Effects of duration of zilpaterol hydrochloride and days on the finishing diet on carcass cutability, composition, tenderness, and skeletal muscle gene expression in feedlot steers

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ABSTRACT: Preselected carcasses (n = 112) from feedlot steers fed zilpaterol hydrochloride (ZH; 8.33 mg/kg, DM basis) in a serial slaughter experiment were evaluated to determine the effects of ZH upon carcass cutability, composition, and tenderness. A 4 × 4 factorial arrangement of treatments in a completely random design was used with days on ZH (0, 20, 30, and 40 d before slaughter with a 3-d withdrawal) and days on the finishing diet (DOF; 136, 157, 177, and 198 d). No relevant ZH duration × slaughter group interactions were detected (P > 0.05) for carcass cutability, composition, or tenderness data. Exposure to ZH increased the lean yield of 22 of the 33 subprimals evaluated with every subprimal within the round showing increased cutability (P ≤ 0.04). Carcass fat was decreased, whereas carcass protein and moisture were increased due to ZH (P < 0.01). Lengthening the ZH feeding period did not result in additive gains in subprimal yield or chemical composition (P > 0.05). Warner-Bratzler shear force analysis of the LM indicated that ZH caused a toughening effect (P < 0.01) regardless of the length of the aging period (7, 14, or 21 d). Extending the ZH dose duration caused a linear increase in Warner-Bratzler shear force at 7 (P = 0.06) and 21 d (P < 0.01) of aging. Within 10 min postmortem, samples (n = 48) were collected from the semimembranosus muscle for RNA isolation from 4 randomly selected steers from each treatment within the 157, 177, and 198 d slaughter groups. Feeding ZH did not alter β1- or β2-adrenergic receptor (AR), calpastatin (CAL), IGF-I, or myosin heavy chain (MHC) isoform I mRNA abundance (P > 0.10). There was a ZH duration × DOF interaction (P < 0.01) for the expression of MHC-IIa and -IIX. Expression of MHC-IIa was decreased in every ZH treatment within the 177 and 198 DOF groups (P < 0.02). Expression of MHC-IIx was increased in the 20-d ZH group in the 157 DOF group (P = 0.03), and the 40-d ZH group in the 177 (P = 0.10) and 198 (P = 0.03) DOF groups. There was a tendency for a linear decrease in CAL mRNA abundance as ZH duration increased (P = 0.07), and there was a linear increase in β2-AR (P = 0.03) and CAL (P < 0.01) mRNA abundance as DOF increased. Collectively, the data indicate that ZH may influence net protein turnover by decreasing MHC-IIa mRNA transcription and possibly increasing MHC-IIx. Furthermore, a ZH feeding duration of 20 d appeared to be adequate for capturing lean yield benefits while limiting tenderness losses.

Key words: β-adrenergic agonist, carcass cutout, days on feed, myosin, steer, zilpaterol hydrochloride

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INTRODUCTION

Clenbuterol, cimaterol, L-644,969, ractopamine hydrochloride (RAC), and zilpaterol hydrochloride (ZH) are members of a class of synthetic growth enhancers known as β-adrenergic agonists (β-AA), which have generally been shown to improve feed efficiency, carcass weight gain, and carcass composition in beef cattle (Ricks et al., 1984; Miller et al., 1988; Moloney et al., 1990; Chikhoun et al., 1993; Avendaño-Reyes et al., 2006; Winterholler et al., 2007). Although these compounds vary in the degree of their response, the most consistent biological effect is their resulting increase in skeletal muscle tissue (Johnson, 2004). Cimaterol (Vestergaard et al., 1994) and clenbuterol (Miller et al., 1988) have been documented to cause a hypertrophic increase in muscle fiber diameter. Unfortunately, due to the resulting hypertrophic increase in muscle fiber diameter,
meat tenderness has been shown to be compromised with β-AA usage (Avendano-Reyes et al., 2006; Gruber et al., 2008).

Chung and Johnson (2007) theorize that β-AA do not promote additional recruitment of DNA from satellite cells lying adjacent to muscle cells, but cause the existing nuclei to improve their efficiency through transcriptional activity to increase protein accretion. In swine fed RAC, most evidence points toward a shift in protein synthesis due to the increased expression of specific myosin isoforms, which favor the larger, glycolytic muscle fiber phenotype (Depreux et al., 2002; Gunawan et al., 2007). Current models in cattle explaining ZH gains in protein accretion have yet to be fully elucidated. The objectives of the current study were to determine the effect of ZH feeding duration and days on the finishing diet (DOF) on 1) carcass cutout yield and composition, 2) tenderness, and 3) skeletal muscle gene factors that could be responsible for altering protein turnover, attenuating the ZH growth response with prolonged exposure, or both.

MATERIALS AND METHODS

All procedures involving live animals were approved by the Texas Tech University Animal Care and Use Committee.

Methodology outlining cattle management procedures, design of the experiment, and general feedlot and carcass performance results were described in detail by Vasconcelos et al. (2008). Briefly, 560 steers (British and British × Continental) were randomized across 16 treatments (5 steers/pen; 7 pens/treatment) in a 4 × 4 factorial completely randomized design. The factors were duration of ZH (8.33 mg/kg; Zilmax, Intervet Schering Plough Animal Health, DeSoto, KS) feeding (0, 20, 30, and 40 d) and DOF (137, 157, 177, and 198 d).

Carcass Analyses

Carcass Sampling. Seven steers per treatment (n = 112; one steer per pen) were preselected before duration of ZH treatment application for further carcass cutout analyses from the original 560 steers. Black-hided steers were preselected based upon carcass ultrasound measurements taken by personnel from Cargill Cattle Feeders (Lockney, TX) 1 wk before the initiation of treatment diets for each slaughter group. Within each slaughter group, steers were preselected to be as homogenous as possible for BW, LM area, 12th-rib fat, and percent intramuscular fat across all treatment groups to reduce the initial inherent variation across treatments for carcass composition before initiation of ZH feeding treatments. Following conventional slaughter procedures at the Cargill facility in Friona, TX, the right side of each carcass from each of the preselected cutout steers (n = 112) was transported under refrigeration to the Texas Tech University Gordon W. Davis Meat Science Laboratory, Lubbock, for further carcass cutout fabrication analyses. At 48 h postmortem, the left side of each carcass was followed through the plant fabrication floor. The whole rib section (Institutional Meat Purchase Specifications; IMPS # 107; USDA, 1990) and boneless strip loin (IMPS #180) was obtained, vacuum sealed, and sent to the Texas Tech University Gordon W. Davis Meat Science Laboratory for storage at 2 to 4°C until proximate analyses, and Warner-Bratzler shear force (WBSF) analyses were performed.

