pens/treatment). Treatments consisted of 1) control (CON), 2) zilpaterol hydrochloride (ZH; Zilmax, Intervet Inc., Millsboro, DE; 8.30 mg/kg), 3) Revalor-S (RS, Intervet Inc.; 120 mg of trenbolone acetate and 24 mg of estradiol-17β), or 4) ZH and RS (ZH+RS). There was no interaction of treatment on the expression of MHC-IIA mRNA. There was a tendency for RS to increase ($P = 0.08$) and a tendency for the ZH to decrease ($P = 0.08$) the expression of MHC-IIA mRNA. Error bars are SEM.

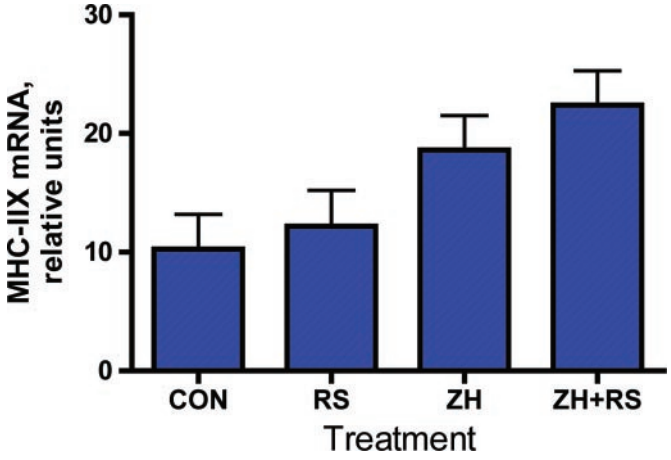

Figure 5. Type IIX myosin heavy chain (MHC-IIX) gene expression in bovine semimembranosus muscle collected from feedlot steers 10 min postslaughter. Four animals per pen were used in the analysis (6 pens/treatment). Treatments consisted of 1) control (CON), 2) zilpaterol hydrochloride (ZH; Zilmax, Intervet Inc., Millsboro, DE; 8.30 mg/kg), 3) Revalor-S (RS, Intervet Inc.; 120 mg of trenbolone acetate and 24 mg of estradiol-17β), or 4) ZH and RS (ZH+RS). There was no interaction of treatment on the expression of MHC-IIX mRNA. Administration of ZH increased ($P < 0.01$) the expression of MHC-IIX mRNA for the ZH and the ZH+RS treatments; however, there was no effect of RS administration. Error bars are SEM.

fastest, most glycolytic myosin fiber type of mRNA expressed in bovine skeletal muscle. Previous studies have shown a decrease in abundance of slower fiber types IIA and IIX MHC protein and an increase in abundance of the fiber type IIB MHC in porcine LM and semitendinosus muscle due to β-AA administration (Depreux et al., 2002). In other studies, rats were administered clenbuterol, and soleus muscle fiber types of MHC showed a transition from a slower, anaerobic to faster, aerobic fiber types (Zeman et al., 1988; Polla et al., 2001). One study showed the transition in muscle fiber types as it relates to increasing physiological maturity in cattle. Another study by Lefaucheur et al. (2004) analyzed differences in myosin isoforms and muscle fiber types in pig breeds that differ dramatically in muscle growth by hypertrophy. This study found Meishan pigs, which have less hypertrophy characteristics, had a greater abundance of MHC-I isoforms and slow oxidative fibers and a decreased abundance of MHC types IIA, IIX, and IIB isoforms and fewer fast oxidative glycolytic and fast glycolytic fiber types when compared with the Large White pigs, which exhibited greater muscle hypertrophy characteristics (Lefaucheur et al., 2004). In another study, cull cows were utilized to measure the effects of TBA and RH on types I and II LM fibers (Gonzalez et al., 2007). Gonzalez et al. (2007) found that TBA and RH increased the cross-sectional area and fiber diameter of type I fibers with the greatest increase measured in the TBA+RH treatment; however, there was no effect on the type II fibers. The lack of effect on the type II fibers could be attributed to not separating the different isoforms of the type II fibers, as well as the effect of the level of physiological maturity on the capacity for muscle hypertrophy (Gonzalez et al., 2007). Research was conducted to analyze the effects of RH on skeletal muscle gene expression in pigs (Gunawan et al., 2007). They found that RH differentially induced expression of the type IIB MHC gene (the fastest, most glycolytic isoform in swine skeletal muscle) at the expense of the other isoforms. The altered expression of mRNA for MHC due to ZH administration suggests that ZH is capable of altering the transcription of various MHC mRNA toward those that may result in larger muscle fibers.

