Effect of Zilpaterol and Ractopamine on biometric parameters and muscle fiber thickness in Pelibuey lambs

Efecto del zilpaterol y la ractopamina sobre los parámetros biométricos

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ABSTRACT. The objective of this study is to evaluate the effect of β-agonists (BA), Zilpaterol Hydrochloride (ZH), and Ractopamine Hydrochloride (RH) on the biometric parameters and the muscle fiber thickness in Pelibuey lambs (n = 6). The control group was fed a standard diet (16% protein content and 12 MJ per day); treatment groups received a standard diet supplemented with ZH or RH for 37 days and was assessed presence of BA in samples of muscle tissues, and urine. The effect of Daily Weight Gain (DWG) and Food Conversion Rate (FCR) on muscle fiber thickness was determined by Scanning Electron Microscopy (SEM) and Digital Image Analysis (DIA). Results showed an increase of 30% in DWG in the ZH group, compared with RH. While FCR was lower in ZH (3.83) than in RH (4.85). In both BA treatments, the withdrawal time was sufficient to minimize drug concentration in meat. In contrast with the control group, muscle fiber thickness increased in BA treated groups (ZH = 69.2%, and RH = 15.6%). In conclusion, ZH displayed a higher carcass yield in Pelibuey lambs.
Key words: β-adrenergic, digital image analysis, meat, Pelibuey sheep.

RESUMEN. El objetivo de este estudio fue evaluar el efecto de los β-agonistas (BA), Clorhidrato de Zilpaterol (ZH) y el Clorhidrato de Ractopamina (RH) sobre los parámetros biométricos y el grosor de la fibra muscular en corderos Pelibuey (n = 6). El grupo control recibió una dieta estándar (DS) (16% de contenido de proteína y 12 MJ por día), mientras que los grupos de tratamiento recibieron DS+ZH o DS+RH durante 37 días. Se evaluó la presencia de BA en tejido muscular y orina, determinó el aumento de peso diario (DWG), la tasa de conversión de alimentos (FCR) sobre el grosor de la fibra muscular, mediante microscopía electrónica de barrido (SEM) y análisis de imagen digital (DIA). Los resultados mostraron 30% más en DWG en el grupo ZH, que en RH. Mientras que la FCR fue menor en ZH (3.83) que en RH (4.85). El tiempo de supresión aplicado fue suficiente para que los grupos ZH y RH no mostraran residuos. En contraste con el grupo de control, el grosor de las fibras musculares aumentó en los grupos tratados con BA (ZH = 69.2% y RH = 15.6%).
Palabras clave: β-adrenérgico, Análisis Digital de imágenes, carne, Borrego pelibuey.

INTRODUCTION

Meat is one of the most nutritious foods due to its supply of high biological value proteins, lipids, B-complex vitamins, and minerals like iron, zinc, and phosphorus (McAfee *et al*. 2010). In the last decades, the increase of the worldwide population has generated a higher demand for meat products (fresh, refrigerated, frozen, or processed) despite their high economic cost. The most consumed meat products, in descending order, are beef, lamb, mutton, poultry and pork (Boler *et al*. 2012, Sans and Combris 2015). To meet the consumers' demand, countries such as Mexico, Brazil, and the United States of America (USA) have approved the use of growth promoters in livestock to increase carcass yield (Giannetti *et al*. 2016, Parr *et al*. 2016, Chu *et al*. 2017). β-agonists (BA) are highly utilized in the meat industry, including Zilpaterol Hydrochloride (CA) and Ractopamine Hydrochloride (RH) however, Clenbuterol Hydrochloride (CC) remains unregulated by food safety authorities (Mondragón *et al*. 2010, Montes-Nino *et al*. 2017, Ma *et al*. 2018).

Due to their anabolic effects, BA at high doses can increase lean muscle mass in animals (Whipple *et al*. 1990, Domínguez-Vara *et al*. 2009). These compounds are analogs of adrenaline and noradrenaline, derived from phenylethanolamines, thus differ in structure from the endogenous catecholamines in the aromatic ring and the amino group (Giannetti *et al*. 2016, Parr *et al*. 2016). These present powerful pharmacological activity in humans acting as bronchodilators and tocolytic agents (Al-Majed *et al*. 2017), but when administered to livestock species, they promote growth and weight gain (Parr *et al*. 2016). β-2 adrenergic receptor agonists such as clenbuterol and cimaterol, produce the greatest effect on muscle growth (Parr *et al*. 2016); RH binds to both β-1 and β-2 adrenergic receptors and produces similar results (Rikard-Bell *et al*. 2009). Zilpaterol or terbutaline also improve the growth rate and the feed efficiency of lambs under thermo-neutral conditions (Avendaño-Reyes *et al*. 2011, Avendaño-Reyes *et al*. 2018).

The repartition action of BA-increasing muscle proteins and decreasing adipose tissue deposition-has positive effects on body weight gain, feed conversion ratio (FCR), and muscle growth (Mersmann 1998, Fang *et al*. 1999), depending on the animals' characteristics such as species, breed, age, etcetera.

