Evaluation of the biochemical and hematological profiles of feedlot hair sheep after the supplementation with generic zilpaterol hydrochloride

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Abstract:

This study aimed to evaluate zilpaterol hydrochloride (ZH, generic) supplementation on fattening hair sheep, using hematological and biochemical variables as health status indicators. A total of 32 hair lambs (Dorper x Pelibuey) were grouped by initial weight and randomly assigned into four treatments: T1= basal diet (control group), T2= basal diet supplemented with ZH at 0.10 mg·kg^{-1} of LW d^{-1} (Grofactor®, Virbac México, Guadalajara, Mexico), T3= basal diet supplemented with ZH at 0.20 mg·kg^{-1} of LW d^{-1}, and T4= basal diet supplemented with ZH at 0.30 mg·kg^{-1} of LW d^{-1}. Blood samples were collected on days 1, 15, and 30 of the study. The hematological profile was determined in fresh blood samples; metabolites, electrolytes, and hormones were determined in serum samples. The study followed a randomized complete block experimental design, using an orthogonal polynomial analysis to determine the trend of the responses at the different concentrations of ZH. Cholesterol and urea levels were higher ($P<0.05$) in T3 than in T2. Furthermore, the mean corpuscular hemoglobin concentration was higher ($P<0.05$) in T1 than in T3; the red blood cell distribution width was higher ($P<0.05$) in T2 and T3 than in T4. The Na levels and the number of platelets showed a linear trend ($P <0.05$) to decrease and increase, respectively, as ZH levels increased. A quadratic trend was observed ($P<0.05$) in mean corpuscular hemoglobin concentration and red blood cell distribution width with increasing dose of ZH (generic). The remaining variables did not show significant trends at ZH levels (generic). The values of the biochemical and hematological profiles were within the reference range, which suggests that the addition of ZH did not alter the health status of fattening lambs.

Key words: Hemoglobin, Lambs, Metabolites, Electrolytes, Zilpaterol hydrochloride.

In Mexico, sheep farming focused on meat production is growing considerably, but animal demand for supply is greater than what is produced, translating into imports of live cattle and carcasses from the United States, New Zealand, and Australia, mainly[1]. Hair sheep have been an alternative to cover these meat demands since they require less care than wool breeds. Additionally, hair sheep can adapt, reproduce, and produce under any production system. This species maintains lamb production throughout the year; however, it has the disadvantage of limited weight gain[2]. Therefore, farmers use growth

promoters to obtain more efficient animals for meat production[3]. The beta-adrenergic agonists (β-AA) are an attractive strategy that has positively impacted the production of fattening sheep[4].

A study that compared different β-AAs (zilpaterol hydrochloride, ractopamine hydrochloride, terbutaline, isoproterenol, etc.) concluded that zilpaterol hydrochloride (ZH) is a good option for lamb fattening[5]. However, results are contradictory in terms of performance[4,6] and carcass characteristics[6,7]. Since ZH supplementation promotes physiological, metabolic, hormonal, and hematological changes, the health of the animal can be compromised[8]. Additionally, the use of β-AA in ruminants and its negative effects on animal welfare have been one of the most important concerns in the livestock sector[9]. ZH supplementation has focused on animal performance and carcass characteristics; meanwhile, its effects on health and welfare are cared for trivially[10]. Although cattle supplemented with β-AA showed higher morbidity rates at the end of fattening[11], few studies have evaluated the effects of β-AA on animal health and included the analysis of biochemical and hematological components. This study aimed to evaluate the effect of different doses of a generic ZH on hematological and biochemical variables in feedlot-finished hair sheep.

The study was performed during the fall-winter season of 2015 in the Sheep Experimental Unit of the Instituto de Ciencias Agrícolas of the Universidad Autónoma de Baja California, in Valle de Mexicali, Baja California, México (32.8º N, 114.6º W). The climatic conditions of this region are similar to those found in the Sonoran Desert, defined by an extremely dry and warm climate, with a maximum temperature during summer ≥ 42 °C and a minimum temperature during winter ≤ 0 °C; the annual mean precipitation is 85 mm[12].

