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Keywords: β-agonist; vitamin D; ruminant; growth performance; carcass traits; meat quality

Article

Effects of Zilpaterol Hydrochloride with a Combination of Vitamin D_3 on Feedlot Lambs: Growth Performance, Dietary Energetics, Carcass Traits, and Meat Quality

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Simple Summary: Zilpaterol hydrochloride (ZH) is an additive used to enhance growth performance and yield carcass in ruminants. However, its use can lead to a reduction in meat quality, resulting in tougher meat. Meat tenderness is a crucial quality trait that affects consumer acceptability, satisfaction, and repeat purchase. Studies have shown that supplementing ruminants with Vitamin D_3 (D3) before slaughter can improve meat tenderness. It is believed that supplementing cattle treated with ZH with D3 can mitigate the negative effects of ZH and improve meat quality. In this study, we aimed to evaluate the combined effect of ZH and D3 on growth performance, energy efficiency, carcass traits, and meat quality of feedlot lambs. ZH supplementation improved growth, energy efficiency, and carcass quality, but resulted in tougher meat. In contrast, supplementing with D3 led to an improvement in meat pH, but did not affect reducing toughness. Additionally, when both ZH and D3 were used together, growth and energy efficiency were negatively impacted. however, D3 supplementation failed to improve the tenderness of the meat produced by ZH in feedlot lambs.

Abstract: The study evaluated the impact of supplementing ZH in combination with D3 on growth performance, energy efficiency, carcass traits, and meat quality of feedlot lambs. Thirty-two Dorper x Katahdin cross lambs (37.3±5.72 kg) were utilized in a 29 d experiment in a complete randomized block design with a 2x2 factorial structure consisting of two levels of ZH for 26 d (0 and 0.20 mg /kg PV^{-1}) and two levels of D3 for 7 d (0 and $1.5x10^6$ IU/d^{-1}). ZH improved ($P \leq 0.05$) average daily gain (ADG) and feed efficiency by 9.9% and 17.8%, respectively, as well as hot carcass weight (HCW) and dressing carcass by 4.3% and 2.6%, respectively. ($p \leq 0.03$). However, ZH increased ($p<0.01$) muscle pH and Warner-Bratzler shear force (WBSF) (2.5 and 23.0%, respectively). D3 supplementation negatively affected ($P \leq 0.02$) dry matter intake (DMI) (last 7 d) and ADG by 15.7% and 18.1%. On the other hand, D3 improved the pH of the *longissimus* muscle (LM) by 1.7% ($p=0.03$) without affecting WBSF. When D3 was supplemented in combination with ZH, it was observed that meat quality was improved by reducing muscle pH compared to lambs treated only with ZH. However, D3 did not improve meat tenderness produced by ZH supplementation.

Keywords: β-agonist; vitamin D; ruminant; growth performance; carcass traits; meat quality

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1. Introduction

Beta-agonists are commonly used in beef production to increase carcass yield by promoting muscle protein synthesis and reducing body and carcass fat[1]. However, studies have shown that the use of ZH (Beta-agonist) can have a negative impact on meat quality by increasing the meat's toughness measurement [2–5]. Several factors can contribute to an increase in WBSF values resulting from ZH supplementation. One of the reasons is the alteration of the activity of the myofibrillar enzyme system, which may occur due to a decrease in calpain concentrations and an increase in the activity of calpastatin [6,7]. Likewise, previous studies indicate that hypertrophy and an increase in muscle fiber diameter [8] and a decrease in intramuscular fat increases WBSF [9]. Meat tenderness is a crucial quality trait that affects consumer acceptability, satisfaction, and repeat purchase [10]. Different strategies have been implemented that improve this characteristic with variable effectiveness. The addition of D3 to feed for a short period of time prior to slaughter is one of the alternatives that have demonstrated an improvement in meat tenderness [11]. D3 supplemented to feed before slaughter increases intramuscular calcium concentration [12–15] and activates calpain enzymes that degrade myofibrillar proteins [16], leading to improve the meat tenderness [17–19]. Therefore, oral supplementation with supra-nutritional doses of D3 seems to be an alternative to enhance meat tenderness in cattle treated with ZH [15]. Therefore, the objective of this experiment was to determine the effect of the addition of D3 in diets with ZH in feedlot lambs on growth performance, dietary energetics, carcass characteristics, and meat quality.

2. Materials and Methods

2.1. Handling and Facilities

The study was carried out in the Experimental Growth Unit for Small Ruminants at the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autónoma de Sinaloa (24.7721 N, -107.3545 W). All live animal handling was conducted in accordance with the Official Mexican norms for animal care[20–22] and of the Institutional Committee for the Care and Use of Animals of the Veterinary Medicine and Animal Science Faculty (CICUA-FMVZ/17-10-2016).

2.2. Experimental Diets

The study utilized a complete randomized block design with a 2x2 factorial structure, incorporating pre-slaughter supplementation of two levels of ZH over 26 d (0 and 0.20 mg/kg LW-1) and two levels of D3 over 7 d (1.5 x 106 IU/lamb/d-1). ZH (ZILMAX®) was provided at a total dose of approximately 9 mg/lamb daily for 26 d, followed by a three-d withdrawal period. Additionally, D3 (Microvit D3®) was introduced to feed during the final 7 d leading up to slaughter. The diet was formulated based on cracked corn and soybean meal with a crude protein content of 14.5% and 1.43 Mcal/Neg (Table 1). It was provided twice a day, with a morning-to-afternoon service percentage of total feed intake of 30:70 (0900 and 1500 h). Before the morning feed, the bunk feeders were checked 30 minutes in advance to assess the previous day's intake. Any excess feed was removed, weighed, and recorded. This information was then used to adjust the afternoon feed to ensure a rejection rate of less than 7.0%.

Table 1. Formula of experimental diets.

Ingredients	Inclusion, %
Sudangrass hay	14.5
Cracked corn	66.0
Soybean meal	6.0
Grease trap waste	2.0
Molasses cane	8.0
Urea	1.0
Trace mineral salt	2.5

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Nutritional composition[a]	
Dry matter, %	89.0
Crude protein, %	14.5
NE_m, Mcal/kg/Dry matter	2.10
NE_g, Mcal/kg/Dry matter	1.43

[a]Derived from tabular values based on ingredient composition of the experimental diet.

