Protein level and β2-adrenergic agonist supplementation on growth performance and dietary energy of hairy lambs

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SUMMARY
Zilpaterol hydrochloride (ZH) is a β2-adrenergic agonist used as a feed additive during fattening phase that increases growth performance and carcass in lambs by increasing tissue protein accretion. However, information about the effects of high protein level in diet on the responses to ZH in feedlot diets for lambs is unknown. For this reason, a study was conducted to evaluate the interaction of two dietary protein levels (15 and 18% CP, dry matter basis) and two dietary ZH levels (0 and 6 mg/kg of diet dry matter) on growth performance, dietary energy, and carcass dressing percentage of finishing lambs. For the above, 40 Pelibuey × Katahdin (37.8±2.2 kg) crossbred intact male lambs were used in a feeding trial which lasted 33 days. Lambs were grouping to 5-pen blocks (two lambs per pen, 5 replicas per treatment). Both diets contained similar energy concentration (~2.05 Mcal NE_m/kg). At the final of feeding trial, all lambs were slaughtered and dressing percentage was determined. No interactions were detected between protein level and ZH supplementation. Increasing crude protein level from 15 to 18% did not affect (P≥0.18) dry matter intake (DMI), average daily gain (ADG), gain to feed ratio (GF), and dietary net energy (NE). Inclusion of ZH in diets did not affect (P=0.17) DMI, but increased (P<0.01) ADG (16.2%), increasing GF and dietary NE (16 and 10.5%, respectively). Protein level did not affect (P≥0.78) carcass weight or carcass dressing percentage, while ZH supplementation increased both carcass weight (4.2%, P<0.01) and dressing percentage (2.59%, P=0.04) independent of protein level. The β2-adrenergic agonist zilpaterol hydrochloride is a tool to enhance growth performance and carcass in finishing lambs. The increase in protein level from 15 to 18% during the late phase of finishing (i.e., 30 d) did not improve growth performance or carcass weight in response to ZH supplementation.

KEY WORDS
Zilpaterol hydrochloride, Feeding strategy, Feedlot lambs, Performance, Carcass.

INTRODUCTION

Zilpaterol hydrochloride is a β2-adrenergic agonist. When administered at the rate of 4 to 8 mg/kg diet during the last 20-40 days of fattening it increases muscle accretion and hence, improving growth performance, efficiency of dietary energy utilization, and carcass traits in fattening lambs [1]. However, the magnitude to the response ZH supplementation varies depending dosage level [2,3], supplementation period [4], withdrawal period [5], slaughter weight [6], type of ZH (patented or generic) [7], and environmental factors [8]. In spite that ZH supplementation increase rate of protein accretion [9,10], studies are lacking on the effects of supplementing higher levels of PC than those recommended when lambs are finished with supplemental ZH. In absence of ZH supplementation, growth performance of hairy lambs during the finishing phase is not enhanced when level of CP supplementation exceeds 14% [11-13]. In an earlier study performed with Bonsmara steers (278 kg of initial weight), O'Neill *et al.* [14] did not observed difference in growth performance of ZH supplemented steers receiving diets containing 10, 12, or 14% CP during the final 35 d before slaughter. However, steers in their study were harvested at a 328 kg, representing only 79% of they mature weight (415 kg) [15]. This could mask the possible interactions of ZH and protein level, in as much as the effects of ZH on composition of gain are more pronounced as final weight approach mature final weight. In the region of North of Mexico, feedlot lamb systems usually fatten by periods from 60 up to 90 days. During the last stage of fattening (~40-50 d) lambs are fed finishing diets ranging from 2.00 to 2.20 Mcal NE_m/kg, and containing around 15% CP. The objective of this experiment was to evaluate the possible interaction of CP level and supplemental ZH during the late finishing phase. Accordingly, an experiment was conducted

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evaluating the effect of two CP levels, a conventional level (15% of diet DM) and high-protein level (18% CP of diet DM), and zilpaterol hydrochloride (0 vs 6 mg/kg diet DM) on growth performance, efficiency of dietary energy utilization, and dressing percentage of hairy lambs slaughtered at 45 kg of body weight.

