


# Effect of supplementing zilpaterol hydrochloride during different periods on energetics, growth performance, carcass traits and fatty acid profile in meat of finishing lambs

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## Abstract

To determine the effect of adding  $\beta_2$  agonist zilpaterol hydrochloride during different periods on growth performance, dietary energetic, carcass traits and fatty acid profile, forty male lambs Pelibuey  $\times$  Katahdin ( $37.70 \pm 0.83$  kg) were blocked by body weight and randomly assigned to pens. Treatments consisted in: 1) control, no zilpaterol supplementation, 2) zilpaterol supplementation for 20 d (ZIL20), 3) zilpaterol supplementation for 30 d (ZIL30), and 4) zilpaterol supplementation for 40 d (ZIL40). Compared with control treatment, zilpaterol supplementation increased final live weight (FLW) (3.5 %;  $P = 0.0563$ ), average daily gain (ADG, 14.8 %;  $P = 0.0598$ ) and gain:feed ratio (10.3 %;  $P = 0.0324$ ). As well as hot carcass weight (6.1 %;  $P = 0.0064$ ), dress out percentage (2.6 %;  $P = 0.0139$ ) and *Longissimus dorsi* muscle area (13.7 %;  $P = 0.0015$ ). Compared to control group, zilpaterol increased ( $P = 0.0413$ ) observed vs. expected dietary of NEm (net energy requirements for maintenance) and NEg (net energy requirements for growth) (5.7 and 8.1 %, respectively). Twenty days of zilpaterol treatment improved FLW (4.4 %,  $P = 0.0566$ ) and tended to improve ADG (17.4 %,  $P = 0.0718$ ) when compared to control group. Only intramuscular fat showed a positive linear effect ( $P = 0.0006$ ) in stearic fatty acids proportion and negative linear effect ( $P = 0.0585$ ) in araquidonic fatty acid proportion. We conclude that zilpaterol hydrochloride improved variables of growth performance, energy retention and carcass traits with noticeable effects from 20 d of zilpaterol supplementation.

**Keywords:**  $\beta$ -adrenergic agonist; Feedlot lambs; Carcass characteristics; Fatty acids.

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## Study contribution

$\beta$ -adrenergic agonists are widely investigated in large ruminants, since these compounds improve growth performance and yield carcass by increasing muscle protein synthesis. Multiple factors such as the dose, withdrawal and supplementation period of these technologies have been investigated in beef cattle production with the purpose of improving the profitability of their use. There is little scientific information on the use of different days on feed of zilpaterol hydrochloride in feedlot lambs. The reduction of the zilpaterol supply period of 10 days with respect to the 30 days recommended, and obtaining similar yield carcass, represent 33 % of cost savings for additive use. Zilpaterol hydrochloride supplementation for 20 days improves growth efficiency and yield carcass similarly to 30 or 40 days.

## Introduction

Adrenergics  $\beta$ -agonists are synthetic compounds with a chemical structure similar to catecholamines that bind to cell membrane  $\beta$ -receptors on its surface and produce biological effects.<sup>(1)</sup> Zilpaterol hydrochloride is a  $\beta_2$ -agonist feed additive approved for use in feedlot cattle in México.<sup>(2)</sup> In lambs, zilpaterol supplementation at a dose of 0.15 mg/kg of live weight per day during the last 30 days before slaughter improved average daily gain (12.5-40.0 %) and feed efficiency (18.3-23.9 %) as well as hot carcass weight (5.0-6.6 %) and dressed carcass yield (1.7-4.8 %).<sup>(1,3)</sup>

For economical and practical purposes, supplementation period of zilpaterol in livestock industry could be variable and considering that responses of  $\beta$ -agonists are temporary, because exposure to a constant dose to the receptor will eventually cause acute desensitization or inactivation of receptor-mediated signaling.<sup>(4)</sup> In feedlot cattle a study shown that there were no substantial differences on growth performance between 20 d compared to 30 or 40 d of supplementation.<sup>(5)</sup>

In opposite, has been detected a linearly increase on feed efficiency when zilpaterol supplementation goes from 20 to 40 d.<sup>(6)</sup> Even so, both reports indicate carcass change mainly related to fat and muscle composition. Since zilpaterol supplementation have shown similar results in growth performance and carcass of feedlot lambs, therefore it has been expected that increase the length of zilpaterol administration have the same results, however, to our knowledge no information is available regarding the period duration of zilpaterol supplementation in lambs. There is currently a concern about cardiovascular disease in consumers. Some studies in ruminants showed that  $\beta$ -agonists modify the fatty acid profile of intramuscular fat.<sup>(7,8)</sup>

The hypothesis is that growth performance and carcass traits have a response as of 20 d of zilpaterol administration in feedlot lambs. The aim of this study was to determine the effect of zilpaterol supplementation period on growth performance, dietary energetics, carcass traits and fatty acid profile in finishing lambs.