2.3. Calculations

Feed samples were collected daily for dry matter analysis[23]. To evaluate the effect of the treatments on carcass performance expressed as ADG and feed efficiency, the final live weight (FLW) adjusted to carcass weight was calculated as HCW/overall average of dressing carcass (55.5%) for all treatments. Assuming that DMI 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: $\boldsymbol{DMI, kg/d} = \left(\frac{\boldsymbol{ME}}{\boldsymbol{2.06}}\right) + \left(\frac{\boldsymbol{EG}}{\boldsymbol{1.40}}\right)$ Where ME (energy required for maintenance, Mcal/d) = $\boldsymbol{ME} = \boldsymbol{56xSBW^{0.75}}$, SBW, kg = (shrunk body weight, $\boldsymbol{BW^{0.96}}$), EG (energy gain, Mcal/d)= $\boldsymbol{276xADGxSBW^{0.75}}$ and NEm and NEg are 2.06 and 1.40 Mcal/kg, respectively, derived from tabular values based on ingredient composition of the experimental diet [24]. A coefficient of 276 was estimated assuming a mature weight for Pelibuey × Katahdin male lambs of 115 kg [24]. Dietary Net Energy was estimated utilizing the quadratic formula [25], $\boldsymbol{x} = \frac{\boldsymbol{-b-\sqrt{b2-4ac}}}{\boldsymbol{2c}}$

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

2.4. Carcass Traits and Primary Cuts

Three days before the processing of lambs at the slaughterhouse, ZH supplementation was removed from the diets. D3 was withdrawn from the diets 24 h before slaughter, which corresponds to the fasting time before transport to the slaughterhouse. At the end of the growth test, their FLW was recorded and subsequently transported to the slaughterhouse facilities for carcass obtain. In this stage, the weight of the HCW was measured, and the percentage of the dressing carcass was subsequently calculated. The carcasses were stored in a cold room (2° C) for 24 h before being transported to the meat cutting room. The carcasses were divided longitudinally by the center of the vertebrae, subsequently recorded the thickness of back fat (Cadena-a020®) and area of LM using a grid positioned in the cross-section (between 12-13th rib) of the left half carcass. To determine the quantity of perirenal and pelvic fat, it is manually removed from the carcass, and the weight is recorded and subsequently expressed as a percentage of cold carcass weight. The half carcasses were divided into primary cuts, their weight was recorded, and the percentage of each cut was calculated with respect to cold carcass weight. Primary cuts were obtained in accordance with the guidelines of North American Meat Processors Association (NAMP)[26]. The front quarter of the carcass was divided into: neck, foreshank (208D) shoulder (207), rib (209A), rack (204), and breast (209); while the hind quarter into: flank (232E), loin (232A), and leg (233A)[26].

2.5. Meat Quality and Fatty Acid Profile

The LM was obtained from the primary cut of the loin of all lambs, after removing the bone and fat, It was then divided into two samples and packaged under vacuum, 1) 50 g sample (-20°C) for the determination of the fatty acid profile, and 2) sample of ~ 500 g (2°C) for the determination of meat quality characteristics. This last sample was refrigerated for 7 d (2°C), subsequently the pH (Delta track-ISFET pH101), the color (L*, luminosity, a*, redness, b*, yellowness) were evaluated. With a Konica-Minolta CR10 colorimeter, chromaticity with a spectrophotometer, and tenderness using the WBSF technique (Lloyd Instruments).

Determination of fatty acids Samples of 500 mg dry meat were deposited in Pyrex tubes with Teflon stoppers. To each sample, 2 mL hexane and 3 mL 5% methanolic hydrochloric acid were added, and the mixture was shaken carefully for 1 min to homogenize the sample. It was then placed

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in a 70 °C water bath for 45 min. After cooling for 20 min at room temperature, 5 mL 6% potassium carbonate and 1 mL hexane were added. The samples were then shaken for 1 min and centrifuged at 1500 rev min−1 (200g) for 5 min. The supernatant was then immediately transferred to sterile Pyrex tubes with 1 g sodium sulfate. Again, the samples were shaken for 1 min and centrifuged for 5 min at 1500 rev min−1 (200g) to finally extract the supernatant with Whatman (grade 597) filter paper. These samples were put into Eppendorf vials and stored at −40 °C until analysis. The chromatograph (Perkin Elmer, model Clarus 500, Waltham, MA, USA) at working conditions was supplied with capillary column 100 m × 0.25 mm × 0.2 µm (SUPELCO TM-2560), nitrogen carrier gas was used, and oven temperature was maintained at 140 °C for 5 min with increases of 4 °C per min up to 240 °C. The injector and detector were maintained at 260 °C. The peaks were identified according to the retention times of the methyl ester standards (SUPELCO37, FAME MIX analytical Sigma–Aldrich).

2.6. Statistical Analysis

The following data were analyzed as part of a randomized complete block design: growth performance, dietary energetics, carcass traits, and primary cuts components. The meat quality variables data were analyzed as a completely randomized design. The experiment used a factorial arrangement 2 x 2, with two levels of ZH (0 and 0.20 mg/kg LW d^{-1}) and two levels of D3 (0 and $1.5x10^6$ IU/$lamb^{-1}$), considering a pen as the experimental unit. The SAS program's MIXED procedure was used to analyze variables (SAS Inst. Inc., Cary, NC). The fixed effect was based on treatments, while the pen was considered as the random component. Treatment effects were tested as follows: a) ZH level inclusion b) D3 level inclusion and c) ZH x D3 interaction. To observe interaction responses, a comparison of multiple means was done using the Tukey test on some variables. For the comparisons of treatment means (all figures), the following abbreviations were used: zilpaterol (ZIL), vitamin D3 (VIT), and zilpaterol plus vitamin D3 (ZIL+VIT). The analyses were considered significant if the p-value was ≤0.05 and showed a tendency if the probability value was p≤0.1.

