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# Impact of a leptin single nucleotide polymorphism and zilpaterol hydrochloride on growth and carcass characteristics in finishing steers

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**ABSTRACT:** A total of 4,178 steers (mean initial BW = 403.9 ± 16.04 kg) were used to test the interactive effects, if any, of leptin R25C genotypes (CC, CT, or TT) and zilpaterol hydrochloride (ZH) feeding duration on growth performance and carcass traits. Steers were blocked by arrival at the feed yard, genotyped for the leptin SNP, allotted to genotype-specific pens (90 steers/pen), and assigned randomly within genotype and block to 0 or 21 d of dietary ZH. All pens within a block were slaughtered on the same day (132.1 ± 10.9 d on feed). Final BW of steers fed ZH was 6.0 kg heavier ( $P = 0.008$ ), and ZH-fed steers had greater ( $P = 0.003$ ) ADG than steers not fed ZH. Feeding ZH decreased DMI in steers with increased frequency of the T allele (9.67, 9.53, and 9.28 kg/d for CC, CT, and TT, respectively), but DMI increased with the frequency of the T allele (9.68, 9.90, and 10.1 kg for CC, CT, and TT, respectively) when ZH was not fed (leptin genotype × ZH,  $P = 0.011$ ). At the conclusion of the study, ultrasonic fat was greatest for TT steers (11.4 ± 0.28 mm) and least ( $P = 0.003$ ) for CC steers (11.0 ± 0.25

mm). Regardless of ZH-feeding duration, TT steers produced a greater ( $P = 0.006$ ) percentage of USDA yield grade (YG) 4 or higher carcasses (5.4 vs. 2.7%) and a lesser ( $P = 0.006$ ) percentage of YG 1 carcasses (17.7 vs. 26.8%) than CC steers. In addition, ZH-fed steers produced a greater ( $P < 0.001$ ) percentage of USDA YG 1 carcasses (25.9 vs. 16.2%) and a lesser ( $P < 0.001$ ) percentage of YG 4 or higher carcasses (1.6 vs. 6.0%) than steers fed the control diet. Marbling scores and the percentage of carcasses grading USDA Choice and Prime were greater in TT than CC steers when fed diets devoid of ZH, but both marbling and quality grades did not differ among leptin genotypes when fed ZH for 21 d (leptin genotype × ZH,  $P \leq 0.03$ ). The amount of HCW gain tended to be less ( $P = 0.095$ ) for steers of the TT genotype (12.7 kg) than either CC (16.3 kg) or CT (17.0 kg) genotypes. Results indicated that leptin R25C genotype impacted most traits associated with fatness whereas feeding ZH for 21 d affected HCW and ADG positively but impacted feed intake, marbling, and USDA quality grades negatively.

**Key words:** feedlot cattle, leptin single nucleotide polymorphism, quality grades, yield grades, zilpaterol hydrochloride

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## INTRODUCTION

A cytosine (C) to thymine (T) transitional SNP located in exon 2 of the obese gene (*LEP*), which codes for the hormone leptin, is associated with fatness (Buchanan et al., 2002) and carcass yield grade in feedlot

cattle (Kononoff et al., 2005). This functional SNP results from an arginine to cysteine substitution and is commonly abbreviated as LEP R25C. The LEP R25C SNP is believed to impact steer fatness by affecting the function of the leptin molecule.

The beef industry uses  $\beta$ -adrenergic agonists ( $\beta$ -AA) to promote lean tissue growth. These compounds shift nutrient use away from adipose tissue and toward carcass lean tissue (Etherton and Smith, 1991). Two  $\beta$ -AA approved for use in the U.S. feedlot industry are ractopamine hydrochloride (Optaflexx; Elanco Animal Health,

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Greenfield, IN) and zilpaterol hydrochloride (**ZH**; Zilmax; Merck Animal Health, De Soto, KS). Although the mechanism of action is not fully elucidated, when  $\beta$ -AA are administered orally to feedlot cattle, an increase in live BW gain is usually observed (Montgomery et al., 2009a). This increase in lean muscle tissue mass is usually coupled with a decrease in carcass fat (Vasconcelos et al., 2008) because the action of  $\beta$ -AA is to decrease lipogenesis and increase lipolysis (Mersmann, 1998). Although an array of hormones is believed to be responsible for these effects, these metabolic modulations are at least, in part, achieved through the action of leptin. Leptin is known to affect fatty acid oxidation through 5' adenosine monophosphate-activated protein kinase-activated protein kinase (**AMPK**; Minokoshi et al., 2002), a pathway also known to be affected by  $\beta$ -AA; so, the extent of carcass fatness of beef cattle may depend on the extent to which leptin and ZH interact. Therefore, the objective of this experiment was to determine the interactive effect, if any, of LEP R25C genotype and ZH on the growth performance and carcass characteristics of feedlot cattle.