## Materials and methods

### *Ethical statement*

Animal management procedures were performed within the guidelines of locally approved techniques for animal use and care.<sup>(9)</sup> Humanitarian care of animals during mobilization of animals.<sup>(10)</sup> Technical specifications for production, care and use of laboratory animals<sup>(11)</sup> and ethics, biosafety and animal welfare standards of the Comité Institucional para el cuidado y uso de animales (CICUA) of the Universidad Autónoma de Sinaloa (Protocol: CICUA-FMVZ/01-03-2023).

### *Location and experimental setup*

This trial was conducted at the Universidad Autónoma de Sinaloa, Unidad de Engorda Experimental para Ovinos II, located in Culiacán, Sinaloa, México (24° 46' 13N and 107°21' 14W). Culiacán is about 55 m above sea level and has a dry tropical climate. Sixty Pelibuey×Katahdin crossbred male lambs were received at the research unit eight weeks before to start of the experiment in order to adapt them to feed.

### *Experimental procedures and feeding regimen*

Upon arrival animals were identified, weighed with a hook scale with 50 g precision (TORREY-CRS200); treated for endoparasites (SAGUAYMIC PLUS, Microsules Lab), hemoparasites (IMIZOL, MSD Animal Health) and injected with 1×10<sup>6</sup> IU of vitamin A (Synt-ADE, FortDodge, Animal Health). After the adaptation period, sick lambs or those with abnormal feeding behavior were discarded, from those left the heaviest forty lambs were selected ( $37.7 \pm 0.83$  kg) and used in a 40 d feeding trial plus a 2 d zilpaterol withdrawal to evaluate the effect of zilpaterol supplementation period on feedlot performance, dietary energetics, carcass traits and fatty acids profile of hair-breed lambs.

The animals were blocked by live weight (LW) into five uniform weight groups (block) and assigned to 20 pens (2 lambs/pen). Pens were 6 m<sup>2</sup> with overhead shade, automatic waterers and had 1 m long fence line fed bunk. Treatments were randomly assigned within each block: 1) control, no zilpaterol supplementation, 2) zilpaterol supplementation for 20 d (ZIL20), 3) zilpaterol supplementation for 30 d (ZIL30), and 4) zilpaterol supplementation for 40 d (ZIL40). All lambs received an *ad libitum* diet (Table 1) based on cracked corn (1.30 Mcal NEg/kg and 13.7 % CP) and were fed twice daily at 09:00 and 16:00 h in a 25:75 proportion (as feed basis). Treatments with zilpaterol supplementation initiated on different days of trial (10 d difference by zilpaterol treatment) in order to allow that all lambs finish their feeding period at the same time.

**Table 1.** Ingredients and composition of basal diet fed to lambs

Item	Diet composition (% DM)
Ingredient(% of dry matter)	
Cracked corn	60.0
Soybean meal	12.0
Sudan grass hay	16.0
Molasses cane	9.5
Trace mineral salt	2.5
Chemical composition (% DM basis)	
Crude protein	13.7
NDF	18.8
Calculated net energy (Mcal/kg of DM basis)	
Maintenance	1.95
Gain	1.30

Trace mineral salt contained: CP, 50 %; calcium, 28 %; phosphorous, 0.55 %; magnesium, 0.58 %; potassium, 0.65 %; NaCl, 15 %; vitamin A, 1 100 IU/kg; vitamin E, 11 IU/kg; Chemical composition based on tabular net energy (NE) values for individual feed ingredients.<sup>(12)</sup>