MATERIAL AND METHODS

This experiment was conducted at the Universidad Autónoma of Sinaloa Feedlot Lamb Research Unit, located in the Culiacán City (24° 46' 13" N, 107° 21' 14" W; 55 m a.s.l), México. Culiacán City has a tropical climate. Average daily minimum and maximum air temperatures during the trial were 24.9 and 36.8°C (average=31.1°C), with an average relative humidity of 49.9% (minimum and maximum of 67.6 and 32.3%, respectively). All animal management procedures were conducted according to the Mexico Federal guidelines for animal use and care and approved by the Ethics Committee of the Faculty of Veterinary Medicine and Zootechnics, Autonomous University of Sinaloa (Approval Protocol #01232021).

Animals, Treatments, and Sample Analysis

Forty-eight Pelibuey × Katahdin crossbred intact male lambs were received at research facilities 60-d prior to the start of the experiment. Lambs were treated for endoparasites (Closantil, 5%, CHINOIN LAB, México City, México), and injected with 1×10^6 IU vitamin A (Synt-ADE®, Fort Dodge, Animal Health, México). Over a 3-week period, lambs were gradually adapted to the finishing diet (15% CP, no supplemental ZH; Table 1). Subsequently, lambs were group-fed the finishing diet an additional 40 d prior to starting the experiment. At the start of the experiment, lambs were individually weighed (electronic scale; TORREY TIL/S: 107 2691, TOR REY electronics Inc., Houston TX, USA). From the original group of 48 lambs, 40 lambs (37.8 ± 2.2 kg of initial weight, BW) were selected, based on the uniformity of weight and general condition, for use in the experiment, and were assigned withing five weight grouping (blocks) to 20 pens (two lambs/pen and 5 replicas per treatment). Individual pens were 6 m^2 with overhead shade, automatic waterers, and 1-m fence-line feed bunks. Dietary treatments (Table1) consisted of two dietary protein levels (15 and 18% CP, DMB) and two dietary zilpaterol hydrochloride (ZH; (Zilmax® MSD Salud Animal Mexico, Santiago Tianguistenco, Mexico) levels (0 and 6 mg/kg diet dry matter). All diets were formulated to meet or exceed the requirements for growing-finishing lambs [16]. The four dietary treatments were randomly assigned to pens within each weight block in a randomized complete block design. The experiment lasted 33 days. Supplemental ZH was hand-weighed using a precision balance (Ohaus, mod AS612, Pine Brook, NJ), and premixed for 5 min with the mineral-protein supplement before incorporation into the complete mixed basal diet using a 2.5 m^3 capacity paddle mixer (mod 30910-7, Coyoacán, México). To avoid cross contamination among treatments, the mixer was thoroughly cleaned between each treatment. Zilpaterol hydrochloride was supplemented for 30 d followed by a 3-d preharvest withdrawal in which lambs continued to receive the corresponding treatment without ZH. Lambs were allowed ad libitum access to both dietary treatments and clean water. Fresh feed was provided twice daily at 0800 and 1400 h at approximately 30:70 proportion of the daily registered intake (as feed basis). Daily feed allotments to each pen were adjusted to allow for approximately 5% residual feed remaining in the feed bunk at the time of the morning feeding. Feed bunks were checked between 0740 and 0750 h each morning, and residual feed was collected and weighed for the determination of feed intake. Adjustments in daily feed delivery were made at the afternoon feeding. Lambs were individually weighed at the beginning of the trial and at the end of the experiment (day 33). The initial shrunk body weight (SBW) was determined as full body weight × 0.96 (adjustment for gastrointestinal fill). Upon completion of the study, all lambs were weighed following an 18-h fast (feed but not drinking water was withdrawn) to

Table 1 - Dietary composition of experimental diets fed to lambs.