3. Results

3.1. Growth Performance

The main effects of ZH and D3 on the growth performance variables in lambs are observed in Table 2. Supplementation with ZH did not alter the average FLW and DMI during the entire trial, or DMI during the last 7 d of the trial. However, ZH reduced DMI as a percentage of the average LW by 6.5% (p=0.04). Likewise, ADG, total weight gain, and feed efficiency experienced an improvement of (P=0.05) 9.9, 9.8, and 17.8%, respectively. When analyzing growth performance components considering the FLW adjusted-carcass, lambs treated with ZH showed significant improvement (p<0.01) in FLW, ADG, and feed efficiency (4.3, 28.2 and 36.8 %, respectively).

Vitamin D_3 supplementation did not modify DMI overall. However, during the last 7 d of the experiment, DMI was reduced by 15.7% (P=0.02) due to D3 supplementation. Additionally, the use of D3 resulted (p=0.04) in a 2.7% reduction of FLW, ADG, and total LW gain were also negatively affected by 18.1% and 18.0% respectively (p<0.01). As a result, the combination of lower LW gains and DMI reduction led to a decrease (p<0.01) in feed efficiency by 25.9%. While D3 supplementation did not impact FLW carcass-adjusted, it did negatively affect ADG and feed efficiency carcass-adjusted by 17.0% and 25.5%, respectively (p<0.01).

Table 2. Main effects of zilpaterol and vitamin D_3 on growth performance on feedlot lambs.

	Treatments[a]							
	ZH		**D3**			**p-value**		
Variables	**0**	**0.20**	**0**	**1.5**	**EEM**	**ZH**	**D3**	**ZIL*VIT**
Days on trial								
Replicas	4	4	4	4				
Live weight								

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Initial	37.32	37.32	37.20	37.44	0.261	1.00	0.53	0.81
Final	44.39	45.09	45.36	44.12	0.369	0.21	0.04	0.12
DMI								
Daily, g	1240	1180	1180	1230	0.027	0.13	0.24	0.57
As % of LW	3.06	2.86	2.88	3.04	0.05	0.04	0.08	0.28
Last 7 d, g/d	1330	1260	1400	1180	0.05	0.41	0.02	0.62
Weight gain								
Daily, g	243	267	281	230	0.007	0.05	<0.01	0.01
Total, kg	7.07	7.76	8.15	6.68	0.222	0.05	<0.01	0.01
Feed efficiency								
DMI/Gain	5.14	4.54	4.28	5.39	0.166	0.03	<0.01	0.03
Gain/DMI	197	232	242	187	0.007	0.01	<0.01	0.01
Adjusted Carcass[b]								
FLW	43.90	45.78	45.43	44.25	0.409	0.01	0.07	0.07
ADG, g	227	291	283	235	0.009	<0.01	<0.01	<0.01
DMI/ADG	5.76	4.24	4.43	5.56	0.210	<0.01	<0.01	<0.01
ADG/DMI	182	249	242	189	0.008	<0.01	<0.01	<0.01

[a] ZH, Zilpaterol hydrochloride, (0 and 0.20 mg/kg LW^{-1}) 26 d of supplementation plus 3 d of withdrawal; D3, Vitamin D_3, (0 and 1.5 x 10^6 IU/lamb/d^{-1}) 7 d of supplementation plus 1 d of withdrawal. [b] Growth performance components calculated from FLW-Adjusted carcass.

The ZIL treatment was shown to have significant interaction with VIT in growth performance variables ($p \leq 0.03$), such as ADG, total LW gain, and feed efficiency. Likewise, in those variables calculated from FLW carcass-adjusted, showed an interaction between ZH and D3 ($P<0.01$). The effects of the treatments on ADG and feed efficiency are shown in Figures 1 and 2. ZIL supplementation improved ADG by 22.8% ($p<0.01$) compared to the control group. However, lambs treated with ZIL+VIT showed similar performance to the control group. Similarly, feed efficiency was enhanced by 33.1% ($p<0.01$) with ZIL supplementation, but this positive effect was nullified when supplemented ZIL+VIT.

Figure 1. Effect of zilpaterol and vitamin D_3 on ADG of feedlot lambs

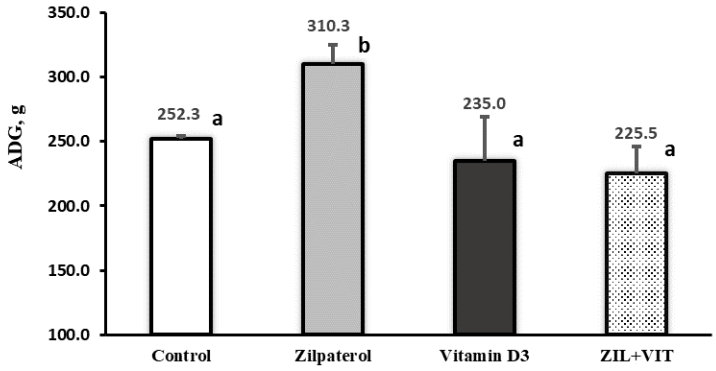
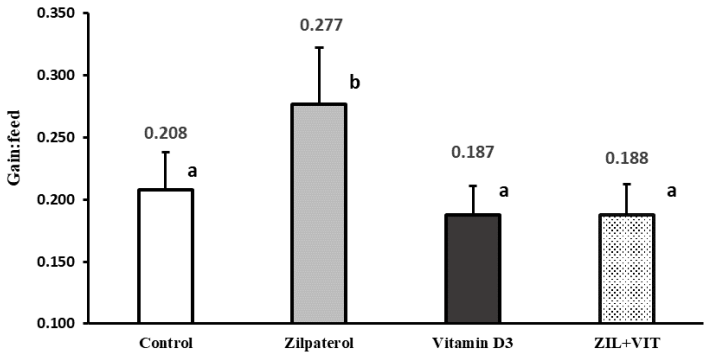


Figure 2. Effect of zilpaterol and vitamin D_3 on feed efficiency on feedlot lambs