Lambs treated with zilpaterol was weighed at the beginning of supplementation and average LW was estimated for dose calculation. To ensure the consumption of the total daily dose, 0.20 mg/kg LW of zilpaterol was added in a 50 g of ground diet and incorporated into the feed offered during the morning. At the end of the trial, the average LW of each group treated with zilpaterol was obtained and the average daily intake of zilpaterol was calculated. Feed bunks were evaluated visually before morning feeding between 08:40 and 08:50 h to determine the quantity of feed remaining from the previous day. Daily feed allotments to each pen were adjusted to allow for minimal (< 5.0 %) feed accumulation. Adjustments (increase or decrease) in daily feed delivery were allotted to afternoon feeding. Feed samples were collected daily for DM analysis.<sup>(13)</sup>

Assuming that Dry matter intake (DMI) intake is related to energy requirements and dietary NEm, it is expected that the DMI can be estimated from average ADG and LW values according to the following equation:

$$\text{DMI, kg/d} = \left( \frac{\text{ME}}{1.95} \right) + \left( \frac{\text{EG}}{1.30} \right)$$

Where ME (energy required for maintenance, Mcal/d) = ME = 56×SBW<sup>0.75</sup>, SBW (shrunk body weight), kg = BW<sup>0.96</sup>, EG (energy gain, Mcal/d)= 276×ADG×SBW<sup>0.75</sup><sup>(12)</sup> and NEm and NEg are 1.95 and 1.30 Mcal/kg, respectively (derived from tabular values based on ingredient composition of the experimental diet).<sup>(12)</sup> A coefficient of 276 was estimated assuming a mature weight for Pelibuey×Katahdin male lambs of 115 kg.<sup>(12)</sup> Dietary NE was estimated utilizing the quadratic formula:<sup>(14)</sup>

$$x = \frac{-b - \sqrt{b^2 - 4ac}}{2c}$$

Where:

$x = NEm$ ,  $a = -0.41$ ,  $ME$ ,  $b = 0.877 EM + 0.41 DMI + EG$ , and  $c = -0.877 DMI$ .

After 42 d feeding trial, lambs were transported to a slaughterhouse 24 h before slaughter and were only offered water. Following slaughter hot carcass weight (kg) was obtained with a hook scale and led to a chiller room. To observe the effect of treatments on carcass performance expressed as ADG and gain:feed ratio, carcass-adjusted final body weight (BW) was calculated as hot carcass weight (HCW)/overall average dressing percent (51.3 %) for all treatments. HCW was obtained for all lambs after slaughter, calculating carcass dress out percentage. After carcasses were chilled for 24 h at 4 °C, the following measurements were obtained from cold carcasses: Fat thickness over the 12<sup>th</sup> rib, perpendicular to the *Longissimus dorsi* muscle with a vernier caliper (CADENA-A020®, México), *Longissimus dorsi* muscle area, between the 12<sup>th</sup> and 13<sup>th</sup> rib was measured using a plastic grid, and kidney, pelvic and heart fat was removed, weighed (kg) and reported as a percentage of final live weight.

All carcasses (40 units) were divided longitudinally into two equal parts; untrimmed wholesale cuts were obtained from the left side according to the IMPS fresh lamb-USDA and calculated as a percentage of hot carcass weight, each wholesale cut except the head were dissected into muscle, bone and fat components. Tissue components were weighted and means from muscle, fat and bone were expressed in percentages in relation to cold carcass weight.

Samples (50 g) of *Longissimus dorsi* muscle samples were collected from the left side of twenty carcasses (1 sample/pen), samples were frozen (-18 °C) and transported to the lab for fatty acid analysis of intramuscular fat. Samples were thawed (4 °C) and ground to homogenize them utilizing a modified method<sup>(15)</sup> was used to carry out lipid extraction from muscle samples and percentage of intramuscular fat was determined by total fat extracted from the previously weighted samples. A sonotransesterification process was conducted to characterize fatty acids profile.<sup>(16)</sup> Fatty acid composition was determined by gas chromatography (Agilent 6890N)) utilizing a mass spectrometer (Agilent 5973) and column Omega Wax 250.