## MATERIALS AND METHODS

### *Steers Experimental Design and Measures*

All experimental protocols and procedures were in compliance with Federation of Animal Science Societies (FASS, 2010) guidelines for the care and use of cattle in agricultural research. In a randomized complete block design, a total of 4,178 British  $\times$  continental crossbred steers (initial BW of  $403.9 \pm 16.04$  kg) were blocked by arrival at the feedyard (Cactus Research, Cactus, TX) into 8 blocks, each consisting of 6 contiguous pens. To fill each block,  $900 \pm 100$  steers were procured from a number of commercial sources and subsequently transported to the feedyard. The treatment structure was a  $3 \times 2$  factorial, consisting of 3 leptin R25C genotypes (CC, CT, or TT) and dietary inclusion of ZH for 0 (**0-ZH**) or 21 d (**21-ZH**). Within a few days after arrival at the feedyard, each steer was uniquely identified with a serial number ear tag, vaccinated with a modified-live viral vaccine (Vista 3; Merck Animal Health), implanted with Revalor-S (120 mg trenbolone acetate + 24 mg estradiol; Merck Animal Health), treated for parasites with 1% Ivomec (Merial Animal Health), and had an ear tissue sample taken for LEP R25C SNP genotype determination. Once leptin genotype was ascertained, genotypes were merged with their respective individual steer identification numbers in an Excel (Microsoft Corp., Redmond, WA) worksheet and sorted into CC, CT, and TT subgroups. Within each of the 8 arrival blocks, there were 2 pens of steers (90 steers/pen) for each leptin genotype, and within arrival blocks and genotype, pens were assigned randomly to either 0 or 21 d of ZH feeding before slaughter. Yet, for

1 block, the available number of TT genotypes restricted the number and was only 166 (83 steers/pen). Following the spreadsheet randomization process, treatments were assigned randomly to pens within a block, and steers were physically sorted into their respective treatment groups based on their individual steer identification number and the corresponding treatment assignment.

At the time of the physical sort, each steer was weighed individually and given a pen identification tag, and fat thickness was measured ultrasonically. Before feeding the following morning, each pen was group weighed on a platform scale to establish 0-d BW for performance calculations. At the initiation of the study, cattle were fed a diet that contained 60% concentrate and gradually transitioned to the finishing diet within 40 d of the start of the experiment. The finishing diet contained (DM basis) 71.6% steam flaked corn, 8.5% corn dried distillers grains and solubles, 10.1% corn silage, 4.2% beet molasses blend, 1.6% animal fat, and 4.0% mineral and vitamin supplement. This diet is typical of those fed in high plains commercial feedlots and was formulated to meet or exceed nutrient requirements of growing–finishing steers (NRC, 1996). Diets were also formulated, on a DM basis, to contain 33.3 mg/kg Rumensin (Elanco Animal Health) and 11.0 mg/kg Tylan (Elanco Animal Health). During the last 21 d before slaughter, half of the steers were fed 8.3 mg/kg of ZH. The quantity of feed delivered and removed from feedbunks was recorded daily to calculate DMI and G:F.

Fat thickness was measured ultrasonically using a diagnostic ultrasound unit (SSD-500V; Aloka Co., Ltd., Tokyo, Japan) equipped with a 20-cm, 3.5-MHz linear array transducer (Aloka Co., Ltd.). Given the nature of the study, these measures could not be taken on exactly the same number of days on the experiment for each steer, but all measures within block were collected on the same day (d 0,  $65 \pm 2.9$ ,  $105 \pm 9.4$ , and  $132 \pm 10.0$ ). Ultrasound fat thickness was measured as described by Perkins et al. (1992). The Beef Imaging Analysis (**BIA**; Designer Genes, Harrison, AR) software program (version 2.1.4) was used to measure 12th rib fat thickness for each image, and the technician either accepted or rejected the calculated thickness of fat. Each steer's fat thickness was recorded on the hard drive and exported to an Excel spreadsheet.

All treatments within a block were slaughtered on the same day ( $132.1 \pm 10.9$  d on feed) at a USDA-Food Safety and Inspection Service-inspected facility (Tyson Foods, Inc., Amarillo, TX). After a 36-h chill, carcass quality, and yield grade data (USDA, 1997) were collected by West Texas A&M University (Canyon, TX) personnel, and USDA quality grades (**QG**) and yield grades (**YG**) were assigned by USDA graders. In addition, HCW, 12th rib fat thickness, LM area, and KPH were used to calculate YG for each carcass. Marbling, 12th rib fat depth, LM area, and KPH data (USDA, 1997)

were also recorded for each carcass. Empty body weight (**EBW**) and the percentage of EBW that was fat (**%EBF**) were also calculated as outlined by Tedeschi et al. (2004). Hot carcass weight gain was calculated as HCW – (initial BW × 0.58).