Carcass Cutout. On the given production day, scales (model CW-11, Ohaus Corp., Pine Brook, NJ) were calibrated by weighing known weights to ensure accuracy. Cold carcass weight for each side was determined as they entered the fabrication floor. Carcasses were grouped by fours with one carcass from each duration of ZH treatment representing each group. The fabrication order of each carcass was chosen at random within a group to eliminate any potential confounding results based on carcass fabrication order. Each primal was fabricated into subprimal cuts according to the IMPS as outlined by the North American Meat Processors guidelines (NAMP, 2007) and trimmed to fat levels mimicking boxed beef (approximately 7 mm). Fabricated subprimals collected from the chuck and brisket included the shoulder clod (IMPS # 114C), shoulder tender (IMPS # 114F), pectoral meat (IMPS # 115D), chuck roll (IMPS # 116A), chuck tender (IMPS # 116B), boneless whole brisket (IMPS # 120), and boneless short ribs (IMPS # 130). Fabricated subprimals collected from the rib and plate included the blade meat (IMPS # 109B), lip-on ribeye roll (IMPS # 112A), outside skirt (IMPS # 121C), inside skirt (IMPS # 121D), back ribs (IMPS # 124), and deep pectoral. Fabricated subprimals collected from the loin and flank included the boneless strip loin (IMPS #180), flank steak (IMPS # 193), defatted full tenderloin (IMPS # 189A), and cutaneous omobrachialis. Fabricated subprimals collected from the round included the peeled knuckle (IMPS # 167A), top inside round (IMPS # 169), flat outside round (IMPS # 171B), eye of round (IMPS # 171C), heel bottom round (IMPS # 171F), boneless top sirloin butt (IMPS # 184), boneless bottom sirloin flap (IMPS # 185A), boneless bottom sirloin ball tip (IMPS # 185B), boneless bottom sirloin tri-tip (185C), and hind shank meat. Additionally, kidney knob fat, total fat, and total bone, and 90/10, 80/20, and 50/50 trimmings were collected and weighed. Ground beef trim ratios were visually assessed by fabricators simulating industry practices. Subprimal weights were divided by the carcass side weight and multiplied by 100 for expression as a percentage of the cold carcass weight. For validation purposes, the weight of every subprimal and trim was cumulatively added to ensure that weighing errors had not occurred. Total cutout yield ranged from 99.15 to 100.2% for the 112 measured carcasses.
Purge. After carcass cutout data were obtained from the right side of each carcass, the boneless strip loin (IMPS # 180) was labeled, vacuum packaged, and aged for 6 or 7 d at 4°C. Fabrication of carcasses from each slaughter group required 2 consecutive production days; thus, boneless strip loins were vacuum packaged and aged for 6 or 7 d before purge analysis. No bias existed in purge analysis due to aging length because treatment representation was proportional due to the fabrication order described previously. On d 7 postmortem (based on the first fabrication production day), each strip loin was removed from the refrigerator and weighed while still in the package. Each strip loin was removed from the vacuum package, blotted with a towel to remove surface moisture, and weighed to acquire the actual boneless strip loin weight. Each vacuum package was dried in an oven at 90°C for 2 h after which the bag was weighed. Percent purge was calculated as the weight of the boneless strip loin in the vacuum packaging minus the total weight of the dried bag and boneless strip loin divided by the weight of the boneless strip loin in the vacuum packaging and multiplied by 100.

Proximate Analyses. The collected whole rib sections (IMPS # 107) from the left side of the carcass were dissected into 9-10-11th rib sections to estimate carcass chemical composition according to procedures outlined by Hankins and Howe (1946). The 9-10-11th rib sections were homogenized using a Robocoupe Blixer 6V (Robot Coupe USA Inc., Ridgeland, MS). Random subsamples were then exposed to liquid nitrogen and blended into a powder. Each powdered sample was analyzed in triplicate for collagen, protein, fat, and moisture content according to AOAC (1990) techniques. Moisture content was determined by drying a 4-g sample at 100°C for 16 h in a drying oven. Protein content was determined from a 1-g sample using a Leco (model FP-2000, St. Joseph, MO). Fat content was determined by ether extraction on a 4-g sample. Warner-Bratzler Shear Force. At d 7 postmortem, each collected boneless strip loin (IMPS # 180) from the left side of the carcass was cut into 2.54-cm-thick steaks. Three steaks from each boneless strip loin (IMPS # 180) were randomly allotted to be aged for 7 (frozen that day), 14, or 21 d at 2 to 4°C and then frozen at −20°C. At a later date, steaks were thawed for 24 h at 4°C and then cooked on a belt grill to a medium degree of doneness (internal temperature of 68 to 71°C). Steaks were cooled at 4°C for 24 h before shearing. Six 1.3-cm diameter cores were taken from each steak parallel to the orientation of the muscle fibers. Each core was sheared perpendicular to the muscle fiber using a WBSF instrument (GR Electric Mfg., Manhattan, KS). The 6 subsample WBSF values were then averaged for statistical analysis.

Gene Expression Analyses

Muscle sample collection, RNA isolation, determination of RNA integrity, reverse transcription, and realtime quantitative PCR techniques were applied according to procedures outlined by Winterholler et al. (2007).

Muscle Sample Collection. Within 10 min postmortem, 10-g muscle samples (n = 48) were collected from the semimembranosus from 4 steers selected randomly per treatment (not carcasses selected for cutout analyses). Samples were not available from the initial slaughter group (137 DOF). Each muscle sample was immediately snap frozen in liquid N and transported to the Texas Tech University Gordon W. Davis Meat Laboratory at Texas Tech University on dry ice and then stored at −80°C. At a later date, samples were shipped to Kansas State University, Manhattan, on dry ice and again placed in storage at −80°C.

RNA Isolation. Isolation of RNA was completed through several steps. A 200-ng subsample from each sample was placed in a steel mortar bowl with liquid nitrogen and physically crushed using a pestle. After the liquid nitrogen evaporated, 2 mL of TRI reagent (Sigma-Aldrich, #93289, St. Louis, MO) was added to solubilize cellular membranes and thus release intracellular components. After each substance had melted, the aqueous fluid was pipetted off into 1-mL aliquots into two 1.5-mL microcentrifuge tubes and allowed to stand at room temperature for 5 min. Each tube was then combined with 200 µL of chloroform, vortexed for 15 s, and held at room temperature for 5 min. Each tube was centrifuged for 15 min at 12,000 × g at room temperature. Centrifugation isolated the RNA to the upper, clear, aqueous phase of the fluid. This portion was pipetted off into a new microcentrifuge tube and combined with 500 µL of isopropanol. After standing for 5 min at room temperature, each tube was centrifuged at 12,000 × g for 10 min to precipitate the RNA into a pellet. The isopropanol was poured off, 1 mL of 70% ethanol was added, each tube vortexed, and then placed in storage at −80°C until analyzed.

Determination of RNA Integrity. To verify the integrity of the isolated RNA, a 1% agarose-formaldehyde gel was prepared. One microcentrifuge tube per sample containing the isolated RNA was removed from the freezer and centrifuged, placing the RNA pellet at the bottom of the microcentrifuge tube. The supernatant was removed, and the remaining ethanol was allowed to air dry. The RNA pellet was then diluted with 30 µL of nuclease-free water, and a 3-µL subsample was pipetted into a separate 0.5-mL microcentrifuge tube. Two microliters of etidium bromide was added to the RNA subsample and incubated at 65°C for 10 min to dissolve the RNA pellet. Underneath a hood, the prepared 1% agarose-formaldehyde gel was placed in an electrophoresis tray, and a running buffer consisting of 480 mL of deionized water, 60 mL of 3-morpholinopropanesulfonic acid (10×), and 60 mL of formaldehyde were applied to the tray. Finally, 5 µL of sample was loaded into each well, and the gel was run for 1 h at 100 V. Upon completion, 18S and 28S rRNA bands were visualized to validate the integrity of total RNA.
Reverse Transcription. Total RNA concentration for each sample was determined at an absorbance of 260 nm using a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, LLC, Wilmington, DE). Total RNA concentration (1 µg) was standardized across all samples before reverse transcription procedures. Total RNA was reverse transcribed into cDNA using Taqman Reverse Transcription Reagents and MultiScribe Reverse Transcriptase (Applied Biosystems, Foster City, CA). The GeneAmp PCR System 9700 (Applied Biosystems) was set at 25°C for 10 min, 37°C for 60 min, and 95.5°C for 5 min. Random hexamers were used as the primer during cDNA synthesis.

Real-Time Quantitative PCR. The relative abundance of mRNA for each gene of interest was determined using real-time quantitative PCR techniques. The genes evaluated included β1-adrenergic receptor (AR), β2-AR, IGF-I, calpastatin, and myosin heavy chain (MHC) isoform I, IIa, and IIx. The custom forward and reverse primers and probes utilized for the gene of interest and ribosomal protein S9 are outlined in Table 1. Commercia lly available eukaryotic 18S RNA (Applied Biosystems; GenBank, X03205) served as an endogenous control for MHC-I and –IIa. TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM of the appropriate forward and reverse primers, 200 nM of the appropriate detection probe, and 1 µL of cDNA mixture were combined in triplicate on a 96-well plate. Complementary DNA was amplified using an ABI Prism 7000 Sequence Detection System (Applied Biosystems) set at 40 cycles of 95°C for 15 s and 60°C for 1 min. The threshold line was manually set within the geometric phase of the logarithmic chart to determine the cycle threshold value of each targeted gene. Each gene of interest was normalized against the endogenous control listed above, and relative mRNA abundance was reported as an arbitrary value.