### Statistical analysis

Growth performance, energetics, carcass traits and wholesale cut yields data were analyzed as a randomized complete block design considering a pen as the experimental unit. The SAS PROC MIXED is the procedure in SAS program used to analyze variables. The fixed effect consisted of treatment and pen as the random component. Fatty acids muscle variables were analyzed as a randomized complete block design, with the individual lamb being the experimental unit. The MIXED procedure in the SAS program<sup>(17)</sup> was used to analyze variables. The contrasts: control vs. zilpaterol treatments, control vs. ZIL20 were tested. Moreover, effects were tested for linear and quadratic components of the zilpaterol supplementation level. Contrast and orthogonal polynomials were considered significant when the P value was  $\leq 0.05$  and tendencies were identified when the P value was  $> 0.05$  and  $\leq 0.10$ .

**Table 2.** Effect of zilpaterol supplementation on growth response in feedlot lambs

Item	Days on zilpaterol <sup>a</sup>				SEM	P value			
	C	ZIL20	ZIL30	ZIL40		C vs. Z	C vs. ZIL20	Lin	Qua
Days on test	42	42	42	42					
Pens	5	5	5	5					
ILW, kg	37.72	37.90	37.78	37.69	0.83	0.72	0.44	0.80	0.45
FLW, kg	49.02	51.18	50.98	50.20	0.86	0.05	0.05	0.35	0.74
Carcass-adjusted FLW, kg	48.14	51.36	50.93	51.06	0.95	< 0.01	< 0.01	0.75	0.72
TLW gain, kg	11.29	13.28	13.20	12.51	0.36	0.06	0.07	0.42	0.71
ADG, g	269	316	315	298	8.98	0.05	0.07	0.42	0.69
DMI, g/d	1464	1604	1523	1473	0.03	0.27	0.08	0.12	0.81
DMI % of LW	3.39	3.61	3.42	3.35	0.05	0.53	0.13	0.10	0.63
Gain:Feed	0.183	0.197	0.207	0.203	0.005	0.03	0.17	0.54	0.40
Carcass-adjusted performance									
ADG, g	248	320	313	318	9.62	< 0.01	0.01	0.93	0.77
Gain:Feed	0.169	0.200	0.208	0.217	0.007	0.02	0.05	0.46	0.98

Initial Live Weight (ILW), Final Live Weight (FLW), Total Live Weight (TLW).

<sup>a</sup>Zilpaterol supplementation at dose of 0.20 mg/kg LW. C= No zilpaterol supplementation. ZIL20= Zilpaterol supplementation for 20 d. ZIL30= zilpaterol supplementation for 30 d. ZIL40= zilpaterol supplementation for 40 d. C vs. Z= Control vs. All zilpaterol treatments contrast. C vs. 30= Control vs. 30 d of zilpaterol supplementation. 20 vs. 30= 20 d of zilpaterol supplementation vs. 30 d of zilpaterol supplementation. L= Linear orthogonal polynomial Q= Quadratic orthogonal polynomial.

## Results

The effects of different zilpaterol treatments on growth performance and estimated dietary energetics of finishing lambs are shown in [Table 2](#) and [Table 3](#). Daily zilpaterol intake averaged 0.214, 0.203 and 0.196 mg/kg LW for ZIL20, ZIL30 and ZIL40 respectively, in close agreement with projected dose (0.20 mg/kg LW/d). All treatments with zilpaterol supplementation (control vs. zilpaterol) showed positive responses on growth performance variables when compared with control treatment, such as a greater average final live weight (3.5 %,  $P = 0.0563$ ), improve of ADG (14.8 %,  $P = 0.0598$ ) and gain:feed ratio (10.3 %,  $P = 0.0324$ ). Positive feed efficiency response was the product of not showing changes in DMI of lambs ( $P \geq 0.2757$ ) with improvement in body daily gain. Twenty days of zilpaterol treatment improved final live weight (4.4 %,  $P = 0.0566$ ) and tended to improve ADG (17.4 %,  $P = 0.0718$ ) when compared to control group (Control vs. ZIL20). However, no differences were observed in gain:feed ratio ( $P = 0.1785$ ). In both contrasts tested (control vs. all zilpaterol treatments and C vs. ZIL20), growth performance calculated from adjusted final live weight showed improvements in ADG (27.8 % and 29 %;  $P \leq 0.006$ , respectively) and feed efficiency (23 % and 18.3 %, respectively;  $P \leq 0.0232$ ). The average of all zilpaterol groups improved  $P = 0.0413$  the estimated dietary energy of NEm (5.7 %), NEg (8.1 %) and reduced observed:expected DMI ratio (4.9 %) compared with control group. Likewise, when comparing Control and ZIL20, similar results  $P = 0.0303$  were obtained in NEm, NEg and observed:expected DMI ratio (3.03, 5.1 and 2.6 %, respectively).



