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ORIGINAL ARTICLE

Dietary supplementation with a zilpaterol and natural additive blend on cut yield and meat quality of fattening hair lambs under conditions of high ambient temperature

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ABSTRACT. Forty-eight Pelibuey × Katahdin male lambs (90 ± 18 d age; 25.12 ± 3.79 kg LW) were fed for 70 d under conditions of high ambient heat load (average Temperature Humidity Index (THI) = 80.6 ± 3.2) to test feed additive strategy supplementation in a high-energy diet, as follows: 1) no feed additives (control), 2) a blend of essential oils (EO) plus vitamin D3 offered throughout 70 d of fattening (EOD3), 3) zilpaterol hydrochloride (ZH) offered during the final 35 d of fattening, and 4) a combination of EOD3 with ZH (EOD3+ZH). The experimental design used was a randomized complete-block design in a 2 × 2 factorial arrangement of treatments (ZH and EOD3 factors). The ambient temperature and relative humidity during the experiment were 29.2 ± 3.4 °C and 73.1 ± 14.1 % (THI = 80.6 ± 3.2), respectively. These ambient conditions represent a high ambient heat load that can compromise the productivity and quality of the final product. There were no interaction effects ($P > 0.38$) on the shoulder muscle tissue composition and whole cuts. Except for a reduction in drip loss (interaction, $P = 0.05$) and an increase in the intensity of yellow color (b^*), which was increased (interaction, $P < 0.01$), the combination of EOD3+ZH did not affect the rest of the meat quality variables. EOD3 supplementation alone had no effects on meat characteristics but decreased the water-holding capacity by 2.9% ($P < 0.01$) and increased drip loss by 2.14% vs. 1.65% ($P = 0.04$) at 24 h. Lambs supplemented with ZH alone showed an increase ($P < 0.01$) in lean tissue (4.6%), whole cut of leg percentage (3.6%), and a^* color value (17%) ($P = 0.02$), but reduced thawing by 50% ($P < 0.03$). It was concluded that the EOD3 blend supplemented alone or combined with ZH to fatten lambs under high ambient heat load conditions had no effects on tissue composition and whole cuts and had a small effect on meat quality improvements. ZH supplementation affected fat deposition and meat yield without affecting the meat shear force.

Keywords: essential oils, vitamin D3, sheep, meat characteristics, β-agonist, heat stress.

INTRODUCTION

Worldwide, a significant proportion of feedlot lambs are finished in semi-arid, tropical, and subtropical climates (Morris, 2017). For most of the year, these regions experience high ambient heat loads (HAHL; Temperature Humidity Index (THI) > 79). Although Pelibuey lambs are more tolerant to HAHL than cattle, their productivity efficiency can be compromised when fattened under these conditions (Vicente-Pérez *et al*., 2020). Metabolic adjustments for body heat dissipation negatively impact the efficiency of energy utilization for weight gain; however, the mechanisms for protein accretion and glycogen reserves are also affected (NRC, 2007; Al-Dawood, 2017). Furthermore, HAHL promotes an increase in cellular oxidative stress, which is one of the most important factors favoring negative effects on meat quality (Chen *et al*., 2022). Therefore, meat yield and quality can be affected in fattening cattle exposed to high ambient temperatures (Alam *et al*., 2024). Therefore, several strategies, including the use of feed additives, have been tested to mitigate the negative effects of HAHL on productivity and final product quality. Zilpaterol hydrochloride (ZH, a β-agonist receptor) is extensively used as a feed additive to improve feedlot performance by increasing muscle accretion and energy efficiency during the late finishing phase (Ortiz *et al*., 2016). Its benefits occur even in animals fattened under HAHL conditions (Barnes *et al*., 2019). Thus, the use of ZH is a strategy to mitigate the negative effects of HAHL on lamb fattening (Estrada-Angulo *et al*., 2024). Although some studies have reported no changes in meat quality with ZH supplementation (López-Baca *et al*., 2019), most studies have attributed negative effects of ZH on meat quality. In this sense, ZH supplementation can affect lamb meat quality, mainly by reducing its lightness and tenderness (Partida *et al*., 2015; Leyva-Medina *et al*., 2024). Therefore, the negative effects of HAHL on meat quality could be exacerbated when lambs are supplemented with ZH under high ambient heat load conditions. In contrast, the use of certain natural feed supplements can improve the meat quality. In this regard, essential oil (EO) (such as thymol, eugenol, vanillin, guaiac, and limonene) supplementation reduces cellular oxidative stress and improves meat quality (He *et al*., 2023). Similarly, vitamin D3 supra-supplementation in the finishing phase has been reported to increase meat tenderness according to some studies (Swanek *et al*., 1999; Montgomery *et al*., 2002). To our knowledge, there is no scientific information available on the possible interaction of ZH and natural supplements on the meat quality of lambs finished under high ambient heat load conditions. Therefore, it can be expected that the blending of a mixture of essential oils with vitamin D3 (EOD3) can be a strategy to improve some parameters of meat quality of lambs fattened under HAHL and supplemented with ZH. Therefore, the objective of this experiment was to determine whether the EOD3 blend can counteract the negative impacts of ZH on muscle tissue, whole cuts, and meat quality of hairy lambs fattening under HAHL conditions. We hypothesized that supplementation with a combination of EOD3 and ZH could have additive effects on improving meat yield and quality in lambs finished under high ambient heat load conditions.

MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee of the Universidad Autónoma de Sinaloa (protocol no.04292023).

The meat samples used in this study were obtained from slaughtered lambs from a feeding trial conducted at the Universidad Autónoma de Sinaloa Feedlot Lamb Research Unit in Culiacán, Mexico. The results of feedlot performance and carcass characteristics have been published previously (Estrada-Angulo *et al*., 2024). Therefore, ethical practices, lamb management and feeding, as well as slaughter process and carcass management, were exhaustively described by Estrada-Angulo *et al*. (2024).

Forty-eight Pelibuey × Katahdin (¼ Pelibuey and ¾ Katahdin; initial shrunk weight = 25.12 ± 3.8 kg, age = 3 ± 0.6 months) crossbred intact male lambs were fed for 70 d to evaluate the treatment effects. Lambs were placed in individual pens (12 lambs per treatment and 2 lambs per pen). The description of diets, method of additive inclusion in diets, and management of experimental units prior to and during the experiment were previously described by Estrada-Angulo *et al*. (2024).

Dietary treatments consisted of a corn-based finishing diet (final diet composition=14.2% crude protein, 15.4% neutral detergent fiber, and 2.10 Mcal of net energy for maintenance/kg) supplemented with additives as follows: 1) no feed additives (Control); 2) 150 mg of essential oil blend plus 0.10 mg of 25-hydroxy-Vit-D3/kg diet offered throughout the 70-d experimental period (EOD3); 3) control diet fed during the first 35 d and zilpaterol hydrochloride (ZH) supplementation at 6 mg/kg diet offered during the final 35 d of the experiment (32 d with ZH with a withdrawal 3 d before harvest); and 4) supplementation of both strategies: treatment EOD3 for 70 d (entire experimental period) and treatment ZH for the last 35 d of the experimental period with its 3 d of withdrawal prior to harvest (EOD3+ZH).

The source of ZH was Zilmax® (MSD Salud Animal México, Santiago Tianguistenco, Mexico). The sources of the blended oils (EO) and 25-hydroxy-Vit-D3 (D3) used were CRINA-Ruminant® and Hy-D® (DSM Nutritional Products, Basel, Switzerland). CRINA-Ruminant® contains a standardized mixture of essential oils, including thymol, eugenol, vanillin, guaiac, and limonene. The dosage level of supplemental EO was based on prior studies, in which positive responses in the growth performance of finishing lambs were observed at 150 mg EO/kg diet (Artega-Wences *et al*., 2021; Estrada-Angulo *et al*., 2021). The dosage level of D3 was based on positive responses in growth performance and carcass in lambs (Escobedo-Gallegos *et al*., 2023) and feedlot cattle (Acedo *et al*., 2019; Mendoza-Cortez *et al*., 2022), where levels of supplementation ranged between 0.002 and 0.006 mg D3/kg live weight (LW). The source of ZH was Zilmax® (MSD Salud Animal México, Santiago Tianguistenco, México). Once the feeding trial was finished, all lambs were slaughtered on the same day, following the Mexican federal guidelines for animal use and care (NOM-051-ZOO-1995, NOM-033-ZOO-1995).