Statistical Analyses

All data were initially evaluated for satisfaction of model assumptions. The Kolmogorov-Smirnov test statistic was used to evaluate whether the residuals originated from a normally distributed population. If the normality assumption was violated, then a histogram was plotted. If the histogram also appeared to be skewed, then the data were submitted to a log-transformation. If the variances between treatments were heterogeneous, the Repeated/Group option (group = duration of ZH × DOF) within the MIXED procedure (SAS Inst. Inc., Cary, NC) was utilized to evaluate data. Percentage subprimal yield data, percentage purge, chemical composition, and gene expression data were analyzed as a 4 × 4 (4 × 3 for gene expression factors) completely random design with the MIXED procedure. Frequency distributions of carcases within WBSF tenderness categories (<3.0, ≤4.3, and >4.9 kg) were analyzed as binomial proportions using the GLIMMIX procedure of SAS. One steer was subsampled per pen so that in every analysis, animal was treated as the experimental unit. The duration of ZH, DOF, and the ZH × DOF interaction were treated as fixed effects. Specific orthogonal contrasts were constructed to test (1) control vs. the mean of the 3 ZH-fed groups; (2) linear effects of duration of ZH and DOF; and (3) quadratic effects of duration of ZH and DOF. If appropriate, the LSMEANS/PDIFF option of SAS was used to separate means between simple effects.

Warner-Bratzler shear force data were analyzed as a split-plot with duration of ZH and DOF serving as the factors of interest in the main-plot, and days postmortem was treated as the sub-plot. Initially, the error term in each plot was a summation of the error term for each factor in the plot and their associated interactions. If a Levene’s test indicated that heteroskedasticity existed, then adjustments were made by implementing procedures outlined by Brown and Forsythe (1974) to test model significance. A duration of ZH × days postmortem and a DOF × days postmortem interaction existed in the sub-plot. Consequently, duration of ZH and DOF were each held constant to test the aging effect. When the simple-effect test for each factor level proved significant, then linear and quadratic contrasts were constructed as described above to evaluate the trend of the aging curve. Evaluation of the main-plot factors was performed by holding days postmortem constant and calculating separate error terms for each specific contrast of interest (those described above) using SPSS (Chicago, IL).

RESULTS AND DISCUSSION

Carcass Cutout

With the exception of #171F heel meat (P = 0.01), there were no days on ZH × DOF interactions detected (P > 0.05) for any of the subprimals evaluated for yield. Evaluation of the #171F heel meat simple-effect means indicated that the interaction was based upon the magnitude of LS mean differences and not the direction. Therefore, main-effect LS means and differences were reported for all subprimals including #171F heel meat. Overall, ZH could be characterized as having a strong response on carcass cutout because 22 of the 33 subprimal yields evaluated displayed a positive difference (P < 0.05) between the control group and the ZH treatment groups combined (Table 2). Although ZH manifested its effect within every whole primal region in the carcass, the most consistent ZH effect was seen in the round where ZH increased the percentage yield of every subprimal recorded. More specifically, ZH increased the cutout yield of #167A peeled knuckle, #169 top inside round, #171B bottom round flat, #171C eye of the round, #171F heel meat (P < 0.01), #184 top sirloin butt (P = 0.02), #185A bottom sirloin butt flap (P = 0.04), #185B bottom sirloin butt ball tip (P = 0.02),
Our results are in agreement with Hilton et al. (2009) and Kellermeier et al. (2009) in that carcass cutout yield was most pronounced in the hindquarter of ZH-supplemented cattle. Similar to the current report, ZH increased the yield of #167A knuckle, #169 top round, #171B outside round, #171C eye of the round, #184 top sirloin butt (Hilton et al., 2009; Kellermeier et al., 2009), and #185B bottom sirloin butt (Hilton et al., 2009). In the current study, the chuck recorded yield increases in #114C chuck shoulder clod (P < 0.01), #114F chuck shoulder tender (P = 0.02), #116A chuck roll (P = 0.03), and #116B chuck mock tender (P < 0.01). In comparison, Hilton et al. (2009) and Kellermeier et al. (2009) only observed a ZH yield effect on the #114C chuck shoulder clod and #116B chuck mock tender. Within the rib and plate region, ZH increased the yield of #109B rib blade meat, #121D inside skirt, and pastrami (P < 0.01). Hilton et al. (2009) also observed a yield increase in #109B rib blade meat. Although no change in #112A ribeye roll (P = 0.29) was reported in the current experiment, there was an observed numerical increase in yield and a documented tendency for increased yield by Kellermeier et al. (2009) and Hilton et al. (2009), respectively. Within the loin and flank region, ZH increased the yield of #193 flank steak and #189A defatted full tenderloin (P < 0.01). In agreement with our results, Hilton et al. (2009) saw a ZH increase in #193 flank steak, and Hilton et al. (2009) and Kellermeier et al. (2009) noted an increase in #189A defatted full tenderloin. In contrast to the current study, these authors did see a ZH increase in yield of #180 strip loin. As expected, there was a ZH decrease in the percentage of kidney fat, fat, and bone (P < 0.01). No differences were found in the proportion of 90/10, 80/20, and 50/50 trimmings (P ≥ 0.09). Conflicting reports by Hilton et al. (2009) and Kellermeier et al. (2009) show an increase in 90/10 trimmings. In agreement, these authors also detected a decline in total trimmable fat (Hilton et al., 2009; Kellermeier et al., 2009) and bone (Kellermeier et al., 2009).

Beyond evaluating the ZH effect on carcass yield, contrasts were constructed to determine the potential benefit of increasing the length of the ZH dose duration. A linear increase in yield of #171C eye of the round was observed (P = 0.05) as ZH dose duration increased. A quadratic response was observed for #112A

### Table 1. Sequences of bovine-specific PCR primers and TaqMan probes utilized for determination of mRNA for β1- and β2-adrenergic receptors (β-AR); calpastatin; myosin heavy chain-I, -IIa, and -IIx (MHC); IGF-I; and ribosomal protein S9 (RPS9)

| Item | Sequence (5′ to 3′) |
|------|-------------------|
| β1-AR (accession # AF188187) | Forward GTGGACCGGCTGGGAGTAT<br>Reverse TGACACACAGGGTCTCAATGC<br>TaqMan probe 6FAM-CTCCTTCTGCGAGCTCTGGACCTC-TAMRA |
| β2-AR (accession # NM_174231) | Forward CAGCTCCAGAAGATCGACAAATC<br>Reverse CTGCTCCACTTGACTGACGTTT<br>TaqMan probe 6FAM-AGGGCCGCTTCCCATGCCC-TAMRA |
| Calpastatin (accession # X67333) | Forward CCCCTGATCAACTTCTTCGACG<br>Reverse TGCAGTTCTCCCTACAGGTTTTATTTCT<br>TaqMan probe 6FAM-TCGGGCAAAGACACGCTGACA-TAMRA |
| MHC-I (accession # AB059400) | Forward CCCACTTCCTCCCTGATCCACTAC<br>Reverse TCTCCGGGTACGGATGACTAC<br>TaqMan probe 6FAM-TCTCTTCAAGCCTATCACCATGTCAT-TAMRA |
| MHC-IIa (accession # AB059398) | Forward CCCGGCCCAACTCTT<br>Reverse TCGCAGCTCTCCCAT<br>TaqMan probe 6FAM-TCTCTTCAAGCCTATCACCATGTCAT-TAMRA |
| MHC-IIx (accession # AB059399) | Forward GGCCACCTTTCCTCCCTAT<br>Reverse CGGACGGGGAGGATGAG<br>TaqMan probe 6FAM-TCTCTTCAAGCCTATCACCATGTCAT-TAMRA |
| IGF-I (accession # X15726) | Forward TGTGATTCTCTTGAGCAAGGG<br>Reverse AGCAGGGCCGATAGAAGG<br>TaqMan probe 6FAM-TCTCTTCAAGCCTATCACCATGTCAT-TAMRA |
| RPS9 (accession # DT860044) | Forward GAGCTGAGCCGGACTCC<br>Reverse GGTCGAGGCGGGACTC<br>TaqMan probe 6FAM-TCTCTTCAAGCCTATCACCATGTCAT-TAMRA |

1Primers were verified for their specificity by Winterholler et al. (2008).
2Primers were verified for their specificity by Baxa (2008).
ribeye roll ($P = 0.04$) in which yield increased in the 30-d ZH group but then returned to a similar level in the 40-d ZH group as the 20-d ZH group. Kidney fat also responded quadratically ($P = 0.03$) in that it was most elevated in the 30-d group and the most reduced in the 40-d group. Otherwise there were no detected linear or quadratic responses in yield across the 20, 30, and 40-d ZH dose duration treatments ($P > 0.05$). Results strongly suggest that no additional gains in carcass yield are achieved by feeding ZH longer than 20 d.