**Table 3.** Effect of zilpaterol supplementation on dietary energetics in feedlot lambs

Item	Days on zilpaterol <sup>a</sup>				SEM	P value			
	C	ZIL20	ZIL30	ZIL40		C vs. Z	C vs. ZIL20	Lin	Qua
NE diet (Mcal/kg)									
Maintenance	1.92	1.99	2.07	2.05	0.025	0.04	0.04	0.38	0.34
Gain	1.28	1.34	1.41	1.39	0.02	0.04	0.04	0.38	0.34
Obs to exp NE									
Maintenance	0.99	1.02	1.07	1.05	0.013	0.04	0.04	0.38	0.34
Gain	0.98	1.03	1.08	1.07	0.017	0.04	0.04	0.38	0.34
Obs to exp DMI	1.01	0.97	0.93	0.92	0.014	0.04	0.04	0.36	0.36

<sup>a</sup>Zilpaterol supplementation at dose of 0.20 mg/kg/LW. C= No zilpaterol supplementation. ZIL20= Zilpaterol supplementation for 20 d. ZIL30= zilpaterol supplementation for 30 d. ZIL40= zilpaterol supplementation for 40 d. C vs. Z= Control vs. All zilpaterol treatments contrast. C vs. 30= Control vs. 30 d of zilpaterol supplementation. 20 vs. 30= 20 d of zilpaterol supplementation vs. 30 d of zilpaterol supplementation. L= Linear orthogonal polynomial Q= Quadratic orthogonal polynomial.

The effects of zilpaterol treatment period on lamb's carcass characteristics are shown in Table 4. On average, all zilpaterol treatment increased ( $P = 0.0064$ ) hot carcass weight (6.1 %,  $P=0.0064$ ), carcass yield (2.6 %,  $P=0.0139$ ), *Longissimus dorsi* muscle area (13.7 %,  $P=0.0015$ ) and muscle:fat ratio (29.4 %,  $P=0.0015$ ) compared to control group. Otherwise, dorsal fat thickness was reduced (27.6 %,  $P = 0.0388$ ), without changes in pelvis-renal fat and intramuscular fat ( $P > 0.3691$ ). Same manner, comparing control lambs with ZIL20 treatment, showed again similar responses in carcass traits and carcass composition variables.

The effect of different periods of zilpaterol supplementation on wholesale cut yields (Table 5) show that zilpaterol treatment reduced the percentage of neck (10.5 %,  $P = 0.0027$ ) and rack (6.0 %,  $P = 0.0546$ ) primal cuts. Likewise, when comparing the control group and ZIL20 lambs, only neck primal cut was reduced (9.4 %  $P = 0.0153$ ).

The results of the effects of zilpaterol on fatty acid proportion in intramuscular fat are presented in Table 6. On average zilpaterol treatment (control vs. Z) reduced the stearic fatty acid proportion (17.6 %,  $P = 0.0163$ ) and tended to increase linoleic acid proportion (48.0 %,  $P = 0.0662$ ).

Orthogonal polynomials were applied to days on feed zilpaterol treatments (ZIL20, ZIL30 and ZIL40) and showed in Table 5. No effects of linear contrast were observed for variables of growth performance, dietary energetics, and wholesale cut yields. Nevertheless, there is a positive linear trend for percentage of muscle tissue composition ( $P = 0.0673$ ) and muscle:bone ratio ( $P = 0.0959$ ). Intramuscular fat showed a positive linear effect ( $P = 0.0006$ ) in stearic fatty acids proportion and negative linear effect ( $P = 0.0585$ ) in araquidonic fatty acid proportion.