After sacrifice, the lambs were skinned, and the gastrointestinal organs were separated from the carcass. The carcasses were then chilled in a cooler at –2 to 1 °C for 24 h. After the carcasses were chilled, each was split into two halves. The left side was fabricated into wholesale cuts without trimming, according to the North American Meat Processors Association guidelines (IMPS, 2014). Neck, Rack (IMPS204), breast (IMPS209), shoulder (IMPS2010), and ribs (IMPS209_A) were obtained from the foresaddle, and loins (IMPS231), flank (IMPS232), and leg (IMPS233) were obtained from the hind saddle. The weight of each cut was subsequently recorded. The shoulder tissue composition was assessed using physical dissection, as described by Luaces *et al*. (2008).

For meat quality evaluation, *longissimus* muscle (LM) steaks (13-cm thick) from the left half of each carcass (six per treatment) were removed between the 12th and 13th rib interface, preserved immediately on dry ice, and shipped to the Meat Quality Laboratory, where they were vacuum packaged, frozen at –20°C, and stored for subsequent meat quality trait analysis. The measured variables included water holding capacity (WHC), color, drip loss at 24 and 48 h, cook loss (CL), and Warner-Bratzler shear force (WBSF). The color values L* (lightness), a* (redness), and b* (yellowness) were determined using a Minolta CR-410 spectrophotometer (Konica Minolta Camera Co., Ltd., New Jersey, USA). The steaks obtained from the rib were thawed for 24 h at 4 °C, and the thawing value was calculated using the following formula: thawing (%) = [(initial weight – final weight)/initial weight] × 100. From the thawed ribeye steaks, 10 cm thick pieces were cut and broiled on an electric grill (George Foreman Grill, Model

GR2120B), until they reached an internal temperature of 70 °C, followed the procedure described by Lopez-Carlos *et al*. (2014) and calculated as: Cooking loss (%) = [(initial weight – final weight)/initial weight] × 100. After cooking, the steaks were allowed to cool at 4°C for 24 h. To obtain the WBSF values, six cores measuring 1×1×3 cm were taken from each cooked steak parallel to the orientation of the muscle fibers. WBSF measurements (kg/cm^2) were determined using a Lloyd texturometer (Lloyd Instruments, Fareham, Hampshire, UK) equipped with Warner-Bratzler shear blades at a crosshead speed of 50 mm/min. The water-holding capacity (WHC) was determined using a modified compression technique from the method termed press-juice, in which 0.3 g of a meat sample was positioned between two layers of filter paper (Whatman No. 1, Cytiva, Wilmington, DE, USA) and two plaques of acyclic Plexiglas (15 × 15 cm), and subjected to a consistent pressure of 10 kg for 20 min (Tsai & Ockerman, 1981). The WHC was expressed as the percentage of weight loss compared to the initial weight. This was calculated as [(initial weight – final weight)/initial weight] × 100. Drip loss was measured using the technique described by López-Carlos *et al*. (2014).

Data were analyzed as a randomized complete-block design in a 2 × 2 factorial arrangement of treatments (ZH and EOD3 supplementation alone or combined) using the MIXED procedure of SAS (2007) software (v. 9.3), considering the initial weight as the blocking criterion and pen as the experimental unit, with treatment and block as fixed effects and the experimental unit (pen) within the treatment as a random effect. The statistical model for the trial was as follows:

$$Yijk=\mu+Wi+\alpha j+\beta k+(\alpha\beta)jk+\epsilon ijk$$

where $Yijk$ is the response variable, μ is the common experimental effect, Wi is the block effect, αj is the Factor A (ZH) effect, βk is the Factor B (EOD3) effect, $(\alpha\beta)jk$ is the interaction (ZH×EOD3), and ϵijk is the random error. The treatment effects were separated into the following orthogonal contrasts: 1) non-additive vs. EOD3; 2) non-additive vs. ZH; and 3) EOD3 × ZH interaction. For all measured variables, treatment interactions were considered when $P \leq 0.05$. Treatment means were separated using the "honestly significant difference test" (Tukey's HSD test). In all cases, contrasts were considered significant when $P \leq 0.05$.

RESULTS

It is important to note that the current report is complementary to the report by Estrada *et al*. (2024), which included growth performance, dietary energetics, visceral mass, gene expression, and carcass characteristics such as hot carcass weight (HCW), dressing percentage (DP), LM area, fat thickness, and kidney-pelvic-heart fat. Regarding carcass characteristics, the same report highlighted that the slaughter weights were 43.75, 45.06, 45.97, and 48.22 kg for the control, EOD3, ZH, and combination EOD3 + ZH, respectively. The main carcass responses reported by Estrada-Angulo *et al*. (2014) were that compared to the controls, lambs that were fed with EOD3 showed greater HCW (4%, $P < 0.01$), while ZH supplementation increased HCW (6%, $P < 0.01$), dressing percentage (1.7%, $P = 0.04$), and reduced fat thickness by 14.7% ($P = 0.03$) and kidney-pelvic-hearth fat by 16.3% ($P < 0.01$). No treatment interactions were detected by these researchers for any of the carcass measurements. The results of the present study are presented in accordance with the previous relevant clarifications.