However, BA administered at high doses may cause toxic reactions in consumers, for example, distal tremors, palpitations, headaches, facial erythema, nausea, tachypnea-dyspnea, vomit, and colic (Brambilla *et al*. 2000, Lefaucheur 2010). Therefore, BA must be removed from the animals' diet at least 72 h before slaughter to ensure that all residues are excreted in the urine (Álvarez-Fernández *et al*. 2011). Trade guidelines have changed through the years, the European Community demand BA-free products (FEEDAP 2009); a directive that the USA, Canada, and Mexico have adopted. To comply with industry criteria, some analytical detection methods have been used: High-Performance Liquid Chromatography (HPLC), High-Performance Liquid Chromatography-Mass spectrometry (HPLC-MS), and Liquid Chromatography with Electrospray Ionization and Tandem Mass Spectrometry (LC-ES/MS /MS) (Williams *et al*. 2004, Montes-Nino *et al*. 2017, Avendaño-Reyes *et al*. 2018).Few reports exist on the effects of BA on muscle fibers and the effectiveness of withdrawal time in sheep (Hernández-Martínez *et al*. 2014, Costa-Lima *et al*. 2015, Dávila-Ramírez *et al*. 2015). The objective of this study is to evaluate the effect of Zilpaterol hydrochloride (ZH), and Ractopamine hydrochloride (RH) on biometric parameters and muscle fiber thickness in Pelibuey lambs.

MATERIALS AND METHODS

Experimental Animal Testing

Eighteen Pelibuey male lambs aged 2.5 months and weighing 18 $\pm$ 1 kg were acquired from Rodeo Ranch in Jalapa municipality, state of Tabasco, in south-eastern Mexico. Animals were dewormed with closantel 10% (Digavet, Belgium), 1 mL oral albendazole per 10 kg of body weight (Pfizer, USA) and treated with an intramuscular injection of vitamins A, D and E (2 mL 10 kg^{-1} of bodyweight, Pfizer, USA) following the technical specifications of the products and per accordance with the UK Animals (Scientific

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Procedures) Act 1986, EU Directive 2010/63/EU for animal experiments, the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the Mexican Official Standards (SAGARPA 1995, SAGARPA 2014).

Lambs were fed with a standard diet (SDiet) -12 MJ per day with 16% protein- formulated with milled corn, whole wheat, soy paste, grass, coconut paste, molasses, and a supplement of minerals and vitamins (AFRC 1993). Water was provided ad libitum through 5 L dispensers. After being weighed, animals were randomly divided into three groups of six lambs each, according to the BA they received: SDiet or negative control, ZH group = SDiet+ZH (Zilmax, Merck Sharp & Dohme Corp, USA), and RH group = SDiet+RH (Lapi-Racto. REG. SAGARPA Q-20183-158, Lapisa). Dosage of growth promoters was given in accordance to the NOM-EM-015-Z00-2002 (SAGARPA 2002) and the technical specifications of the product (C115 g ZH, and 125 g RH). The experiment lasted 40 days: growth promoters were supplied daily for 37 days plus the withdrawal period of 72 h. All feeding was withdrawn 12 h before slaughter. The response variables were final live weight gain (FW), average daily weight gain (ADG), feed conversion ratio (FCR), and feed consumption for 40 days. In addition, urine samples were taken from each animal (days 0, 10, 20, 30, and 40) and placed in sterile assay tubes, frozen immediately by using liquid nitrogen and stored at -20 ^{o}C until analysis.

All lambs were slaughtered following the Mexican Official Standard on animal welfare, NOM-033-ZOO-1995 (SAGARPA 2014). For each animal, 350 g of different cuts from the hindquarter were stored at - 20 ^{o}C until analysis.

Extraction of BA from muscle samples

To extract the BA, 100 g of muscle was blended with 40 mL of acetonitrile and 20 mL of isopropanol during 30 s. NaCl (1.2 g) was added to the blend, mixed on vortex for 2 min, supplemented with 4 g of Na_2SO_4 and 0.5 g of $MgSO_4$ and mixed again for 2 min (Water, 2012). Lastly, the samples were centrifuged (Hermle Model Z323K, Germany) at 1960 g for 10 min. The supernatant was dried using a rotary evaporator (Büchi R-215, Labortechnik, Switzerland). The pellet was dissolved in 1 mL of 0.1% formic acid, sonicated for 2 min, passed through cartridges (Oasis MCX 3 cc Vac Cartridge, 60 mg Sorbent per cartridge, 30 μm, Waters, USA) and filtered (Membrane Filter, 0.45 μm and 0.22 μm pore size, Merck Millipore, Ireland) (Water 2012).

Determination of BA in urine and meat samples

Identification and quantification of growth promoters (BA) were determined through HPLC using a five-point calibration curve of the standards in the following concentrations for each group: ZH (0.47, 0.23, 0.11, 0.05, 0.02 g mL^{-1} diluted in 50:50 methanol: water), and RH (0.66, 0.33, 0.16, 0.08, 0.04 g mL^{-1} diluted in 50:50 methanol: water) (Sumano *et al.* 2002). After extraction, samples were analyzed according to the USDA (2012). Urine samples were injected directly after filtration (0.45 μm). The standards were filtered (0.45μm) and injected into the HPLC (HPLC 1200 (Agilent Technologies, Germany), at 4°C, in a C-18 column (ZORBAX Eclipse column, XDB 80Å C18, 4.6 x 150 mm, 5 μm, Agilent, USA), with a flow of 20 μL min^{-1} at 210 nm to 280 nm wavelengths, using as mobile phase (A) water and 1% formic acid, and as a gradient (B) acetonitrile and 1% formic acid (Waters 2012).

