Meat Quality in Katahdin Lamb Terminal Crosses Treated with Zilpaterol Hydrochloride

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Abstract

The effect of Zilpaterol hydrochloride (ZH) supplementation (0 vs. 0.15 mg/kg live weight) on the meat quality was evaluated in Katahdin x Charollais (32 KCh) and Katahdin x Dorper (28 KD) crosses. Lambs were fed a totally 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. The breed of the sire (BS) did not affect most of the meat traits, only KCh crosses had higher color values (L* = 34.0 ± 0.6 vs. 35.6 ± 0.6 ; a* = 13.4 ± 0.3 vs. 14.9 ± 0.4 ; b* = 5.6 ± 0.3 vs. 6.6 ± 0.4 ; h* = 20.8 ± 0.9 vs. 23.0 ± 0.09 ; C* = 14.7 ± 0.4 vs. 16.4 ± 0.5), more fat (11.2 ±0.5 vs. 11.9 ± 0.5) and less protein (21.3 ±0.1 vs. 21.8 ± 0.0) than KD. ZH meat had lower values (P < 0.001) than meat from the control animals: L* (31.9 ± 0.6 vs. 37.7 ± 0.6), a* (12.9 ± 0.4 vs. 15.5 ± 0.4), h* (15.1 ± 0.9 vs. 28.6 ± 0.9) and C* (13.5 ± 0.5 vs. 17.7 ± 0.8). ZH increased shear force on meat (5.4 ± 0.2 vs. 3.7 ± 0.2 kgf), and produced less fat (9.7 ± 0.5 vs. $12.5\pm0.5\%$) and bone (23.2 ± 0.3 vs. $24.6\pm0.2\%$), but more muscle (65.8 ± 0.5 vs. $61.2\pm0.4\%$). Zilpaterol hydrochloride use in lamb production caused leaner yield and more protein retention, at the expense of reducing meat sensory qualities.

Keywords: Lamb, Meat quality, Sensory traits, Zilpaterol hydrochloride

1. Introduction

According to Food and Agriculture Organization (FAO, 2014), lamb production increased at an average rate of 1.12% per year during last decade. This moderate growth was due mainly to two factors, the first was the reduction of flocks in several countries caused by the presence of severe droughts in America, Australia, Africa and Middle East (Garniere, 2010); and the second, was the increase in grain prices caused by the use of maize for the biofuels industry (ICTSD, 2008). Nevertheless, for 2025 it is expected a growth of 2.5% annually in lamb production driven by a substantial lamb consumption increase in developing countries (OECD-FAO, 2013).

Mexico is not a highly lamb consumer (738 g / person / year) country (SAGARPA, 2005); however, to satisfy a demand for at least 750g of lamb/person/year is necessary to import more than 31 000 tons of sheep meat / year (SIAP, 2014). This situation provides an opportunity for Mexican sheep producers to increase their lamb production by more than 60% to meet current needs. During recent years, several specialized breeds in meat production had been introduced into the country to improve animal performance, carcass conformation and carcass finishing degree (Hernandez et al., 2009; Partida et al., 2009; Rios et al., 2011). Particularly, the crossing of hair ewes with males specialized in meat production, showed comparative advantages and generated different quality options to meet Mexican market demands (Bores et al., 2007; Rios et al., 2011; Vazquez et al., 2011). In some of these studies, Katahdin, Charollais and Dorper breeds were highlighted as the most efficient breeds in terms of growth and carcass traits (Bores et al., 2007; Macias et al., 2010; Vázquez et al., 2011; Partida et al., 2010). Beyond these improvements, the overall increase of slaughter weight (> 45 kg) has been considered a real possibility to reach the objective of increasing production. Literature indicates that an increase in slaughter weight is accompanied by an increase in the total fat quantity (Bianchi et al., 2006; Warris, 2003) and a reduction of body muscle proportion (Partida et al., 2011; Hopkins, 2005). Therefore, recent research has focused on