Item	Dietary treatments [a]			
	CP15	CP15+ZH	CP18	CP18+ZH
Ingredient composition, % of DM				
Corn grain cracked	71.00	71.00	64.00	64.00
Sudangrass hay	7.00	7.00	7.00	7.00
Soybean meal	11.50	11.50	18.50	18.50
Cane molasses	8.00	7.875	8.00	7.875
Zilmax (zilpaterol hydrochloride)	0.00	0.125	0.00	0.125
Mineral-protein supplement [b]	2.50	2.50	2.50	2.50
Net energy concentration, Mcal/kg of DM [c]				
EN_m, Mcal/kg	2.06	2.06	2.05	2.05
EN_g, Mcal/kg	1.40	1.40	1.39	1.39
Nutrient composition, % of DM[d]				
Crude protein	14.97	14.97	17.94	17.94
Neutral detergent fiber	14.09	14.09	13.87	13.87
Ether extract	3.18	3.18	3.13	3.13

DM: dry matter; Mcal: megacalorie; EN_m: Energy for maintenance; EN_g: Net energy for gain

[a] The source of zilpaterol (ZH) used was Zilmax® (MSD Salud Animal Mexico, Santiago Tianguistenco, Mexico), according to the label, the product contains 4.8% ZH. Thus, the dosage of 125 mg of product/kg diet corresponds to a dietary ZH concentration of 6 mg/kg diet (as feed basis);

[b] Mineral-protein supplement contained (%): Ca, 13.58%; P, 0.40%; CP 50.5%; NaCl, 18.0%, Mg 1.0%; K, 0.71%; Co, 5.6 ppm; Cu, 20.4 ppm; Mn, 1,674 ppm; Fe, 2759 ppm; Zn, 2900 ppm.

[c] Based on tabular net energy (NE) values for individual feed ingredients (NRC 2007).

[d] Dietary composition, with exception of rumen degradable intake protein which was estimated from NRC (2007) was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other.

obtain final SBW. Feed samples were subjected to the following analyses: DM (oven drying at 105°C until no further weight loss; method 930.15) and CP (N× 6.25, method 984.13) and ether extract (method 934.01) according to AOAC [17]. Neutral detergent fiber (NDF) was determined following procedures described by Van Soest *et al.* [18] (corrected for NDF-ash, incorporating heat stable -amylase using Ankom Technology, Macedon, NY).

Calculations

Estimates of ADG, and dietary net energy were based on the initial SBW and the final SBW (day 33 fasted live weight). Average daily gain was computed by subtracting the initial SBW from the final SBW and dividing the result by the number of days on feed. Feed efficiency was computed as ADG/ daily DMI and expressed as gain-to-feed ratio (GF). One approach for the evaluation of efficiency of dietary energy utilization in growth-performance trials is the ratio of observed-to-expected DMI and observed-to-expected dietary NE. Based on the estimated diet NE concentration and measures of growth performance, there is an expected energy intake. This estimation of expected DMI is performed based on the observed ADG, average SBW, and NE values of the diet (Table 1): expected DMI, kg/d = $(EM/EN_m) + (EG/EN_g)$, where EM (energy required for maintenance, Mcal/d) = $0.056 \times SBW^{0.75}$, EG (energy gain, Mcal/d) = $0.276 \times ADG \times SBW^{0.75}$, and NEm and NEg is the NE contained in the experimental diets, those values were calculated based on the ingredient composition of the basal diet (Table 1). The coefficient (0.276) was taken from the NRC [19] assuming a mature weight of 113 kg for Pelibuey × Katahdin male lambs. The observed dietary net energy was calculated using the EM and EG values, and DMI observed during the experiment by means of the quadratic formula:

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

where: $x = NE_m$, Mcal/kg, a =-0.41EM, b = 0.877 EM + 0.41 DMI + EG, and c = -0.877 DMI [20]

Carcass Data

All lambs were harvested on the same day. Lambs were stunned (captive bolt), exsanguinated, and skinned. The gastrointestinal organs were removed and hot carcass weight (HCW) was immediately recorded. Dressing percentage was calculated as HCW/final fasted live weight.

Statistical Analysis

Growth performance data (gain, DMI, gain efficiency, and dietary energetics) and carcass data were analyzed as a randomized complete block design in a 2×2 factorial arrangements of treatments using the MIXED procedure of SAS software [21], considering the initial weight as the blocking criterion and pen as the experimental unit. Treatment effects were separated into the following orthogonal contrasts: (1) 15% CP vs. 18%CP; (2) non additive vs ZH; and (3) CP× ZH interaction. Contrasts were considered significant when the p-value was ≤0.05.