Light microscopy

Micrographs of the meat samples from each treatment group were taken through optical microscopy. Samples were cut in 5 mm sections and placed in 35 mL of fixative FAA (30 mL of 40% formaldehyde, 150 mL of ethanol, 15 mL of acetic acid, and 105 mL of distilled water) for 72 h. Afterwards, they were washed and dehydrated with water: ethanol (3:1, 1:1, 1:3) and absolute ethanol, for 40 min. Samples were placed in xylol: ethanol (3:1,1:1,1:3) until absolute xylol was reached, during 20 min; then embedded in paraffin: xylol, set in a microscope slide, and stained with haematoxylin-eosin. Longitudinal and transverse sections were observed under LEYCA RAM optical microscope at 60X, 150X, and 300X (Arzate-Vázquez *et al.* 2011).

Scanning Electron Microscopy (SEM)

Using a scalpel blade no. 10, muscle tissue cuts were made to obtain longitudinal and transversal sections (1.0 x 1.0 x 0.5 cm). These were fixed in 4% formaldehyde and 25% glutaraldehyde, later washed, and dehydrated with water: ethanol (25:100). Finally, the sample sections were dried until critical point in an SPI-Module Sputter (SPI-supplies, West Chester, PA), carbon coating equipment at 34-35 ^{o}C and 1200 psi, and covered with gold for 40 s (Estrada-Solis *et al*. 2016). Micrographs were taken at 5 kV through scanning electron microscope (JSM-7800F, JEOL, Japan).

Digital Image Analysis (DIA)

Ten micrographs were analyzed with ImageJ software (1.52q, National Institutes of Health, USA) to determine muscle thickness. The scale was set to 2.55 pixels/μm based on SEM imaging (5 kV, 200X magnification) previously obtained, and the images threshold was automatically adjusted by the software (approximately 100). Average muscle fiber thickness was determined from a minimum of 20 muscle fibers per micrograph using ImageJ software measuring tool.

Statistical analysis

The results of biometric parameters and muscle fiber thickness were analyzed with an ANOVA test using Sigma Plot 12.5 software (Systat Software, USA) and reported as average $\pm$ standard error. The contrast of means was performed through the Tukey Test, assuming a significance level of 0.05.

RESULTS

***In vivo* assessment of β-agonists in lambs**

Table 1 shows a greater DWG in the animals fed with BA (93 and 49% for ZH and RH, respectively), compared with the control group, during the 37 days of BA treatment. DWG pairwise comparisons between all three groups were statistically significant ($p \leq 0.05$). FCR was lower in ZH in contrast to RH and the control group ($p \leq 0.05$). FE was higher in the ZH group (26.00%) than in the RH group (20.63%), ($p \leq 0.05$).

Kinetics and urinary excretion of β-agonists

Firstly, to detect BA on muscle samples, the UV spectrum of each standard was obtained using a UV detector of 190-380 nm; maximum wavelengths for standard samples were: ZH = 210 nm, 8.9 min retention time and RH = 280 nm, 12.49 min retention time.

Secondly, the concentration of BA excreted in urine was evaluated. Higher urinary excretion of BA occurred in the first 10 days of treatment (Table 2). At day 40, the excreted amount of BA was minimal (ZH = 0.14 mg mL^{1} and RH = 0.0039 mg mL^{-1}), indicating that a pre-slaughter withdrawal period of 72 h allows proper elimination of ZH and RH.

Determination of β-agonist in meat samples

Analysis of meat samples through HPLC reported the absence of ZH and RH residues, probably due to their urinary excretion and the withdrawal period (72 h) before slaughter.

Optical microscopy of lamb samples

Optical microscopy allowed to characterize different cellular structures of lamb samples - to assess the effect of BA on their lipid content. Optical micrographs of the transverse sections of the lamb samples reflect the intensity of lipolysis in the BA treatment groups, compared to the control group (Figure 1).

SEM of lamb samples

Figure 2 displays morphological aspects of lamb meat samples in the treated groups. Elongated muscle fibers surrounded by dense connective tissue (epimysium) were identified in groups ZH and RH (1). While the control group, showed contracted fibers with a corrugated appearance (2). this effect may indicate stress on the lambs during slaughter. Abundant connective tissue was detected in ZH treatment samples (3).

Muscle thickness

200 muscle fibers were measured with DIA

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Table 1. Biometric parameters of lambs fed with a control diet and BA-supplemented diet over a 40-day experiment.