evaluating various compounds that modify animal metabolism and improve lean carcass yield (Beermann, 2009; Dikeman, 2007; Sillence, 2004). Specifically, β -adrenergic agonists (β -AA) increase protein synthesis and lipid degradation (Yang & Mc Elligott, 1989; Mersman, 1998); however, studies in sheep have shown inconsistent results on body composition (Brand et al., 2013; Mondragon et al., 2010; Salinas et al., 2006). When we compared the results of recent studies that evaluated different β -AA (zilpaterol hydrochloride, ractopamina hydrochloride, terbutaline, metaprotenerol, isoprotenerol, BRL35135A, BRL26830, etc.) we found that zilpaterol hydrochloride was the best option for small ruminant (Mohammandi et al., 2006; Dickeman, 2007; Nourozi et al., 2008; López et al., 2010; López et al., 2011). In addition, Zilpaterol hydrochloride (Zilmax®, Intervet México, México City), is a β -AA that produces extremely weak pharmacological actions in man, and are so rapidly metabolized and cleared from the animal's body, that it is virtually impossible to regard them as potential causes of drug poisoning in human beings, even after consuming meat products derived from animals medicated with these drugs (Sumano et al., 2002). Zilpaterol hydrochloride is a β -AA commercially available in México (Zilmax®, Intervet México, México City), but registration and commercial use are approved in other 25 countries, such as the United States (NADA 141-258; FDA, 2006), Canada (Canadian Food Inspection), Brazil, Colombia, Peru, the Republic of South Africa South, Kazakhstan, Kore and others.

Nourozi et al. (2008) found agonists to have different effects depending on the type of adipose tissue. They reduce the amount of visceral fat (Samadi et al., 2013) and intramuscular fat (Mondragon et al., 2010), but do not affect subcutaneous fat (Yang and Mc Elligott, 1989; Coleman et al., 1988; Estrada et al., 2008). These effects on fat can modify meat taste and tenderness. Furthermore, β -AA also alter color, pH and texture (Davila et al., 2013; Mondragon et al., 2010; Salinas et al., 2006). All this indicates that use of β -AA on sheep production may substantially alter the organoleptic quality of meat. Given the above, the aim of this study was to evaluate the effect of the zilpaterol hydrochloride on meat quality in lambs from terminal crossbreeding of Katahdin females mated to males Charollais and Dorper.

2. Materials and Methods

2.1. Animal Management and Experimental Design

Lambs were raised in the municipality of Colón, Ouerétaro, 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). A 200-head Katahdin ewe flock of 51 ± 18 months of age and 6 ± 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). A commercial diet (creep feeding) was given to the lambs from birth to weaning $(78 \pm 6 \text{ days})$; 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 ± 13 days) under similar management conditions, which included a totally mixed ration (sorghum 47.2%, molasses 20.0%, dry alfalfa 11.0%, corn stover 8.0%, canola 6.5%, soybean paste 4.0%, mineral and urea mix 3.3%) with 14% crude protein (CP), 2.9 Mcal of EM/kg MS and water ad libitum. The same mixed ration was used in the fattening stage, for both treatments, 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. Work was done with the support of a cooperating producer under ordinary commercial conditions for sheep production. Following Intervet recommendations, ZH supplementation was withdrawn three days before slaughter. At the end of the fattening period, 10 animals from each group were randomly selected for slaughter. The lambs were slaughtered at Federal Inspection Type Facility No. 412 at San José El Alto, Querétaro, according to the industrial processes and procedures for animal welfare that have been established by federal authorities.

2.2 Meat Instrumental and Sensory Evaluation

After slaughter, hot carcasses were weighed and cooled at 4 °C for 24 h. Then, carcasses were divided in two along the backbone. Later, the rack was removed from the left side cutting from the 4th to the 12th thoracic vertebra and measures were taken. Final pH_{24h} was measured at the 12th thoracic vertebra, 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. In addition, Hue (h*) and Chroma (C*) were calculated. Before taking the measurements, meat

samples were allowed to bloom for 1 h at 12 ± 1 °C. To determine meat composition (percentage of moisture, protein, intramuscular fat and ash) the 9th or 10th rib eye steak was extracted of the loin, and the official methods of analysis (AOAC, 2002) used. The left shoulder was separated from each carcass following the methodology of Vergara (2005). Shoulders were thawed for 24 h under refrigeration and weighed before being dissected (muscle, fat and bone) using Boccard methodology (1976). Waste (fascia, nerves tendons, vessels, etc.) was recorded with the bone. Indirect water holding capacity (water loss) was determined on the Longissimus dorsi muscle (MLD) by compression method and expressed as percentage of released juice (Pla, 2005). Briefly, a 0.30±0.05 g loin sample, between the 6-8th rib, was placed on a No. 4 Whatman filter paper, previously weighed. The sample covered with glass was pressed under a 2.25 kg weight for 5 min, afterwards the filter paper was weighed again and the percentage of juice loss from the sample was calculated based on weight difference (Pla, 2005). Shear force (SF) was determined according to Honikel (1998), but ageing period was only 24h. The MLD steaks were thawed for 24h and cooked on a double plaque broiler to an internal temperature of 70 °C, which was monitored with iron-constantan thermocouples (Omega Engineering Inc., Stamford, USA) and a recording portable thermometer. Upon reaching the desired internal temperature, steaks were removed from the broilers and allowed to cool to room temperature (20-25 °C). Subsequently, from each steak, a minimum of six, 1.27-cm-diameter cores were obtained parallel to the longitudinal orientation of muscle fibers. Cores were cut perpendicularly using a texture analyzer TA. XT Plus (Stable Micro Systems). To quantify the texture of the raw meat, we measured stress of compression at 20% (C20) and at 80% (C80) of maximum compression (N/cm2) using a modified compression device that avoids transversal elongation of the sample (Lepetit & Culioli, 1994).