The ambient temperature and relative humidity (RH) during the experiment are shown in Table 1. Based on temperature and RH, the minimum and maximum calculated average values of THI during the experiment were 79.62 and 81.26, respectively. The average THI (80.4 ± 3.2) was within the range (79–84) coded as "Danger" (Mader *et al*., 2006). The daily maximal THI exceeded 80 for an average of 4.7 h during 52 of the 70 days of the experiment. These ambient conditions are expected to compromise energy intake and thus lamb growth performance (Silanikov, 2000).

No interaction effects were observed on shoulder muscle tissue composition or lean-to-fat ratio ($P \geq 0.52$). Similarly, EOD3 supplementation alone did not affect muscle composition ($P \geq 0.51$). Lambs that received ZH showed an increase of 4.7% in the lean tissue composition and a tendency ($P = 0.07$) to reduce fat tissue composition by 8.1%, increasing ($P = 0.03$) the lean-to-fat ratio by 13.8% (Table 1). The effects of ZH were not modified (increased or decreased) by the combination with EOD3.

The treatment effects on whole cuts (as a percentage of cold carcass weight (CCW)) are presented in Table 2. No interaction effects were observed for whole cuts ($P \geq 0.23$). Compared to the control group, lambs that received EOD3 supplementation had very similar values in whole cuts, whereas ZH supplementation promoted a 5.0% increase in hindquarter ($P = 0.02$) as a result of the increase 3.6% in leg cut; however, the combination of EOD3+ZH did not improve the response of ZH when supplemented alone.

Except for drip loss at 24 h and the intensity of yellow color (b*), the combination of EOD3+ZH did not affect most evaluated variables (Table 3). Drip loss was significantly higher in meat of EOD3 supplemented lambs, but this effect disappeared when it was combined with ZH (interaction, $P = 0.05$). In contrast, the intensity of the yellow color (b*) notably increased when both additives were combined (interaction, $P < 0.01$). EOD3 supplementation alone had minimal effects on meat characteristics, but decreased WHC and increased drip loss at 24 h. ZH supplementation alone increased the lean tissue ($P < 0.01$) and percentage of whole cut of leg ($P < 0.01$) and reduced the thawing percentage ($P < 0.01$) and a* color value ($P = 0.02$) (Table 4).

Table 1.
Ambient temperature (T_a), relative humidity (RH), and calculated temperature-humidity index (THI)[1] were recorded every hour and expressed as a weekly average.

Week	Mean Ta (°C)	Max Ta (°C)	Min Ta (°C)	Mean RH (%)	Max RH (%)	Min RH (%)	Mean THI1	Max THI	Min THI
1	30.18 ± 3.8	30.54 ± 3.9	29.83 ± 3.6	72.73 ± 12.9	74.18 ± 12.4	70.99 ± 13.4	81.93 ± 3.9	82.74 ± 4.2	81.14 ± 3.5
2	28.42 ± 2.9	28.80 ± 3.0	28.04 ± 2.7	80.58 ± 11.3	82.18 ± 10.7	79.01 ± 11.8	80.47 ± 3.0	81.33 ± 3.4	79.67 ± 2.7
3	29.78 ± 3.1	30.10 ± 3.2	29.47 ± 3.0	75.04 ± 12.6	76.60 ± 12.1	73.58 ± 12.9	81.79 ± 3.1	82.55 ± 3.3	81.09 ± 3.0
4	30.21 ± 2.4	30.51 ± 2.6	29.88 ± 2.3	73.44 ± 9.7	74.87 ± 9.5	72.12 ± 9.7	82.33 ± 2.5	83.04 ± 2.8	81.64 ± 2.3
5	30.00 ± 3.3	30.38 ± 3.4	29.66 ± 3.2	72.23 ± 13.3	73.75 ± 13.0	70.61 ± 13.5	81.63 ± 3.3	82.47 ± 3.5	80.88 ± 3.1
6	29.20 ± 3.4	29.56 ± 3.4	28.80 ± 3.3	70.10 ± 16.0	72.02 ± 15.4	68.36 ± 16.4	79.96 ± 2.8	80.82 ± 3.1	79.11 ± 2.5
7	30.77 ± 3.8	31.18 ± 3.8	30.33 ± 3.7	68.34 ± 15.5	70.44 ± 15.2	66.38 ± 15.6	82.01 ± 3.1	83.00 ± 3.3	81.06 ± 3.0
8	27.46 ± 3.5	27.81 ± 3.6	27.12 ± 3.4	76.10 ± 15.5	77.62 ± 15.0	74.53 ± 16.0	78.13 ± 3.2	78.88 ± 3.5	77.40 ± 3.0
9	28.39 ± 3.8	28.80 ± 3.9	27.99 ± 3.8	72.84 ± 16.9	74.53 ± 16.5	71.17 ± 17.2	79.02 ± 3.4	79.89 ± 3.7	78.18 ± 3.3
10	27.29 ± 4.1	27.73 ± 4.1	26.86 ± 3.9	69.97 ± 17.0	71.70 ± 16.6	68.18 ± 17.0	76.94 ± 3.9	77.84 ± 4.1	76.08 ± 3.7
Mean	29.17 ± 3.4	29.54 ± 3.5	28.80 ± 3.3	73.14 ± 14.1	74.79 ± 13.6	71.49 ± 14.3	80.42 ± 3.2	81.26 ± 3.5	79.62 ± 3.0

