

## Effect of zilpaterol hydrochloride on the carcass characteristics of Katahdin lamb terminal crosses

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### Abstract

Zilpaterol hydrochloride (ZH) supplementation (0 vs. 0.15 mg/kg live weight) was evaluated based on the carcass characteristics of Katahdin x Charollais (32 KCh) and Katahdin x Dorper (28 KD) crosses. Lambs were fed a mixed ration with 14% crude protein (CP) and 2.9 Mcal EM/kg DM. Data were analyzed using a completely randomized 2 x 2 factorial design: 2 genotypes (KCh and KD) and 2 ZH levels (0 and 0.15 mg/kg live weight). No interaction was found between ZH and the genotypes. Zilpaterol hydrochloride increased dressing percentage from  $52.1 \pm 0.3$  to  $53.7 \pm 0.4\%$  ( $P < 0.001$ ). Animals that received ZH supplementation increased ( $P < 0.001$ ) the area of their *Longissimus dorsi* (Ld) by 18.5% and had 7.5% more muscle, 6.0% less bone and 22.4% less fat compared with control lambs ( $P < 0.05$ ). The breed of the sire had no effect on any of the variables studied. Final pH, fat thickness, conformation and linear carcass measurements did not change with ZH supplementation.

**Keywords:** Lamb; Zilpaterol; Carcass quality; Crossbreeding; Katahdin; Charollais; Dorper.

### Introduction

Over the last decade, the Mexican sheep industry has mainly focused on meat production and grew at an annual rate of 5.11% from 38,196 t in 2002 to 57,692 t in 2012 (SIAP, 2012). Consequently, lamb imports have decreased by almost 75% (SIAP, 2012; SAGARPA, 2013). Although this growth has been important, there is a need for an additional 32,500 t of lamb annually to satisfy the demand of the Mexican population for at least 750 g of lamb/person/year. Many producers in Mexico were encouraged to invest in highly meat efficient breeds and to satisfy the market demand using either pure breeds or crosses under highly intensive systems. Recent studies have evaluated several crosses and found higher growth performance and better carcass characteristics than in native breeds (Hernández *et al.*, 2009; Partida *et al.*, 2009; Ríos *et al.*, 2011). Crossing hair-type ewes with meat rams generated products with different qualities to satisfy the Mexican market (Bores *et al.*, 2007; Ríos *et al.*, 2011; Vázquez *et al.*, 2011). Some studies have highlighted Katahdin, Charollais and Dorper breeds as the most

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productive based on their growth rate and carcass characteristics (Bores *et al.*, 2007; Vázquez *et al.*, 2011).

Raising slaughter weight is vital to increasing carcass yield and the size of high value cuts (Solomon *et al.*, 1980), but this leads to an accumulation of fat deposits in carcasses (Hopkins, 2005; Warris, 2005). To increase lean yield, different metabolic modifiers, especially  $\beta$ -adrenergic agonists ( $\beta$ -AA) compounds, have been used (Beerman *et al.*, 1995; Dikeman, 2003; Sillence, 2004). When we compared the results of recent studies that evaluated different  $\beta$ -AA (ZH, ractopamine hydrochloride, terbutaline, metaprotenerol, isoproterenol, BRL35135A, BRL26830, etc.), we found that ZH was the best option for small ruminants, which is consistent with the results of several authors (Mohammadi *et al.*, 2006; Dikeman, 2007; Nourozi *et al.*, 2008; López *et al.*, 2010; López *et al.*, 2011). However, the results have been inconsistent (Salem, 2013) or contradictory in terms of animal performance (Salinas *et al.*, 2004; Mondragón *et al.*, 2010; López *et al.*, 2011) and carcass characteristics (Shackelford *et al.*, 1992; Koohmaraie *et al.*, 1996; Salinas *et al.*, 2006; Mondragon *et al.*, 2010). It has been hypothesized that a possible interaction between  $\beta$ -AA and genotype could be the reason for these inconsistencies (Mohammadi *et al.*, 2006). Different regulatory pathways for lipid metabolism among breeds and the role of  $\beta$ -AA in reducing fat deposits could vary with receptor selectivity (Nourozi *et al.*, 2008). These possible interactions between  $\beta$ -AA and genotype may also affect muscle protein synthesis and retention (Mondragon, 2008) and modify the hypertrophy that occurs in different muscles, which could vary with breed (Beerman, 2002).