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3.2. Dietary Energetics

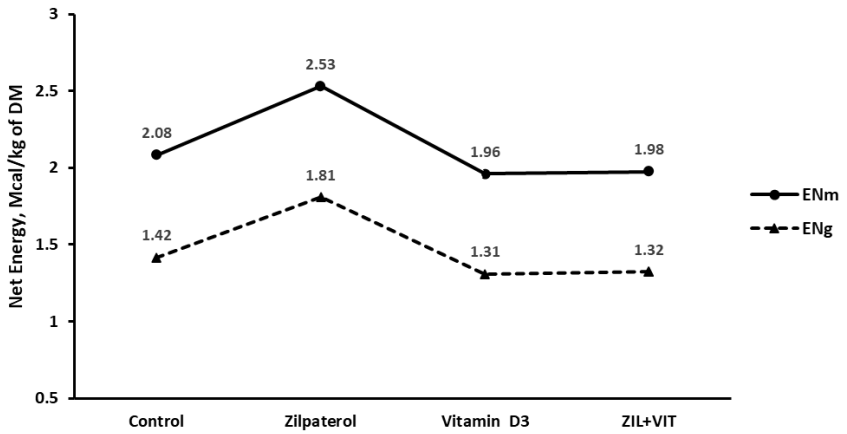
Table 3 outlines how to calculate dietary energetics. During the trial, dietary energetics improved with ZH supplementation, as determined by LW gain and DMI. ZH significantly improved dietary NEm and NEg by 11.3% and 14.7%, respectively ($p<0.01$). These improvements are reflected in the observed/expected ratio for DMI, NEm, and NEg, which increased by 11.3%, 11.4%, and 14.7%, respectively ($p\leq0.01$). The inclusion of D3 in the diet of lambs for 7 d prior to slaughter had an adverse effect on the calculation of dietary energy derived from LW gain and DMI during the experiment. This resulted in a significant reduction ($p<0.01$) of NEm and NEg by 14.7% and 18.6%, respectively. Additionally, there was a decrease ($p<0.01$) of 20.0% in observed:expected DMI ratio efficiency. All calculations used to estimate dietary energy from LW gain and DMI showed (Figure 3) a negative interaction response ($p\leq0.02$). Despite the positive response in growth performance components, the addition of ZIL+VIT to the diet did not improve energy efficiency.

Table 3. Main effects of zilpaterol and vitamin D_3 on dietary energetic of feedlot lambs.

	Treatments[a]							
	ZH		D3			p-value		
Variables	**0**	**0.20**	**0**	**1.5**	**EEM**	**ZH**	**D3**	**ZIL*VIT**
Net Energy								
NEm Mcal/Kg/DM	2.02	2.25	2.30	1.96	0.047	<0.01	<0.01	0.01
NEg Mcal/Kg/DM	1.36	1.56	1.61	1.31	0.042	<0.01	<0.01	0.01
Obs/exp DMI[b]	1.06	0.94	0.90	1.08	0.025	0.01	<0.01	0.02
Obs/exp NE[c]								
Maintenance	0.96	1.07	1.09	0.93	0.022	<0.01	<0.01	0.01
Gain	0.95	1.09	1.12	0.92	0.029	<0.01	<0.01	0.01

[a] ZH, Zilpaterol hydrochloride, (0 and 0.20 mg/kg LW^{-1}) 26 d of supplementation plus 3 d of withdrawal; D3, Vitamin D_3, (0 and 1.5 x 10^6 IU/lamb/d^{-1}) 7 d of supplementation plus 1 d of withdrawal. [b] Observed/expected ratio of DMI. [c] Observed/expected ratio of Net energy.

Figure 3. Effect of treatments on dietary energetics of feedlot lambs



3.3. Carcass Traits and Primary Cuts

The results of the treatments on carcass traits are shown in Table 4. ZH administration for 26 d followed by a 3-d withdrawal period resulted in significant improvements ($p\leq0.03$) in HCW, dressing

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percentage, and LM area by 4.3%, 2.6%, and 6.7%, respectively. However, adipose tissue characteristics such as fat thickness and perirenal-pelvis fat remained unchanged. On the other hand, D3 did not affect dressing carcass percentage, LM area, fat thickness, or perirenal-pelvis fat percentage. However, there was a tendency (p=0.07) for D3 to negatively impact HCW by 2.54%. Additionally, there was a trend (p=0.07) of interaction between ZIL+VIT resulting in negative effects on HCW (Figure 4). The primary meat cuts components (Table 5) were not impacted by either ZH (p>0.35) or D3 (p>0.27) treatments. Furthermore, no interactions between the treatments were identified (p>0.13).

Table 4. Main effects of zilpaterol and vitamin D_3 on carcass traits of feedlot lambs.

	Treatments[a]							
	ZH		D3			p-value		
Variables	**0**	**0.20**	**0**	**1.5**	**EEM**	**ZH**	**D3**	**ZIL*VIT**
HCW, kg	24.40	25.45	25.25	24.60	0.227	0.01	0.07	0.07
Dressing, %	54.85	56.31	55.53	55.62	0.248	<0.01	0.80	0.39
LM area, cm^2	13.56	14.47	14.20	13.83	0.254	0.03	0.32	0.95
Fat thickness, mm	2.60	2.44	2.46	2.58	0.084	0.21	0.32	0.75
Perirenal-pelvis fat, %	2.87	2.66	2.86	2.67	0.176	0.43	0.46	0.78

[a] ZH, Zilpaterol hydrochloride, (0 and 0.20 mg/kg LW^{-1}) 26 d of supplementation plus 3 d of withdrawal; D3, Vitamin D_3, (0 and 1.5 x 10^6 IU/lamb/d^{-1}) 7 d of supplementation plus 1 d of withdrawal.

Figure 4. Effect of treatments on hot carcass weight of feedlot lambs

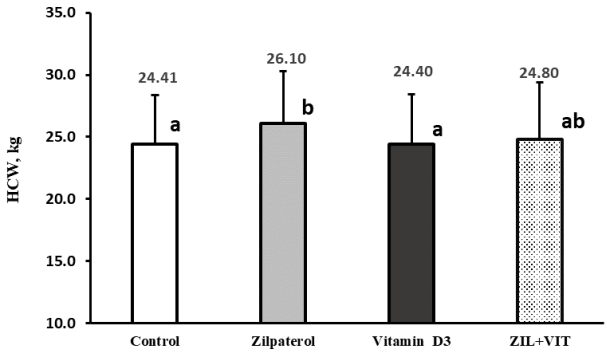

Table 5. Main effects of zilpaterol and vitamin D_3 on primary cuts of feedlot lambs.