A total of 32 (Dorper x Pelibuey) F1 male hair lambs were used with an average weight of 29.3 ± 0.22 kg and age between 5 and 6 months. Groups of four lambs were formed according to their initial weight; these groups were randomly assigned to one of four treatments: T1= basal diet (control group); T2= basal diet supplemented with ZH at 0.10 mg·kg^{-1} of LW d^{-1} (Grofactor®, Virbac México, Guadalajara, Mexico); T3= basal diet supplemented with ZH at 0.20 mg·kg^{-1} of LW d^{-1}; and T4= basal diet supplemented with ZH at 0.30 mg·kg^{-1} of LW d^{-1}. Lambs were housed in individual pens (1.0 x 1.5 m) provided with drinking and feeding troughs, and shade. Feed was offered twice a day (0700 and 1500 h) in a 40:60 ratio. The diet consisted of ground wheat grain (60%), alfalfa hay (17.5%), wheat straw (11%), soybean flour (7%), soybean oil (2%), limestone (1%), dicalcium phosphate (1%), and common table salt (0.5%). This formulation provides 15% of PC and 2.9 Mcal of EM kg^{-1} of MS[13]. To ensure ZH consumption, the daily dose of the product was mixed in 30 g of ground wheat grain and offered during the morning before providing the basal diet. On d 30, ZH was withdrawn from the diet following the

instructions provided by the manufacturer. The experiment lasted 47 d (15 for adaptation, 30 for fattening, and 2 for withdrawal).

To analyze metabolites, electrolytes, and hematological components, we collected blood samples in 10 and 4 ml Vacutainer tubes by jugular venipuncture. Blood was collected in the morning (0600 h), under fasting conditions, at the initial, intermediate, and final phases of fattening (days 1, 15, and 30). Fresh blood samples were used for the hematological analysis using an automated equipment (Auto Hematology Analyzer, MINDRAY, BC-2800 Vet; Shenzhen, China). The blood collected in 10 ml tubes was centrifuged at 3,500 rpm at 10 °C for 15 min. Then, serum was separated in duplicate in 2 ml vials and stored at -20 °C for subsequent glucose (Glu), cholesterol (Cho), urea (Ur), triglycerides (Trig), total protein (TP), electrolytes (Na, K, and Cl), thyroxine (T_4) and triiodothyronine (T_3) hormone analysis. Metabolites were measured with a blood chemistry analyzer (Model DT-60, Johnson Co.; High Wycombe, UK); electrolytes were determined using an automated equipment (Electrolyte Analyzer LW E60A; Landwind Medical; Shenzhen, China). Hormone determination was performed using a Thunderbolt® Analyzer (Davis, CA, USA) for ELISA and chemiluminescence (CLIA) assays.

Responses were analyzed following a randomized complete block design. An orthogonal polynomial analysis was performed to determine the response trend through β-AA levels. Significance was declared at a probability of $P \leq 0.05$ using the PROC MIXED of SAS. Since the cubic trend was not significant for any of the analyzed variables, it was omitted from the result tables. All data were processed using the Statistical Analysis System software[14].

Table 1 shows the results of ZH supplementation on the blood metabolites of fattening lambs. The levels of Cho and Ur were higher ($P<0.05$) in T3 than in T2. The levels of Trig, Glu, and TP were not affected ($P>0.05$) by ZH supplementation. These values fall within the reference interval for sheep[15,16]. Similar results were reported by López-Carlos et al[6]; they found no differences in TP, Glu, or Trig when supplementing with similar ZH doses using Dorper x Katahdin sheep. Other authors[17] reported that ZH supplementation at 0.20 mg·kg^{-1} of LW d^{-1} in wool sheep did not modify blood levels of Glu, Trig, TP, or Cho, finding only a decrease in Ur levels. It has also been reported that ZH supplementation in cattle does not modify blood metabolites[8,18]. Hatefi et al[19] found that ZH supplementation decreases Glu and Cho plasma levels in male goats without affecting Trig levels.

β-AA administration immediately increases gluconeogenesis, which in turn increases plasma Glu levels[8]. However, it is estimated that due to a decrease in tissue sensitivity,

the initial increase in Glu concentration returns to normal as the administration time increases[20]. This effect could have been present in this study, where metabolite levels returned to their normal concentrations. This is confirmed by the fact that the metabolites associated with the energy and protein status were not affected by the supplementation with different doses of ZH, indicating that the lambs did not modify or compromise their homeostasis or metabolism. Therefore, the use of Grofactor® β-AA may have no adverse health effects on sheep. However, to date, few studies have been performed on the effect of β-AA on blood metabolites, and the results obtained are still inconsistent[19].

Table 1: Serum metabolite concentrations in hair sheep in response to different doses of zilpaterol hydrochloride

	ZH dose (mg·kg^{-1} of LW d^{-1})				SEM	Effects	
	0	0.10	0.20	0.30		L^2	Q^3
Cho, mg/dL	49.4^{ab}	46.7^a	55.4^b	50.5^{ab}	3.02	0.36	0.71
Trig, mg/dL	26.0	26.7	25.8	26.4	1.40	0.95	0.98
Glu, mg/dL	64.3	62.6	65.7	61.8	4.12	0.83	0.82
TP, mg/dL	6.80	6.85	6.82	7.03	0.108	0.17	0.47
Urea, mg/dL	38.3^{ab}	35.4^a	40.9^b	38.5^{ab}	1.69	0.40	0.91

^{ab} Averages with different letters in the same row indicate statistical difference ($P<0.05$).
SEM= standard error of the mean; L= linear; Q= quadratic.