Therefore, the objective of this research was to evaluate the effect of ZH on the carcass characteristics of terminal Katahdin x Charollais and Katahdin x Dorper crosses to provide alternatives for increasing meat production.

## Materials and Methods

### *Animals, housing and management*

Lambs were raised in the municipality of Colón, Qro., located at 20°41'40.62" north latitude and 100°00'53.52" west longitude (Google, 2013). The weather in this region is mildly dry with an annual rainfall of 450 mm and a temperature range of 15-19 °C (García, 1981). Lamb finishing was carried out at the Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal (INIFAP) in Ajuchitlán, Qro., located 2 km from where the lambs were raised. A 200-head Katahdin ewe flock of  $51 \pm 18$  months of age and  $6 \pm 2$  births was used for this study. Ewes were separated into 2 groups, synchronized using intravaginal progesterone sponges (Progestpon®, Syntex, S. A., México) followed by an FSH application (Folligon®, Merck, Sharp & Dohme, México), and then inseminated (laparoscopy) using fresh semen from 5 unrelated Charollais (Ch) and 5 Dorper rams (D).

From birth to weaning ( $78 \pm 6$  days), lambs were creep-fed, and after weaning, ram lambs were selected for further study and divided into 2 groups (32 KCh and 28 KD). Both groups went through a growing stage ( $56 \pm 13$  days) under similar management conditions, which included a totally mixed ration with 14% crude protein (CP), 2.9 Mcal of EM/kg MS (Table 1) and water *ad libitum*.

**Table 1.** Composition of the fattening diet.

Ingredient	%
Sorghum grain	47.2
Sugarcane molasses	20.0
Alfalfa hay	11.0
Corn stover	8.0
Canola meal	6.5
Soybean meal	4.0
Mineral and urea mix	3.3
<b>Total</b>	<b>100.0</b>
Calculated analysis	
Metabolizable energy, Mcal/kg DM	2.9
Crude protein, %	14.0

The diet with 6 ppm ZH (treated group; equivalent to 0.15 mg/kg LW/day, approximately) was fed to the animals for 30 days. Three days before slaughter, ZH supplementation was withdrawn.

Once through the growing stage, lambs were regrouped for further treatment. The same mixed ration used in the growing stage was fed to the animals both without ZH (control group) and with 6 ppm ZH (Zilmax®, Intervet, S. A. de C. V., México) (treated group; equivalent to 0.15 mg/kg LW/day, approximately) for 30 days. The experimental groups were as follows: Group 1 = 14 KD with ZH; Group 2 = 14 KD without ZH; Group 3 = 16 KCh with ZH and Group 4 = 16 KCh without ZH. At the end of the fattening period, 10 animals from each group were randomly selected to be slaughtered. According to laboratory recommendations, ZH supplementation was withdrawn three days before slaughtering in order to avoid any risk to consumers; 90% of ZH at this dose is excreted in the urine and feces after 48 h (Zilmax®, Intervet, S. A. de C. V., México). Zilpaterol hydrochloride produces extremely weak pharmacological actions in man, and it is so rapidly metabolized and cleared from the animal's body that it is virtually impossible to regard it as a potential cause of drug poisoning in human beings, even after consuming meat products derived from animals medicated with this drug and when no withdrawal period is enforced (Sumano *et al.*, 2002).

The lambs were slaughtered at Federal Inspection Type Slaughterhouse No. 412 at San José El Alto, Querétaro, following the industrial processes and procedures for animal welfare that have been established by federal authorities. Work was done with the support of a cooperating producer under ordinary commercial conditions for sheep production.