[1]THI = 0.81 × ambient temperature + [(relative humidity × (ambient temperature – 14.4)] + 46.4. THI code (normal THI < 74; alert 75–79; danger 79–84; and emergency > 84).

Table 2.
Treatment effects on tissue composition of lamb meat.

	Treatment[1]				Main effects						Interaction	
					EOD3 (mg/kg DM)			ZH (mg/kg DM)			EOD3×ZH	
Shoulder composition (%)	Control	EOD3	ZH	ZH+EOD3	0	150	*P*-value	0	6.2	*P*-value	SEM	*P*-value
Lean	61.09	60.14	63.4	63.45	62.26	61.7	0.51	60.61	63.45	< 0.01	0.112	0.50
Fat	18.01	18.53	16.6	16.91	17.34	17.7	0.67	18.27	16.79	0.07	0.010	0.90
Bone	19.80	20.02	19.2	18.90	19.53	19.4	0.88	19.91	19.08	0.10	0.006	0.52
Lean to fat ratio	3.39	3.25	3.81	3.75	3.60	3.50	0.69	3.32	3.78	0.03	0.199	0.87

[1]Control, basal diet without feed additives; EOD3, 150 mg/kg DM (dry matter) of essential oil blend with 0.08 mg/kg DM diet of 25-hydroxy-Vit-D3 offered during all experimental period (DSM Nutritional Products, Basel, Switzerland); ZH, control diet during the first 35 d and zilpaterol hydrochloride supplementation at 6.2 mg/kg DM diet offered during the final 35 d of the experiment (33 d with ZH with a withdrawal 3 d at moment of harvest; MSD Salud Animal Mexico, Santiago Tianguistenco, México); ZH+EOD3, EOD3 treatment was offered during the first 35-d of the experiment, in the final 35 d of the experiment the treatments EOD3 and ZH were combined. Supplemental ZH was withdrawn 3 d before harvest.

Control, basal diet without feed additives; EOD3, 150 mg/kg DM (dry matter) of essential oil blend with 0.08 mg/kg DM diet of 25-hydroxy-Vit-D3 offered during all experimental period (DSM Nutritional Products, Basel, Switzerland); ZH, control diet during the first 35 d and zilpaterol hydrochloride supplementation at 6.2 mg/kg DM diet offered during the final 35 d of the experiment (33 d with ZH with a withdrawal 3 d at moment of harvest; MSD Salud Animal Mexico, Santiago Tianguistenco, México); ZH+EOD3, EOD3 treatment was offered during the first 35-d of the experiment, in the final 35 d of the experiment the treatments EOD3 and ZH were combined. Supplemental ZH was withdrawn 3 d before harvest.WHC, water holding capacity; WBSF, Warner-Bratzler shear force

Control, basal diet without feed additives; EOD3, 150 mg/kg DM (dry matter) of essential oil blend with 0.08 mg/kg DM diet of 25-hydroxy-Vit-D3 offered during all experimental period (DSM Nutritional Products, Basel, Switzerland); ZH, control diet during the first 35 d and zilpaterol hydrochloride supplementation at 6.2 mg/kg DM diet offered during the final 35 d of the experiment (33 d with ZH with a withdrawal 3 d at moment of harvest; MSD Salud Animal Mexico, Santiago Tianguistenco, México); ZH+EOD3, EOD3 treatment was offered during the first 35-d of the experiment, in the final 35 d of the experiment the treatments EOD3 and ZH were combined. Supplemental ZH was withdrawn 3 d before harvest.

