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Research Article

ZILPATEROL-HCL (ZH) ALTERS MYOSIN HEAVY CHAIN (MHC) MRNA ABUNDANCE IN FINISHING STEERS

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ABSTRACT
This experiment investigated the effects of zilpaterol-HCl and the steroidal implant Revalor-S (RS; 120 mg trenbolone acetate and 24 mg estradiol-17β) on finishing steer performance and the mRNA concentration of β-adrenergic receptors (β-AR) types I and II, calpastatin, 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 either 0 or 8.3 mg 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 harvest, 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 β2-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 prior to harvest, 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 live and carcass gain in finishing feedlot steers. 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.

Keywords: *Zilpaterol-HCl (ZH), mRNA, Myosin, Steers*

INTRODUCTION
Zilpaterol-HCl (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 sub-types of β-AR (β1, β2, and β3) on most mammalian cells with β2-AR being the most abundant receptor sub- type in bovine skeletal muscle and adipose tissue (Sillence and Matthews, 1994). Zilpaterol-HCl can bind to both the β1-AR and β2-AR, with a greater affinity for β2-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 the combination of trenbolone acetate (TBA) and estradiol-17β (E2) 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 indicates that TBA + E2 implantation increases 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 the feedlot steers in the United States. The purpose of this study was to investigate the effects of ZH administration in combination with a steroidal implant containing TBA and E2 on steer performance and

the mRNA abundance for β1-AR; β2-AR; calpastatin; and myosin heavy chain (MHC) types-I, -IIA, and -IIX.

MATERIALS AND METHODS

The following experiments were a collaboration of Kashan/Tehran Biology Medical center (KBMC). 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).

Animals

English X 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 Kashan/Tehran in soil- surface pens. At approximately d -61, all animals were administered a Component TE-IS (16 mg estradiol and 80 mg TBA; Vetlife Inc., West Des Moines, IA) implant. Prior to 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 EID 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 x 2 factorial design with the main effects of feeding ZH (0 or 8.38 mg/kg; Zilmax; Intervet, Inc., Millsboro, DE) for the last 30 d on feed with a 3 d withdrawal (in accordance with FDA regulations), and a terminal implant of Revalor-S (RS; 120 mg TBA and 24 mg E2; Intervet, Inc., Millsboro, DE) 91 d before harvest 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) or 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 a ground corn-based premix (Table 1). When the study was conducted 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, Indianapolis, IN); therefore, during the ZH feeding period, these feed additives were removed from the diets of the 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 approximately equal distribution of the day's 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 the industry standard, 4% pencil shrink, was used to calculate final BW. All animals were transported approximately 193 km to a commercial abattoir to be harvested.

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 N2 and shipped to Kashan Biology Medical center (KBMC) 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 N2. The samples were homogenized by using a sterile pestal in liquid N2, 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, St. Louis, MO) was added, and the samples were centrifuged for 15 min at 12,000 x g at room temperature. After centrifugation, the upper aqueous phase was removed and transferred to a new microcentrifuge tube. Isopropanol (Sigma, St. Louis, MO) was added, and incubated at room temperature for 5 min. Then the samples were centrifuged for 10 min at 12,000 x 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 -80ºC. 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 260nm. 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 ribosomal RNA (18S). Total RNA (1 µg) was then reverse-transcribed to produce the first-strand complementary DNA (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 β1-AR, β2-AR, calpastatin, MHC-I, MHC-IIA, and MHC-IIB quantitative gene expression relative to the quantity of ribosomal protein S9 (RPS9) in total RNA isolated from muscle tissue. Measurements of the relative quantity of cDNA was performed by using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 900 nM of the appropriate forward and reverse primers, 200 nM of the appropriate TaqMan detection probe, and 1 µL of the cDNA mixture. The bovine-specific β1-AR, β2-AR, calpastatin, MHC-I, MHC-IIA, and MHC-IIB forward and reverse primers and TaqMan detection probes (Table 2) were synthesized by using published GenBank sequences. Custom RPS9 (Genbank Accession No. DT860044) rRNA primers and probes were used as an endogenous control. The ABI Prism 7000 detection system (Applied Biosystems, Foster City, CA) 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 β1-AR, β2-AR, calpastatin, MHC-I, MHC-IIA, and MHC-IIB. The data were expressed as relative units.

Statistical Analysis

Data were analyzed as a 2 x 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 harvest. 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).

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 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 to skeletal protein; however it does have a negative impact on carcass quality grade due to results show that ZH improves performance, and enhances carcass protein accumulation without stimulating DMI, leading to improved efficiency with which dietary nutrients are converted to 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).

