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Effect of the time of supplementation of Zilpaterol hydrochloride on residues in muscle, liver, and kidney of feedlot hair lambs

Efecto de tiempo de suplementación de Clorhidrato de Zilpaterol sobre residuos en músculo, hígado y riñón de corderos de pelo en finalización



Montaño-Gómez Martín^{*1} , Vega-Cazares Miguel¹ , Mellado-Bosque Miguel² ,
Chirino-Romero Juan¹ , Villa-Angulo Rafael³ , Márquez-Salazar Dolores¹ 

¹Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autónoma de Baja California. México. ²Departamento de Nutrición Animal, Universidad Autónoma Agraria Antonio Narro Saltillo, México. ³Instituto de Ingeniería, Universidad Autónoma de Baja California. México. *Responsible and Corresponding author: Montaño-Gómez Martín. Instituto de Investigaciones en Ciencias Veterinarias de la Universidad Autónoma de Baja California. Fraccionamiento Laguna Campestre, Mexicali, Baja California, México. CP 21386. E-mail: mmontano5@yahoo.com, miguevega@yahoo.com.mx, melladomiguel07@gmail.com, octavio.chirino@uabc.edu.mx, rafael.villa@uabc.edu.mx, marquezd@uabc.edu.mx

ABSTRACT

Zilpaterol hydrochloride (ZH) supplementation is marketed for cattle only, and its use has not been widely adopted as growth promoter in other species. The objective of this study was to assess the residual ZH in various tissues of feedlot hair lambs administered with this growth promoter. Forty Intact male lambs were randomly assigned to one of the following four treatments: ZH for 0, 10, 20, and 30 d before the end of the fattening period. All lambs were fed a basal diet and slaughtered 48 h after the feeding trial. Regarding muscle, a linear increase ($P \leq 0.05$) in ZH concentrations with increasing days of administration was observed (0.0, 0.19, 0.26, and 0.64 ng/g for 0, 10, 20, and 30 d of administration). For the liver, a linear increase ($P \leq 0.05$) in ZH concentrations with increasing days of administration was also observed (0.0, 0.18, 0.27, and 0.44 ng/g for 0, 10, 20, and 30 days of administration). Higher values of ZH were found in the kidney with 0.0, 0.49, 0.66, and 0.71 ng/g for 0, 10, 20, and 30 days of administration). It was concluded that, regardless of tissue, residues of ZH increased with increasing days of administration of this growth promoter. Also, the observed levels of ZH were lower than those established by SENASICA (Mexico) for cattle, and the values presented in the present study could be the basis for establishing the maximum permissible limits of this growth enhancer in various tissues of sheep.

Keywords: $\beta 2$ adrenergic agonists, zilpaterol hydrochloride, residues, meat.

RESUMEN

La suplementación con hidrocloreto de zilpaterol (HZ) se comercializa solo para el ganado y su uso no ha sido ampliamente adoptado como promotor del crecimiento en otras especies. El objetivo de este estudio fue evaluar el HZ residual en varios tejidos de corderos de pelo finalizados en corral de engorde a los que se administró este promotor de crecimiento. Cuarenta corderos machos intactos se asignaron aleatoriamente a uno de los siguientes cuatro tratamientos: HZ durante 0, 10, 20 y 30 días antes del final del período de engorde. Todos los corderos fueron alimentados con una dieta basal y sacrificados 48 h



después de la prueba de alimentación. En cuanto al músculo, se observó un aumento lineal ($P \leq 0.05$) en las concentraciones de HZ con el aumento de los días de administración (0.0, 0.19, 0.26 y 0.64 ng/g para 0, 10, 20 y 30 d de administración). Para el hígado, también se observó un aumento lineal ($P \leq 0.05$) en las concentraciones de HZ con el aumento de los días de administración (0.0, 0.18, 0.27 y 0.44 ng / g durante 0, 10, 20 y 30 días de administración). Se encontraron valores más altos de HZ en el riñón con 0.0, 0.49, 0.66 y 0.71 ng / g a los 0, 10, 20 y 30 días de administración). Se concluyó que, independientemente del tejido, los residuos de ZH aumentaron con el aumento de los días de administración de este promotor de crecimiento. Asimismo, los niveles observados de ZH fueron inferiores a los establecidos por el SENASICA (México) para bovinos, y los valores presentados en el presente estudio podrían ser la base para establecer los límites máximos permisibles de este potenciador del crecimiento en diversos tejidos de ovinos.