	Treatments[a]							
	ZH		D3			p-value		
Primary cuts[b]	**0**	**0.20**	**0**	**1.5**	**EEM**	**ZH**	**D3**	**ZIL*VIT**
Neck[c]	5.14	4.97	4.99	5.12	0.147	0.49	0.57	0.13
Foreshank-208D	17.7	17.6	17.7	17.5	0.160	0.80	0.42	0.67
Rib-209A	7.96	8.15	8.03	8.08	0.121	0.49	0.87	0.81
Rack-204	9.22	9.46	9.39	9.29	0.091	0.27	0.62	0.92
Breast-209	4.12	3.99	4.16	3.94	0.175	0.75	0.59	0.89
Shoulder-207	8.96	9.27	9.02	9.22	0.252	0.60	0.74	0.90
Flank-232E	6.15	6.24	6.30	6.08	0.094	0.65	0.27	0.40

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Loin-232A	9.47	9.5	9.45	9.53	0.115	0.91	0.80	0.98
Leg-233A	31.2	30.7	30.8	31.1	0.293	0.35	0.47	0.33

[a] ZH, Zilpaterol hydrochloride, (0 and 0.20 mg/kg LW^{-1}) 26 d of supplementation plus 3 d of withdrawal; D3, Vitamin D_3, (0 and 1.5 x 10^6 IU/lamb/d^{-1}) 7 d of supplementation plus 1 d of withdrawal. [b] NAMP, North American Meat Processors Association number item. [C] Not included in NAMP's list as a primary cut.

3.4. Meat Quality and Fatty Acid Profile

The main effects of the treatments on meat quality characteristics are shown in Table 6. ZH supplementation resulted in significant changes in meat quality, causing a 7.0% reduction (p=0.02) in lightness (L*) compared to untreated lambs. Conversely, ZH increased (p<0.01) the meat pH, WBSF, and drip loss by 2.5%, 21.5%, and 23.0%, respectively. ZH did not impact the intensity of redness (a*) or yellowness (b*) color. The addition of D3 supplementation improved meat color characteristics, increasing (p≤0.02) lightness (L*), redness (a*), and yellowness (b*) intensity by 8.0%, 6.7%, and 17.0%, respectively. Additionally, D3 improved (P=0.03) the decrease in meat pH by 1.7% compared to the untreated lambs but did not affect WBSF values.

Table 6. Main effects of zilpaterol and vitamin D_3 in meat quality characteristics of feedlot lambs.

	Treatments[a]							
	ZH		**D3**			**p-value**		
Variables	**0**	**0.20**	**0**	**1.5**	**EEM**	**ZH**	**D3**	**ZIL*VIT**
Color[b]								
L*	33.33	30.99	30.92	33.41	0.683	0.02	0.01	0.54
a*	16.37	15.80	15.56	16.61	0.540	0.16	0.01	<0.01
b*	14.08	13.17	12.55	14.69	0.644	0.32	0.02	0.20
pH	5.59	5.73	5.71	5.61	0.032	<0.01	0.03	<0.01
WBSF[c], kg	2.39	2.94	2.62	2.71	0.090	<0.01	0.43	0.09
Water drip loss, %	12.77	15.82	13.03	15.56	0.540	<0.01	<0.01	0.91

[a] ZH, Zilpaterol hydrochloride, (0 and 0.20 mg/kg LW^{-1}) 26 d of supplementation plus 3 d of withdrawal; D3, Vitamin D_3, (0 and 1.5 x 10^6 IU/lamb/d^{-1}) 7 d of supplementation plus 1 d of withdrawal. [b] L*, Lightness; a*, Redness; b*, Yellowness. [c] WBSF, Warner Bratzler Shear Force.

In Figures 5 and 6, the interaction (P<0.01) in the variables of redness intensity (a*) and meat pH with the combined supplementation of ZIL+VIT is observed. ZIL treatment had a negative effect (p<0.01) on the intensity of redness, decreasing it by 11.5% compared to the control group. However, when combined with VIT, the redness intensity of lambs treated with ZIL+VIT improved by 11.2% compared with ZIL, although there was no significant difference compared to other treatments. Similarly, ZH maintained high meat pH values (p<0.01) concerning control, ZIL, and VIT treatments, if compare ZIL to the control group, pH was 5.3% higher. However, when supplementing ZIL+VIT the final pH of the meat decreased by 4.6% with compared to lambs treated with ZIL alone. Finally, lambs treated with ZIL+VIT had similar final pH to the control and VIT groups. Additionally, there was an interaction between ZIL and VIT in the muscle's WBSF. Contrary to expectations, the ZIL+VIT treatment led to higher WBSF values than the Control and VIT treatments, indicating increased tenderness (Figure 7).

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Figure 5. Effect of treatments on redness (a*) of *Longissimus* dorsi of feedlot lambs

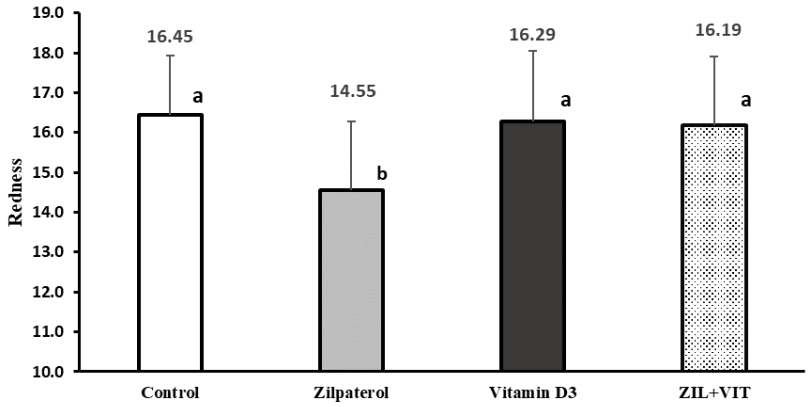


Figure 6. Effect of treatments on pH (a*) of *Longissimus* dorsi of feedlot lambs