Table 3.
Treatment effects on whole cuts in lambs

	Treatment				Main effects						Interaction	
					EOD3 (mg/kg DM)			ZH (mg/kg DM)			EOD3×ZH	
Whole cuts (% of CCW)	Control	EOD3	ZH	ZH+EOD3	0	150	*P*-value	0	6.2	*P*-value	SEM	*P*-value
Forequarter	40.32	40.33	40.62	40.78	40.47	40.56	0.81	40.33	40.70	0.29	0.337	0.83
Neck	8.77	8.29	7.96	8.16	8.37	8.22	0.58	8.54	8.06	0.10	0.265	0.23
Shoulder IMPS206	8.25	8.12	8.48	8.32	8.36	8.22	0.50	8.19	8.40	0.33	0.295	0.96
Rack IMPS204	6.49	6.49	6.60	6.87	6.54	6.68	0.30	6.49	6.73	0.09	0.182	0.32
Breast IMPS209	4.37	4.27	4.49	4.31	4.43	4.28	0.39	4.32	4.40	0.62	0.231	0.79
Ribs IMPS209_A	6.82	7.01	6.86	7.03	6.83	7.01	0.21	6.92	6.94	0.87	0.189	0.96
Hindquarter	37.78	37.17	38.47	38.32	38.13	37.74	0.28	37.48	38.39	0.02	0.341	0.51
Loin IMPS231	7.50	7.40	7.52	7.40	7.51	7.40	0.94	7.45	7.46	0.93	0.163	0.92
Flank IMPS232	6.23	6.40	6.37	6.38	6.30	6.38	0.73	6.32	6.37	0.85	0.357	0.74
Leg IMPS233	23.47[b]	23.97[b]	24.51[a]	24.65[a]	23.99	24.31	0.13	23.72	24.58	< 0.01	0.196	0.38

Table 4.
Treatment effects on meat quality in lambs.

Item	Treatments				Main effects						Interaction	
					EOD3 (mg/kg DM)			ZH (mg/kg DM)			EOD3×ZH	
	Control	EOD3	ZH	ZH+EOD3	0	150	*P*-value	0	6.2	*P*-value	*P*-value	SEM
WHC (%)	87.13[ab]	85.09[ab]	87.26[a]	84.20[b]	87.19	84.65	< 0.01	86.11	85.73	0.66	0.56	0.609
Drip loss (%)												
24 h	1.72[b]	2.68[c]	1.58[a]	1.59[a]	1.65	2.14	0.04	2.20	1.59	0.01	0.05	0.232
48 h	0.65	0.61	0.41	0.72	0.53	0.66	0.31	0.63	0.57	0.61	0.21	0.132
Thawing (%)	5.10[b]	5.31[b]	2.28[a]	2.96[a]	3.69	4.13	0.46	5.20	2.62	< 0.01	0.70	0.422
Cooking loss (%)	15.70	17.62	15.91	14.96	15.81	16.29	0.66	16.66	15.44	0.28	0.20	0.779
WBSF (kg/cm2)	2.44	2.61	2.36	2.58	2.41	2.59	0.29	2.52	2.48	0.78	0.88	0.174
Color												
L*	46.82[a]	45.45[ab]	42.71[b]	44.66[ab]	44.76	44.91	0.91	46.13	43.54	0.06	0.25	1.296
a*	12.90	12.73	15.00	15.12	13.95	13.93	0.98	12.82	15.06	0.02	0.87	0.638
b*	9.06	8.36	6.43	9.22	7.74	8.83	0.06	8.72	7.87	0.12	< 0.01	0.380

DISCUSSION

The ambient temperature and relative humidity (RH) during the experiment were 29.2 ± 3.4 °C and 73.1 ± 14.1 %, respectively. Based on temperature and RH, the calculated average value of THI during the experiment was 80.4 ± 3.2 (Dikmen & Hansen, 2009), which was within the range (79–84) coded as "Danger" (Mader *et al*., 2006). These ambient conditions represent a high ambient heat load that can compromise the productivity efficiency and quality of the final product. According to Al-Dawood (2017), high ambient heat load can affect meat quality characteristics. This phenomenon can be explained by the high thermal sensation, which leads to an increase in adrenaline in the bloodstream, causing peripheral vasodilation and muscle glycogenolysis, thereby decreasing the rate of lactic acid formation after death and maintaining high pH levels in the post-mortem muscle. This condition triggers a reaction that favors dark-cut presentation, negatively affecting the meat quality (Rana *et al*., 2014).