RESULTS

No morbidity or mortality was observed during the experimental period. No interactions were detected between protein level and ZH supplementation in any of variables measured. Based on the average BW and DM intake observed in this experiment, the CP intakes were 11.02 and 12.62 g CP/kg $BW^{0.75}$ for CP15 and CP18, respectively. Protein level did not affect (p≥0.18) DMI, ADG, GF, or dietary NE. The expected extra-caloric effect due to increased metabolizable protein (MP) intake was not apparent. The daily intake of ZH averaged 7.3 mg ZH/lamb (0.17 mg ZH/kg BW), which is considered within the optimal dosage range. Supplemental ZH did not affect (P=0.17) DMI, but increased (P<0.01) ADG (16.2), GF (16%) and dietary NE (10.5%). Protein level did not affect(P≥0.78) carcass weight or carcass dressing. Supplemental ZH increased both carcass weight (4.2%, P<0.01) and dressing percentage (2.6%, P=0.04).

DISCUSSION

Crude protein level and ZH supplementation interaction

Supplementation with ZH consistently increases muscle accretion in ruminants when administered at dose of 4-8 mg/kg diet [2, 22]. Increased tissue protein accretion is reflected in increased metabolism rate. Hatefi *et al.* [9] observed that in castrated male goats (29.93 kg initial BW), ZH supplementation (0.20 mg ZH/kg BW) during a 30-d period increased plasma insulin, and thyroids hormones, increasing plasma volume, and cardiovascular and respiration rates. Barnes *et al.* [10] reported that ZH supplementation of lambs (49.9 kg initial BW) fed a high-energy finishing diet increased 9% fiber muscle mass and muscle glucose oxidation. Notwithstanding enhancements in muscle growth and metabolism we did not detect an appreciable interaction between CP level and ZH supplementation. Likewise, O'Neill *et al.* [14] did not observe an effect of dietary protein level (10, 12, or 14% CP) on growth performance of Bonsmara steers supplemented with 0.15 mg ZH/kg BW during the final 35 d prior to harvest. However, steers in that study were harvested at only 79% of estimated mature final weight (415 kg) [15]. We hypothesized that light body weight of Bonsmara steers at slaughtered in the experiment of O'Neill *et al.* [14] might have masked the possible interactions of ZH and protein level, since ZH effects on rate and composition of gain are more pronounced as cattle reach or exceed chemically mature final weight. However, based on our current findings it appears that effect of supplemental ZH less dependent on CP level in conventional (15% CP) finishing diets. During this late (last 30 d) finishing phase, metabolizable protein level may already markedly exceed requirements for growth.

Crude protein level effect

Protein level effects on growth performance in fattening lambs have been extensively investigated [12,13,20]. Protein deficiencies may be reflected in both decreased microbial protein synthesis and bypass protein reaching the small intestine, affecting energy utilization [23]. Dietary CP levels lower than 14% in short-term (i.e., <28 d) finishing lambs decreases the efficiency of dietary energy utilization [20]. But increasing CP level beyond 14% did affect growth performance, dietary energy, or in carcass traits in long-term finishing period (>56 d) [13]. As the protein supply exceeds the requirements for growth, the energy intake is the primary limiting factor affect growth rate and efficiency [24]. Machado da Rocha *et al.* [25] observed

Table 2 - Growth performance, dietary energetic and dressing percentage of lambs finished with isoenergetic diets contained two levels of CP and supplemented with 0 or 6 mg of hydrochloride//kg diet.

	15% CP		18% CP			p-value		
Item	0	ZH	0	ZH	SEM	CP	ZH	CP×ZH
Live weight, kg								
Initial	37.92	37.72	37.90	37.71	0.166	0.38	0.28	0.55
Final	45.42	47.13	45.69	46.54	0.486	0.74	0.02	0.40
Weight gain, kg/d	0.227	0.282	0.237	0.272	0.013	0.98	<0.01	0.45
DM intake, kg	1.200	1.232	1.118	1.201	0.039	0.18	0.17	0.54
Gain to feed ratio	0.189	0.228	0.193	0.227	0.004	0.90	<0.01	0.56
Diet energy, Mcal/kg								
Maintenance	2.03	2.27	2.04	2.26	0.023	0.93	<0.01	0.83
Gain	1.37	1.58	1.38	1.57	0.020	0.93	<0.01	0.83
Observed-to-expected diet NE								
Maintenance	0.99	1.10	0.99	1.10	0.011	0.72	<0.01	0.85
Gain	0.99	1.12	0.99	1.13	0.015	0.82	<0.01	0.89
Observed-to-expected DMI	1.01	0.90	1.01	0.89	0.102	0.69	<0.01	0.75
Hot carcass weight, kg	26.26	28.03	26.49	27.62	0.181	0.76	<0.01	0.59
Dressing percentage	57.80	59.47	57.94	59.35	0.370	0.38	0.04	0.58