Table 3 presents the DOF main effect upon percentage subprimal yield. The DOF strongly influenced carcass cutout yields; 24 of the 33 cuts measured had a linear or quadratic response. Unlike the ZH treatment effect in which the round was most consistently affected, DOF affected every whole primal region relatively equally. Within the round, yield decreased linearly as DOF increased in #169 top inside round ($P < 0.01$), #171C eye of the round ($P = 0.01$), #184 top sirloin butt, #185A sirloin butt flap, and #185C sirloin butt tri-tip ($P < 0.01$). Advancing DOF caused a quadratic response in #171F heel meat in that yield was greatest in the 157-d group and shank meat in that yield was greatest in the 136-d group, least in the 177-d group, and intermediate in the final 198-d group ($P < 0.01$). In the chuck, as DOF increased there was a linear decrease in yield of #115D pectoral meat, #116A chuck roll ($P$

### Table 2. The main effect of duration of zilpaterol hydrochloride (ZH) on the percentage yield of subprimal cuts

| Subprimal2 | Days on ZH3 | SEM4 | Contrast5 |
|------------|------------|------|-----------|
|            | 0  | 20  | 30 | 40 | 0 vs. ZH | L | Q |
| Chuck and brisket | | | | | | | |
| 114C Chuck shoulder clod | 4.98 | 5.27 | 5.17 | 5.35 | 0.07 | <0.01 | 0.42 | 0.12 |
| 114F Chuck shoulder tender | 0.25 | 0.28 | 0.27 | 0.27 | 0.01 | 0.02 | 0.76 | 0.70 |
| 115D Pectoral meat | 0.57 | 0.57 | 0.58 | 0.63 | 0.03 | 0.30 | 0.13 | 0.45 |
| 116A Chuck roll (3 × 4) | 5.33 | 5.55 | 5.45 | 5.58 | 0.08 | 0.03 | 0.81 | 0.21 |
| 116B Chuck mock tender | 0.79 | 0.84 | 0.83 | 0.86 | 0.02 | <0.01 | 0.50 | 0.38 |
| 120 Brisket whole, boneless | 3.28 | 3.45 | 3.37 | 3.41 | 0.06 | 0.08 | 0.60 | 0.43 |
| 130A Chuck short ribs, boneless | 0.99 | 0.99 | 0.98 | 1.04 | 0.02 | 0.67 | 0.16 | 0.25 |
| Rib and plate | | | | | | | |
| 109B Rib blade meat | 1.44 | 1.53 | 1.63 | 1.56 | 0.04 | <0.01 | 0.55 | 0.06 |
| 112A Ribeye roll (2 × 2) | 3.11 | 3.12 | 3.24 | 3.15 | 0.04 | 0.29 | 0.65 | 0.04 |
| 121C Outside skirt | 0.42 | 0.41 | 0.42 | 0.41 | 0.01 | 0.48 | 0.91 | 0.74 |
| 121D Inside skirt | 0.58 | 0.61 | 0.64 | 0.64 | 0.02 | <0.01 | 0.27 | 0.52 |
| 124 Rib back ribs | 1.16 | 1.10 | 1.13 | 1.14 | 0.02 | 0.09 | 0.15 | 0.83 |
| Deep pectoral | 0.40 | 0.47 | 0.46 | 0.44 | 0.02 | <0.01 | 0.27 | 0.88 |
| Loin and flank | | | | | | | |
| 180 Strip loin (0 × 1) | 3.13 | 3.10 | 3.13 | 3.07 | 0.05 | 0.69 | 0.64 | 0.50 |
| 193 Flank steak | 0.49 | 0.52 | 0.53 | 0.53 | 0.01 | <0.01 | 0.68 | 0.82 |
| 189A Full tenderloin | 1.55 | 1.73 | 1.72 | 1.75 | 0.03 | <0.01 | 0.67 | 0.55 |
| Cutaneous omobrachialis | 0.79 | 0.79 | 0.82 | 0.82 | 0.22 | 0.40 | 0.37 | 0.58 |
| Round | | | | | | | |
| 167A Knuckle, peeled | 2.18 | 2.38 | 2.34 | 2.37 | 0.06 | <0.01 | 0.94 | 0.61 |
| 169 Top inside round | 5.42 | 5.71 | 5.81 | 5.84 | 0.09 | <0.01 | 0.29 | 0.73 |
| 171B Bottom round flat | 3.94 | 4.22 | 4.27 | 4.32 | 0.06 | <0.01 | 0.23 | 0.94 |
| 171C Eye of the round | 1.52 | 1.63 | 1.64 | 1.72 | 0.03 | <0.01 | 0.05 | 0.45 |
| 171F Heel meat | 1.17 | 1.27 | 1.25 | 1.27 | 0.02 | <0.01 | 0.86 | 0.43 |
| 184 Top sirloin butt | 2.90 | 2.97 | 3.07 | 3.10 | 0.05 | 0.02 | 0.13 | 0.64 |
| 185A Sirloin butt flap | 0.91 | 0.98 | 0.97 | 0.93 | 0.02 | 0.04 | 0.11 | 0.58 |
| 185B Sirloin butt ball tip | 0.88 | 1.00 | 1.08 | 1.03 | 0.06 | 0.02 | 0.71 | 0.38 |
| 185C Sirloin butt tri-tip | 0.63 | 0.70 | 0.75 | 0.73 | 0.02 | <0.01 | 0.16 | 0.12 |
| Shank meat | 1.33 | 1.41 | 1.41 | 1.41 | 0.03 | 0.01 | 0.99 | 0.89 |
| Trimmings | | | | | | | |
| 50/50 trimmings | 8.88 | 8.43 | 8.63 | 8.56 | 0.24 | 0.13 | 0.71 | 0.68 |
| 80/20 trimmings | 3.94 | 4.35 | 4.20 | 4.38 | 0.19 | 0.09 | 0.90 | 0.48 |
| 90/10 trimmings | 4.36 | 4.81 | 4.70 | 4.62 | 0.23 | 0.21 | 0.58 | 0.98 |
| Kidney fat | 2.88 | 2.55 | 2.74 | 2.42 | 0.11 | <0.01 | 0.45 | 0.03 |
| Fat | 13.15 | 11.53 | 11.17 | 10.73 | 0.49 | <0.01 | 0.25 | 0.94 |
| Bone | 16.60 | 15.71 | 15.61 | 15.91 | 0.23 | <0.01 | 0.54 | 0.46 |

1. Table values are expressed as a percentage of chilled carcass weight.
2. Institutional Meat Purchase Specifications number (USDA, 1990) followed by the common name of the subprimals.
3. Treatment diets were formulated to contain no ZH (0 d) or ZH (8.33 mg/kg, DM basis) for the final 20, 30, or 40 d before slaughter.
4. Standard error of treatment means, n = 28 carcasses/main-effect mean.
5. Observed significance levels of orthogonal contrasts: 0 vs. ZH = control vs. ZH-fed steers; L = linear response of days on ZH; Q = quadratic response of days on ZH. With the exception of 171F Heel meat ($P = 0.01$), no days on ZH × days on feed interactions were detected ($P > 0.05$). Subjective evaluation of the interactive means indicated that the occurrence of the interaction should not limit the reporting of main-effect means.
Chemical Composition

There was not a days on ZH × DOF interaction \((P ≥ 0.29)\) for collagen, protein, fat, and moisture content. Total carcass chemical composition results are displayed in Table 4. Carcasses from cattle fed ZH had a lesser percentage of fat and a greater percentage of protein and moisture \((P < 0.01)\). No linear or quadratic responses were detected as days on ZH increased \((P ≤ 0.29)\). Results from Hilton et al. (2009) and Kellermeier et al. (2009) are in agreement with the current study in that ZH caused the estimated percentage of carcass fat to increase and the percentage of protein and moisture to decrease in steers. In contrast, Leheska et al. (2009) reported that dissection of the entire right side of ZH steer and heifer carcasses showed the carcasses were not different in carcass fat but did contain a greater percentage of protein. Zilpaterol hydrochloride heifers also exhibited an increase in carcass moisture, but steers did not. Dose duration (20 or 40 d on ZH) did not interact with any chemical composition variable (Leheska et al., 2009). Nevertheless, the current study confirms the potency of ZH to increase muscle accretion and consequently to reduce the percentage of carcass fat. Plus, no additional improvements in carcass chemical composition are derived through extending the feeding period of ZH beyond 20 d. Furthermore, the degree of the ZH repartitioning effect is independent of the maturity status of the animal.