## Discussion

Improvements in weight gain and feed efficiency,<sup>(18)</sup> and no effect on DMI<sup>(1)</sup> has been the most consistent response to zilpaterol supplementation. Nonetheless, some studies involving lambs resulted in marked increases in DMI,<sup>(19)</sup> while other

**Table 4.** Effect of zilpaterol supplementation on carcass traits in feedlot lambs

	Days on zilpaterol <sup>a</sup>					P value			
Item	C	ZIL20	ZIL30	ZIL40	SEM	C vs. Z	C vs. ZIL20	Lin	Qua
HCW (kg)	24.70	26.34	26.13	26.19	0.49	< 0.01	< 0.01	0.75	0.72
Dressing (%)	50.38	51.45	51.29	52.17	0.38	< 0.01	0.01	0.90	0.75
LM area (cm <sup>2</sup> )	17.06	19.80	19.56	18.65	0.37	< 0.01	< 0.01	0.07	0.52
Fat thickness (mm)	1.41	1.05	0.93	1.06	0.08	0.03	0.11	0.97	0.48
Pelvis-renal fat (%)	2.28	1.96	1.82	2.18	0.15	0.47	0.52	0.69	0.61
Intramuscular fat (%)	2.81	2.64	2.13	2.04	0.23	0.36	0.68	0.37	0.72
Composition (%)									
Muscle	60.98	63.51	66.34	66.88	0.79	< 0.01	0.16	0.06	0.42
Fat	18.22	15.36	14.50	14.99	0.66	0.04	0.14	0.79	0.58
Bone	20.19	19.69	18.67	18.66	0.31	0.08	0.52	0.20	0.45
Muscle:fat ratio	3.46	4.34	4.60	4.50	0.18	0.01	0.08	0.68	0.59
Muscle:bone ratio	3.04	3.25	3.56	3.59	0.08	< 0.01	0.22	0.09	0.39

<sup>a</sup>Zilpaterol supplementation at dose of 0.20 mg/kg LW. C= No zilpaterol supplementation. ZIL20= Zilpaterol supplementation for 20 d. ZIL30= zilpaterol supplementation for 30 d. ZIL40= zilpaterol supplementation for 40 d. C vs. Z= Control vs. All zilpaterol treatments contrast. C vs. 30= Control vs. 30 d of zilpaterol supplementation. 20 vs. 30= 20 d of zilpaterol supplementation vs. 30 d of zilpaterol supplementation. L= Linear orthogonal polynomial Q= Quadratic orthogonal polynomial.

**Table 5.** Effect zilpaterol supplementation on wholesale cut yields (%)

	Days on zilpaterol <sup>a</sup>					P value			
Item	C	ZIL20	ZIL30	ZIL40	SEM	C vs. Z	C vs. ZIL20	Lin	Qua
Front quarter	53.76	52.89	52.74	52.50	0.27	0.10	0.25	0.66	0.96
Rear quarter	46.23	47.10	47.26	47.48	0.27	0.10	0.25	0.66	0.96
Neck	4.35	3.94	3.89	3.82	0.06	< 0.01	0.01	0.43	0.96
Chuck blade	19.28	19.03	19.30	19.56	0.18	0.96	0.66	0.43	0.99
Rib	7.86	8.35	7.79	7.86	0.13	0.66	0.23	0.26	0.40
Rack	10.40	9.82	9.88	9.61	0.12	0.04	0.11	0.57	0.61
Breast	1.77	1.69	1.91	1.78	0.05	0.84	0.65	0.57	0.22
Shoulder	9.15	10.05	9.95	9.87	0.17	0.74	0.93	0.75	0.99
Flank	6.38	5.96	6.02	6.09	0.07	0.06	0.07	0.36	0.99
Loin	9.15	9.52	9.59	9.66	0.10	0.11	0.26	0.69	0.99
Full leg	30.69	31.62	31.63	31.72	0.28	0.11	0.20	0.92	0.96

<sup>a</sup>Zilpaterol supplementation at dose of 0.20 mg/kg LW. C= No zilpaterol supplementation. ZIL20= Zilpaterol supplementation for 20 d. ZIL30= zilpaterol supplementation for 30 d. ZIL40= zilpaterol supplementation for 40 d. C vs. Z= Control vs. All zilpaterol treatments contrast. C vs. 30= Control vs. 30 d of zilpaterol supplementation. 20 vs. 30= 20 d of zilpaterol supplementation vs. 30 d of zilpaterol supplementation. L= Linear orthogonal polynomial Q= Quadratic orthogonal polynomial.