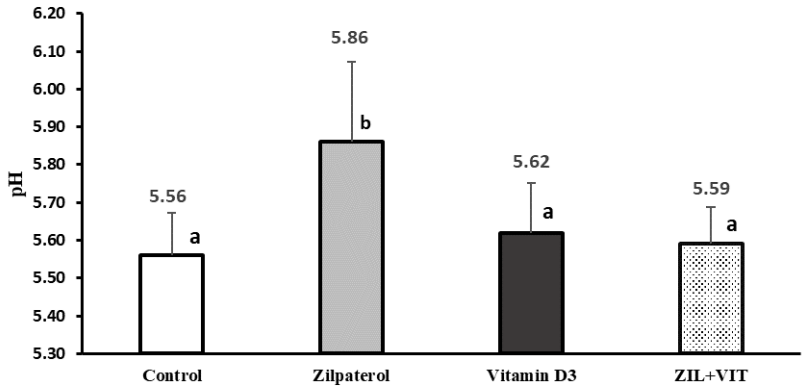
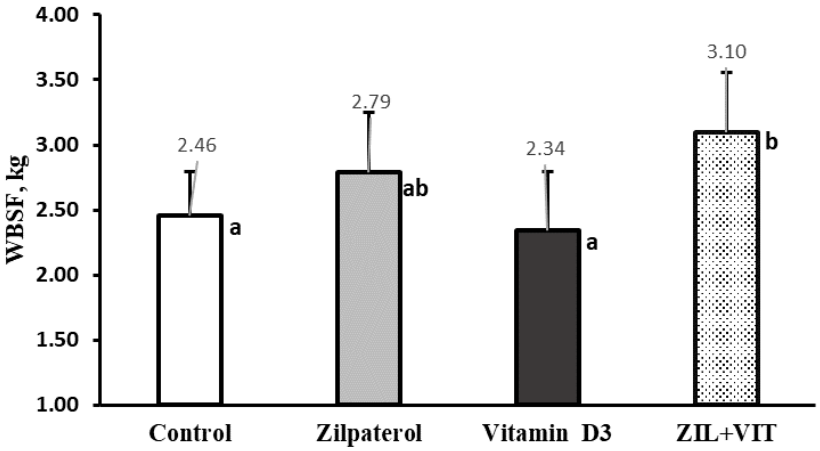


Figure 7. Effect of treatments on WBSF of Longissimus muscle.



The main effects of the treatments on the fatty acid profile of the intramuscular fat of LM are shown in Table 7. Lambs treated with ZH showed a 19.4% reduction in the percentage of palmitoleic fatty acid in intramuscular fat compared to untreated lambs (p=0.10). However, no significant changes were observed in the rest of the analyzed fatty acids due to treatment with ZH. On the other hand, supplementation with D3 resulted in a reduction (p=0.05) of 51.8% in the percentage of linolenic

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fatty acid in the samples analyzed. Additionally, there was a trend (p=0.10) towards a 5.7% reduction in oleic fatty acid in the intramuscular fat of lambs treated with D3 compared to untreated ones.

Table 7. Main effects of zilpaterol and vitamin D_3 on fatty acids profile of meat of feedlot lambs.

	Treatments[a]							
	ZH		D3			p-value		
Fatty acid	**0**	**0.20**	**0**	**1.5**	**EEM**	**ZH**	**D3**	**ZIL*VIT**
C14:0 Myristic	4.13	3.03	3.59	3.56	0.511	0.15	0.97	0.55
C14:1 Myristoleic	0.30	0.20	0.25	0.25	0.056	0.22	0.93	0.30
C16:0 Palmitic	31.04	29.70	29.42	31.32	0.779	0.24	0.11	0.29
C16:1 Palmitoleic	2.31	1.86	2.09	2.08	0.180	0.10	0.96	0.45
C18:0 Stearic	11.41		11.96	12.04	0.567	0.16	0.92	0.44
C18:1 Oleic	46.29	47.88	48.49	45.68	1.120	0.33	0.10	0.03
C18:2 Linoleic	4.12	4.48	3.90	4.70	0.710	0.72	0.44	0.30
C18:3 Linolenic	0.22	0.18	0.27	0.13	0.044	0.46	0.05	0.96
C20:0 Arachidonic	0.14	0.04	0.01	0.19	0.108	0.50	0.23	0.50
Saturated	46.58	45.34	44.98	46.94	0.901	0.34	0.15	0.10
Unsaturated	53.41	54.65	55.01	53.05	0.901	0.34	0.15	0.10
Unsat/Satur	1.15	1.21	1.23	1.13	0.041	0.39	0.14	0.09

[a] ZH, Zilpaterol hydrochloride, (0 and 0.20 mg/kg LW^{-1}) 26 d of supplementation plus 3 d of withdrawal; D3, Vitamin D_3, (0 and 1.5 x 10^6 IU/lamb/d^{-1}) 7 d of supplementation plus 1 d of withdrawal.

An interaction response (p=0.03) between ZIL and VIT was observed in the percentage of oleic fatty acid of intramuscular fat. Figure 7 shows that treatment with VIT reduced the percentage of oleic fatty acid in intramuscular fat by 13.1% compared to the control group. However, this effect did not occur when supplemented with ZIL. Furthermore, an interaction trend (p=0.10) was observed with VIT supplementation, resulting in a reduction of unsaturated fatty acids and an increase in saturated ones (Figure 8). These effects were not observed in the samples analyzed from lambs supplemented with ZIL+VIT.