### *Treatment, slaughter and carcass measurements*

After slaughter, hot carcasses were weighed and cooled at 4 °C for 24 h, and carcass classification was then performed according to the Norma Mexicana NMX-FF-106-SCFI-2006. To evaluate conformation, the original scale (Excellent, Good and Deficient) was modified to the following values: Excellent (+) = 9; Excellent = 8; Excellent (-) = 7; Good (+) = 6; Good = 5; Good (-) = 4; Deficient (+) = 3; Deficient = 2; Deficient (-) = 1. This was done to create more classification options, as in the European classification system (SEUROP). Following evaluation, linear measurements (cm) were taken, including rump perimeter (at the level of the trochanters of both femurs), thorax internal depth (maximum distance between the sternum and the dorsal part of the carcass at the sixth thoracic vertebra) and carcass length (from the last cervical vertebra to the last sacral vertebra). The compactness index was calculated (weight (kg)/carcass length (cm)) following the methodology described by Ruiz de Huidobro *et al.* (2005).

Carcasses were divided into equal parts (right and left middle-carcass) by cutting along the backbone, and the rack was then removed from the left middle-carcass by a cut from the 4<sup>th</sup> to the 12<sup>th</sup> thoracic vertebrae. At the 12<sup>th</sup> thoracic vertebra, meat color, fat thickness and area *Longissimus dorsi* (Ld) were measured, and pH was measured using a portable pH meter with a penetration probe (Hanna Instruments, HI-99163, Woonsocket, RI). The instrumental color (CIELAB) of the meat and fat (kidney fat) were measured using a Minolta CR-410 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan); the D65 illuminant was selected with

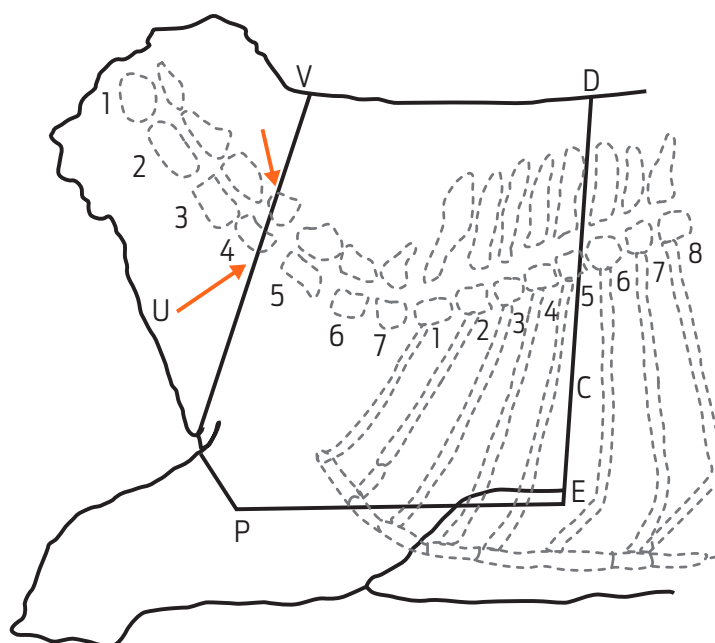
an angle of 2° and a 2-cm measurement approach. Before taking the measurements, meat samples were allowed to bloom for 1 h at  $12 \pm 1$  °C. Each *Ld* area was drawn on paper and calculated using a digital planimeter (Planix 6, Tamaya Technics Inc. Tokyo, Japan). Fat thickness was measured using a Vernier caliper.

The left shoulder was separated from each carcass following the methodology of Vergara (2005), which involves a cut along the four lines shown in Fig. 1. Starting at point V, the cutting passes over the spinous apophysis of the 4<sup>th</sup> cervical vertebra (point U) across the neck, and then a cut to the tip of the chest (point P) must be done before continuing parallel to the ventral midline to point E, which is located between the 5<sup>th</sup> and 6<sup>th</sup> costochondral joints. The posterior boundary line (line D-E) runs perpendicular to the dorsal line and passes through point C between the 5<sup>th</sup> and 6<sup>th</sup> cervical vertebrae. The upper boundary is formed by the V-D line that runs over the backbone. The shoulders were vacuum-packaged and kept at -18 °C for further dissection.