Indicator	Control	ZH	RH
IW (kg)	18.03 ± 0.13^{a}	17.94 ± 0.38^{a}	18.01 ± 0.02^{a}
FW (kg)	25.3 ± 0.84^{c}	31.94 ± 0.13^{a}	29.01 ± 0.07^{b}
DWG (g)	181.66 ± 19.32^{c}	350.00 ± 18.91^{a}	270.00 ± 20.08^{b}
FCR	7.83 ± 0.66^{c}	3.83 ± 0.20^{a}	4.85 ± 0.22^{b}
FE%	13.63 ± 1.53^{c}	26.25 ± 1.16^{a}	20.63 ± 0.79^{b}

IW = Initial weight, FW = Final weight, DWG = Daily weight gain, FCR = Feed Conversion Ratio, FE = Food efficiency, ZH = Zilpaterol Hydrochloride, RH = Ractopamin Hydroclorhide. Results correspond to the mean of n = 9 ± SE. Different letters in the same row indicate statistically significant differences (Tukey Test, $p < 0.05$).

Table 2. Kinetics of BA present in the urine of lambs treated with two growth promoters.

Day \ Treatment	ZH (μg/mL)	RH (μg/mL)
10	$1.86 \pm 0.003^{c,A}$	$3.06 \pm 0.005^{a,A}$
20	$1.81 \pm 0.005^{b,B}$	$0.88 \pm 0.005^{c,B}$
30	$1.09 \pm 0.003^{b,C}$	$0.38 \pm 0.003^{c,C}$
40	$0.14 \pm 0.005^{b,D}$	$0.0039 \pm 0.009^{c,D}$

ZH = zilpaterol hydrochloride, RH = ractopamine hydrochloride. Results correspond to the mean of n = 9 ± SE. Different small letters in the same row indicate statistically significant differences (Tukey Test, $p < 0.05$) between treatments. Different capital letters in the same column indicate statistically significant differences (Tukey Test, $p < 0.05$).

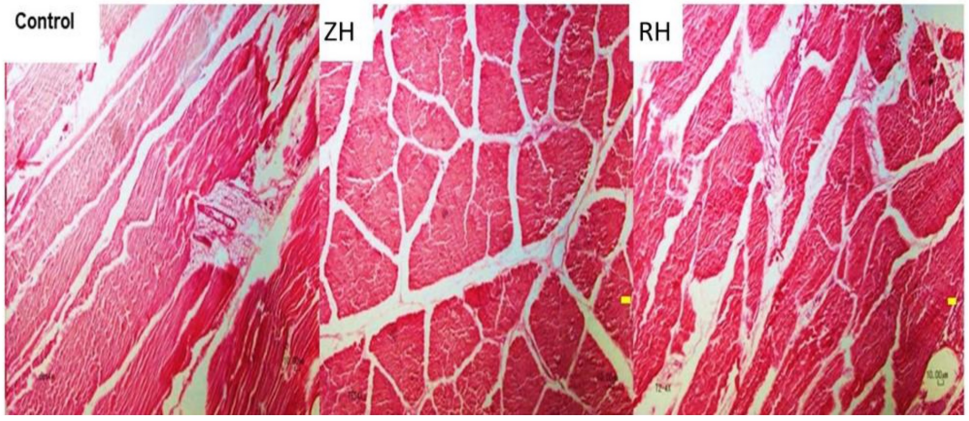

Figure 1. Kinetics of BA present in the urine of lambs treated with two growth promoters.

software using the longitudinal-section micrographs obtained by SEM. Results show that muscle fiber thickness may be proportional to drug bioavailability. This seems to be correlated with the observations under optical microscopy (Figure 1) in which RH treatment might increase fat content in lamb samples. A deeper study is currently in progress to assess differences in fatty acids content of the samples in the BA-treated groups.

DISCUSSION

In vivo assessment of β-agonists in lambs

Positive effects in DWG were observed from day 13 of BA supplementation due to their capacity to stimulate muscle hypertrophy (Mersmann 1998) through inhibiting proteolysis and lipogenesis. stimulating protein synthesis, and redirecting energetic substrates towards muscle accretion (Mills 2002, Chu *et al.* 2017, Claffey *et al.* 2018). In the

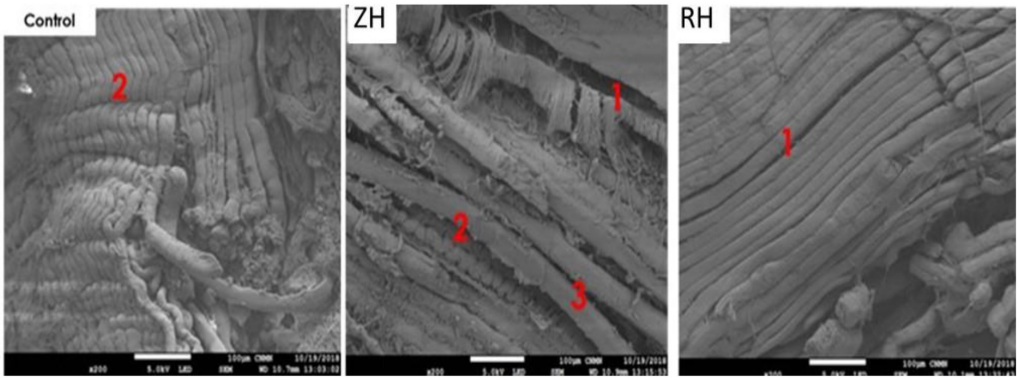

Figure 2. Scanning electron longitudinal-section micrographs of lamb samples in Control group, Zilpaterol Hydrochloride (ZH) group, and Ractopamine Hydrochloride (RH) group, 200X magnification (bar scale corresponds to 100 μm). (1) muscle fibers surrounded by their dense connective tissue; (2) contracted fibers with corrugated appearance; (3) connective tissue.