2.3 Sensory Evaluation

The *M. Longissimus lumborum* was used for sensory evaluation. Each steak was thawed for 24h, wrapped in aluminum foil and cooked on a double plaque broiler until 70 °C was reached at the geometrical center. Upon reaching the desired internal temperature, muscles were removed from the broiler and portioned into cubes of uniform dimensions (2 cm³) and kept warm until evaluation. A consumer affective test was performed using a seven point hedonic scale from 1) I dislike it very much; to 7) I like it very much. In sample preparation and present samples to panelists, Campo (2005) methodology was used. A total of 60 panelists from different sex, age and cultural background participated in the study. Each panelist was presented four samples and asked to score each sample for flavor, tenderness, juiciness and overall desirability.

2.4 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). 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, maternal age, etc., the weight of the animals at the beginning of the finishing (ZH) period was used as a covariate (Steel & Torrie, 1980). Water retention capacity (WRC) and chemical composition data were analyzed with the Cochran-Mantel-Haenszel test and meat pH were analyzed for normality using the Shapiro-Wilk test. Results from the sensory evaluation were analyzed by the Kruskal-Wallis test. All tests were performed at a 95% confidence level.

3. Results and Discussion

Two level interactions were not significant (P > 0.05) for any of the studied traits, so results are presented for the main effects (sire breed and zilpaterol hydrochloride level). Table 1 shows effects of sire breed (SB) and zilpaterol hydrochloride (ZH) on instrumental meat quality. The final pH was not affected by SB (P = 0.61) or by ZH supplementation (P = 0.49).

Variable	Sire breed			Zilpaterol hydrochl		
Variable	Charollais	Dorper	P value	0	0.15	P value
pH 24 h	5.7±0.4	5.7±0.5	0.605	5.7±0.0	5.7±0.5	0.490
Water loss ¹	26.6±0.6	28.1±0.7	0.122	27.1±0.6	27.6±0.7	0.634
Meat Color						
L*	34.0±0.6	35.6±0.6	0.024	37.7±0.6	31.9±0.6	0.001
a*	13.4±0.3	14.9±0.4	0.007	15.5±0.4	12.9±0.4	0.001
b*	5.6±0.3	6.6±0.4	0.001	3.7±0.4	8.4±0.3	0.435
h*	20.8±0.9	23.0±0.9	0.019	28.6±0.9	15.1±0.9	0.001
C*	14.7±0.4	16.4±0.5	0.004	17.7±0.8	13.5±0.5	0.001
Texture:						
Shear force (kgf)	4.9±0.2	4.3±0.2	0.068	3.7±0.2	5.4±0.2	0.001
Compression 20% (N/cm ²)	5.6±0.2	5.8±0.2	0.390	5.6±0.2	5.7±0.9	0.218
Compression 80% (N/cm ²)	21.8±0.7	23.1±0.8	1.460	22.4±0.7	22.5±0.6	1.480
Total load (N)	27.3±0.8	28.8±0.9	1.408	28.1±0.9	28.1±0.9	0.100

Table 1	. Effects	of sire	breed a	nd zilpa	terol hv	ydrochlor	ide on	instrumental	meat c	juality	$(Mean \pm EE)$
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2-way interactions were not significant (P > 0.05) for any traits. ¹Water loss expressed as juice expelled (%). L* = lightness; a* = red index; b* = yellow index; h* = Hue (tone); C* = Chroma (saturation). Color scale: Red index (a*) = 60 (red) to -60 (green); yellow index (b*) = 60 (yellow) to -60 (blue); lightness (L*) = 0 (black) to 100 (withe); h* = arctan (b*/a*) (0 to 360 degrees); C* = $\sqrt{(a*)^2 + (b*)^2}$ (0 to 200).