### Steer Genotyping

A modified Y-Text ear tagger (Y-Text Corp, Cody, WY) was used to capture a 2 mm- diameter ear tissue biopsy into a tissue/DNA collection tag (Quantum Genetix Canada Inc, Saskatoon, SK, Canada). Samples were stored at –20°C until the time of extraction. Each collection tag was cut open, tissue was removed and placed in individually labeled 1.5-mL microcentrifuge tubes, and 75 µL of fresh 0.2 M NaOH solution was added. Samples were vortexed for 10 s before incubation at 65°C for 15 min. Samples were then neutralized with 125 µL of a solution containing 1.6% (vol/vol) concentrated HCL and 0.1 M Tris. Samples were vortexed and subsequently diluted 1:10 with sterile distilled water in a 96 well microplate. Low yielding samples from the initial extraction method were further purified in the MagNA Pure LC instrument with MagNA Pure LC DNA Isolation Kit I (Roche Applied Science, Mannheim, Germany).

Genotyping was performed in the LightCycler 2.0 real-time PCR instrument (Roche Applied Science). The following oligonucleotide sequences were used: forward primer 5' AAG GAA AAT GCG CTG T 3', reverse primer 5' ACG GTT CTA CCT CGT C 3' (Integrated DNA Technologies, Coalville, IA), anchor probe 5' GGC CCT ATC TGT CTT ACG GGA GG-fluorescein 3', and sensor probe 5' LC Red640-GTG CCC ATC CGC AAG G-C3 blocker 3' (IT Biochem, Salt Lake City, UT). Each 10-µL reaction contained 4.5 µL 2x PCR Master Mix (catalog K0171; Fermentas, Burlington, ON), 0.4 µM of each forward and reverse primer, 0.15 µM of each anchor and sensor probe, 2.25 mM MgCl<sub>2</sub>, 200 ng/µL human high density lipoprotein (**HDL**; Biomedical Technologies Inc., Stoughton, MA), and 3% (v/v) dimethyl sulfoxide (**DMSO**; Sigma-Aldrich, St. Louis, MO). For each reaction, 1.0 µL of extracted sample was used as template DNA. The PCR conditions consisted of an initial denaturation at 95°C for 2 min followed by an amplification program of 45 cycles of 95°C for 2 s, 58°C for 10 s, and 72°C for 10 s. Sample genotypes were determined from a subsequent melting program of 95°C for 0 s, 40°C for 2 min, and continuous fluorescent detection over a temperature increase to 75°C at a ramp rate of 0.2°C/s. Real-time PCR data were acquired and analyzed using LightCycler software (Roche Applied Science) version 4.0.

### Statistical Analysis

Data were analyzed as a randomized complete block design, with pen as the experimental unit and treatments in a 3 × 2 factorial arrangement. The ANOVA was generated using the mixed models procedure of SAS (SAS Inst. Inc., Cary, NC), with treatments and associated interactions considered to be fixed effects whereas pen(block) was considered to be a random effect. The statistical model was  $Y_{ijkl} = \mu + \rho_i + \alpha_j + \beta_k + (\alpha\beta)_{jk} + e_{ijk}$ , in which  $Y_{ijkl}$  = observation for the  $j$ th treatment ration within the  $i$ th block at the  $k$ th measurement,  $\rho_i$  = random effect of the  $i$ th block,  $\alpha_j$  = fixed effect of the  $j$ th ZH treatment,  $\beta_k$  = fixed effect for leptin genotype, and  $e_{ijk}$  = normally identical and independently distributed error term. The interaction between leptin genotype and ZH treatment,  $(\alpha\beta)_{jk}$ , was also included as a fixed effect. The proportion of cattle grading USDA Low Choice or greater was analyzed as a binomial proportion using PROC GLIMMIX of SAS, with block as the random effect. Ultrasound fat thickness data were also analyzed as repeated measures using PROC MIXED of SAS, with day of ultrasonic measurement the repeated variable in the model statement. Least squares means were generated and separation was accomplished via pairwise comparisons of the main or interactive effects (PDIF option) when a significant ( $P \leq 0.05$ )  $F$ -test was noted. For each steer, a nonlinear exponential model was also fitted to the longitudinal measurements of fat thickness as described by Brethour (2000). The nonlinear regression (NLIN) procedure of SAS was used to fit the nonlinear exponential function  $Y = Ae^{(kt)}$ , in which  $Y$  = projected fat,  $A$  = the initial fat,  $k$  = rate of increase (Brethour, 2000), and  $t$  = time in days. Mean model parameters were then tested using the statistical model as described previously.