present study, DWG was higher than reported by López-Carlos *et al*. (2010) who fed sheep a diet of 28% alfalfa and 47.65% yellow corn, which supplied 22 and 9% of raw protein into the diet, respectively. Differences in weight gain can be caused by multiple factors, for instance, the quality and amount of dietary protein, duration of treatment, sex, and hygiene (Parr *et al*. 2016, Tang *et al*. 2016, Rivera-Villegas *et al*. 2019). DWG reflects lean muscle mass gain, because BA act on the calpain-calpastatin complex, diminishing protein degradation (Dávila-Ramirez *et al*. 2015, González-Ronquillo and Angeles-Hernández 2017, Smith *et al*. 2019). These results are consistent with Resendiz *et al*. (2013), which declared that a high FE is an indicator of the efficiency and profitability of productive systems.

Kinetics of β-agonists excretion in urine

According to Waters (2012) and Morales-Trejo *et al*. (2013), selective analyses can reliably identify growth promoters in biological matrices of ruminants such as liver, meat, and urine. BA absorption depends on the administration pathway; drug levels in plasma and blood are higher after oral dosing than intramuscular administration. After reaching effective concentrations in muscle, BA are excreted into the urine (Meza-Márquez *et al*. 2012). Similarly to present findings, Van Hoof *et al*. (2005) reported urinary excretion of RH and ZH in calves; the maximum elimination rate (12.6 μg mL^{-1}) of both compounds occurred after the first 5 days of treatment. For this reason, urine is a reliable biological matrix to detect and quantify the elimination rate of BA (Van Hoof *et al*. 2005).

In all species, BA are mostly excreted through urine (50-58%), but a smaller portion are eliminated through feces (5-30%), and milk (0.9-3%) as non-metabolized products (Ma *et al*. 2018). Sumano *et al*. (2002) indicated that BA are bio transformed and rapidly inactivated by Catechol-O-Methyltransferase (COMT) enzyme, which methylates the hydroxyl groups on the aromatic ring. RH is metabolized by hepatic glucuronidation; hence, it is not a substrate for COMT.

Determination of β-agonists in meat samples

Lamb samples at day 40 were BA-free, after the withdrawal period recommended in the technical specifications for using ZH and RH. Mondragón *et al*. (2010), López-Carlos *et al*. (2011) and Avendaño-Reyes *et al*. (2018), agree that BA are fully eliminated from meat samples after 72 h.

Optical microscopy of lamb samples

Giannetti *et al*. (2016) indicated that the decrease of lipids or lipolysis in sheep -produced by lipases that hydrolyze triacylglycerols to glycerol and free fatty acids-facilitates glycolysis, oxidation, and the tricarboxylic acid cycle, which can derive in the synthesis of proteins. Vahedi *et al*. (2014) manifested

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that the addition of BA to the diet of ruminants increases muscle mass and decreases adipose tissue (depending on the cut area).

SEM of lamb samples

Meat has a complex structural organization, and its components impact its quality (Lu *et al*. 2000). Moreover, meat morphological structure indicates the post-mortem treatment to which the animal was subjected, this may modify quality attributes, such as tenderness (Li *et al*. 1999). Romero-Peñuela *et al*. (2011) stated that stress can only be measured by biomarkers or cortisol levels. The present study's findings revealed that in ZH groups the sarcoplasm was more compressed (F), compared with RH and control, which showed open basal cisterns that function as calcium storage. After the death of the animal, calcium is released to activate endogenous enzymes such as calpains and catechins that improve meat tenderness (Rubio-Lozano *et al*. 2011).

Muscle fiber thickness

The greater muscle fiber thickness was observed in the ZH group, which may be due to the bioavailability of the treatments. RH bioavailability ranges between 45-50% in ruminants, and 88% in pigs; while ZH bioavailability is 80% in ruminants (Sumano *et al*. 2002, Estrada-Solis *et al*. 2016). Results suggested that muscle fiber thickness is correlated with the yield in weight gain. as reported by Chulayo and Muchenje (2016), who described that the use of growth promoters in ruminants improved protein synthesis, hence, greater muscle thickness. As observed in Table 3, the percentage of increase in fiber thickness was higher after ZH treatment (69.2%) than after RH intervention (15.6%) compared to control. The reason beneath this significant difference is directly related to the increase that RH produced in FW respect to the control, this is, RH increased 14.7% the weight of the sheep, but such increase seems to be explained not only by the increase in muscle fiber thickness because the end percent increase of this parameter was the lowest after RH treatment.

Table 3. Statistical difference found in muscle fibre thickness and DWG between treatments.