It is known that the response of ZH in cattle is mediated through the β-AA binding and activation of β-AR. Zilpaterol hydrochloride functions mainly though the β2-AR which predominates in skeletal muscle

and adipocytes in cattle (Birkelo, 2003). Once the receptors are activated, intracellular actions are mediated through cyclic AMP and subsequent activation or deactivation of key enzymes. The net effect is to direct nutrients in support of enhanced protein synthesis (Birkelo, 2003). It has been demonstrated that β-AA promote triacylglycerol degradation in the adipocyte and block fatty acid synthesis in vitro (Mersmann, 1998). Research conducted in vivo with heifers showed that clenbuterol administration reduced adipocyte cell volume, suggesting the depression in lipid deposition was due to the inhibition of pivotal enzymes necessary for de novo fatty acid biosynthesis (Smith *et al.*, 1987). Our understanding of the primary method with which ZH elicits a biological response supports the results observed in the current study, as well as the concept that ZH acts as a repartitioning agent to disrupt the ability of adipose tissue to utilize nutrients, making more nutrients available for muscle hypertrophy. The cattle receiving the combination ZH+RS treatment had the greatest increase in ADG and G: F that appear to be additive when compared to the individual treatments with ZH or RS. The ZH+RS treatment also had an additive increase in HCW, LM and dressing percentage when compared to individual treatments of ZH or RS. Marbling scores, 12th rib fat depth, and KPH were additively decreased for the ZH+RS treatment when compared to individual treatments of ZH or RS. There is little published research available that compares the effects of implants and ZH in cattle, however, these results are supported by similar studies that found increases in ADG, G: F, HCW, dressing percentage, and LM area; decreases yield grade, 12th rib fat depth, and marbling scores in finishing steers that were administered RS (120 mg TBA and 24 mg E2) 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 TBA and 28 mg estradiol benzoate) 60 d before ZH administration (Avendaño-Reyes *et al.*, 2006). These results support our findings that steroid implant 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 prior to 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.

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 the HKG RPS9 was the most stable relative to the various experimental types of hepatic tissue (Janovick-Guretzky *et al.*, 2007). Following 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 β1-AR, β2-AR, and Calpastatin mRNA Concentrations

The mRNA concentrations of β-AR and calpastatin are shown in Figures 1 through 2.3. There was no effect from any treatments on the expression of β1-AR mRNA. The ZH treatment increased (P = 0.01) mRNA expression of the β2-AR, however there was no other treatment effects on the β2-AR. No significant treatment effects were observed for calpastatin mRNA concentrations. These findings are similar to a study by Sissom *et al.*, (2007) in heifers initially implanted with 80 mg TBA and 8 mg E2, and re-implanted with 200 mg TBA 96 d prior to feeding the β-AA, ractopamine-HCl (RH), for 28 d. These authors reported no change in β1-AR mRNA levels, and a tendency to increase β2-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 β1-AR mRNA levels. However there was a tendency to increase β2-AR mRNA levels (Winterholler *et al.,* 2007). They further determined in vitro that primary bovine muscle cultures, in response to RH, showed an increase ($P < 0.001$) in the β2-AR mRNA level during differentiation, but no effect on β1-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 β2-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 β2-AR was nearly 1000 times greater than the expression of β1-AR, which suggests that the β2-AR is the most abundant β-AR sub-type in the semimembranosus muscle of steers. This corresponds with previous research that determined the β2-AR mRNA is the most abundant mRNA sub-type in semimembranosus of heifers and steers (Sissom *et al.,* 2007; Winterholler *et al.,* 2007) as well as β2-AR being the most abundant receptor in bovine LM and peri-renal adipose tissue (Sillence and Matthews, 1994).

Table 1: Composition and analyzed nutrient content (DM basis) of the finishing diet1

	Treatments[1]	
Item	Control [2]	ZH [3]
Flaked corn	27.55	27.55
High moisture corn	20.00	20.00
Wet corn gluten feed	25.00	25.00
Tallow	2.95	2.95
Corn silage	19.60	19.60
Finisher supplement[4]	3.65	3.65
Control microingredients[5]	1.25	-
ZH microingredients[6]	-	1.25
Nutrient levels, 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-HCl (ZH) administration until harvest*
[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 at 30.86 mg/kg of diet DM and tylosin at 9.70 mg/kg of diet DM*
[6] *Added at the time of diet preparation to provide zilpaterol hydrochloride at 8.38 mg/kg of diet DM*

Effect of ZH and RS on Semimembranosus Muscle Type-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 2. Through 2.6.

Table 2: Sequences for bovine-specific PCR primers and Taqman probes for β1- and β2-adrenergic receptors; calpastatin; types I, IIA, and IIX myosin heavy chain mRNA; and 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
Calpastatin (accession no. X67333)	
Forward	CCCTGGATCAACTTTCTGACAGT
Reverse	TGACTTTATCCTCTACAGGTTTATTCTCA
TaqMan probe	6FAM-TCGGGCAAAGACAGCCTGATCCA-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

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. There was an increase (P < 0.01) in the expression of MHC-IIX mRNA as a result of the ZH treatment, but the RS treatment had no effect. 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 longissimus and semitendinosus muscles, due to β-AA administration, (Dupreux *et al.,* 2002). Within the bovine specie 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 is the fastest, most glycolytic myosin fiber type of mRNA expressed in cattle. For this reason we did not run an analysis on the expression of type IIB MHC mRNA. In another study rats were administered clenbuterol, and soleus

muscle fiber types of MHC showed a transition from a slower, anaerobic to faster, aerobic fiber types (Polla *et al.,* 2001; Zeman *et al.,* 1988).