Fig. 7. Effect of treatments on oleic fatty acid from *Longissimus* of feedlot lambs

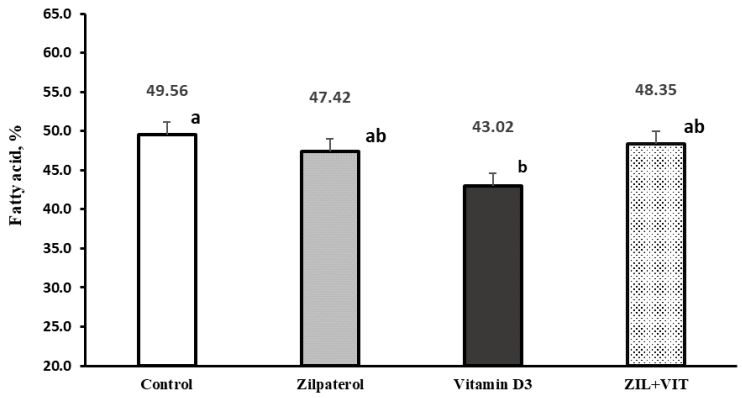

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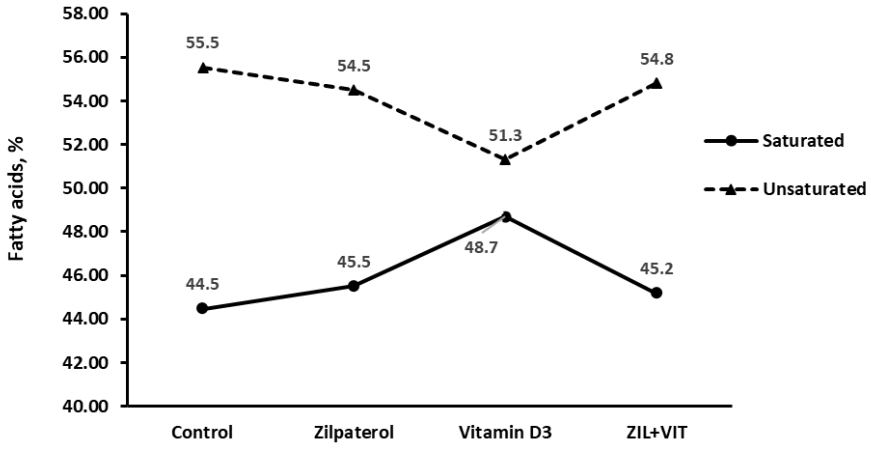
Figure 8. Effects of treatments on fatty acids of *Longissimus* of feedlot lambs



4. Discussion

4.1. Growth Performance, Energetics, and Carcass Traits

According to [27], the primary impact of ZH on ruminant production is linked to the enhancement of both LW and carcass yield. This is attributed to an increase in muscle protein synthesis and retention, which occurs concurrently with the suppression of lipogenesis and an increase in lipolysis. ZH activates a biological mechanism that improves growth performance indicators and energy retention due to higher muscle tissue concentration compared to adipose tissue. This is confirmed by calculations to estimate the energy intake of the diet [24,25], which indicates an apparent increase in the NEm, NEg, and DMI observed/expected ratio. Multiple studies have demonstrated the effectiveness of ZH in lamb production. For instance, research conducted with ZH in lambs with LW, doses, and results similar to those of the present study has shown improvements in growth indicators [28–31]. These studies have reported an increase in ADG, feed efficiency, and energy retention provided by the diet (12.5-25.0%, 13.0-36.0%, and 9.5-35.0%, respectively). Similarly, the results in lambs on carcass quality traits from some authors [29,32,33] coincide with those presented in our study. These studies indicate that the effects of ZH supplementation are observed in the carcass quality, such as an increase in the HCW (7.0-11.2%), dressing carcass (2.0-8.8%), LM area (11.2-15.4%), and the reduction of perirenal-pelvic fat (21.7-26.1%).

Vitamin D_3 acts as a precursor of 1,25-dihydroxycholecalciferol, a hormone involved in calcium and phosphorus homeostasis [34]. Previous studies have shown that feeding supra-nutritional doses of D3 in ruminants can cause hypercalcemia, reduced DMI, and body weight [18]. In a study by [18], steers were supplemented with $5x10^6$ IU of D3 for 10 d during the ZH supplementation stage (21 d), resulting in a significant decrease in ADG, DMI, and feed efficiency by 47.6%, 11.5%, and 52.0% respectively. Similarly, [12] observed reductions in ADG and feed efficiency of up to 25.1% and 23.0%, respectively, when testing the supplementation of $5x10^6$ IU of D3 in steers for 24 d. Our findings align with these results, where administering D3 led to an 18.1% reduction in ADG and a 25.9% reduction in feed efficiency over a period of 29 d, although the overall DMI was not affected. However, DMI during the last 7 d was reduced by 15.7%.

Studies with supra-nutritional doses of D3 supplementation were carried out in short periods before slaughter. This approach should ensure that the carcass quality is not compromised by DMI reduction due to D3 supplementation. However, some studies suggest that there may be a slight impact on the quality of the carcass. Studies have shown that supplementing D3 in steers and lambs before slaughter can result in a significant reduction in weight and fat thickness. [18] found that supplementing $5x10^6$ IU of D3 for 10 d led to a reduction in HCW and back fat thickness by 3.8% and 16.3%, respectively. [35] reported that supplementing 6 million IU of D3 for 4-6 d prior to slaughter resulted in a reduction in FLW by 4.7%, HCW by 5.3%, and back fat thickness by 19.1%. Similarly,

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[36] observed a 19.0% reduction in adjusted fat thickness in lambs when supplementing 7.5x10^6 IU of D3 for 4 d before slaughter, without any changes in dressing carcass and LM area.

On the contrary, a study conducted by[12] observed a 20.2% increase in back fat thickness in steer carcasses when feeding 5x10^6 IU of D3, without affecting HCW and dressing carcass. However, providing high doses of D3 may lead to a reduction in DMI, which can reduce the supply of nutrients for body maintenance and result in catabolism of fat tissue. This can ultimately lead to a reduction in the USDA yield grade. Nonetheless, other studies[12] did not show this pattern of results when providing D3. Although the negative effect of D3 on DMI, ADG, and feed efficiency is an expected response, ZH supplementation could compensate for this effect. The negative interaction is the result of the inability of ZH to compensate for the reduction in DMI. It seems that ZH cannot have a favorable response in growth performance when the energy available in the diet is reduced due to low DMI.

4.2. Meat Quality

Meat tenderness is a crucial quality trait that affects consumer acceptability, satisfaction, and repeat purchase [10]. The muscle hypertrophy produced by ZH supplementation consequently increases the toughness of the meat. Various factors can contribute to an increase in WBSF values due to ZH supplementation. One reason for this is the alteration of the myofibrillar enzyme system's activity, which may occur due to decreased calpain concentrations and increased calpastatin activity [6,14,37]. Research has also shown that an increase in muscle fiber diameter and hypertrophy [8] and a decrease in intramuscular fat levels [9] can raise WBSF values. The calpain-calpastatin proteolytic system has been identified as the key factor that determines meat tenderness.