Cho= cholesterol; Trig= triglycerides; Glu= glucose; TP= total protein.

Table 2 shows the electrolyte and thyroid hormone levels after supplementation with different doses of ZH. ZH administration significantly reduced ($P<0.01$) Na serum levels, showing a linear trend with increased ZH levels in the diet. Serum levels of Cl, K, T$_3$, and T$_4$ were not affected ($P>0.05$) by the different treatments. β-AAs are considered excellent nutrient redistributors for skeletal muscle formation and fat deposit reduction in the carcass[21]. However, β-AAs may affect the concentration of various biochemical components involved in muscle and adipose tissue development[22]. The quadratic effect observed in Na concentrations depended on the increasing levels of ZH in the diet. The addition of 0.10 mg of ZH decreased Na in serum, while the 0.20 mg dose of ZH maintained serum Na levels. Moreover, the addition of 0.30 mg of ZH considerably decreased Na levels. Although there was a significant difference in the serum Na levels between doses, all values are within the normal reference interval for sheep[15,16], which suggests that the treated animals maintained their osmotic pressure and acid-base equilibrium without any major stress symptoms.

K plays an important role in the regulation of water inside and outside the cell. Therefore, maintaining optimal plasma K levels is essential for proper body function. However, Buntyn et al[23] found that ZH supplementation decreases blood K levels, resulting in an

increase in lean muscle deposition during ZH supplementation. This study obtained similar results; the lowest K level occurred at the highest ZH level. We also observed that control sheep had higher K concentrations compared to those treated with 0.30 mg·kg^{-1}. Frese et al[8] reported that supplementation with ZH and ractopamine hydrochloride did not modify K levels compared to the control in finishing steers. The decrease of serum Na levels after β-AA ingestion has not been reported in sheep. However, equines have been reported to decrease blood Na levels after β-AA ingestion in very extreme cases, either due to loss during profuse sweating or by impaired renal Na transport; hypochloremia may even be associated with endogenous glucocorticoid release or renal failure[24], an effect that possibly did not occur in this study. Moreover, other studies[8,23] reported that finishing steers and heifers had similar Na concentrations after ZH and ractopamine hydrochloride supplementation; these results were attributed to the availability and consumption of free access to water.

Table 2: Serum electrolyte and thyroid hormones concentrations in hair sheep in response to different doses of zilpaterol hydrochloride

	ZH dose (mg·kg^{-1} of LW d^{-1})				SEM	Trend	
	0	0.10	0.20	0.30		L	Q
Na, mmol	139.7 ^{a}	138.2^{ab}	138.5^{ab}	137.1^b	0.584	<0.01	0.94
Cl, mmol	113.3	114.3	113.3	113.9	0.489	0.95	0.84
K, mmol	6.29 ^{a}	5.85^{ab}	6.31 ^{a}	5.57^b	0.181	0.10	0.51
T$_3$, ng/ml	1.40	1.19	1.32	1.25	0.728	0.33	0.38
T$_4$, ng/ml	2.92	3.06	2.40	2.29	0.343	0.10	0.74

SEM= standard error of the mean; L= linear; Q= quadratic.

K= potassium; Na, sodium; Cl= chlorine; T$_3$= triiodothyronine; T$_4$= thyroxine.

^{ab} Means with different letters in the same row indicate statistical difference ($P<0.05$).

Previous studies have observed that T$_4$ and T$_3$ hormones are not affected during acute or chronic treatments with β-AA[25]; in this study, was also found no effects after ZH supplementation for 30 days. However, Hatefi et al[19] reported that T$_3$ and T$_4$ levels increased after chronic administration of different β-AA in goats, which could be explained by the increase in lipolysis. Thyroid hormones are widely related to carbohydrate metabolism, which includes glucose absorption and mobilization to the adipose and muscle tissues, increasing glycolysis, gluconeogenesis, and insulin levels[26], favoring the increase in lipolysis. However, studies regarding the effects of the administration of β-AA on blood biochemical components and hormones remain inconsistent and scarce. The concentration of circulating blood components indicates the physiological equilibrium and health status of the animal. However, external agents can modify the optimal concentration of these components and, consequently, result in a physiological disequilibrium, affecting animal well-being. Therefore, these components are important indicators of the physiologic and pathologic status of organisms. Table 3

shows the concentration of blood variables after supplementation with different doses of ZH.

Table 3: Concentration of blood components in hair sheep in response to different doses of zilpaterol hydrochloride

SEM= standard error of the mean; L= linear; Q= quadratic.