General Conclusion

These results indicate that ZH improved animal performance and increased lean tissue accumulation in finishing steers. When the steroidal implant RS was administered in finishing steers before inclusion of ZH in the diet, there were additive improvements on performance and lean carcass characteristics. The cattle receiving the combination ZH+RS treatment had the greatest increase in ADG and G:F and appear to be additive when compared with the individual treatments with ZH or RS. The ZH+RS treatment also had an additive increase in HCW, LM, and dressing percentage when compared with individual treatments of ZH or RS. Marbling scores, 12th-rib fat depth, and KPH were additively decreased for the ZH+RS treatment when compared with individual treatments of ZH or RS. In addition, there was an increase in the expression of β_2-AR mRNA due to ZH administration. Finally, ZH feeding elicited a differential response in the mRNA abundance of MHC by causing a transition away from slower fiber types and increasing faster fiber types, which could be responsible for larger skeletal muscle fiber diameters in carcasses from cattle fed ZH.

LITERATURE CITED

Avendaño-Reyes, L., V. Torres-Rodríguez, F. J. Meraz-Murillo, C. Pérez-Linares, F. Figueroa-Saavedra, and P. H. Robinson. 2006. Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84:3259–3265.

Bartle, S. J., R. L. Preston, R. E. Brown, and R. J. Grant. 1992. Trenbolone acetate/estradiol combination in feedlot steers: Dose-response and implant carrier effect. J. Anim. Sci. 70:1326–1332.

Beermann, D. H. 2002. Beta-Adrenergic receptor agonist modulation of skeletal muscle growth. J. Anim. Sci. 80:E18–E23.

Birkelo, C. P. 2003. Pharmaceuticals, direct-fed microbials, and enzymes for enhancing growth and feed efficiency of beef. Vet. Clin. Food Anim. 19:599–624.

Chikuni, K., S. Muroya, and I. Nakajima. 2004. Myosin heavy chain isoforms expressed in bovine skeletal muscle. Meat Sci. 67:87–94.

Depreux, F. F. S., A. L. Grant, D. B. Anderson, and D. E. Gerrard. 2002. Paylean alters myosin heavy chain isoform content in pig muscle. J. Anim. Sci. 80:1888–1894.

Elam, N. A., J. T. Vasconcelos, G. Hilton, D. L. VanOverbeke, T. E. Lawrence, T. H. Montgomery, W. T. Nichols, M. N. Streeter, J. P. Hutcheson, D. A. Yates, and M. L. Galyean. 2009. Effect of zilpaterol hydrochloride duration of feeding on perfor-

mance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87:2133–2141.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Fed. Anim. Sci. Soc., Savoy, IL.

Gonzalez, J. M., J. N. Carter, D. D. Johnson, S. E. Ouellette, and S. E. Johnson. 2007. Effect of ractopamine-hydrochloride and trenbolone acetate on longissimus muscle fiber area, diameter, and satellite cell numbers in cull beef cows. J. Anim. Sci. 85:1893–1901.

Guiroy, P. J., L. O. Tedeschi, D. G. Fox, and J. P. Hutcheson. 2002. The effects of implant strategy on finished body weight of beef cattle. J. Anim. Sci. 80:1791–1800.

Gunawan, A. M., B. T. Richert, A. P. Schinckel, A. L. Grant, and D. E. Gerrard. 2007. Ractopamine induces differential gene expression in porcine skeletal muscles. J. Anim. Sci. 85:2115–2124.

Janovick-Guretzky, N. A., H. M. Dann, D. B. Carlson, M. R. Murphy, J. J. Loor, and J. K. Drackley. 2007. Housekeeping gene expression in bovine liver is affected by physiological state, feed intake, and dietary treatment. J. Dairy Sci. 90:2246–2252.

Johnson, B. J., P. T. Anderson, J. C. Meiske, and W. R. Dayton. 1996. Effect of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers. J. Anim. Sci. 74:363–371.