Before dissection, shoulders were thawed for 24 h under refrigeration, and they were then weighed and the components (muscle, fat and bone) were removed (Boccard, 1976). Waste (fascia, nerves tendons, vessels, etc.) was recorded with the bone and reported as bone + waste.

### Statistical analysis

Data were analyzed using a completely randomized 2 x 2 factorial design: 2 genotypes (KCh and KD) and 2 ZH levels (0 and 0.15 mg/kg). Sex effect was excluded from the analysis because the study was conducted in a commercial facility where only males were available. The general lineal model procedure in SAS 9.1.3 (SAS, 2008) was used, and the separation of means was performed using Tukey's test. To eliminate the variability generated by secondary effects, such as birth type, ma-



**Figure 1.** Separation of the left shoulder [normalized method defined by Boccard and Dumont (1955) and modified by Colomer-Rocher *et al.*, (1988)].

ternal age, etc., the weight of the animals at the beginning of the finishing (ZH) period was used as a covariate (Steel and Torrie, 1980). Carcass conformation data were analyzed with the Cochran-Mantel-Haenszel test, and shoulder composition and meat pH were analyzed for normality using the Shapiro-Wilk test. All tests were performed at a 95% confidence level.

## Results and Discussion

There was no genotype x ZH regimen interaction for any of the carcass variables ( $P > 0.05$ ), which may imply that  $\beta$ -AA exerted the same effects on both genotypes. This is in agreement with the results from Montoya *et al.* (2011), who found no effect of ZH (0.15 mg/kg LW) on the growth performance and carcass characteristics of Pelibuey x Blackbelly and Pelibuey x Dorset crosses. Therefore, ZH could be used in different terminal crosses with similar results. Table 2 shows the effect of sire breed and ZH supplementation on lamb carcass characteristics. Mean slaughter weight,  $47.8 \pm 3.2$  kg, was not affected by any of the treatments ( $P = 0.917$ ), and hot carcass weight ( $25.4 \pm 0.5$  kg) was not affected by sire breed ( $P = 0.560$ ) or ZH administration ( $P = 0.197$ ). Dressing percentage was not affected by genotype averaging,  $52.9 \pm 0.4\%$  ( $P = 0.466$ ), but ZH supplementation increased carcass dressing from  $52.1 \pm 0.3$  to  $53.7 \pm 0.4\%$  ( $P = 0.001$ ). Average carcass conformation,  $7.0 \pm 0.2$  (equivalent to Excellent (-)), was not affected by genotype or ZH use, and rump perimeter, carcass length and thorax depth were not affected by any of the treatments. On the other hand, the leg length of KCh lambs was 5% longer than that of KD lambs ( $P = 0.005$ ), and the leg length of ZH lambs was 3.3% longer than that of the control lambs ( $P = 0.001$ ).

Zilpaterol hydrochloride significantly ( $P = 0.001$ ) increased carcass yields by 3.1%, which translated into an additional 0.7 kg in the ZH treated animals in accordance with several studies that used either ZH (Vergara, 2006; Estrada, *et al.*, 2008; López *et al.*, 2010) or ractopamine (López *et al.*, 2010; Romero, 2011).  $\beta$ -AA products increase muscle by increasing protein and decreasing muscle catabolism (Mersman, 1998; Beerman, 2002), thus decreasing body fat (Yang y McElligott, 1989). Generally speaking, muscle hypertrophy could explain the muscle mass gain obtained through  $\beta$ -AA administration. However, each muscle responds according to its fiber type and proportion (Beerman, 2002), and gain is more evident in type II and mixed oxidative-glycolytic type fibers (Li *et al.*, 2000).