To our knowledge, this is the first report on the combination of EOD3+ZH on lamb meat characteristics; however, the absence of the effect of EOD3 on tissue composition is consistent with previous reports in which EO was supplemented alone (Estrada-Angulo *et al*., 2021) or in combination with D3 (Escobedo-Gallegos *et al*., 2023). Supplemental ZH increased the lean tissue proportion (4.7%, $P < 0.01$) and tended to reduce fat tissue proportion

(8.1%, $P = 0.07$), increasing the lean-to-fat ratio by 13.8 % ($P = 0.03$). The response to ZH supplementation in tissue composition is well known, and ZH has been shown to change the gain composition by increasing the proportion of muscle and decreasing fat deposition (Ortiz *et al*., 2016; Rivera-Villegas *et al*., 2019; Robles *et al*., 2024). This effect is associated with changes in cellular metabolism by stimulating lipolysis through the activation of the lipase-sensitive hormone and by inhibiting the synthesis and esterification of fatty acids in triglycerides, limiting lipogenesis, and thus redistributing energy towards muscle formation (Mersmann 2002; Cayetano-De-Jesús *et al*., 2020).

The absence of effects on the whole cut proportion in lambs is a common response when EO alone or in combination with D3 is supplemented in lambs (Arteaga-Wences *et al*., 2021; Estrada-Angulo *et al*., 2021) or when D3 is supplemented alone (Ibraheem *et al*., 2022). This can be explained by the main effects of supplemental EO on changes in the rate of ruminal fermentation and its antioxidant and anti-inflammatory effects, which improve the energy utilization efficiency but do not affect carcass traits (Dorantes-Iturbide *et al*., 2022; Escobedo-Gallegos *et al*., 2023). Combining ZH with EOD3 had no effect on improving this variable.

Lambs fed with ZH showed an increase in hindquarter proportion (38.39 vs. 37.49%) mainly due to the increase of 1.3% ($P < 0.01$) in the proportion of leg cuts. β-Adrenergic receptors in muscle tissues differ in number, affinity, and specificity (Chikhou *et al*., 1993). ZH stimulates muscle development, especially in the hindquarter, mainly due to the higher concentration of β-2 receptors in the lamb's biceps femoris (Koohmaraie *et al*., 1991; Ekpe *et al*., 2000). Likewise, previous studies (Macías-Cruz *et al*., 2010; López-Carlos *et al*., 2012) reported a greater effect of ZH supplementation on leg cuts (as a percentage of CCW), increasing the weight of the hindquarter.

Generally, meat consumers perceive meat quality based on certain attributes, which are defined by properties such as tenderness, color, flavor, water retention capacity, and fat content (Półtorak *et al*., 2017); among these properties, tenderness and color stand out as the most relevant characteristics (Carvalho *et al*., 2019). Therefore, any nutritional strategy that modifies these characteristics can impact the greater or lesser acceptance of meat by consumers. Except for drip loss and the intensity of yellow color (b^*), contrary to our hypothesis, the combination of EOD3 and ZH did not affect most of the variables evaluated for meat quality. Compared with the control group, drip loss at 24 h increased by 55.8% (2.68 vs. 1.72) when EOD3 was supplemented, and this effect was dramatically reversed (drip loss = 1.59) when EOD3 was combined with ZH (interaction, $P = 0.05$). The effect of the treatments on drip loss did not differ at 48 h. The water retention capacity of meat products is a quality attribute of utmost importance that influences product yield and has economic and quality implications (Cheng & Sun, 2008). The capacity of meat to retain water (own or added water) during the application of external forces is directly related to the density of muscle fibers (Carvalho *et al*., 2019).