There was not effect of genetic group (P > 0.05) or ZH in water loss (WL) averaging $27.4 \pm 0.7\%$ of expelled juice. Water loss is a variable associated to meat juiciness and values between 25-35% are consistent with previous results (Partida et al., 2012) and they point out juicy meat. These results are consistent with those of studies that evaluated two β -AA on finishing lambs where not significant effects were observed on pH, hold water capacity and water loss (Brand et al., 2013; López et al., 2010). However, present results contrast partially with those of Dávila et al. (2013) that showed pH values of 6.2 in hair lamb treated with ZH, which is a value that would correspond to a dry, firm and dark meat (Vergara, 2005). Other studies showed that β -AA administration reduces pH drop after slaughter due to anabolic metabolism (Gregory, 1998; Moloney & Beermann, 1996).

Meat color was affected by SB and ZH (P < 0.05). KD had slightly higher (P < 0.024) lightness (L*), redness (a*), yellowness (b*), hue (h*) and Chroma (C*) values than KCh. On the other hand, ZH meat had lower (P < 0.001) L*, a*, h* and C* values than meat from the control animals. Literature review indicates that the effect of ZH on meat color has not been consistently shown. Different factors contribute to this inconsistency, such as final pH and temperature (Sañudo et al., 1989), lower concentration of heme pigment (Ferreira & Bastos, 1994; Warris et al., 1990), small quantity of oxy-myoglobin (Ramos & Silveira, 2002). Some studies using sheep have found no effect of ZH on color variables (Avendaño et al., 2006; Romero, 2011; Berge et al., 1990), 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 high in energy rations (Wulf & Wide, 1999).

No differences (P > 0.05) due to SB or ZH administration were observed in any compression parameters. Shear force (SF) was not affected by SB (P > 0.05), but ZH produced meat 46% hardest than the control treatment (P = 0.001), which agrees with tenderness data from the sensory evaluation (see below). The decrease in meat tenderness in animals treated with ZH has also been observed by other researchers (Kim et al., 1987; Koohmaraie et al., 1996; Ketchmar et al., 1987; Davila et al., 2013; Mondragón et al., 2010). Meat softness is the most important organoleptic property for consumers (Koohmaraie, 1994) and a value around 3.7 kgf is considered very soft meat, while 5.4 kgf correspond to hard meat (Wheeler et al., 1997). According to literature, in meat that not have been aged for more than 24h, the ZH effect on the meat hardness could be explained by different factors such as: intramuscular fat decrease (Holmer et al., 2009; Mondragón et al., 2010), increase in the muscle fibers diameter (Buttery et al., 2000), increase in amount of connective tissue and the number of fast twitch fibers type II (Maloney & Allen, 1996).

Table 2 shows effect of SB and ZH on shoulder tissue composition. Sire breed did not affect (P > 0.05) fat, bone

and muscle percentages, but ZH administration produced 22.4% less fat (P < 0.001), 5.7% less bone (P < 0.004) and 7.5% (P < 0.001) more muscle in the shoulder than the control ones.

Variable -	Sire	Sire breed			Zilpaterol hydrochloride (mg/kg LW)		
	Charollais	Dorper	P value	0	0.15	P value	
Fat	11.2±0.5	11.9±0.5	0.968	12.5±0.5	9.7±0.5	0.001	
Bone	23.8±0.3	24.0±03	0.307	24.6±0.2	23.2±0.3	0.004	
Muscle	63.4±0.4	63.7±0.5	0.752	61.2±0.4	65.8±0.5	0.001	
Ratio:							
Muscle : fat	5.7±0.2	5.4±0.1	0.860	4.9±0.1	6.8±0.2	0.001	
Muscle : bone	2.7±0.1	2.7±0.0	0.530	2.5±0.1	2.8±0.1	0.010	

Table 2. Effects of sire breed and zilpaterol hydrochloride on shoulder tissue composition (%) on lamb (Mean \pm SE)

2-way interactions were not significant (P>0.05) for any traits.