Treatment	Mean muscle thickness (μm)	increase of thickness (%)	increase of FW (%)
Control	14.3 ± 0.99^{D}	-	-
ZH	24.2 ± 1.10^{A}	69.2 + 7.6	26.2 ± 0.51
RH	16.54 ± 0.52^{C}	15.6 ± 3.6	14.7 ± 0.27

DWG = Daily weight gain, Control, ZH = Zilpaterol Hydrochloride, RH = Ractopamin Hydroclorhide groups. Results correspond to the mean of n = 9 $\pm$ SE. Different letters in the same column indicate statistically significant differences (Tukey, p < 0.05).

Since the same diet was provided to all lambs, differences in muscle fiber thickness and weight gain depended only on the type of BA that was supplied; like the results of Giannetti *et al*. (2016) and Rivera-Villegas *et al*. (2019) on using ZH in cattle to promote weight gain. Furthermore, RH treatment increased muscle mass and fat simultaneously, probably due to the rapid metabolism of this BA (Sumano *et al*. 2002).

CONCLUSIONS

Utilizing BA at the dosage recommended by the manufacturers ensures the obtention of BA-free meat products, as they are efficiently excreted through urine. When comparing the treatment groups, ZH gave better results in weight gain and muscle fiber thickness. The BA, and particularly ZH, proving that it is more apt to achieve better performance lamb carcass yield. Using SEM and DIA, the effect that BAs have on the thickness of the muscle fiber was evidenced, showing that these techniques can be a tool in the evaluation of the quality of the meat if what you are looking for is to confirm its organic quality.

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LITERATURE CITED

AFRC (1993) Energy and protein requirements of ruminants. CAB International. Wallingford, UK. 122p.

Álvarez-Fernández E, Alonso-Calleja C, García-Fernández C, Rosa-Capita R (2011) Prevalence, and antimicrobial resistance of Salmonella serotypes isolated from poultry in Spain: Comparison between 1993 and 2006. International Journal of Food Microbiology 153: 281-287.

Al-Majed AA, Khalil NY, Khbrani I, Abdel-Aziz HA (2017) Clenbuterol hydrochloride. In: Profiles of drug substances, excipients and related methodology. Harry G (ed) Brittain Academic Press. London UK. pp: 91-123.

Arzate-Vázquez I, Chanona-Pérez JJ, Perea-Flores MJ, Calderón-Domínguez G, Moreno-Armendáriz MA, Calvo H, Godoy-Calderon S, Quevedo R, Gutiérrez-López G (2011) Image processing applied to classification of avocado variety Hass (*Persea americana* Mill.) during the ripening process. Food and Bioprocess Technology 4: 1307-1313.

Avendaño-Reyes L, Macias-Cruz U, Álvarez-Valenzuela FD, Águila-Tepato, Torrentera-Olivera NG, Soto-Navarro SA (2011) Effects of zilpaterol hydrochloride on growth performance, carcass characteristics, and wholesale cut yield of hair-breed ewe lambs consuming feedlot diets under moderate environmental conditions. Journal Animal Science 89: 4188-4194.

Avendaño-Reyes L, Torrentera-Olivera N, Correa-Calderón A, López-Rincón G, Soto-Navarro S, Rojo-Rubio R, Gerra-Liera JE, Macias-Cruz U (2018) Daily optimal level of a generic beta-agonist based on zilpaterol hydrochloride for feedlot hair lambs. Small Ruminant Research 165: 48-53.

Boler D, Shreck A, Faulkner DB, Killefer J, McKeith FK, Homm JW, Scanga JA (2012) Effect of ractopamine hydrochloride (Optaflexx) dose on live animal performance, carcass characteristics and tenderness in early weaned beef steers. Meat Science 92: 458-463.

Brambilla G, Cenci T, Franconi F, Galarini R, Macrı A, Rondoni F, Strozzi M, Loizzo A (2000) Clinical and pharmacological profile in a clenbuterol epidemic poisoning of contaminated beef meat in Italy. Toxicology Letters 114: 47-53.

Chu L, Zheng S, Qu B, Geng S, Kang X (2017) Detection of β-agonists in pork tissue with novel electrospun nanofibers-based solid-phase extraction followed ultra-high performance liquid chromatography/tandem mass spectrometry. Food Chemistry 227: 315-321.

Chulayo AY, Muchenje V (2016) Effects of animal class and genotype on beef muscle nanostructure, pH, colour and tenderness. South African Journal 112: 1-9. DOI: 10.17159/sajs.2016/20150056.

Claffey NA, Fahey AG, Gkarane V, Moloney AP, Monahan FJ, Diskin MG (2018) Effect of forage to concentrate ratio and duration of feeding on growth and feed conversion efficiency of male lambs. Translational Animal Science 2: 419-427.

Costa-Lima BR, Suman SP, Li S, Beach CM, Silva TJ, Silveira ETF, Bohrer BM, Boler DD (2015) Dietary ractopamine influences sarcoplasmic proteome profile of pork *Longissimus thoracis*. Meat Science 103: 7-12.

Dávila-Ramírez J, Macías-Cruz U, Torrentera-Olivera N, González-Ríos H, Peña-Ramos E, Soto-Navarro S, Avendaño-Reyes L (2015) Feedlot performance and carcass traits of hairbreed ewe lambs in response to zilpaterol hydrochloride and soybean oil supplementation. Journal of Animal Science 93: 3189-3196.