**Table 6.** Effect of zilpaterol supplementation on intramuscular fatty acids proportion feedlot lambs

	Days on zilpaterol <sup>a</sup>					P value			
Item	C	ZIL20	ZIL30	ZIL40	SEM	C vs. Z	C vs. ZIL20	Lin	Qua
Palmitic, C16:0	25.19	26.22	24.1009	22.74	0.72	0.76	0.40	0.17	0.83
Palmitoleic, C16:1(9)	1.71	1.96	2.10	1.79	0.10	0.26	0.37	0.55	0.42
Stearic, C18:0	18.38	17.60	12.92	14.91	0.78	0.01	0.97	0.01	< 0.01
Oleic, C18:1(9)	43.97	44.21	43.28	42.74	0.60	0.57	0.70	0.86	0.81
Linoleic, C18:2 (9,12)	7.52	7.54	12.81	13.05	0.91	0.06	0.89	0.10	0.39
Linolenic, C18:3 (9,12,5)	0.29	0.23	0.60	0.82	0.10	0.22	0.87	0.13	0.98
Araquidonic, C20:4	2.94	2.25	4.09	3.95	0.36	0.70	0.26	0.05	0.19

<sup>a</sup>Zilpaterol supplementation at dose of 0.20 mg/kg LW. C= No zilpaterol supplementation. ZIL20= Zilpaterol supplementation for 20 d. ZIL30= zilpaterol supplementation for 30 d. ZIL40= zilpaterol supplementation for 40 d. C vs. Z= Control vs. All zilpaterol treatments contrast. C vs. 30= Control vs. 30 d of zilpaterol supplementation. 20 vs. 30= 20 d of zilpaterol supplementation vs. 30 d of zilpaterol supplementation. L= Linear orthogonal polynomial Q= Quadratic orthogonal polynomial.

studies in cattle have reported a decrease.<sup>(20)</sup> A study was conducted on lambs with an average weight of 28.9 kg in order to test the effect of zilpaterol treatment in periods of 0, 14, 28 and 42 d, similar to what is seen in this study, no changes in DMI were observed, however, after comparing treated animals to control the authors observed similar responses in final live weight, increasing ADG and feed efficiency.<sup>(21)</sup>

Calculations of dietary NE concentration from DMI and growth performance allow evaluation of growth promoters as  $\beta$ -agonists, which modify the apparent diet energy by increasing muscle protein synthesis or reducing its elimination, this effect is shown as an increase in the apparent increase in energy retention (NE<sub>m</sub> and NE<sub>g</sub>) per unit of DMI in lambs with zilpaterol supplementation.<sup>(4)</sup> Similar to what is observed in this study, some authors have reported 10.9 to 23.4 % improvements in observed:expected DMI ratio utilizing zilpaterol in lambs.<sup>(23, 24)</sup>

The most important effect of zilpaterol supplementation is the increase in hot carcass weight and the reduction of body fat, thereby, improving dressed carcass yield and modifying some carcass quality traits. An increase in hot carcass weight, dressed carcass yield and *Longissimus dorsi* muscle area (7, 2.4 and 15.4 %, respectively) was observed,<sup>(23)</sup> results similar to the ones found in the present study.

The increase in muscle mass and reduction of adipose tissue is an effect known as energy exchange, which can occur due to an increased activity in protein synthesis, as well as a reduction in protein degradation and lipolysis due to the action of zilpaterol in interaction with  $\beta_2$  adrenergic receptors of the muscle fibers and adipocytes.<sup>(24)</sup>

Published results agree with the findings of the present study. A investigation has reported a reduction in the proportion of neck wholesale cuts and a tendency in the increase of loin wholesale cut proportion (18.9 and 8.28 %, respectively) in lambs supplemented with zilpaterol.<sup>(25)</sup> However, other researches did not find an effect on primary cuts of lamb's carcasses after the treatment of zilpaterol for 28 d.<sup>(26)</sup> The fiber composition of the muscle might determine responsiveness to  $\beta$ -agonist.<sup>(27)</sup>

Some studies have been conducted to evaluate the effect of different  $\beta$ -agonist on fatty acid profile of body fat, an experiment was conducted to evaluate the effect of zilpaterol supplementation in crossbreed steers on fatty acid profile and found an increase of heneicosanoic acid (C21:0) this being the only adipose tissue fatty acid affected by zilpaterol supplementation.<sup>(28)</sup> A decrease in palmitic fatty acid (C16:0) of subcutaneous fat without affecting other fatty acids when a  $\beta$ -agonist was supplemented in steers.<sup>(29)</sup>