## RESULTS AND DISCUSSION

### Live BW Gain and Fat Thickness Growth

There were no ( $P \geq 0.230$ ) leptin genotype × ZH interactions for any live performance trait. Initial and final BW ( $P \geq 0.528$ ) as well as ADG ( $P = 0.418$ ) and G:F ( $P = 0.170$ ) were not affected by leptin genotype (Table 1). This is similar to Nkrumah et al. (2004) who tested the impact of LEP R25C SNP and observed that slaughter weight, ADG, and G:F were similar across genotypes. In addition, Montgomery et al. (2009a,b) reported feeding ZH for 21 d before slaughter increased BW by an average of 6.0 kg. Steers fed ZH for 21 d also had greater ( $P \leq 0.003$ ) ADG and G:F than steers not fed ZH. Moreover, DMI increased ( $P < 0.05$ ) with the frequency of the T allele over the last 21 d on feed when fed diets devoid of ZH; however, among ZH-fed steers, DMI actually decreased ( $P < 0.05$ ) with the frequency of the T allele (leptin genotype × ZH,  $P = 0.011$ ;

**Table 1.** Main effects of leptin R25C genotype and zilpaterol hydrochloride (ZH) feeding duration on live steer performance and ultrasound-measured 12th rib fat thickness

Item	Leptin genotype			SEM <sup>1</sup>	ZH inclusion, <sup>1</sup> d		SEM	P-value <sup>2</sup>		
	CC	CT	TT		0-ZH	21-ZH		GT	ZH	I
Number of pens	16	16	16		24	24				
Initial BW, kg	396.6	396.8	398.3	5.13	398.0	396.5	5.09	0.528	0.282	0.892
Final BW, kg	607.6	608.5	606.7	5.51	604.6 <sup>a</sup>	610.6 <sup>b</sup>	5.40	0.792	0.008	0.389
ADG, kg/d	1.62	1.63	1.60	0.027	1.59 <sup>a</sup>	1.65 <sup>b</sup>	0.025	0.418	0.003	0.404
DMI, <sup>3</sup> kg/d	10.2	10.3	10.3	0.100	10.3	10.2	0.095	0.330	0.124	0.230
G:F, kg/kg/day	0.159	0.158	0.155	0.0027	0.154 <sup>a</sup>	0.161 <sup>b</sup>	0.0025	0.170	<0.001	0.790
Ultrasound fat thickness, <sup>4</sup> mm										
d 0	3.48 <sup>a</sup>	3.52 <sup>ab</sup>	3.61 <sup>b</sup>	0.126	3.55	3.53	0.124	0.050	0.611	0.909
d 65	7.58 <sup>a</sup>	7.86 <sup>b</sup>	8.02 <sup>b</sup>	0.173	7.92	7.72	0.166	0.004	0.061	0.689
d 105	9.73 <sup>a</sup>	9.90 <sup>ab</sup>	10.31 <sup>b</sup>	0.171	10.08 <sup>a</sup>	9.88 <sup>b</sup>	0.164	<0.001	0.055	0.790
d 132	11.0 <sup>a</sup>	11.2 <sup>ab</sup>	11.5 <sup>b</sup>	0.254	11.4 <sup>a</sup>	11.0 <sup>b</sup>	0.254	0.003	0.002	0.964
Mean	9.19 <sup>a</sup>	9.42 <sup>b</sup>	9.72 <sup>b</sup>	0.148	9.32 <sup>a</sup>	9.58 <sup>b</sup>	0.141	<0.001	0.004	0.733
AFG, <sup>5</sup> mm/d	0.0568 <sup>a</sup>	0.0585 <sup>ab</sup>	0.0599 <sup>b</sup>	0.00216	0.0599 <sup>a</sup>	0.0569 <sup>b</sup>	0.00211	0.027	0.003	0.976

<sup>a,b</sup>Within a row and main effect of leptin genotype, least squares means lacking a common superscript letter differ,  $P < 0.05$ .

<sup>1</sup>Dietary inclusion of zilpaterol hydrochloride for 0 (0-ZH) or 21 d (21-ZH).

<sup>2</sup>Probability value for the main effect of leptin genotype (GT) and duration of ZH feeding (ZH) as well as the interactive (I) effect of GT and ZH.

<sup>3</sup>Daily DMI recorded before dietary administration of ZH.

<sup>4</sup>Analyzed as a repeated measure using the 4 observation d ( $0 \pm 0.0$ ,  $65 \pm 2.9$ ,  $105 \pm 9.4$ , and  $132 \pm 10.0$  d).