Subcutaneous fat thickness ( $3.4 \pm 0.2$  mm) was not affected by sire breed ( $P = 0.372$ ) nor by ZH supplementation ( $P = 0.413$ ). There was no effect of genetic group on *Ld* area ( $P = 0.116$ ), but this variable increased by 17.2% under the ZH treatment ( $P = 0.001$ ). ZH-treated animals had a larger *Ld* area; a possible explanation is an increase in muscle protein and a decrease in protein degradation (Mersman, 1998). The area of the *Ld* has been correlated with total carcass muscle (Stanford *et al.*, 1995; Hopkins *et al.*, 1996) and prime cuts (Bianchi *et al.*, 2000), which represent 25-50% of the total carcass price (Gómez, 2013). Therefore, treatment with ZH may be advisable from an economic point of view.

Table 3 shows the effect of sire breed and ZH use on the tissue components of the lamb shoulder. There was no effect of genetic group on shoulder tissue composition ( $P > 0.05$ ), but the shoulders from ZH-treated lambs had 7.5% more muscle,



**Table 2.** Effects of sire breed and zilpaterol hydrochloride inclusion on lamb carcass characteristics (mean  $\pm$  SE).

Variable	Sire breed			Zilpaterol hydrochloride (mg/kg LW)		
	Charollais	Dorper	P value	0	0.15	P value
Slaughter weight (kg)	47.9 $\pm$ 0.6	48.0 $\pm$ 0.6	0.917	47.9 $\pm$ 0.6	48.0 $\pm$ 0.6	0.997
Hot carcass weight (kg)	25.5 $\pm$ 0.4	25.2 $\pm$ 0.5	0.560	25.0 $\pm$ 0.4	25.7 $\pm$ 0.5	0.197
Cold carcass dressing (%)	53.1 $\pm$ 0.3	52.7 $\pm$ 0.4	0.466	52.1 $\pm$ 0.3	53.7 $\pm$ 0.4	<b>0.001</b>
Carcass conformation	7.0 $\pm$ 0.2	6.9 $\pm$ 0.2	0.168	6.9 $\pm$ 0.2	7.0 $\pm$ 0.2	0.181
Rump perimeter (cm)	68.5 $\pm$ 0.4	67.9 $\pm$ 0.5	0.337	68.6 $\pm$ 0.5	67.2 $\pm$ 0.4	0.594
Internal thorax depth (cm)	26.5 $\pm$ 0.3	26.2 $\pm$ 0.3	0.423	26.5 $\pm$ 0.3	26.2 $\pm$ 0.3	0.138
Length of carcass (cm)	65.7 $\pm$ 0.4	65.1 $\pm$ 0.4	0.188	65.1 $\pm$ 0.4	65.7 $\pm$ 0.4	0.184
Length of leg (cm)	38.0 $\pm$ 0.3	36.2 $\pm$ 0.3	<b>0.005</b>	37.7 $\pm$ 0.3	36.5 $\pm$ 0.3	<b>0.001</b>
Compactness index	0.38 $\pm$ 0.0	0.38 $\pm$ 0.0	0.429	0.38 $\pm$ 0.0	0.38 $\pm$ 0.0	0.940
SF thickness (mm)	3.5 $\pm$ 0.20	3.3 $\pm$ 0.2	0.372	3.4 $\pm$ 0.2	3.3 $\pm$ 0.2	0.413
<i>L. dorsi</i> area (cm <sup>2</sup> )	18.8 $\pm$ 0.4	17.9 $\pm$ 0.5	0.116	16.9 $\pm$ 0.4	19.8 $\pm$ 0.5	<b>0.001</b>

LW = live weight.

SF = subcutaneous fat.

Conformation: 9 = Excellent(+); 8 = Excellent; 7 = Excellent(-); 6 = Good(+); 5 = Good; 4 = Good(-); 3 = Deficient(+); 2 = Deficient; 1 = Deficient(-).