The concentration of collagen changed quadratically \((P = 0.04)\) as DOF increased in that collagen density increased from 137 to 175 DOF and then returned to similar levels by 198 DOF. The percentage of fat increased linearly \((P < 0.01)\) as DOF increased. The concentration of protein changed quadratically \((P < 0.01)\) and in cutaneous omobrachialis \((P = 0.04)\) in which yields remained constant until the 198-d group in which yields were reduced. A linear increase occurred \((P = 0.04)\) in kidney fat as DOF increased. A quadratic response \((P = 0.04)\) was noted in #193 flank steak, in which yield greatest in the 136-d group and then remained constant. A quadratic response was also seen in #189A full tenderloin \((P < 0.01)\) and in cutaneous omobrachialis \((P = 0.04)\) in which yields remained constant until the 198-d group in which yields were reduced. A linear increase occurred \((P = 0.04)\) in kidney fat as DOF increased. A quadratic response was seen in 90/10 \((P = 0.05)\) and 80/20 \((P = 0.03)\) trimmings, total trimmable fat \((P = 0.04)\), and bone \((P < 0.01)\). In general, 80/20 trimmings and total trimmable fat were increased and 90/10 trimmings were decreased with increasing DOF. Total bone yield was greatest in the 136-d group, least in the 177-d group, and intermediate in the 157- and 198-d group. Collectively, these compositional findings are consistent with those from Vasconcelos et al. (2008) in that numerically USDA yield grade increased with increased DOF. Van Koevering et al. (1995) noted a linear increase in numerical USDA yield grade brought about by a linear increase in 12th-rib fat thickness and HCW with no statistical increase in LM area as DOF increased. All together, it is logical that in general subprimal yield was continually diminished with increasing DOF.

Warner-Bratzler Shear Force

There was not a days on ZH × DOF interaction \((P ≥ 0.50)\) for WBSF. As shown in Table 4, the control group \((0 \text{ d})\) had less WBSF values at all aging periods \((7, 14, \text{ and } 21 \text{ d})\) than cattle fed ZH \((P < 0.01)\). The toughening effect corresponds with the majority of reports with the feeding of β-AA to cattle (Vestergaard et al., 1994; Avendaño-Reyes et al., 2006; Gruber et al., 2008; Hilton et al., 2009; Kellermeier et al., 2009; Leheska et al., 2009). Among the ZH fed treatments, the 20-d group had a numerically less WBSF value at every postmortem period. At 7-d postmortem, there was a tendency \((P = 0.06)\) for WBSF to linearly increase as ZH dose duration was extended. At 14-d postmortem, no linear \((P = 0.71)\) or quadratic \((P = 0.13)\) responses were observed dependent upon ZH dose length. At 21-d postmortem, there was a linear increase \((P < 0.01)\) in WBSF as ZH dose duration increased. Collectively, the results strongly suggest that ZH causes a toughening effect and that 20 d was the most favorable ZH dosage period of ZH beyond 20 d. Furthermore, the degree of the ZH repartitioning effect is independent of the maturity status of the animal.
A linear aging response was detected for the 0
(P = 0.03), 20, and 30-d ZH treatments (P < 0.01). A quadratic aging response was detected in the 40-d ZH
treatment (P = 0.01), in which WBSF was decreased
by 1.0 kg from 7 to 14 d of aging and then by only 0.15
kg from 14 to 21 d of aging. The results would indicate
that an aging curve exists (a decrease in WBSF as days
postmortem increases) for ZH-treated carcasses. Never-
theless, the magnitude of the decrease in WBSF varied
across treatments. From 7 to 21 d postmortem, there
was a decrease in WBSF in the control steers by only
0.42 kg, whereas the ZH treatments (20, 30, and 40 d)
recorded decreases of 0.89, 1.06, and 1.15 kg, respec-
tively. Although the ZH treatments exhibited acceler-
ated decreases in WBSF with aging vs. the control, as
already established, they were still not able to return to
control WBSF levels by 21 d of aging.

Tenderness has been identified by consumers as the
most important palatability trait (Platter et al., 2003).
Consumers can discern differences between categories
of tenderness based on WBSF and are willing to pay
a premium for improved tenderness (Boleman et al.,
1997). Consequently, several scientists have attempted
to set forth tenderness thresholds that divide steaks
into more or less acceptable categories (Shackelford et
al., 1991; Miller et al., 2001; Vote et al., 2003). Specifi-
cally, Miller et al. (2001) outlined WBSF thresholds by
which consumers deemed New York strip steaks accept-
able from a tenderness standpoint. Steaks recording a
WBSF value <3.0 kg were perceived to be acceptable

Table 3. The main effect of days on the finishing diet (DOF) on the percentage yield of subprimal cuts1

| Subprimal1                           | DOF2 | SEM1  | Contrast3 |
|--------------------------------------|------|-------|-----------|
|                                      | 136  | 157   | 177       | 198       |
| Chuck and brisket                    |      |       |           |           |
| 114C Chuck shoulder clod             | 5.29 | 5.21  | 5.16      | 5.11      |
| 114F Chuck shoulder tender           | 0.26 | 0.27  | 0.27      | 0.28      |
| 115D Pectoral meat                   | 0.62 | 0.63  | 0.54      | 0.55      |
| 116A Chuck roll (3 × 4)              | 5.74 | 5.61  | 5.21      | 5.35      |
| 116B Chuck mock tender               | 0.86 | 0.84  | 0.81      | 0.81      |
| 120 Brisket whole, boneless          | 3.38 | 3.32  | 3.53      | 3.28      |
| 130A Chuck short ribs, boneless      | 1.00 | 0.99  | 1.02      | 0.99      |
| Rib and plate                        |      |       |           |           |
| 109S Rib blade meat                  | 1.46 | 1.49  | 1.61      | 1.60      |
| 112A Ribeye roll (2 × 2)             | 3.07 | 3.20  | 3.24      | 3.10      |
| 121C Outside skirt                   | 0.45 | 0.43  | 0.41      | 0.38      |
| 121D Inside skirt                    | 0.66 | 0.61  | 0.61      | 0.58      |
| 124 Rib back ribs                    | 1.14 | 1.14  | 1.13      | 1.12      |
| Deep pectoral                        | 0.42 | 0.44  | 0.48      | 0.44      |
| Loin and flank                       |      |       |           |           |
| 180 Strip loin (0 × 1)               | 3.26 | 3.06  | 3.11      | 3.00      |
| 193 Flank steak                      | 0.55 | 0.50  | 0.51      | 0.51      |
| 189A Full tenderloin                 | 1.75 | 1.74  | 1.74      | 1.52      |
| Cutaneous omobrachialis              | 0.83 | 0.82  | 0.83      | 0.73      |
| Round                                |      |       |           |           |
| 167A Knuckle, peeled                 | 2.33 | 2.34  | 2.32      | 2.27      |
| 169 Top inside round                 | 5.95 | 5.68  | 5.63      | 5.53      |
| 171B Bottom round flat               | 4.18 | 4.32  | 4.14      | 4.11      |
| 171C Eye of the round                | 1.68 | 1.66  | 1.59      | 1.58      |
| 171F Heel meat                       | 1.14 | 1.35  | 1.26      | 1.20      |
| 184 Top sirloin butt                 | 3.23 | 2.98  | 3.01      | 2.83      |
| 185A Sirloin butt flap               | 0.99 | 0.95  | 0.96      | 0.88      |
| 185B Sirloin butt ball tip           | 1.00 | 0.98  | 0.95      | 0.97      |
| 185C Sirloin butt tri-tip            | 0.75 | 0.71  | 0.70      | 0.65      |
| Shank meat                           | 1.63 | 1.28  | 1.23      | 1.41      |
| Trimmings                            |      |       |           |           |
| 50/50 trimmings                      | 8.51 | 8.51  | 8.55      | 8.93      |
| 80/20 trimmings                      | 3.44 | 4.30  | 4.51      | 4.61      |
| 90/10 trimmings                      | 5.74 | 4.61  | 4.11      | 4.02      |
| Kidney fat                           | 2.42 | 2.72  | 2.70      | 2.73      |
| Fat                                 | 9.10 | 11.45 | 12.84     | 13.19     |
| Bone                                | 17.21| 15.63 | 15.20     | 15.78     |