Palabras clave: agonistas adrenérgicos β_2 , clorhidrato de zilpaterol, residuos, carne.

INTRODUCTION

The meat industry is continually looking for feed additives to promote fast and efficient growth of livestock, as well as to improve the carcass yield and quality of farm animals (Smith *et al.*, 2020). One feed additive used in sheep is zilpaterol hydrochloride (ZH), a β -adrenergic agonists (β -AA) used as growth promoter and improver of carcass characteristics of feedlot lambs (Brand *et al.*, 2013; Macías-Cruz *et al.*, 2013). On the same manner ZH affect the ruminal microbiome of wethers (Dufy *et al.*, 2018). Activation of β -receptors in muscle and fat results in decreased lipogenesis increased lipolysis, and increased muscle accretion or the combination of these effects (Abney *et al.*, 2007; Scramlin *et al.*, 2010; Miller *et al.*, 2012; Johnson *et al.*, 2014). Also, β -AA reduce the frequency and intensity of ruminal contractions (Leek, 2001), which are important to digestion, and these growth promoters increase absorption in the digestive tract (McIntyre & Thompson, 1992) and the amount of gram-negative bacteria species which are vital for fermentation (Walker & Drouillard, 2012). These changes in ruminant digestion attributed to β -AA may lead to changes in VFA production. In addition, β -AA may influence the production of VFA directly to increase the efficiency of digestion and provide more energy to the animal.

Currently, the only β -AA approved for livestock in the United States is ractopamine HCl (RHCl), a β_1 agonist, and ZH, a β_2 agonist, (Delmore *et al.*, 2010; Boler *et al.*, 2012). β -AA have been approved for use as a growth promoter in food-animal production in several countries including Mexico (Johnson *et al.*, 2013).

Because β -AA can promote skeletal muscle growth without increasing serum hormone concentration, both swine and cattle producers have broadly adopted this technology to increase meat production (Centner *et al.*, 2014). However, there is concern regarding the potential harm of β -AA for human consuming meat from animals treated with these growth promoters (Centner *et al.*, 2014). For this reason, China, the European Union, and Russia



have constrained and banned the use of β -AA, and the importation of meat with detectable concentrations of ractopamine (Bories *et al.*, 2009).

In FDA analyzes for beta-agonist residues in porcine and bovine meat, positive results are rarely found, but even in such cases, the levels found are below the maximum residue limits established by the FDA itself and the International Food Code Commission for safe human consumption. At the same time, no foodborne illness or human side effects have been reported that are attributable to the use of approved beta-agonists in the production of meat products.

In Mexico, the use of β -AA as feed additive for livestock is allowed and the maximum residue limits have been established exclusively for cattle, contemplating 10 ng/g in muscle tissue, 12 ng/g in kidney, and 15 ng/g in liver. Therefore it was considered pertinent to ascertain the residues of β -AA in feedlot lambs. The objective of the present trial was to evaluate the effect of the time of supplementation of ZH on residues of this growth promoter in muscle, liver, and kidney of feedlot hair lambs.

MATERIALS AND METHODS

The experiment was carried out in the experimental area for small ruminants of the Veterinary school of the Autonomous University of Baja California, in Mexicali, Mexico. The climate in this region is arid dry with extreme temperatures, both in winter and summer (0 °C to 50 °C), and the average annual rainfall is 80 mm (INEGI, 2018).

Treatment of experimental lambs

The procedures and handling of lambs were carried out based on the following Mexican Standards for the care and handling of animals: NOM-051-ZOO-1995 (humane attention to animals during mobilization), NOM-024-ZOO-1995 (provisions on animal health and characteristics during animal transportation), NOM-033-ZOO-1995 (humane slaughter of domestic and wild animals) and NOM-EM-015-ZOO-2002 (technical provisions for the control of the use of beta-agonists in animals).

Animals and pre-experimental management

Forty four-months old Dorper x Katahdin male lambs were used, which had a live bodyweight of 34.6 ± 2.4 kg at the beginning of the experiment. The study lasted 52 days, of which 20 days were for adaptation and 32 days for the experimental period. During the adaptation period, all lambs were dewormed (Ivermectin; Sanfer Laboratorio, Mexico City; 0.5 ml/animal), received vitamins (Vigantol ADE Fuerte; Bayer, Mexico City, Mexico; 1



ml/animal), and were housed in individual pens (1.2 x 2.0 m) equipped with a trough, feeder and shade. Lambs were adapted to the basic diet (Table 1).