Two-way interactions were not significant for any trait.

**Table 3.** Effects of sire breed and zilpaterol hydrochloride inclusion on shoulder tissue (%) of lambs (mean  $\pm$  SE).

Variable	Sire breed			Zilpaterol hydrochloride (mg/kg LW)		
	Charollais	Dorper	P value	0	0.15	P value
Muscle	63.4 $\pm$ 0.4	63.7 $\pm$ 0.5	0.752	61.2 $\pm$ 0.4	65.8 $\pm$ 0.5	<b>0.001</b>
Bone + waste	23.8 $\pm$ 0.3	24.0 $\pm$ 0.3	0.307	24.6 $\pm$ 0.2	23.2 $\pm$ 0.3	<b>0.004</b>
Fat	11.2 $\pm$ 0.5	11.9 $\pm$ 0.5	0.968	12.5 $\pm$ 0.5	9.7 $\pm$ 0.5	<b>0.001</b>
Muscle/fat	5.7 $\pm$ 0.2	5.4 $\pm$ 0.1	0.860	4.9 $\pm$ 0.1	6.8 $\pm$ 0.2	<b>0.001</b>
Muscle/bone + waste	2.7 $\pm$ 0.1	2.7 $\pm$ 0.0	0.530	2.5 $\pm$ 0.1	2.8 $\pm$ 0.1	<b>0.010</b>

LW = live weight.

Two-way interactions were not significant for any trait.

6.0% less bone and 22.4% less fat than those from control lambs ( $P < 0.005$ ). These results indicate that muscle:fat (6.8:1) and muscle:bone (2.8:1) ratios improved. In summary, ZH supplementation increased the final amount of shoulder meat by 0.240 kg, which is equivalent to 1.6 kg of additional carcass meat.

The effect of ZH on carcass composition has been widely reported (Dikeman, 2007; Leheska *et al.*, 2009; Montgomery *et al.*, 2009; Shook *et al.*, 2009) both in hair (Salinas *et al.*, 2004; Salinas *et al.*, 2006; Estrada *et al.*, 2008; Bores *et al.*, 2010; López *et al.*, 2010) and wool breeds (Pringle *et al.*, 1993; Mondragón *et al.*, 2010). Although ZH decreased shoulder fat, no effect was found on the subcutaneous measurements. Yang and Mc Elliott (1989) found that the administration of cimaterol hydrochloride decreased total carcass fat and increased non-esterified fatty acids in plasma, which suggests lipid mobilization.

Table 4 shows the lamb *Ld* quality variables. Final mean pH values,  $5.7 \pm 0.3$ , were normal for sheep (Garrido *et al.*, 2005), and they were not affected by genotype or ZH use. KD meat had slightly higher ( $P < 0.05$ ) lightness ( $L^*$ ), red ( $a^*$ ), yellow ( $b^*$ ), hue ( $h^*$ ) and Chroma ( $C^*$ ) values than KCh while ZH meat had lower ( $P < 0.05$ )  $L^*$ ,  $a^*$ ,  $h^*$  and  $C^*$  values than the meat from the control animals. Thus, the meat from the ZH-treated animals was lighter in color than the meat from the control animals but remained within the normal range. ZH use resulted in carcass fat with lower  $L^*$ ,  $b^*$ ,  $h^*$  and  $C^*$  values and higher  $a^*$  ( $P < 0.05$ ) values than the control.

The effect of ZH on meat color has not been consistently shown. Different factors contribute to this inconsistency, such as final pH and temperature variation, and the combination of these 2 factors affects protein denaturation and color (Sañudo *et al.*, 1989). Some studies have found no effect of ZH on color variables (Avendaño *et al.*, 2006; Romero, 2011), but results from Dávila *et al.* (2013) were similar to those of the present study. A possible explanation for the reduced lightness in the meat of ZH-treated sheep is the reduction in the amount of intramuscular fat (marbling), which is white in animals finished with totally mixed rations (Wulf and Wide, 1999).