These data originated better ratios (P < 0.01) muscle/fat (4.9 vs 6.8) and muscle/bond (2.5 vs 2.8) for ZH treated animals. Shoulder tissue composition is highly correlated to body composition (% muscle $r^2 = 0.97$; % fat $r^2 = 0.95$; % bone $r^2 = 0.73$) (Vergara, 2005), so ZH supplementation increased the final amount of shoulder meat by 0.240 kg, which is equivalent to 1.6 kg of additional marketable meat. The effect of ZH on carcass composition has been widely reported on sheep (Salinas et al., 2004; Salinas et al., 2006; Estrada et al., 2008; López et al., 2010; López et al., 2011; Mondragón et al., 2010) and it is agreed on the decrease in amount of fat and the increase on the proportion of muscle by the CZ effect, which can be explained by the cell metabolism modification, favoring protein synthesis and fat degradation (Mersmann 1998; Li et al., 2000). Additional protein, formed with energy derived from lipid catabolism, is deposited within the cells originating muscle hypertrophy (Kim et al., 1987; Maloney et al., 1990). However, every muscle responds according to type and proportion of muscle fibers, being more evident the hypertrophy in type II fast contracting fibers (Bermann, 2002).

SB and ZH affected meat composition (Table 3). Meat from Charollais breeds had slightly more fat (2.8 vs. 2.0%) and less protein (21.3 vs. 21.8%) than Dorper. Also, ZH meat had 40.0% less fat (P<0.004) and 12.0% more protein (P < 0.001) than control meat. Differences in meat composition between breeds may be due to differences in degree of maturity of each breed (Warris, 2003); in the present study, Charollais reached maturity faster than Dorper animals. Boler et al. (2009) and Ferguson (2001) reported more moisture and less fat in carcass originated from ZH treated animals.

Variable	Sir	re breed		Zilpaterol hydrochloride (mg/kg LW)		
%	Charollais	Dorper	P value	0	0.15	P value
Moisture	74.0±0.2	74.3±0.2	0.316	73.9±0.2	74.3±0.2	0.137
Protein	21.3±0.1	21.8±0.1	0.004	20.8±0.1	22.3±0.1	0.001
IM ¹ Fat	2.8±0.2	2.0±0.2	0.006	3.0±0.2	1.8±0.2	0.001
Ash	1.1±0.1	1.0±0.1	0.459	1.0±0.0	1.3±0.5	0.104

Table 3. Effects of sire breed and zilpaterol hydrochloride on meat chemical composition (%) on lamb (Mean \pm EE)

¹ IM = Intramuscular.

In general, consumer rated high (above average) meat in this study (Table 4). Sire breed did not affect meat sensory properties (P > 0.05). ZH meat had lower juiciness (5.2 vs. 4.7), softness (5.4 vs. 4.5), and overall likeness (5.3 vs. 4.9) values than the control treatment (P < 0.01). In sheep, there are few and inconsistent studies

evaluating the effect of CZ on the sensory characteristics of meat. Some researchers have observed ZH administration decreases softness, juiciness, flavor and overall likeness in sensory meat evaluation (Leheska et al., 2008; Hilton et al., 2014; Morón et al., 2002; Ramos and Silveira, 2002), while others have not observed significant differences in sensory data (Dávila et al., 2013; Mondragon et al., 2010; Schroeder, 2004). β -AA affect negatively the aging potential of meat because they increase activity of calpastatins (Hope-Jones et al., 2010) and/or decrease the amount of intramuscular fat (Dávila et al., 2013; Holmer et al., 2009; Mondragón et al., 2010).

Table 4. Effects of sire breed and zilr	aterol hydrochloride on meat sensor	y quality on lamb	$(Mean \pm EE)$
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Variable	Sire breed		Zilpaterol hydrochloride (mg/kg LW)			
	Charollais	Dorper	P value	0	0.15	P value
Flavor	4.9±0.1	5.1±0.1	0.392	5.1±0.1	4.9±0.1	0.098
Juiciness	4.9±0.1	5.0±0.1	0.314	5.2±0.1	4.7±0.1	0.003
Softness	4.8±0.1	5.1±0.1	0.118	5.4±0.1	4.5±0.1	0.001
Overall likeness	5.0±0.1	5.1±0.1	0.725	5.3±0.1	4.9±0.1	0.004

Hedonic scale considered 7 points: 1 = I dislike it very much; 7 = I like it very much. Sensory test involved 60 panelists, of whom 48.3% were men with an average age of 31 years (23-44 years) and the remaining 51.7% were women with an age of 33 years in average (22-51 years).

4. Conclusions

The breed of the sire did not affect most of the meat traits, only Katahdin x Charollais crosses had lower meat color values, more fat and less protein than Katahdin x Dorper. Zilpaterol hydrochloride-treated animals had meat with less lightness, red color, hue and Chroma. Zilpaterol hydrochloride increased shear force on meat, and produced more muscle and less fat and bone. Zilpaterol hydrochloride use in lamb production caused leaner meat and more protein retention, at the expense of reducing meat sensory qualities.

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