Experimental procedure

The lambs were weighed individually on the first day of the experimental period and were assigned to four groups of 10 animals each. The treatments consisted of supplementing the lambs with 10 mg/d of ZH (Zilmax, Intervet / Schering-Plow, Mexico) during the last 0 (T0, control), 10 (T10), 20 (T20), and 30 (T30) days of the finishing period. Lambs were fed daily in the morning (700 h) and the afternoon (1500 h). Water was offered ad libitum and health status was visually verified every day. Feed samples were collected and dried in an oven. The chemical composition of these samples was determined. Two days after the end of the feeding trial all lambs were weighed and slaughtered by the disjuncture method.

Table 1. Ingredients and chemical composition of the diets offered to hair lambs

	Treatments ¹			
	T0	T10	T20	T30
Ingredients, %				
Yellow Corn, flaked	46.00	46.00	46.00	46.00
Alfalfa, hay	26.50	26.50	26.50	26.50
DDGs ²	20.00	20.00	20.00	20.00
Molasses	6.00	6.00	6.00	6.00
ZH, mg/animal/d ³	0.00	10.00	10.00	10.00
Minerals	1.50	1.50	1.50	1.50
Nutrient content, % dry matter basis				
Dry matter	88.96	88.96	88.96	88.96
Metabolizable energy (Mcal/kg)	2.98	2.98	2.98	2.98
Ether extract	4.89	4.89	4.89	4.89
Crude protein	15.78	15.78	15.78	15.78
Neutral detergent fiber	12.48	12.48	12.48	12.48
Acid detergent fiber	4.84	4.84	4.84	4.84
Calcium	0.85	0.85	0.85	0.85
Phosphorous	0.68	0.68	0.68	0.68

¹Treatments were: T0 = Control, no zilpaterol hydrochloride (Z0); T10 = supplementation of zilpaterol hydrochloride the last 10 days of finishing; T20 = supplementation of zilpaterol hydrochloride the last 20 days of finishing; T30 = supplementation of zilpaterol hydrochloride the last 30 days of finishing.

²DDGs = Distiller's' dried grains of corn with solubles.

³ZH = zilpaterol hydrochloride (Zilmax, Intervet/Schering-Plough, Mexico).



Collection and preparation of samples

Liver kidney and meat (masseter muscle) samples were collected at the time of the slaughter and were frozen at -30 °C until their preparation and analysis. The muscle samples were collected after dissecting the heads of the animals, avoiding areas high in fat or connective tissue. For the determination of ZH residues, five animals were randomly sampled per group. Samples were homogenized by grinding. Fatty or connective tissue were removed before grinding. They were kept frozen until the moment of extraction. This was carried out according to the protocol described by the supplier for the different tissues (Bioo Scientific Corp., 2011), using the PowerGen 700™ homogenizer (Fisher Scientific), at the beginning of the extraction in conjunction with the first buffer used, to optimize the process.

Sample analysis

ZH residues were quantified by ELISA analysis using the "MaxSignal® Zilpaterol ELISA Test" kit which is a competitive enzyme immunoassay. For concentration determination, an ELISA plate reader, Eon™ (BioTek Instruments, Inc.) was used, with reading of the plates at 450 nm, as indicated by the supplier. Determinations were made in 96-well microplates in duplicate as well as the standards of the calibration curve, for which two plates were necessary. The absorbance of the wells of the plate was recorded at 450 nm. The calibration curve had a correlation coefficient (R^2) of 0.99 and was performed using the concentrations 0.00, 0.015, 0.20, and 0.50 ng/g. Since the calibration curve is inversely proportional to concentration and has a logarithmic behavior, the supplier recommends adjusting the absolute absorbance values to relative absorbance using the zero standard value (0.0 ng/g) as 100 absorbance. Likewise, to fit a linear equation, data were transformed to natural logarithm. Using Gen5™ Software (BioTek Instruments, Inc.), absorbance of each of the samples were averaged, and these data were used for statistical analysis. The limit for detection was established as 3 times the standard deviation of the blank signal and the limit of quantification (LOQ) as 10 times the value of the standard deviation of the signal or absorbance of the zero standard (blank) (Skoog, 2001).