Intramuscular *Longissimus dorsi* polyunsaturated fatty acids from steers were higher in total unsaturated fatty acids, and lower in total saturated fatty acids,<sup>(8)</sup> these findings are similar to the present experiment where the shift is mainly between stearic (C18:0) and linoleic (C18:2) fatty acids. However, a reduction in total of monounsaturated fatty acids was reported, but not on individual fatty acids proportion of intramuscular fat when zilpaterol was administered.<sup>(7)</sup> Changes in fatty acids of intramuscular and subcutaneous fat is presumably related to an increased rate of lipolysis and subsequent release of fatty acids.<sup>(30)</sup>

There is little information and discussion available about effects of 20 d of zilpaterol supplementation in feedlot lambs. In feedlot cattle, reporting effects in Final LW, ADG, G:F ratio, HCW, dressing percent and LM area,<sup>(6)</sup> the results were similar to those shown in the present study.

Zilpaterol supplementation in lambs was tested 14, 28 and 24 d, although did not compare 1 d of zilpaterol vs. control group, results of positive linear effect in growth performance and carcass characteristics suggest that zilpaterol has a favorable response after two weeks of zilpaterol supplementation.<sup>(21)</sup>

Some authors<sup>(21, 22)</sup> reported a linear effect in DMI increase as zilpaterol treatment days increased, while in this study there was a similar tend effect in this variable. However, both studies report a linear increase in gain:feed ratio. Information regarding the use of zilpaterol during different feeding periods in finishing lambs is scarce. Nonetheless there have been studies carried out in beef cattle where the addition zilpaterol has been evaluated in feeding periods of 20, 30 and 40 d.<sup>(6, 5)</sup> The findings in these studies report a linear increase in gain:feed ratio as treatment days increase in agreement to what is found in this study furthermore, a linear reduction in DMI as treatment days increased has been reported,<sup>(5)</sup> results similar to what is found in this study.

Others authors reported that zilpaterol treatment time increased (0, 14, 28 and 42 d) a linear increase in hot carcass weight, dressed carcass yield and *Longissimus dorsi* muscle area was found in finishing lambs.<sup>(21)</sup>

In cattle utilizing different zilpaterol feeding periods (0, 20, 30 y 40 d) reported a linear increment in hot carcass weight, dressed carcass yield and *Longissimus dorsi* muscle area.<sup>(6)</sup> However in another beef cattle study there was only a linear increase in dressed carcass yield and no changes in hot carcass weight and *Longissimus dorsi* muscle area.<sup>(5)</sup>

The magnitude of  $\beta$ -agonist response varies greatly by factors like age, species, sex, diet and breed.<sup>(31)</sup> Effects of zilpaterol feeding duration has been investigated on beef cattle,<sup>(6, 5)</sup> these results show similar responses to the present experiment, where improvements in growth performance and carcass traits were detected as of the twentieth day of zilpaterol supplementation. Similarly, in this trial no significant differences on linear and quadratic analysis were shown to these variables.

Results on growth performance, carcass characteristics and the estimated energy from diet are widely documented results with  $\beta$ -agonists supplementation. However, for the beef industry, consider the possibility of obtaining similar results from 20 d of zilpaterol supplementation provide, on the one hand, a more profitable process, and the possibility of adjustments to zilpaterol supplementation allowing cattle to be kept in the pens for longer when there are failures in logistics with the slaughterhouses.

## Conclusions

We conclude that zilpaterol hydrochloride improved variables of growth performance, energy retention and carcass traits with noticeable effects from 20 d of zilpaterol supplementation. Nonetheless, in this experiment no effects were observed on linear contrast applied to levels of zilpaterol supplementation, suggesting no difference responses from 20 to 40 d. Growth performance calculated from final live weight with carcass adjusted showed clear improvements, this allow confirm the direct effects of beta agonists adrenergic on muscle and adipose tissues.

## Data availability

All relevant data are within the manuscript and its supporting information files.

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## Conflicts of interest

The authors have no conflict of interest to declare regarding this publication.

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