Table 3: Effects of implanting with Revalor-S (RS) and feeding zilpaterol-HCl (ZH; fed 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 x ZH
Initial BW, 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[4], 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[5]	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
12^{th} 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-HCl (ZH) inclusion in the diet (8.38 mg/kg of feed on a DM basis) for the last 30 days of feed with a 3-d withdrawal
[2] Revalor-S (RS) implantation (120 mg TBA and 24 mg E2) 91 d before harvest
[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 harvest
[4] A 4% shrink was applied to initial and final live weights; dead or removed animals did not contribute to initial or final BW
5300 = Slight; 400 = Small; 500 = Modest.

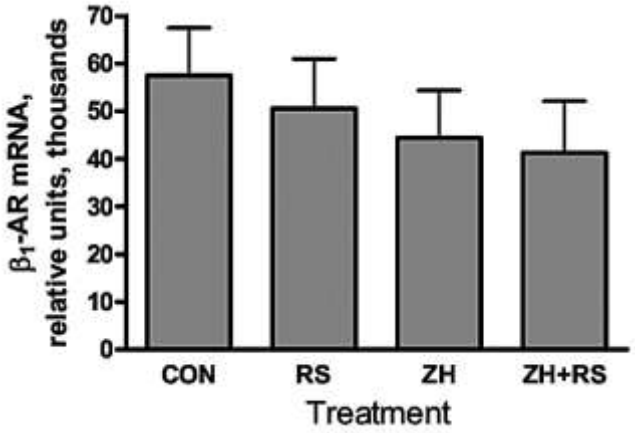

Figure 1: β1-Adrenergic receptor (β1-AR) mRNA abundance in bovine semimembranosus muscle collected from steers 10 min post-slaughter. Four animals per pen were used in the analysis (6 pens/treatment). Treatments consist of 1) control (CON), 2) zilpaterol hydrochloride (ZH; 8.38 mg/kg), 3) Revalor-S (RS; 120 mg TBA and 24 mg E2), or 4) ZH and RS (ZH+RS). There was no main effect or interaction of treatments on the expression of β1-AR mRNA. Error bars are SEM

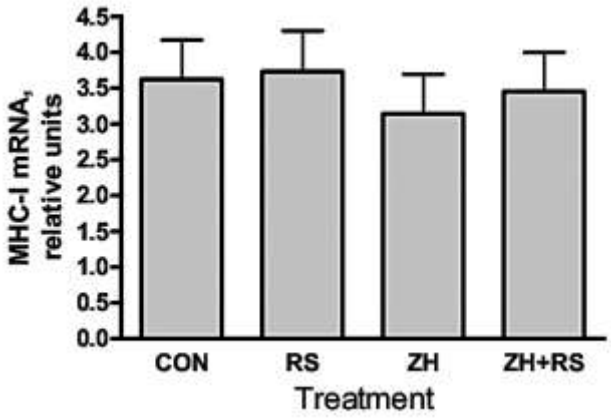

Figure 2: Type I myosin heavy chain (MHC-I) mRNA abundance in bovine semimembranosus muscle collected from feedlot steers 10 min post-slaughter. Four animals per pen were used in the analysis (6 pens/treatment). Treatments consist of 1) control (CON), 2) zilpaterol hydrochloride (ZH; 8.38 mg/kg), 3) Revalor-S (RS; 120 mg TBA and 24 mg E2), 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

One study showed the transition in muscle fiber types as it relates to increasing physiological maturity in cattle. Solomon *et al.,* (1986) used bulls to show that increasing physiological maturity increased slow-twitch-oxidative (SO) fiber followed by a plateau, decreased fast-twitch- oxidative-glycolytic (FOG) fibers, and increased fast-twitch-glycolytic (FG) fibers. Another study by Lefaucheur *et al.,* (2004) analyzed difference 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 lower hypertrophy characteristics, had a greater abundance of MHC-I isoforms and SO fibers and a lower abundance of MHC type -IIA, -IIX, and -IIB isoforms and fewer FOG and FG fiber types when compared to the Large White pigs, which exhibit high muscle hypertrophy characteristics (Lefaucheur *et al.,* 2004). In another study cull cows were utilized to measure the effects of TBA and RH on type -I and -II LM fibers (Gonzalez *et al.,* 2007). They found that TBA and RH increased the cross sectional area (CSA) 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). Another study 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 the translation of more glycolytic, fast twitch fiber type IIX MHC due to ZH administration suggests that ZH is capable of altering protein synthesis; furthermore ZH may decrease the amount of mRNA available for translation of the more oxidative, slower fiber type IIA of MHC.

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 prior to inclusion of ZH in the diet, there were additive improvements on performance and lean carcass characteristics. In addition, there was an increase in the expression of β2-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 a consequence of altered protein synthesis and degradation in bovine skeletal muscle.

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