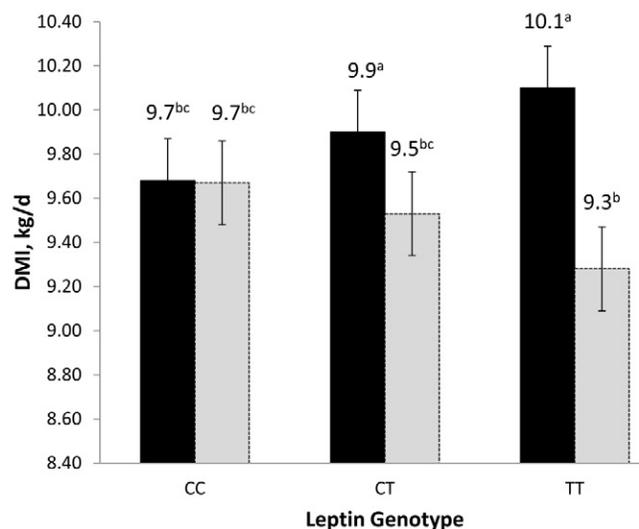
<sup>5</sup>Average fat gain (AFG) = (d-132 fat thickness – d-0 fat thickness)/total days on feed.

Fig. 1). Differences between 0 and 21 d of ZH feeding among the genotypes were 0.82, 0.37, and 0.01 kg/d for steers with TT, CT, and CC genotypes, respectively. When ZH was fed for 40 d Vasconcelos et al. (2008) observed a reduction in DMI of 0.40 kg/d. Interestingly in the current experiment, an interaction between leptin genotype and ZH status was observed on DMI over the last 21 d of the experiment (Fig. 1). The effect of the T allele on DMI in 0-ZH fed steers was expected given that circulating levels of leptin are believed to downregulate feed intake (Houseknecht et al., 1998). Furthermore, if ZH impacts DMI through either a direct tissue effect or an indirect endocrine effect as described by Montgomery et al. (2009a), it is also expected that it would interact with a functional leptin SNP.

Ultrasound-measured fat thickness increased ( $P \leq 0.05$ ) each of the 4 measurement days by leptin genotype, and this effect was independent of ZH feeding (Table 1). At the conclusion of the feeding period, fat thickness ( $P = 0.003$ ) was greatest in TT steers and least in CC steers and these observations, which further supports the positive relationship between T allele and fatness observed in mature beef bulls (Buchanan et al., 2002) and feeder cattle (Nkrumah et al., 2004); however, this relationship had not been examined in finishing cattle fed ZH. The rate of fat gain (AFG) also increased ( $P = 0.027$ ) with the frequency of the T allele. These results are similar to those of Nkrumah et al. (2004), who noted that, when fitted to a linear model, the rate of fat deposition increased with the presence of the T allele. Collectively, these results further support the suggestion that a SNP in the leptin gene

results in functional effects. Although the nature of these effects are not completely understood, it has been speculated that AA change in the leptin molecule may impede receptor binding of leptin or that the unpaired cysteine in the molecule may destabilize the disulfide bridge and affect biological function and ultimately feed intake or energy balance (Buchanan et al., 2002, 2003).

By comparing control and ZH fat thickness values within genotypes in Table 1, mean ultrasound fat thickness at the final (fourth) measurement was reduced ( $P = 0.002$ )



**Figure 1.** The interactive effect ( $P = 0.011$ ) of leptin genotype (CC, CT, and TT) and dietary inclusion of zilpaterol hydrochloride for 0 d (black bars) or 21 d (grey bars) before slaughter on DMI (kg/d) of feedlot steers. Means lacking a common superscript letter differ,  $P < 0.05$ .

**Table 2.** Main effects of leptin R25C genotype and zilpaterol hydrochloride (ZH) on estimated parameters of an exponential function relating ultrasound-measured fat thickness deposition to days on feed

Item	Leptin genotype			SEM	ZH inclusion, <sup>1</sup> d		SEM	<i>P</i> -value <sup>2</sup>		
	CC	CT	TT		0-ZH	21-ZH		GT	ZH	I
Initial fat thickness, <i>A</i> , <sup>3</sup> mm	3.77 <sup>a</sup>	3.87 <sup>a</sup>	3.99 <sup>b</sup>	0.118	3.90	3.86	0.116	0.003	0.390	0.760
Rate of change, <i>k</i> , <sup>3</sup> mm/d	0.0076	0.0077	0.0076	0.0002	0.0077 <sup>a</sup>	0.0075 <sup>b</sup>	0.0002	0.771	0.021	0.605
Days to 10 mm of fat thickness <sup>4</sup>	128 <sup>a</sup>	125 <sup>b</sup>	122 <sup>b</sup>	5.03	123 <sup>a</sup>	129 <sup>b</sup>	4.98	0.002	0.003	0.984
Days to 12 mm of fat thickness <sup>4</sup>	153 <sup>a</sup>	149 <sup>b</sup>	146 <sup>b</sup>	5.51	147 <sup>a</sup>	152 <sup>b</sup>	5.46	0.003	0.001	0.933
Days to 14 mm of fat thickness <sup>4</sup>	173 <sup>a</sup>	169 <sup>ab</sup>	167 <sup>b</sup>	5.95	167 <sup>a</sup>	172 <sup>b</sup>	5.91	0.007	0.001	0.877

<sup>a,b</sup>Within a row and main effect of leptin genotype, least squares means lacking a common superscript letter differ,  $P < 0.05$ .