Variables	ZH dose (mg·kg^{-1} of LW d^{-1})				SEM	Trend	
	0	0.10	0.20	0.30		L	Q
RBC, x 10^{12} L	11.90	12.21	11.32	11.27	0.457	0.16	0.70
Hgb, g/dL	10.68	10.67	10.19	10.25	0.374	0.26	0.91
Hct, %	35.60	36.30	35.00	34.8	1.15	0.48	0.68
MCV, x 10^{15} L	30.21	30.27	31.30	31.90	0.870	0.12	0.78
MCH, Pg	9.10	8.79	8.98	9.24	0.205	0.51	0.17
MCHC, g/dL	29.96 [a]	29.3 [ab]	28.8 [b]	29.2 [ab]	0.303	0.03	<0.05
RDW, %	17.96 [ab]	18.70 [a]	18.62 [a]	17.58 [b]	0.375	0.46	<0.05
Plt, x 10^9 L	445.3	517.0	549.7	575.5	46.07	<0.05	0.65

RBC= red blood cells; Hgb= hemoglobin; Hct= hematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; RDW= red blood cell distribution width; Plt= platelets.
[ab] Averages with different letters in the same row indicate statistical difference (*P*<0.05).

The mean corpuscular hemoglobin concentration was higher (*P*<0.05) in T1 than in T3; the red blood cell distribution width was higher (*P*<0.05) in T2 and T3 than in T4. The mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) values showed a quadratic trend (*P*<0.05), with higher concentrations of MCHC in control lambs compared to those supplemented with Grofactor (Table 3); RDW was also higher in T2 and T3 compared to the other treatments. It was observed a linear trend (*P*<0.05) in platelet levels, in such a way that by increasing ZH levels, platelet concentrations increased. There were no significant differences in the blood components between treatments (*P*>0.05). One possible explanation for the decrease in MCHC is that the use of growth promoters markedly reduces the amount of adipose tissue through lipolysis[18], promoting peripheral vasodilation by adding fatty acids and glycerol to the bloodstream during this process, which causes an increase in blood components over plasma volume[27]. Some studies have shown that the addition of β-AA affects some hematologic components in ruminants[19,28] by modifying physiologic processes, such as vasodilation and cardiovascular action[28]. The cardiovascular effects that occur after β-AA supplementation include the increase of the animal respiratory and heart rates. On the contrary, it has been described that the increase of the respiratory rate leads to an increase in the levels of red blood cell components in the bloodstream[29] due to a high oxygen demand to activate heat dissipation mechanisms by the increase in metabolic heat. Boyd

et al.[30] reported that rumen temperature was lower in steers treated with ZH than those in the control group. The results obtained were attributed to an increase in the respiratory rate of treated animals as a mechanism to reduce the heat accumulated by the increase in ruminal fermentation. Therefore, these results suggest that the addition of 0.10 mg of ZH increased lipolysis; however, adding higher doses would increase metabolic heat production, increasing the animal respiratory rate as a mechanism to maintain a constant body temperature. However, this would result in vasodilation, causing an imminent decrease in red blood cells. Similar results were reported by Hateffi *et al*[19], who found that the supplementation with 20 mg·kg⁻¹ of ZH decreased the hematocrit and hemoglobin values; these results were attributed to the increase of the respiratory rate. However, the effects of β-AA on hematological components are still unclear since few studies have investigated this topic.

The increase in the average number of platelets due to ZH supplementation could be explained by an effect on blood thrombocytosis, increasing platelet concentration as a response to lesions that may increase blood vessels, and the homeostatic imbalance of the organism[16]. Similar results were obtained by Wagner *et al*[24]; they found higher levels of platelets in equines supplemented with 0.17 mg·kg⁻¹ of ZH. However, the number of platelets has not been previously evaluated in ruminants after the administration of β-AA. Nevertheless, the hematological values of all the variables obtained in this study are within the reference interval[31]. Frese *et al*[8] evaluated the effect of adding zilpaterol and ractopamine to the diet of finishing cattle on cardiovascular variables; they concluded that supplementation with these β-AAs did not affect the arrhythmia rate, although there was a slight increase in heart rate, which finally returned to the normal reference interval mentioned in the literature for this type of cattle. However, it is important to continue these studies to confirm if these products represent a risk, both for animal welfare and human health.

In summary, it was observed minimal differences in Cho, Ur, Na, number of platelets, mean corpuscular hemoglobin concentration, and red blood cell distribution width. Nonetheless, all hematological and biochemical variables were within their reference interval after increasing doses of zilpaterol hydrochloride. These results suggest that the experimental animals did not modify their acid-base status or cellular homeostasis, and that their metabolic and physiologic status were not compromised after consuming ZH in their diet. Furthermore, the results demonstrate that no pathologic state was generated in hair sheep after zilpaterol hydrochloride (generic) supplementation.

Acknowledgments and conflicts of interest

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