## Conclusions

The breed of the sire did not affect most of the carcass traits, but Katahdin x Dorper crosses had shorter legs and higher color meat values. Zilpaterol hydrochloride-treated animals had higher carcass yields, and their shoulder meat was leaner with less fat and bone. They also had lighter meat with less red, hue and Chroma values, and a larger *Longissimus dorsi* area. Zilpaterol hydrochloride is a government-authorized and safe option that may help producers improve carcass properties in their animals.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

## Author contributions

José Armando Partida de la Peña: lead the project, designed the experiment, and prepared the manuscript.

Tania Alejandra Casaya Rodríguez: Master's student; collected the samples and analyzed the results for her thesis.

María Salud Rubio Lozano: participated in the laboratory analysis of the meat and the preparation of the English version of this manuscript.

Rubén Danilo Méndez Medina: designed the project, analyzed the results and reviewed the manuscript.

**Table 4.** Effects of sire breed and zilpaterol hydrochloride inclusion on meat quality of lambs (mean  $\pm$  SE).

Variable	Sire breed			Zilpaterol hydrochloride (mg/kg LW)		
	Charollais	Dorper	P value	0	0.15	P value
pH 24 h	5.7 $\pm$ 0.4	5.7 $\pm$ 0.5	0.605	5.7 $\pm$ 0.0	5.7 $\pm$ 0.5	0.490
<b>Meat color</b>						
L*	34.0 $\pm$ 0.6	35.6 $\pm$ 0.6	0.024	37.7 $\pm$ 0.6	31.9 $\pm$ 0.6	<b>0.001</b>
a*	13.4 $\pm$ 0.3	14.9 $\pm$ 0.4	0.007	15.5 $\pm$ 0.4	12.9 $\pm$ 0.4	<b>0.001</b>
b*	5.6 $\pm$ 0.3	6.6 $\pm$ 0.4	0.001	3.7 $\pm$ 0.4	8.4 $\pm$ 0.3	0.435
h*	20.8 $\pm$ 0.9	23.0 $\pm$ 0.9	0.019	28.6 $\pm$ 0.9	15.1 $\pm$ 0.9	<b>0.001</b>
C*	14.7 $\pm$ 0.4	16.4 $\pm$ 0.5	0.004	17.7 $\pm$ 0.8	13.5 $\pm$ 0.5	<b>0.001</b>
<b>Perinephric fat color</b>						
L*	74.6 $\pm$ 0.4	74.9 $\pm$ 0.4	0.385	75.6 $\pm$ 0.4	73.8 $\pm$ 0.4	<b>0.001</b>
a*	1.9 $\pm$ 0.3	2.6 $\pm$ 0.3	0.034	1.1 $\pm$ 0.3	3.5 $\pm$ 0.3	<b>0.038</b>
b*	7.0 $\pm$ 0.2	7.7 $\pm$ 0.2	0.372	9.4 $\pm$ 0.2	5.3 $\pm$ 0.2	<b>0.046</b>
h*	71.8 $\pm$ 1.6	70.5 $\pm$ 1.7	0.634	84.1 $\pm$ 1.5	58.2 $\pm$ 1.8	<b>0.001</b>
C*	7.5 $\pm$ 0.3	8.5 $\pm$ 0.3	0.001	9.6 $\pm$ 0.3	6.2 $\pm$ 0.3	<b>0.001</b>

LW = live weight.

L\* = lightness; a\* = red index; b\* = yellow index; h\* = hue (tone); C\* = Chroma (saturation).

Two-way interactions were not significant for any traits.

Color scale: Red index (a\*) = 60 (red) to - 60 (green); yellow index (b\*) = 60 (yellow) to - 60 (blue); lightness (L\*) = 0 (black) to 100 (white); h\* =  $\arctan(b^*/a^*)$  (0 to 360 degrees); C\* =  $\sqrt{(a^*)^2 + (b^*)^2}$  (0 to 200).

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