<sup>1</sup>Dietary inclusion of zilpaterol hydrochloride for 0 (0-ZH) or 21 d (21-ZH).

<sup>2</sup>Probability value for the main effect of leptin genotype (GT) and duration of ZH feeding (ZH) as well as the interactive (I) effect of GT and ZH.

<sup>3</sup>Nonlinear parameter fitted for each individual steer.

<sup>4</sup>Computed days to 10, 12, and 14 mm of fat thickness based in fitted parameters as extrapolated by the following equation:  $y = [\ln(10) - \ln(x)]/k$ , in which  $y$  is the adjustment from the initial day on feed,  $x$  is the fat thickness measure at the beginning of the experimental period, and  $k$  is the rate of increase (Brethour, 2000).

by 0.4 mm in both CT and TT cattle and 0.5 mm in CC cattle, by ZH feeding (Table 1). The reduction is similar to the impact of ZH on carcass 12th rib fat measures reported previously by Montgomery et al. (2009b). It should be noted that there was a modest difference ( $P = 0.055$ ) in fat thickness between ZH treatment groups before administration of the drug. Although the reason for this differences is largely unknown, it is important to note the mean differences observed on d 2 and 3 (0.18 and 0.22 mm, respectively) were less than that observed on d 4 (0.40 mm); thus, it is plausible that ZH negatively affects accumulation of fat thickness.

Brethour (2000) noted that 25% of feeder cattle were marketed too fat whereas another 25% were marketed too lean, indicating that half of all the feeder cattle sold in the United States were fed either too long or not long enough. Furthermore, Brethour (2000) suggested that ultrasound technology could be used to rapidly estimate carcass characteristics on live cattle and project the time needed to reach a given level of fatness. Therefore, the effects of leptin genotype and ZH feeding duration on the relative rates of fat accretion/deposition were further tested by fitting data to an exponential function on serial within-steer measurements [ $Y = Ae_{(kt)}$ ; Brethour, 2000]. The observed rate coefficients (Table 2) were slightly less than those of Brethour (2000), who reported fat thickness rate coefficients of 0.0117 for a group of 137 Limousin and Simmental steers and 0.0096 for a group of 292 Angus and Angus × Hereford steers. In the present study, patterns of fat accretion were found to be dependent on leptin genotypes and administration of ZH. With respect to leptin genotype, initial fat thickness (parameter  $A$ ) was greatest in steers homozygous for the T allele (3.99 mm) compared to either the CC (3.77 mm) or CT (3.87 mm) genotypes whereas the rate of fat thickness growth ( $k$ ) was not ( $P = 0.771$ ) affected by leptin genotype. The time projected to reach 10, 12, and 14 mm of 12th rib fat thickness was calculated and differed by genotype, with TT steers possible reaching the target fat endpoint of 10 mm 6 d earlier ( $P < 0.01$ ) than

CC steers. Rate of fat growth ( $k$ ) was reduced by ZH ( $P = 0.021$ ), and ZH treatment would increase ( $P = 0.003$ ) the time to reach 10 mm of 12th rib fat thickness as much as 6 d. Similarly, patterns were observed in projections for reaching 12 and 14 mm of fat. These latter observations should be treated with caution because these projections extend beyond the time cattle were on feed ( $132 \pm 10.9$  d) in the current study and projections outside of the modeled range may be unreliable.