Domínguez-Vara IA, Mondragon- Anselmo J, González-Ronquillo M, Salazar-García F, Bórquez-Gastelum JL, Aragón-Martínez A (2009) Los β-agonistas adrenérgicos como modificadores metabólicos y su efecto en la producción, calidad e inocuidad de la carne de bovinos y ovinos: una revisión. CIENCIA ergo-sum 16: 278-284.

E. ISSN: 2007-901X

Estrada-Solís J, Figueroa-Rodríguez KA, Figueroa-Sandoval B, Hernández-Rosas F, Hernández-Cazares AS (2016) Microstructure and physical changes in the Mexican cooked lamb meat barbacoa made with chilled and frozen meat. Meat Science 118: 122-128.

Fang S, Nishimura HT, Takahashi K (1999) Relationship between development of intramuscular connective tissue and toughness of pork during growth of pigs. Journal of Animal Science 77: 120-130.

FEEDAP (2009) Scientific opinion of the panel on additives and products or substances used in animal feed on a request from the European Commission on the safety evaluation of ractopamine. EFSA Journal. 1041: 1-52.

Giannetti L, Ferretti G, Gallo V, Necci F, Giorgi A, Marini F, Gennuso E, Neri B (2016) Analysis of beta-agonist residues in bovine hair: Development of a UPLC-MS/MS method and stability study. Journal of Chromatography B 1036: 76-83.

González-Ronquillo M, Angeles- Hernández JC (2017) Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control 72: 255-267.

Hernández-Martínez M, Gallardo-Velázquez T, Osorio-Revilla G, Almaraz-Abarca N, Castañeda-Pérez E (2014) Application of MIR-FTIR spectroscopy and chemometrics to the rapid prediction of fish fillet quality. CyTA-Journal of Food 12: 369-377.

Lefaucheur L (2010) A second look into fibre typing-relation to meat quality. Meat Science 84: 257-270.

Li J, Tan J, Martz FA, Heymann H (1999) Image texture features as indicators of beef tenderness. Meat Science 53: 17-22.

López-Carlos M, Ramírez R, Aguilera-Soto JA, Plascencia A, Rodríguez H, Aréchiga CF, Rincon R M, Medina-Flores C.A. Gutierrez-Bañuelos H (2011) Effect of two beta adrenergic agonists and feeding duration on feedlot performance and carcass characteristics of finishing lambs. Livestock Science 138: 251-258.

López-Carlos MA, Ramírez RG, Aguilera-Soto JI, Aréchiga CF, Méndez-Llorente F, Rodríguez H, Silva JM (2010) Effect of ractopamine hydrochloride and zilpaterol hydrochloride on growth, diet digestibility, intake and carcass characteristics of feedlot lambs. Livestock Science 131: 23-30.

Lu J, Tan J, Shatadal P, Gerrard DE (2000) Evaluation of pork color by using computer vision. Meat Science 56: 57-60.

Ma L, Nilghaz A, Choi JR, Liu X, Lu X (2018) Rapid detection of clenbuterol in milk using microfluidic paper-based ELISA. Food Chemistry 246: 437-441.

McAfee AJ, McSorley EM, Cuskelly GJ, Moss BW, Wallace JM, Bonham MP, Fearon AM (2010) Red meat consumption: An overview of the risks and benefits. Meat Science 84: 1-13.

Mersmann HJ (1998) Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. Journal of Animal Science 76: 160-172.

Meza-Márquez OG, Gallardo-Velázquez T, Osorio-Revilla G, Dorantes-Álvarez L (2012) Detection of clenbuterol in beef meat, liver and kidney by mid-infrared spectroscopy (FT-Mid IR) and multivariate analysis. International Journal of Food Science & Technology 47: 2342-2351.

Mills S (2002) Implications of feedback regulation of beta-adrenergic signaling. Journal of Animal Science 80: E30-E35.

Mondragón J, Domínguez-Vara IA, Pinos-Rodríguez JM, González M, Bórquez JL, Domínguez A, Mejía ML (2010) Effects of feed supplementation of zilpaterol hydrochloride on growth performance and carcass traits of finishing lambs. Acta Agriculturae Scandinavica, Section A - Animal Science 60: 47-52.

Montes-Nino A, Granja RHMM, Reche KVG, Giannotti FM, de Souza JKG, Ferrari SPG, Dos Santos AD, Wansche ACBA, Salerno AG (2017) Laboratory validation of an LC-MS/MS method for the detection of ractopamine, clenbuterol and salbutamol in bovine and swine muscle at sub-μg kg-1 regulatory limits. Food Additives & Contaminants: Part A, 34: 785-792.

Morales-Trejo F, Vega-y-León S, Escobar-Medina A, Gutiérrez-Tolentino R (2013) Application of high-performance liquid chromatography-UV detection to quantification of clenbuterol in bovine liver samples. Journal of Food and Drug Analysis 21: 414-420.

Parr T, Mareko MH, Ryan KJ, Hemmings KM, Brown DM, Brameld JM. (2016). The impact of growth promoters on muscle growth and the potential consequences for meat quality. Meat Science 120: 93-99.