1Table values are expressed as a percentage of chilled carcass weight.
2Instutional Meat Purchase Specifications number (USDA, 1990) followed by the subprimals common name.
3Steers were finished for a period of 136, 157, 177, and 198 d on the finishing diet before slaughter.
4Standard error of treatment means, n = 28 carcasses/main-effect mean.
5Observed significance levels of orthogonal contrasts: L = linear response of days on zilpaterol hydrochloride (ZH); Q = quadratic response of
days on ZH. With the exception of 171F Heel meat (P = 0.01), no days on ZH × days on feed interactions were detected (P > 0.05). Subjective
evaluation of the interactive means indicated that the occurrence of the interaction should not limit the reporting of main-effect means.
Relative to DOF, WBSF responded quadratically at every aging period \((P < 0.01; \text{Table } 4)\). In general, WBSF was greatest in the 136-d slaughter group; however, evaluation of the interactive means suggested that the nature of the interaction did not appear relevant. Table 4 presents the frequency distribution of carcasses recording a WBSF value ≤4.3 kg by aging period. There was not a days on ZH × DOF interaction \((P \geq 0.32)\) for steaks aged 7 and 14 d. A days on ZH × DOF interaction \((P = 0.01)\) was detected for steaks aged 21 d; however, evaluation of the interactive means suggested that the nature of the interaction did not appear relevant. There was a ZH effect \((P < 0.01)\) across every aging period, in that the control group had a significantly greater proportion of steaks with a WBSF value <3.0 kg. No linear or quadratic responses \((P > 0.39)\) were detected as days on ZH increased. Miller et al. (2001) indicated that steaks with a WBSF value of 4.3 kg were rated as acceptable for tenderness by consumers 86% of the time. Furthermore, Miller et al. (2001) suggested that there was a major transition in the perception of the consumer of tender to tough from 4.3 to 4.9 kg. Figure 3 presents the frequency distribution of carcasses recording a WBSF value ≤4.3 kg by aging period. There was not a days on ZH × DOF interaction \((P \geq 0.11)\) for any aging period. There was a ZH effect \((P < 0.01)\) across every aging period, in that the control group had a significantly greater proportion of steaks with a WBSF value ≤4.3 kg. The proportion of steaks aged 7 d that recorded a WBSF value ≤4.3 kg linearly decreased \((P = 0.03)\) as the ZH feeding duration was extended. Miller et al. (2001) suggested that steaks with a WBSF >4.9 kg had tenderness acceptability ratings of 25%. Figure 4 displays the frequency distribution of carcasses recording a WBSF value >4.9 kg by aging period. There was not a days on ZH × DOF interaction \((P \geq 0.30)\) for steaks aged 7 and 14 d. A days on ZH × DOF interaction \((P = 0.03)\) was detected for steaks aged 21 d; however, evaluation of the interactive means suggested that the nature of the interaction did not appear relevant. There was a ZH effect \((P < 0.01)\) across every aging period, in that the control group had a significantly less proportion of steaks with a WBSF value >4.9 kg. A quadratic response was detected \((P < 0.04)\) as days on ZH increased within the 7 and 14 d aging periods. There was a linear increase \((P = 0.01)\) in the proportion of steaks aged 21 d that recorded a WBSF value >4.9 kg as the ZH feeding duration was extended. In general, the frequency of ZH carcasses recording less desirable WBSF classifications was not mitigated by extending the aging period, but it was improved. Shortening the ZH feeding duration (20 d) appeared advantageous in reducing the proportion of unacceptable steaks in regard to tenderness.

### Table 4. The effect of duration of zilpaterol hydrochloride (ZH) feeding and days on the finishing diet (DOF) on carcass composition, Warner-Bratzler shear force (WBSF), and strip loin purge loss

| Item | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH | Days on ZH |
|------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 9-10-11th rib composition, % | 2.19 | 3.22 | 4.47 | 5.24 | 6.77 | 8.77 | 10.77 | 12.77 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Collagen | 2.22 | 3.32 | 4.47 | 5.24 | 6.77 | 8.77 | 10.77 | 12.77 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Fat | 39.60 | 35.57 | 35.30 | 33.94 | 31.77 | 35.77 | 38.22 | 38.64 | 1.35 | <0.01 | 0.35 | 0.69 | <0.01 |
| Protein | 15.01 | 16.12 | 16.03 | 16.27 | 17.51 | 15.88 | 14.80 | 15.19 | 0.31 | <0.01 | 0.72 | 0.66 | <0.01 |
| Moisture | 45.41 | 48.50 | 48.72 | 49.77 | 51.23 | 48.40 | 46.58 | 46.30 | 0.83 | <0.01 | 0.29 | 0.68 | <0.01 |
| WBSF, kg | 7 d | 2.02 | 3.32 | 4.47 | 5.24 | 6.77 | 8.77 | 10.77 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| 14 d | 3.22 | 4.47 | 5.24 | 6.77 | 8.77 | 10.77 | 12.77 | 14.77 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| 21 d | 4.47 | 5.24 | 6.77 | 8.77 | 10.77 | 12.77 | 14.77 | 16.77 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Purge, % | 1.13 | 1.21 | 1.22 | 1.23 | 1.24 | 1.25 | 1.26 | 1.27 | 0.11 | 0.02 | 0.02 | 0.02 | 0.02 |

1. Treatment diets were formulated to contain no ZH (0 d) or ZH (8.33 mg/kg, DM basis) for the final 20, 30, or 40 d before slaughter.
2. Steers were finished for a period of 157, 177, and 198 d on the finishing diet before slaughter.
3. Standard error of treatment means, n = 28 carcasses/main-effect mean.
4. Observed significance levels of orthogonal contrasts: 0 vs. ZH = control vs. ZH-fed steers; L = linear response of days on ZH or DOF; Q = quadratic response of days on ZH or DOF.
5. Warner-Bratzler shear force estimates of 2.54-cm-thick steaks from the boneless strip loin (IMPS # 180) were assessed after aging for 7, 14, and 21 d postmortem.
6. Purge was calculated as the weight of the strip loin in vacuum packaging minus the total weight of the dried bag and towel dried strip loin divided by the weight of the strip loin in vacuum packaging and multiplied by 100.
was averaged such that the predicted DOF to reach their ideal compositional endpoint for every pen in the experiment was approximately 152 d, leaving the 157-d group 5 d past their predicted endpoint. However, Leheska et al. (2009) noted that attainment of 28% empty body fat was delayed in ZH-fed cattle. Thus, the results from the current study suggest that allowing cattle to achieve an ideal compositional endpoint can have a major impact on reducing WBSF. Carrying steers weeks past their ideal endpoint can result in additive gains in decreasing WBSF, but this result is inconsistent and certainly not of any magnitude when compared with

Figure 1. Effect of days on zilpaterol hydrochloride (ZH) on postmortem changes in Warner-Bratzler shear force (WBSF). Steers were fed ZH (8.33 mg/kg, DM basis) 0, 20, 30, or 40 d before slaughter with a 3-d mandatory withdrawal. After slaughter, a strip loin steak from each carcass was aged for 7, 14, and 21 d. There was a days on ZH × days postmortem interaction (P < 0.01). A linear aging response was detected for the 0- (P = 0.03), 20-, and 30-d ZH treatments (P < 0.01). A quadratic aging response was detected for the 40-d ZH treatment (P = 0.01). The SE of the treatment means (n = 28 carcasses/main-effect mean) equaled 0.23.