that the protein level (14,16,18, and 20%) did not affect DMI, ADG, or GF in Santa Ines lambs fed a high-energy diet during 56 d. Indeed, numerous studies [11,12,13] demonstrate that dietary CP level in excess of 14 to 15% do not further enhance performance of finishing lambs. Estrada-Angulo *et al.* [20] determined that CP requirements of finishing crossbreed Pelibuey lambs was 11 to 12 g CP/kg $BW^{0.75}$. Based on the average BW and DM intake observed in this experiment, the CP intake were 11.02 and 12.62 g CP/kg $BW^{0.75}$, for CP15 and CP18, respectively.

Theoretically, excess intake of CP may provoke an extra caloric effect resulting in greater ADG and carcass weight, but this was not observed in lambs fed with high-energy diets [13,20,26]. The main factor that affecting carcass weight is ADG [27], while the factor affecting dressing percentage (considering that cattle have similar breed, initial weight at fattening, diet energy, days on feed, and fasted time before of slaughter) is composition of gain [28].

Zilpaterol hydrochloride supplementation effect

Due its benefits on growth performance and carcass traits, the beta-agonist zilpaterol hydrochloride (ZH) is feed additive extensively used in feedlots in countries in which it is approved [3]. The impact of ZH supplementation on feedlot lambs and cattle has been extensively studied. Several studies [2,7] demonstrated that ZH supplementation does not appreciably affect DMI in feedlot lambs. Likewise, Lean *et al.* [29] and Castro-Pérez *et al.* [6] did not observe changes in DMI with ZH supplementation of feedlot cattle. In contrast, Cayetano-De-Jesus *et al.* [3] observed decreased DMI, and Ríos-Rincón *et al.* [30] observed increased DMI with ZH supplementation. Reinhardt *et al.* [31] suggested that season and the intake level pattern prior to ZH supplementation may affect DMI when ZH is supplemented.

A meta-analysis study demonstrated that the optimal growth performance, and dietary NE utilization are observed when finishing lambs are supplemented with ZH at a rate of 4 to 8 mg/kg diet during the last 20 to 40 d of fattening [1]. With the optimal dose of ZH, the impact of ZH supplementation on performance can vary by starting live weight, type of diet, and lamb growth potential. In the current experiment, (Pelibuey × Katahdin crossbred intact male) supplemental ZH enhanced ADG (16.2%), GF (16%) and efficiency of dietary NE utilization (10.5%). These improvements are in line with those reported previously using similar dosage levels, type of lambs, and finishing diets [2,7,30]. Results on the current study are likewise consistent with previous reports [32,33]in which zilpaterol supplementation increased HCW and carcass dressing percentages. The increased carcass dressing percentage due to ZH accounted for 49% (22 g/d) of the increase on ADG (45 g/d).

CONCLUSION

It was concluded that zilpaterol hydrochloride is a tool to enhance growth performance and carcass dressing in finishing lambs when supplemented at 6mg/kg diet during the final 30 days prior to harvest. Increasing dietary protein level from 15 to 18% during the late phase of finishing did not improve growth performance, dietary energy utilization, carcass dressing or the magnitude response to ZH supplementation.

Conflict of interest

All authors declare no conflict of interest

Authors Contributions

Authors who meet authorship criteria are listed as authors, and each author certifies that they have contributed sufficiently to the conception and design of this work, as well as the aquire the experimental data and analysis and its interpretation, as well as the writing of the manuscript, to accept public responsibility. According to the authors, the manuscript represents valid research. Moreover, each author certifies that this material or similar material has not been submitted to or published as a full article anywhere else.

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