### Carcass Characteristics and Grades

Even though HCW did not ( $P = 0.432$ ) differ among leptin genotypes, carcasses from TT steers were fatter ( $P = 0.009$ ) than carcasses from CC steers, regardless of ZH feeding (Table 3). Furthermore, neither LM area ( $P = 0.930$ ), KPH fat ( $P = 0.675$ ), nor calculated YG ( $P = 0.284$ ) were affected by leptin genotype; however, the percentage of YG 1 carcasses was greater ( $P < 0.001$ ) and the percentage of YG 4 or greater carcasses was less ( $P = 0.006$ ) from CC steers than CT and TT steers. Additionally, 12th rib fat and calculated empty body fat (EBF) were increased ( $P \leq 0.009$ ) with the frequency of the T allele. Regardless of leptin genotype, feeding ZH for the last 21 d before slaughter produced heavier ( $P < 0.001$ ) HCW, larger ( $P < 0.001$ ) LM areas, less ( $P = 0.001$ ) fat opposite the 12th rib, and lower ( $P < 0.001$ ) calculated YG (Table 3). Moreover, ZH feeding increased ( $P \leq 0.006$ ) the percentages of YG 1 and 2 carcasses and decreased ( $P < 0.001$ ) the percentage of YG 3 and 4 carcasses. Mean calculated YG was also less in carcasses from steers fed ZH ( $P < 0.001$ ) regardless of leptin genotype. The reduction in YG in ZH fed cattle is consistent with the results of Montgomery et al. (2009a,b) and Vasconcelos et al. (2008) and supports the theory that ZH stimulates lipolysis and may also decrease fatty acid synthesis (Mersmann, 1998). When ZH was fed for 20 d, these investigators observed that ZH reduced calculated YG by approximately 0.4 units. The dramatic response on

**Table 3.** Main effects for leptin genotype and zilpaterol hydrochloride (ZH) on carcass characteristics

Item	Leptin genotype			SEM	ZH inclusion, <sup>1</sup> d		SEM	<i>P</i> -value <sup>2</sup>		
	CC	CT	TT		0-ZH	21-ZH		GT	ZH	I
HCW, kg	393.5	394.9	395.2	4.37	387.3 <sup>a</sup>	401.8 <sup>b</sup>	4.33	0.432	<0.001	0.411
12th rib fat, mm	12.0 <sup>a</sup>	12.2 <sup>ab</sup>	12.7 <sup>b</sup>	0.41	12.6 <sup>a</sup>	12.0 <sup>b</sup>	0.40	0.009	0.001	0.179
LM area, cm	93.0	93.3	93.0	0.815	89.1 <sup>a</sup>	97.1 <sup>b</sup>	0.75	0.930	<0.001	0.147
KPH, %	1.92	1.92	1.93	0.018	1.93	1.93	0.017	0.675	0.691	0.692
Calculated yield grade	2.75	2.77	2.83	0.070	2.96 <sup>a</sup>	2.62 <sup>b</sup>	0.067	0.284	<0.001	0.156
EBF, <sup>3</sup> %	28.5 <sup>a</sup>	29.0 <sup>b</sup>	29.3 <sup>b</sup>	0.25	29.4 <sup>a</sup>	28.4 <sup>b</sup>	0.24	<0.001	<0.001	0.553
USDA quality grades, %										
No Roll <sup>4</sup>	2.88	2.94	3.08	0.540	1.87 <sup>a</sup>	4.07 <sup>b</sup>	0.451	0.959	0.001	0.770
Yield grades (YG), %										
YG 1	26.8 <sup>a</sup>	18.8 <sup>b</sup>	17.7 <sup>b</sup>	1.55	16.2 <sup>a</sup>	25.9 <sup>b</sup>	1.31	<0.001	0.001	0.995
YG 2	40.3	45.9	42.6	2.75	39.3 <sup>a</sup>	46.6 <sup>b</sup>	2.45	0.210	0.006	0.357
YG 3	30.2	32.1	34.4	2.83	38.5 <sup>a</sup>	25.9 <sup>b</sup>	2.65	0.246	<0.001	0.129
YG 4 and 5	2.73 <sup>a</sup>	3.28 <sup>a</sup>	5.36 <sup>b</sup>	0.74	5.99 <sup>a</sup>	1.59 <sup>b</sup>	0.660	<0.001	0.006	0.247

<sup>a,b</sup>Within a row and main effect of leptin genotype, least squares means lacking a common superscript letter differ,  $P < 0.05$ .

<sup>1</sup>Dietary inclusion of zilpaterol hydrochloride for 0 (0-ZH) or 21 d (21-ZH).

<sup>2</sup>Probability value for the main effect of leptin genotype (GT) and duration of ZH feeding (ZH) as well as the interactive (I) effect of GT and ZH.

<sup>3</sup>Empty body fat (EBF) =  $17.76207 + (4.68142 \times 12\text{th rib fat, cm}) + (0.01945 \times \text{HCW}) + (0.81855 \times \text{quality grade}) - (0.06754 \times \text{ribeye area})$ .

<sup>4</sup>Included carcasses that graded U.S. Standard as well as carcasses not receiving USDA grades due to skeletal maturity, dark lean color, or other carcass defects.

HCW and LM area observed in the current study demonstrated the potent impact of ZH on muscle growth. In the case of HCW, the response of 14.5 kg observed in the current study is similar to that observed on Montgomery et al. (2009a,b), Scramlin et al. (2010), and McEvers et al. (2012) but less than that reported by Vasconcelos et al. (2008). The response of LM area observed in the current study was similar that that observed by Montgomery et al. (2009a) but greater that that reported by McEvers et al. (2012).