Figure 2. The frequency distribution of carcasses recording a Warner-Bratzler shear force (WBSF) value <3.0 kg at each aging period. Steers were fed zilpaterol hydrochloride (ZH; 8.33 mg/kg, DM basis) 0, 20, 30, or 40 d (see legend) before slaughter with a 3-d mandatory withdrawal. After slaughter, a strip loin steak from each carcass was aged for 7, 14, and 21 d. Miller et al. (2001) indicated that consumer tenderness acceptability was 100% from New York strip steaks with WBSF values <3.0 kg. There was not a days on ZH × days on the finishing diet (DOF) interaction (P ≥ 0.32) for steaks aged 7 and 14 d. A days on ZH × DOF interaction (P = 0.01) was detected for steaks aged 21 d; however, evaluation of the interactive means suggested that the nature of the interaction did not appear relevant. There was a ZH effect (P < 0.01) across every aging period, in that the control group had a significantly greater proportion of steaks with a WBSF value <3.0 kg compared with the ZH treatments. No linear or quadratic responses (P > 0.39) were detected as days on ZH increased within each aging period. The SE of the treatment means (n = 28 carcasses/main-effect mean) equaled 5.36, 5.83, and 5.74 for 7, 14, and 21 d postmortem, respectively.
Figure 3. The frequency distribution of carcasses recording a Warner-Bratzler shear force (WBSF) value ≤4.3 kg at each aging period. Steers were fed zilpaterol hydrochloride (ZH; 8.33 mg/kg, DM basis) 0, 20, 30, or 40 d before slaughter with a 3-d mandatory withdrawal. After slaughter, a strip loin steak from each carcass was aged for 7, 14, and 21 d. Miller et al. (2001) indicated that consumer tenderness acceptability was 86% from New York strip steaks with WBSF values equal to 4.3 kg. There was not a days on ZH × days on the finishing diet (DOF) interaction ($P \geq 0.11$) for any aging period. There was a ZH effect ($P < 0.01$) across every aging period, in that the control group had a significantly greater proportion of steaks with a WBSF value ≤4.3 kg. The proportion of steaks aged 7 d that recorded a WBSF value ≤4.3 kg linearly decreased ($P = 0.03$) as the ZH feeding duration was extended. The SE of the treatment means (n = 28 carcasses/main-effect mean) equaled 7.65, 7.91, and 7.36 for 7, 14, and 21 d postmortem, respectively.

Figure 4. The frequency distribution of carcasses recording a Warner-Bratzler shear force (WBSF) value >4.9 kg at each aging period. Steers were fed zilpaterol hydrochloride (ZH; 8.33 mg/kg, DM basis) 0, 20, 30, or 40 d before slaughter with a 3-d mandatory withdrawal. After slaughter, a strip loin steak from each carcass was aged for 7, 14, and 21 d. Miller et al. (2001) indicated that consumer tenderness acceptability was 25% from New York strip steaks with WBSF values >4.9 kg. There was not a days on ZH × days on the finishing diet (DOF) interaction ($P \geq 0.30$) for steaks aged 7 and 14 d. A days on ZH × DOF interaction ($P = 0.03$) was detected for steaks aged 21 d; however, evaluation of the interactive means suggested that the nature of the interaction did not appear relevant. There was a ZH effect ($P < 0.01$) across every aging period, in that the control group had a significantly less proportion of steaks with a WBSF value >4.9 kg. A quadratic response was detected ($P \leq 0.04$) as days on ZH increased within the 7- and 14-d aging periods. There was a linear increase ($P = 0.01$) in the proportion of steaks aged 21 d that recorded a WBSF value >4.9 kg as the ZH feeding duration was extended. The SE of the treatment means (n = 28 carcasses/main-effect mean) equaled 8.31, 8.05, and 6.27 for 7, 14, and 21 d postmortem, respectively.
the benefits of taking cattle to their ideal endpoint. This conclusion would agree with Zinn et al. (1970). The authors evaluated LM WBSF in cattle fed for 0 to 270 d and slaughtered every 30 d. Cattle fed for 150, 180, and 210 d were more tender than at all other periods. Likewise, May et al. (1992) noted a quadratic effect of DOF upon shear force, in that tenderness was optimized at a certain length of time on feed (112 d in their experiment) and that shear values were greatest before this optimization and worse thereafter but at a lesser degree than the preoptimization period. Van Kovering et al. (1995) noted a tendency \( (P = 0.07) \) for a linear decrease in WBSF as DOF increased.

The repeated measures aspect of the experimental design allowed evaluation of the utility of aging upon WBSF given differences in DOF. There was a DOF × days postmortem interaction \( (P < 0.01) \). As shown in Figure 5, there was a linear aging response \( (P < 0.01) \) noted for every DOF group, such that increasing the aging period linearly decreased WBSF. Although variable, the trend in the range in WBSF decrease from 7 to 21 d postmortem was seemingly irrelevant.

**Purge**

No days on ZH × DOF interaction existed \( (P = 0.64) \) for percentage purge. When compared with the control, there was a tendency \( (P = 0.10) \) for increased purge in ZH-treated cattle (Table 4). There was not a linear \( (P = 0.98) \) or quadratic \( (P = 0.20) \) response noted for changes in purge loss as days on ZH increased. Percentage purge increased linearly \( (P < 0.01) \) as cattle spent a greater number of DOF. Heifers fed RAC did not show changes in purge loss after a 7-d retail case display (Quinn et al., 2008). Carr et al. (2005) indicated that purge loss was increased in enhanced pork loins but decreased in nonenhanced pork loins with 20 mg/kg of RAC. Kellermeier et al. (2009) indicated that cattle fed ZH had increased purge loss vs. control cattle regardless of whether both groups had received a terminal implant or not. Given the transition in chemical composition resulting in an increased percentage of moisture, it is logical that an increase in purge loss may occur when feeding a \( \beta \)-AA such as ZH.

**Semimembranosus Muscle Gene Expression**

There was not a ZH effect \( (P = 0.34) \) or duration of ZH feeding effect \( (P = 0.17) \) upon \( \beta_1 \)-AR mRNA concentration (data not shown). No ZH effect existed \( (P = 0.65) \) upon \( \beta_2 \)-AR mRNA abundance, but it did respond quadratically \( (P = 0.04) \) to ZH dose duration in that \( \beta_2 \)-AR mRNA levels were intermediate at 20 d, least at 30 d, and greatest at 40 d. Other reports of the influence of \( \beta \)-AA on \( \beta \)-AR expression are conflicting. Sissom et al. (2006) and Winterholler et al. (2007) observed a tendency for the \( \beta \)-AA RAC to increase \( \beta_2 \)-AR mRNA and not affect \( \beta_1 \)-AR mRNA abundance in semimembranosus muscle of beef steers and heifers, respectively. In another study, Winterholler et al. (2008) reported that RAC tended to increase \( \beta_1 \)-AR mRNA \( (P = 0.09) \) and had no effect on \( \beta_2 \)-AR mRNA in LM tissue from beef steers. Walker et al. (2007) reported a decrease in \( \beta_1 \)- and \( \beta_2 \)-AR expression in LM tissue from Holstein steers. Baxa (2008) reported that ZH did not change \( \beta_1 \)-AR expression but increased \( \beta_2 \)-AR expression. Cultured bovine myoblast cells exposed to ZH exhibited a decrease in \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-AR mRNA.
abundance and a decrease in β2-AR protein content (Sissom et al., 2007). Nevertheless, these pharmacological results may not be indicative of the physiological response in the animal.

Days on feed did not affect \( P > 0.10 \) the abundance of β1-AR mRNA (data not shown). There was a linear increase \( (P = 0.03) \) in β2-AR mRNA as DOF increased (Figure 6). Similarly, Winterholler et al. (2007) demonstrated the same effect on β2-AR mRNA with advancing days on feed and advancing time in culture in an in vitro model. In contrast to our data, β1-AR mRNA decreased with increased days on feed (Winterholler et al., 2007). Nevertheless, the β2-AR is the most densely populated β-AR subtype on bovine skeletal muscle cells (Silence and Matthews, 1994). Certainly, this fact along with the observed increase in β2-AR with advancing maturity would be favorable for a β-AA with a greater affinity for the β2-AR subtype.