However, some authors suggest that aging can help mitigate the negative impact of meat toughness [38]. The effectiveness of the aging strategy in improving the WBSF of muscles treated with ZH is not consistent. ZH has been shown to increase WBSF in cattle, which has been widely documented [4]. Supplementing with ZH for 20 d resulted in tougher meat (7.0-21.4%) in the LM on different periods of aging. Other studies [39,40] have reported similar increases in WBSF (8.8-29.8%) in the LM with ZH supplementation ranging from 20 to 40 d. The impact of ZH on muscle WBSF in lambs is variable. According to studies by [29,30], supplementing with ZH for 30 d can increase the toughness of the LM by 20.0 to 56.4%, which is consistent with our research showing a 21.5% increase in WBSF. However, other reports by [29,41] have not found any differences in WBSF between lambs treated with ZH and the control group. The final pH of the meat is a crucial factor in determining its quality, and lambs supplemented with ZH have been observed to produce meat with an elevated pH of 3.2 to 8.9%[42]. Our experiment's results align with this result. The findings on the color of lamb meat, when supplemented with ZH, are inconsistent. According to research[29,43] there were no discernible differences in the luminosity (L*), redness (a*), and yellowness (b*) color characteristics of the LM after administering ZH for 28-30 d. However, other studies have reported a decrease in luminosity (L*) and redness (a*) by up to 13.8% and 24.2%, respectively. In the current experiment, ZH only resulted in a 7.0% decrease in luminosity, without any impact on the redness color of the meat.

A higher concentration of calcium in muscle is decisive for the function of this system, which is key to determining the postmortem quality characteristic of meat tenderness [16]. The proteolytic system of the calpain-calpastatin muscle system is a Calcium-dependent enzymatic system. Therefore, it increases the activity of calpain enzymes that degrade myofibrillar proteins [16] and promote meat tenderness [17,18,19]. Vitamin D_3 supplemented before slaughter increases the concentration of intramuscular calcium [12–14], therefore, it could increase the activity of the calpain enzyme system and promote meat tenderness [11]. Studies have shown that D3 supplementation can indeed increase meat tenderness [12–14]. In this regard, studies on cattle, with D3 doses ranging from 1-7 million IU have been shown to reduce WBSF of the LM [14,35]. They used supra-nutritional doses of D3 in the diet for a period ranging from 3 to 7 d before slaughter. Other authors [4,18] have reported that supplementing with 0.5-5 x10^6 IU/d of D3 between 10 and 20 d before slaughter did not affect the WBSF values of meat, even with 14 to 35 d of aging. However,[18] found that treatment of 0.25 x

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10^6 IU/d with D3 for 165 d before slaughter tended to produce tougher meat at 21 d of aging. The findings align with those reported by [36] in their study on lambs supplemented with 750,000 IU/d of D3 for 4 d, which resulted in meat with 11.4% toughness. However, in the current experiment, there was no significant difference observed in the WBSF of muscle samples collected from animals supplemented with D3 for 7 d before slaughter.

Different studies have implemented D3 to mitigate the negative impact of meat toughness caused by ZH supplementation. Authors [14,18] have reported slight improvements in beef tenderness in cattle that were supplemented with ZH and given D3 at doses of 1 to 7 million IU per d for 3 to 6 d. Other studies [40] in steers supplemented with 1-9 x10^6 IU between 3-9 d before slaughter did not reduce meat toughness produced by ZH supplementation. ZH supplementation resulted in tougher meat in 7.0-21.4% of the LM on different aging periods, and the supplementation of 0.5 x 10^6 IU D3 did not reduce the negative effect of zilpaterol [4] In our experiment, supplementing with 1.5 x 10^6 IU of D3 did not result in reduced WBSF values of the LM of feedlot lambs with ZH supplementation, which is similar to the outcome mentioned earlier.

4.3. Fatty Acids

β-agonists have been studied for their effect on the fatty acid profile of ruminants. In a study by [41] lambs treated with ZH showed an increase in polyunsaturated fatty acids in intramuscular fat. In their study on lambs, [44] found that administering Salbutamol, a β-agonist, led to a decrease in saturated fatty acids and an increase in total unsaturated fatty acids. Nonetheless, the research conducted by [42] revealed that the addition of ZH to the diet of lambs did not result in any alteration to the fatty acid composition of the intramuscular fat. This aligns with the findings of our current study, which similarly demonstrated that supplementing lambs with ZH did not impact the overall levels of saturated or unsaturated fatty acids present in intramuscular fat.

Research with D3 in ruminants has focused on calcium metabolism and its effect on meat tenderness. Thus, research on the use of D3 in muscle fatty acid metabolism is scarce. In this sense, the fatty acid profile of meat is better related to vitamin E supplementation because of the oxidative stability of fats [11]. However, in the present experiment, it is observed (Figure 7) that D3 supplementation results in a reduction of oleic fatty acid. Likewise, an interaction was observed, and a change in the metabolism of PUFAs and UFAs in intramuscular fat was noted when the lambs were supplemented with ZIL+VIT. The reason for this interaction is not clear. Probably, the negative effect of DMI by D3 produces this response, which may be altered by the lipid metabolism of ZH in muscle.

5. Conclusions

Supplementation with ZH for 26 d improved growth performance, energy efficiency, and carcass characteristics. However, it negatively affected meat quality by reducing brightness, increasing pH and WBSF. Supplementing D3 for 7 d before slaughter had a negative impact on growth performance and energy efficiency. This was due to a reduction in DMI during the last 7 d of the test. Additionally, D3 seemed to reduce HCW, although no differences in dressing carcass and LM area were noted. Administering D3 at a dose of 1.5 x 10^6 IU/$^{-1}$ for 7 d prior to slaughter in lambs treated with ZH improved meat quality by increasing redness and reducing muscle pH compared to lambs treated with ZH alone. However, D3 did not improve the increase in meat toughness produced by ZH.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The information published in this study is available on request from the corresponding author.

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