Feeding ZH for the last 21 d before slaughter reduced marbling scores and mean QG in CT and TT steers, but marbling scores and QG of CC steers were not affected by ZH feeding (leptin genotype  $\times$  ZH,  $P = 0.027$ ; Table 4). In addition, the percentage of carcasses receiving a QG of U.S. Choice and U.S. Prime increased as the frequency of the T allele increased when steers were fed diets devoid of ZH but in ZH-fed cattle, but there was no difference in the percentage of U.S. Choice or higher carcasses among

the genotypes (leptin genotype  $\times$  ZH,  $P < 0.001$ ; Table 4). Conversely, CT and TT steers fed ZH produced a greater percentage of U.S. Select grade carcasses than all 3 leptin genotypes fed the control diet (leptin genotype  $\times$  ZH,  $P < 0.001$ ). Although feeding ZH has been demonstrated to decrease marbling scores (Montgomery et al., 2009a; McEvers et al., 2012; Vasconcelos et al., 2008) and the proportion of carcasses grading USDA Choice or higher (Montgomery et al., 2009b), the observation that the response may be dependent on the leptin R25C genotype has not been reported previously. The effects of ZH on QG are very similar to the interactive pattern observed on DMI, and it was hypothesized that the effects on carcass QG were largely driven by the impacts on feed intake and energy status, which is known to affect QG positively (Hicks et al., 1990; Cruz et al., 2010). There was a tendency ( $P = 0.095$ ) for an interaction between leptin genotype and ZH on calculated HCW gain (data not shown). The differences within genotype between 0-ZH and 21-ZH on HCW

**Table 4.** The effects of leptin genotype and zilpaterol hydrochloride (ZH) on carcass characteristics and grades<sup>1</sup>

Item	CC genotype		CT genotype		TT genotype		SEM	<i>P</i> -value <sup>2</sup>		
	0-ZH	21-ZH	0-ZH	21-ZH	0-ZH	21-ZH		GT	ZH	I
Marbling score <sup>3</sup>	41.4 <sup>a</sup>	40.7 <sup>a</sup>	42.9 <sup>b</sup>	40.7 <sup>a</sup>	43.4 <sup>b</sup>	40.9 <sup>a</sup>	8.40	0.012	<0.001	0.027
USDA quality grades, %										
U.S. Choice or higher	47.9 <sup>bc</sup>	46.2 <sup>bc</sup>	59.2 <sup>a</sup>	42.3 <sup>bc</sup>	63.8 <sup>a</sup>	42.8 <sup>b</sup>	3.73	0.042	<0.001	<0.001
U.S. Select	49.7 <sup>b</sup>	49.7 <sup>bc</sup>	38.0 <sup>ab</sup>	53.2 <sup>c</sup>	34.3 <sup>a</sup>	52.7 <sup>c</sup>	3.42	0.012	<0.001	<0.001

<sup>a-c</sup>Within a row and main effect of leptin genotype, least squares means lacking a common superscript letter differ,  $P < 0.05$ .

<sup>1</sup>Dietary inclusion of zilpaterol hydrochloride for 0 (0-ZH) or 21 d (21-ZH).

<sup>2</sup>Probability value for the main effect of leptin genotype (GT) and duration of ZH feeding (ZH) as well as the interactive (I) effect of GT and ZH.

<sup>3</sup>Marbling score: based on the following scale: 10 = practically devoid, 20 = traces, 30 = slight, 40 = small, 50 = modest, 60 = moderate, 70 = slightly abundant, 80 = moderately abundant, and 90 = abundant.

gain tended to be less for steers of the TT genotype (12.7 kg) compared to either CC or CT genotypes (16.3 and 17.0 kg, respectively). The gain in BW was less than the gain in HCW, which was observed by Montgomery et al. (2009b) and Holland et al. (2009), who suggested that this difference was due to reductions in DMI and, ultimately, gut fill; however, result of the current study indicated that reductions in DMI of ZH-fed steers represented only a small contribution to HCW. Thus, the reason that HCW gain exceeded BW gain may be either a shift in mass from noncarcass to carcass tissue or more substrate repartitioning in carcass rather than noncarcass tissue (Montgomery et al., 2009b).

Results of the present study support the hypothesis that the extent of carcass fatness of beef cattle may depend on the proper function of leptin and administration of ZH. Although the mechanism of this relationship in feedlot cattle has not been clearly established, it has been suggested that the mechanism is at least in part because of the action of both leptin (Minokoshi et al., 2002) and ZH (Tokach et al., 2010) on AMPK and ultimately fatty acid oxidation.

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