The role of EO when supplemented alone on meat quality has been studied in meta-analyses (Orzuna-Orzuna *et al*., 2022; Dorantes-Iturbide *et al*., 2022). These studies agree that the water retention capacity increases and cooking loss is reduced with EO supplementation. In the present study, the opposite occurred at 24 h with water retention, and cooking loss was not affected when the EO was combined with vitamin D3. Further studies are needed to determine the effect of the combination of EO and D3 on meat quality parameters. Lambs fed EOD3 tended to have increased color intensity b^* (12.34%, $P = 0.06$); however, when EOD3 was combined with ZH, the intensity increased by 32% (interaction effect, $P < 0.01$). Dorantes-Iturbide *et al*. (2022), performed a meta-analysis of 16 studies to correlate the effects of supplemental EO on the color variables of sheep meat. They determined that supplementation with essential oils alone reduced the intensity of color b^* without affecting the color parameters a^* or luminosity (L^*). However, a recent study (Leyva-Medina *et al*., 2024) reported that vitamin D3 supra-supplementation (1.5 × 106 IU/d–1) during the last 7 days of fattening increased the yellow color by 14.5%. This can explain the results of the present study. Although the D3 concentration used in the current experiment was lower, it was consumed over a longer period (70 d). Those same researchers, same as our results, reported that the b^* color of lamb meat was not affected when ZH was supplemented at a rate of 20 mg/kg BW.

It is well known that fattening lambs exposed to high ambient temperatures can have higher meat shear force values (Macías-Cruz *et al*., 2020), mainly due to increased muscle cellular oxidative stress (Chen *et al*., 2022). Zilpaterol supplementation can be used as a strategy to improve performance and meat yield in cattle under HAHL conditions (Mendoza-Cortéz *et al*., 2022; Estrada-Angulo *et al*., 2024). It has been reported that EO supplementation alone reduces cellular oxidative stress in cattle (He *et al*., 2023). On the other hand, vitamin D3 supplementation can increase meat tenderness (Swanek *et al*., 1999; Montgomery *et al*., 2002). Therefore, it can be expected that blending EO with D3 can be a strategy to improve some parameters of meat quality of cattle fattening under HAHL and supplemented with ZH. Surprisingly, no effects of the treatments on WBSF were observed in this experiment. The effects of D3 and ZH on WBSF were contradictory. In this sense, some studies have reported the absence of effects of D3 or ZH on WBSF (Knobel-Graves *et al*., 2016; López-Baca *et al*., 2019; Carrillo-Muro *et al*., 2023), but others have indicated a decrease in WBSF by D3 (Montgomery *et al*., 2002; Foote *et al*., 2004) or an increase in WBSF by ZH (Holmer *et al*., 2009; Carrillo-Muro *et al*., 2021; Leyva-Medina *et al*., 2024). Similar to our results, Knobel-Graves *et al*. (2016), did not observe improvement in meat tenderness in steers supplemented with D3 (12.5 mg/d D3 for 10 d before slaughter) combined with ZH (8.3 mg/kg diet for 20 d) fattened under favorable ambient conditions.

CONCLUSION

Under the circumstances in which this study was carried out, the blend EOD3 supplemented alone or combined with ZH in fattening lambs under high ambient heat load conditions had minimal effects on tissue composition, whole cuts, or meat quality improvements. ZH supplementation increased lean tissue and leg weight (as a percentage of CCW) cut without affecting meat shear force. Apparently, the effects of supplemental ZH on lamb meat characteristics were independent of ambient temperature.

DECLARATIONS

Competing interests statement
The authors declare no conflicts of interest.

Ethics Statement
The meat samples of this report were obtained from the slaughtered lambs from the feeding trial conducted at the Universidad Autónoma de Sinaloa Feedlot Lamb Research Unit located in Culiacán, México (Protocol #04292023) which results of feedlot performance and carcass characteristics it was published previously (Estrada-Angulo *et al*., 2024).

Data availability statement
The data generated and analyzed in this study are available from the corresponding author upon request, indicating the reasons for needing them.

Autor Contributions
A. Estrada-Angulo and A. Plascencia, designed the experimental project, M. Verdugo-Insúa and L. Escobedo-Gallegos were responsible for conducting the fieldwork, E. Ponce-Barraza and A. Barreras performed the capture and statistical analysis of the variables of interest, F. Ríos-Rincón and I. Castro-Pérez were responsible for obtaining the samples and, together with O. Carrillo-Muro, analyzed the samples in the meat laboratory, D. Urías-Estrada and A. Plascencia wrote the manuscript. All authors reviewed and provided feedback to improve the quality of the manuscript.

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