Statistical analysis

Data were subjected to an analysis of variance using a completely randomized design (GLM Procedure of the SAS; SAS Institute Inc., Cary NC., USA). Means were analyzed using orthogonal polynomials, and the Tuckey Test, declaring significance at $P \leq 0.05$. The data were analyzed with the statistical program (2002). Animal was the experimental unit and the model included fixed effects of ZH supplementation and the experimental error as a random term.



RESULTS AND DISCUSSION

The effect of the treatment on residues of ZH is shown in Table 2. Regarding muscle, a linear increase ($P \leq 0.05$) in ZH concentrations was observed as days of supplementation increased. There was a significant difference in ZH concentrations between the control and all other treatments.

Likewise, a linear increase ($P \leq 0.10$) was observed in ZH concentration in the kidney as the days of treatment increased; the control group differed compared to all other treatments. Regarding the liver, a linear increase ($P \leq 0.10$) of ZH residue concentration was observed as days of treatment increased. No significant difference ($p > 0.05$) existed between the control group compared to all other treatments.

Table 2. Effect of time of zilpaterol hydrochloride supplementation on residues of this growth promoter on muscle, kidney and liver

Tejido	Residues of ZH (ng/g)				SEM	P-value	
	T0	T10	T20	T30		T0 vs. Others	Lineal
Muscle	0.0000	0.1908	0.2598	0.6414	0.0707	0.0065	0.0004
Kidney	0.0000	0.4962	0.6666	0.7152	0.1173	0.0438	0.0549
Liver	0.0000	0.1862	0.2740	0.4484	0.0750	0.1270	0.0594

SEM= Estandar error of the mean; ZH= zilpaterol hydrochloride (Zilmax, Intervet/Schering-Plough, Mexico).

T0 = Control, no zilpaterol hydrochloride(Z0); T10 = supplementation of zilpaterol hydrochloride the last 10 days of finishing; T20 = supplementation of zilpaterol hydrochloride the last 20 days of finishing; T30 = supplementation of zilpaterol hydrochloride the last 30 days of finishing.

ZH has a minimum three-day withdrawal period before slaughter to ensure residues in the muscle tissue are eliminated before consumption (Merck, 2017). ZH and other β -adrenergic agonists residues in feedlot produced farm animals can have high trade implications because markets outside the countries where ZH is legally permitted, must comply with ZH residues established by the importing country. In the United States, where the use of ZH is approved for administration to cattle only, maximum residues levels for ZH in beef liver and muscle are 12 and 10 ng/g, respectively. In the case of Mexico, the maximum residues of this growth promoter are 10 ng/g in muscle tissue, 12 ng/g in kidney, and 15 ng/g in liver (SENASICA, 2017). In the case of ZH, residual concentrations in sheep have not been established, but there are reports on ZH concentration in lamb's tissues. A study in sheep have shown that levels of ZH in muscle, liver, and kidney in lambs at different withdrawal times were 1.5 ng/g in muscle, 0.86 ng/g in liver, and 1.10



ng/g in kidney (Shelver & Smith, 2006), which were higher than values found in the present study. It is important to note that the observed levels of ZH in lambs were lower than those established by SENASICA (2017) for cattle. Also, the tissue in which the greatest amount of CZ residues were found was the kidney, which is because ZH is a highly soluble substance in water, therefore its excretion is mainly through the urinary system. In fact, a friendly rapid detection method for ZH is the determination of this growth enhancer in the urine (Shelver & Smith, 2018).

For other ruminants other than cattle and other livestock, SENASICA (2017) establish that animal tissues must present a “not detected” quantity of ZH (zero tolerance limit). Under this criteria, ZH could not be used in sheep in Mexico, which is contradictory because cattle meat can have some ZH residues. It is important to mention that this limit was established basically because of the lack of studies on residues of ZH in sheep meat. Therefore, more studies must be carried out to confirm whether these amounts are dangerous to humans and, if applicable, establish maximum permissible limits that guarantee the health of the consumer.

CONCLUSIONS

The observed residual concentrations of zilpaterol hydrochloride in muscle, kidney, and liver of hair lambs increased linearly with the days of administration of this growth-enhancer. The residues of zilpaterol hydrochloride in various tissues of hair lambs were lower than those established by SENASICA (Mexico) for cattle. Given that this institution has not established formal limits for the use of zilpaterol hydrochloride in sheep, these results are a good point of reference to establish maximum permissible limits for feedlot hair lambs.

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Errata Erratum

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