Reséndiz C, Hernández O, Guerrero I, Gallegos J, Martínez P, Sánchez C (2013) Fattening Pelibuey lambs fed with different alfalfa levels in the diet. Archivos de Zootecnia 62: 457-467.

Rikard-Bell C, Curtis MA, Van Barneveld RJ, Mullan BP, Edwards AC, Gannon NJ, Henman DJ, Hughes PE, Dunshea FR (2009) Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts. Journal of Animal Science 87: 3536-3543.

Rivera-Villegas A, Estrada-Angulo A, Castro-Pérez BI, Urías-Estrada JD, Ríos-Rincón FG, Rodríguez-Cordero D, Barreras A, Plascencia A, González-Vizcarra VM, Sosa Gordillo JS, Zinn RA (2019) Comparative evaluation of supplemental zilpaterol hydrochloride sources on growth performance, dietary energetics, and carcass characteristics of finishing lambs. Asian-Australas Journal of Animal Science 32: 209-216.

Romero-Peñuela MH, Uribe-Velásquez LF, Sánchez-Valencia JA (2011) Biomarcadores de estrés como indicadores de bienestar animal en ganado de carne. Biosalud 10: 13-15.

Rubio-Lozano MS, Martinez-Bruno JF, Hernández-Castro R, Bonilla-Contreras C, Méndez-Medina D, Nuñez Espinosa F (2011) Detection of *Listeria monocytogenes*, *Salmonella* and *Yersinia enterocolitica* in beef at points of sale in Mexico. Revista Mexicana de Ciencias Pecuarias 4: 107-115.

SAGARPA (1995) NOM-051-SCFI-1995. Norma Oficial Mexicana Nom-051-ZOO-1995, Trato Humanitario en la movilizacion de animales México: Diario Oficial de la Federación. http://www.fmvz.unam.mx/fmvz/p_estudios/apuntes_bioet/051zoo_movilizacion.pdf. Data consulted: April 10, 2021.

SAGARPA (2002) NOM-EM-015-Z00-2002. Norma Oficial Mexicana de Emergencia. Especificaciones técnicas para el control del uso de Beta-agonistas en los animales. México: Diario Oficial de la Federación. http://legismex.mty.itesm.mx/normas/zoo/zoo015em-02.pdf. Data consulted: April 10, 2021.

SAGARPA (2014) Proyecto de Modificación a la Norma Oficial Mexicana NOM-033-ZOO-1995, Sacrificio humanitario de los animales domésticos y silvestres. México: Diario Oficial de la Federación. https://www.cuautitlan.unam.mx/descargas/cicuae/normas/Norma033.pdf. Data consulted: April 10, 2021.

Sans P, Combris P (2015) World meat consumption patterns: An overview of the last fifty years 961-2011. Meat Science 109: 106-111.

Smith DJ, Shelver WL, Chakrabarty S, Hoffman TW (2019) Detection and quantification of residues in sheep exposed to trace levels of dietary zilpaterol HCl. Food Additives & Contaminants: Part A 36: 1289-1301.

Sumano H, Ocampo L, Gutiérrez L (2002) Clenbuterol and other b-agonists, are they an option for meat production or a threat for public health? Veterinaria Mexico 33: 137-159.

Tang Y, Gao J, Liu X, Lan J, Gao X, Ma Y, Lin M, Li J (2016) Determination of ractopamine in pork using a magnetic molecularly imprinted polymer as adsorbent followed by HPLC. Food Chemistry 201: 72-79.

E. ISSN: 2007-901X

USDA (2012) Screening and confirmation of beta-agonists by HPLC/MS/MS - CLG-AGON1.04. United States Department of Agriculture- Food Safety and Inspection Service, Office of Public Health Science. 2012. https://www.fsis.usda.gov/sites/default/files/media_file/2020-09/CLG-AGON1.pdf Data consulted: April 10, 2021

Van Hoof N, Schilt R, Van der Vlis E, Boshuis P, Van Baak M, Draaijer A, De Whasch K, Van de Wiele M, Van Hende D, Courtheyn D, De Brabander H (2005) Detection of zilpaterol (Zilmax®) in calf urine and feces with liquid chromatography-tandem mass spectrometry. Analytica Chimica Acta 529: 189-197.

Vahedi V, Towhidi A, Shahneh AZ, Sadeghi M, Zamani F, Dunshea FR (2014) Effects of β-agonist zilpaterol hydrochloride feeding and supplementation period on growth and carcass characteristics of Lori Bakhtiari lambs. Small Ruminant Research 119: 65-71.

Waters (2012) Acquity are registered trademarks of waters corporation, Waters Corporation 2004-2010. Milford, MA, EU. 76p. http://www.vtpup.ZH/common/manual/PrF_hmspektlab_Waters_UPLC_manual_EN.pdf Data consulted: March 22, 2018.

Whipple G, Koohmaraie M, Dikeman M, Crouse J, Hunt M, Klemm R (1990) Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. Journal of Animal Science 68: 2716-2728.

Williams LD, Churchwell MI, Doerge DR (2004) Multiresidue confirmation of β-agonists in bovine retina and liver using LC-ES/MS/MS. Journal of Chromatography B 813: 35-45.