The results would indicate that the dampening of the feedlot and carcass performance response beyond 20 d of ZH feeding reported by Vasconcelos et al. (2008) is not due to a downregulation of the β-AR. Therefore, the performance attenuation observed from prolonged ZH exposure could be due to a desensitization or internalization of the β-AR (Benovic, 2002). Nevertheless, the responsiveness loss may not be related to alterations in β-AR density or receptivity at all. Chung and Johnson (2007) suggested that because β-AA do not activate DNA incorporation from adjacent satellite cells, increases in protein synthesis rates cannot be sustained with prolonged β-AA exposure. Further research needs to be conducted to verify these theories.

No ZH effect \( (P = 0.56) \) or duration of ZH feeding effect \( (P = 0.32) \) on IGF-I mRNA abundance was seen (data not shown). Insulin growth factor-I responded quadratically \( (P = 0.01) \) to DOF in that levels were similar at 157 and 198 d and greater at 177 d. In agreement with the ZH-related results, Parsons et al. (2007) did not detect an influence of the β-AA RAC upon IGF-I mRNA levels in LM or circulating IGF-I plasma concentrations in feedlot cull cows. Walker et al. (2007) observed a decrease in IGF-I mRNA in RAC-fed Holstein steers and Sissom et al. (2007) observed a decrease in IGF-I mRNA in RAC-fed beef heifers implanted with Finaplix-H (Intervet Schering Plough Animal Health). Byrem et al. (1996) demonstrated that the β-AA cimaterol elicited an effect on protein metabolism in the hindlimb of young steers via a close arterial infusion. Collectively, it would appear that this evidence would support the belief that β-AA mediate their effect primarily, and perhaps solely, through direct modulation of the β-AR and not via the endocrine or neural regulatory axes. The direct effect would be the opposite of the indirect effect of anabolic steroids. Pampusch et al. (2003) reported that IGF-I mRNA levels in LM and circulating IGF-I concentrations were greater in Revalor-S (Intervet Schering Plough Animal Health) implanted steers vs. nonimplanted steers. The difference in the mechanistic action behind each of these growth enhancing compounds would aid in explanation of why when utilized together, ZH and estradiol-trenbolone acetate cause additive gains in muscle accretion compared with their use alone (Casey et al., 1997).

There was not a ZH effect upon calpastatin mRNA abundance \( (P = 0.41) \) (data not shown). However, in the current study there was a tendency for calpastatin expression to linearly decrease as days on ZH increased \( (P = 0.07) \); Figure 7). As DOF increased there was a linear increase in calpastatin mRNA levels \( (P < 0.01) \); Figure 7). In contrast to our results, increases in calpastatin, an ante- and postmortem inhibitory agent of proteases, have been shown to be partly responsible for increases in net protein turnover with some β-AA. Kretchmar et al. (1990) and Koohmaraie et al. (1991) reported that lambs fed L-644,969 demonstrated an increase in

![Figure 6. β2-adrenergic receptor (β2-AR) and calpastatin mRNA relative abundance in bovine semimembranosus muscle collected from feedlot steers (n = 48) 10 min postmortem. Steers were on the finishing diet for 157, 177, and 198 d. Total RNA was isolated from skeletal muscle tissue, and mRNA concentration was evaluated using real-time quantitative PCR. Panel A displays the effect of days on the finishing diet on β2-AR mRNA relative abundance. As days on the finishing diet increased, the relative concentration of β2-AR mRNA increased linearly \( (P = 0.03) \). The SE of the treatment means \( (n = 16 \text{ steers/main-effect mean}) \) equaled 26.86. Panel B displays the effect of days on the finishing diet on calpastatin mRNA relative abundance. As days on the finishing diet increased, the relative concentration of calpastatin mRNA increased linearly \( (P < 0.01) \). The SE of the treatment means \( (n = 16 \text{ steers/main-effect mean}) \) is represented by the upward error bars in each panel.](image)
Skeletal muscle is comprised of slow-contracting, characteristics or phenotype of muscle fibers (Johnson, 2004). Skeletal muscle is comprised of slow-contracting, oxidative fibers (type I) and larger, fast-contracting, glycolytic fibers (type II). Depreux et al. (2002) reported that in swine RAC reduced the proportion of MHC-I, -Ia, and -IIax and increased the proportion of IIx in a time- and dose-dependent manner. Gunawan et al. (2007) reported that RAC differentially induces the expression of MHC-IIb (similar to MHC-IIx in cattle) at the expense of other MHC isoforms. In cattle, Miller et al. (1988) found that clenbuterol caused an increase in type II fiber diameter and a decrease in type I fiber diameter compared with untreated heifers. Vestergaard et al. (1994) reported a marked conversion in the proportion of type IIa to type IIb fibers with a pronounced hypertrophy of type IIb fibers and no change in type Ia fibers in cimaterol-administered bulls. Likewise, feeding ZH at a rate of 0.1 mg/kg of BW/d increased the diameter of type I, Ia, and IIb LM muscle fibers while simultaneously decreasing the proportion of type I fibers and increasing the proportion of type IIb fibers (Intervet Study Report, 1995). Feeding ZH at a rate of 0.2 mg/kg of BW/d type increased type Ia and IIb LM fiber diameter, while decreasing the proportion of type Ia fibers and increasing the proportion of type IIb fibers (Intervet Study Report, 1995). Baxa (2008) reported that ZH had a tendency ($P = 0.08$) to decrease MHC-IIa expression and increase ($P < 0.01$) MHC-IIx expression. Collectively, a shift in the transcriptional activity of the MHC isoform (MHC-IIa) indicative of an intermediate muscle fiber type to the MHC isoform (MHC-IIx) responsible for an increased synthesis of proteins in fast glycolytic muscle fibers appears to be the basis for increasing protein accretion in ZH-fed cattle. The current study does not conclusively illustrate this transformation but, combined with past research, contributes to this claim.

Results from the current study supply important conclusions about extending the length of the ZH feeding period and the number of days steers spend on the finishing diet. Feeding cattle to a perceived ideal compositional endpoint is critical for optimizing tenderness in beef, yet pushing cattle beyond this point offers no additional gains while continually compromising sub-primal yields. Apparently, advancing DOF increases the transcriptional activity of $β$-AR and calpastatin. Functionally, the increase in $β$-AR density could only be favorable for a $β$-AA such as ZH. Cattle can be fed ZH without concern for where they are in their feeding period, and a similar degree of ZH carcass yield and composition response can still be expected. It is clear that feeding ZH for only 20 d maximized carcass cutout yield while limiting the ZH toughening effect. Attenuation of the ZH growth response with prolonged feeding is not suppressed by a decrease in $β$-AR transcription. Many factors could be responsible for the increase in net protein turnover gain achieved by ZH, but increased transcription of calpastatin does not appear to be the primary cause and an increased shift toward the transcription of MHC-IIx seems most likely. Continuous investigation into the cellular actions of ZH can...
Figure 8. Myosin heavy chain isoform I (MHC-I), IIa (MHC-IIa), and IIx (MHC-IIx) mRNA relative abundance in bovine semimembranous muscle collected from feedlot steers (n = 48) 10 min postmortem. Steers were on the finishing diet for 157, 177, and 198 d and fed zilpaterol hydrochloride (ZH) for 0, 20, 30, and 40 d. Total RNA was isolated from skeletal muscle tissue, and mRNA concentration was evaluated using real-time quantitative PCR. Panel A displays the simple effect of days on feed (DOF) and days fed ZH on MHC-I mRNA relative abundance. There was no days on feed x days on ZH interaction (P = 0.44). There was no ZH effect (P = 0.96), ZH dose duration effect (P = 0.10), or DOF effect (P = 0.22) on MHC-I mRNA abundance. Simple effect data for MHC-I are presented for descriptive purposes only. Panel B displays the simple effects of days on feed and days fed ZH on MHC-IIa mRNA relative abundance. There was a days on feed x days on ZH interaction (P = 0.05). Within a days on feed group, bars not bearing a common letter differ (a,b,c; P < 0.02) or have a tendency to differ (d,e; P = 0.10). The SE of the treatment means (n = 4 steers/simple-effect mean) is represented by the upward error bar in each panel.

only help aid in development of intervention strategies to minimize its influence on WBSF and still capturing the pronounced positive impact of ZH on skeletal muscle growth.

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