Johnson, B. J., and K. Y. Chung. 2007. Alterations in the physiology of growth of cattle with growth-enhancing compounds. Vet. Clin. Food Anim. 23:321–332.

Johnson, B. J., N. Halstead, M. E. White, M. R. Hathaway, A. DiCostanzo, and W. R. Dayton. 1998. Activation state of muscle satellite cells isolated from steers implanted with a combined trenbolone acetate and estradiol implant. J. Anim. Sci. 76:2779–2786.

Kellermeier, J. D., A. W. Tittor, J. C. Brooks, M. L. Galyean, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, B. J. Johnson, and M. F. Miller. 2009. Effects of zilpaterol hydrochloride with or without an estrogen-trenbolone acetate terminal implant on carcass traits, retail cutout, tenderness, and muscle fiber diameter in finishing steers. J. Anim. Sci. 87:3702–3711.

Lefaucheur, L., D. Milan, P. Ecolan, and C. Le Callennec. 2004. Myosin heavy chain composition of different skeletal muscles in Large White and Meishan pigs. J. Anim. Sci. 82:1931–1941.

Mersmann, H. J. 1998. Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76:160–172.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Pampusch, M. S., B. J. Johnson, M. E. White, M. R. Hathaway, J. D. Dunn, A. T. Waylan, and W. R. Dayton. 2003. Time course of changes in growth factor mRNA levels in muscle of steroid-implanted and nonimplanted steers. J. Anim. Sci. 81:2733–2740.

Plascencia, A., N. Torrentera, and R. A. Zinn. 1999. Influence of the β-agonist, zilpaterol, on growth performance and carcass characteristics for feedlot steers. Proc. West. Sect. Am. Soc. Anim. Sci. 50:331–334.

Platter, W. J., J. D. Tatum, K. E. Belk, J. A. Scanga, and G. C. Smith. 2003. Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability. J. Anim. Sci. 81:984–996.

Polla, B., V. Cappelli, F. Morello, M. A. Pellegrino, F. Boschi, O. Pastoris, and C. Reggiani. 2001. Effects of the β2-agonist clenbuterol on respiratory and limb muscles of weaning rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280:862–869.

Rumsey, T. S., A. C. Hammond, and J. P. McMurtry. 1992. Response to reimplanting beef steers with estradiol benzoate and progesterone: Performance, implant absorption pattern, and thyroxine status. J. Anim. Sci. 70:995–1001.

Salinas-Chavira, J., R. G. Ramirez, M. Domínguez-Muñoz, R. Palomo-Cruz, and V. H. López-Acuña. 2004. Influence of zilpaterol hydrochloride on growth and carcass characteristics of pelibuey lambs. J. Appl. Anim. Res. 26:13–16.

Sillence, M. N., and M. L. Matthews. 1994. Classical and atypical binding sites for β-adrenoceptor ligands and activation of adenylyl cyclase in bovine skeletal muscle and adipose tissue membranes. Br. J. Pharmacol. 111:866–872.

Sissom, E. K., C. D. Reinhardt, J. P. Hutcheson, W. T. Nichols, D. A. Yates, R. S. Swingle, and B. J. Johnson. 2007. Response to ractopamine-HCl in heifers is altered by implant strategy across days on feed. J. Anim. Sci. 85:2125–2132.

Vasconcelos, J. T., R. J. Rathmann, R. R. Reuter, J. Leibovich, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols, and M. L. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim. Sci. 86:2005–2015.

Verhoeckx, K. C. M., R. P. Doornbos, J. Van Der Greef, R. F. Witkamp, and R. J. T. Rodenburg. 2005. Inhibitory effects of the β2-adrenergic receptor agonist ZH on the LPS-induced production of TNF-α in vitro and in vivo. J. Vet. Pharmacol. Ther. 28:531–537.

Winterholler, S. J., G. L. Parsons, C. D. Reinhardt, J. P. Hutcheson, W. T. Nichols, D. A. Yates, R. S. Swingle, and B. J. Johnson. 2007. Response to ractopamine-hydrogen chloride is similar in yearling steers across days on feed. J. Anim. Sci. 85:413–419.

Zeman, R. J., R. Ludemann, T. G. Easton, and J. D. Etlinger. 1988. Slow to fast alterations in skeletal muscle fibers caused by clenbuterol, a β2-receptor agonist. Am. J. Physiol. 254:E724–E732.