



## Effects of two sources of Mexican oregano oil on performance, blood profile, carcass variables, and meat of broilers

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**ABSTRACT** - The current study was conducted to investigate the effects of Mexican oregano essential oil (MOO) extracts from *Lippia berlandieri* Schauer (LBS) and *Poliomintha longiflora* Gray (PLG) on performance, blood profiles, carcass variables, and meat composition of broilers at slaughter. A total of 360 one-day-old Ross broilers were randomly distributed into four dietary treatments with six replicate pens per treatment and 15 birds per pen. The dietary treatments were: a basal diet (control), control + 0.40 g of LBS/kg of feed, control + 0.40 g of PLG/kg, and control + 0.40 g of LBS/kg + 0.40 g of PLG/kg. Results showed that linear, quadratic, and cubic effects of days were significant in the performance variables of broilers. The treatments with LBS and PLG maintained the broiler body weight without increasing feed intake and water intake when compared with the control group. Broilers given LBS+PLG and PLG had increased blood leukocytes, lymphocytes, low-density lipoprotein, and hot carcass yields. In meat composition, treatments with PLG and LBS+PLG presented similar breast protein content compared with the control treatment. Supplementation with these two MOO exhibits positive effects on broiler performance, blood profiles, carcass traits, and meat composition. These two MOO may be promising feed supplements as growth promoters and enhancers of meat quality in broiler production.

Key Words: *Lippia berlandieri* Schauer, meat quality, Mexican oregano, performance, *Poliomintha longiflora* Gray

### Introduction

Resistance of pathogenic bacteria to antibiotics used in broiler production is widely known. For this reason, natural alternatives to antibiotics are being pursued and studied in poultry production. Oregano leaves and oregano essential oils (OEO) as phytochemical feed ingredients exhibit properties such as growth promotion, natural antibiotics, and improved meat quality in chickens (Hong et al., 2012). Several species falling under the common name oregano are found in the genera *Origanum* and *Thymus*, native to Europe, and *Lippia* and *Poliomintha*, originating from the arid Central and North America. The most popular forms under the common name Mexican oregano are *Lippia*

*berlandieri* Schauer and *Poliomintha longiflora* Gray (Rivero-Cruz et al., 2011). *Lippia berlandieri* Schauer is a common shrub found in arid regions of northern Central America, Mexico, and the southwestern United States, while *Poliomintha longiflora* Gray is a spindly shrub restricted to arid north-central Mexico, as well as in Haiti. The dried foliage and inflorescences of both Mexican varieties are used as condiments and as treatment for respiratory and digestive diseases (Rivero-Cruz et al., 2011). The main constituents of essential oils from Mexican oregano include carvacrol, thymol,  $\beta$ -myrcene,  $\alpha$ -terpinene,  $\gamma$ -terpinene, *p*-cymene, and ceneol (Vazquez and Dunford, 2005; Silva-Vazquez et al., 2017).

A number of researchers have evaluated performance and meat quality of broilers given plant extracts (Akbarian et al., 2013; Sharifi et al., 2013; Cho et al., 2014; Park et al., 2014; Starčević et al., 2015) and OEO (Hong et al., 2012; Khattak et al., 2014; Kırkpınar et al., 2014; Küçükylmaz et al., 2014; Ghazi et al., 2015; Silva-Vázquez et al., 2015; Sun et al., 2015; Ghazanfari et al., 2015; Hashemipour et al., 2016; Peng et al., 2016; Méndez-Zamora et al., 2017; Reyer et al. 2017; Chowdhury et al., 2018), demonstrating their

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influence on feed intake, growth enhancement, blood parameters, and meat quality. Silva-Vázquez et al. (2017) indicated that Greek (*Origanum vulgare* L. ssp. *Hirtum*), Turkish (*Origanum onites* L.), Spanish (*Thymus capitatus* L.), and Mexican oreganos (*Lippia graveolens* HBK, *Lippia berlandieri* Schauer, *Lippia palmeri* Watson, and *Poliomintha longiflora* Gray) are important essential oil-producing species. In general, extracts of *Origanum vulgare* L. and *Origanum onites* L., or their mixtures have been studied extensively in broilers. In contrast, comparatively little research has been reported on the effects of Mexican oregano essential oils (MOO) on broiler performance, blood parameters, and meat quality (Méndez-Zamora et al., 2015a,b; Silva-Vázquez et al., 2015; Méndez-Zamora et al., 2017). Silva-Vázquez et al. (2017) proposed that oregano oil and its fractions can be viable alternatives to the synthetic antioxidants widely used in foods and animal feed. Accordingly, it is important to expand research on these very promising MOO for use in poultry production.

The objective of the current study was to investigate the effects of two Mexican oregano essential oils, *Lippia berlandieri* Schauer and *Poliomintha longiflora* Gray, on broiler performance, blood parameters, carcass variables, and meat quality.

## Material and Methods

The research was conducted in Chihuahua City, Chihuahua, Mexico, located between 28°38' N and 106°04' W parallels, at an altitude of 1,440 m, with a mean annual temperature 20 °C, annual precipitation between 200-600 mm, and in a temperate dry climate (INEGI, 2017). Research on broilers was conducted in accordance with animal use committee guidelines (case no. NOM-062-ZOO-1999).

A total of 360 one-day-old Ross 308 mixed-sex broiler chicks (42.68±1.38 g) were randomly assigned to four treatments (diets) in which essential oils from two Mexican oreganos, *Lippia berlandieri* Schauer (LBS) and *Poliomintha longiflora* Gray (PLG), were evaluated: control diet, without MOO; control + 0.40 g of LBS/kg of feed; control + 0.40 g of PLG/kg; and control + 0.40 g of LBS/kg + 0.40 g of PLG/kg. Each treatment consisted of six replicate floor pens (1.25 × 1.25 × 0.70 m), with fresh wood shavings, and 15 broilers in each pen. Mexican oregano essential oils used in the study were purchased from Natural Solutions Company SMI (Jimenez, Chihuahua, Mexico). The MOO composition (Table 1) was obtained by gas chromatography (PerkinElmer Clarus 600 and SQ8

GC/MS; PerkinElmer Inc., Waltham, MA, USA) according to the method of Dunford and Silva (2005).

The starter diet for broilers of 0-21 days of age and finisher diet for broilers of 22-42 days of age were formulated according to NRC (1994) and as used by Silva-Vázquez et al. (2015). The MOO were mixed with canola oil as carrier and added to the basal control diet as previously indicated (Silva-Vázquez et al., 2015). Feed and water were provided *ad libitum*. Husbandry practices were applied according to Roofchae et al. (2011) and Silva-Vázquez et al. (2015). The temperature was set at 34 °C on the first day, followed by 32 °C over the remainder of the first week, then was reduced by 3 °C per week until it reached 23 °C. The relative humidity fluctuated between 25 and 75%. Lighting was provided 22 h/day.

Broiler initial weight (IW; g) was determined at the beginning of the experiment. Broiler body weight (BW), feed intake [FI; (intake of feed per week/number of broilers per pen)], water intake [WI; (intake of water per week/number of broilers per pen)], average daily gain [ADG; (BW<sub>current</sub> - BW<sub>previous</sub>)/day], and feed conversion ratio (FCR; BW/FI) were determined at 7, 14, 21, 28, 35, and 42 days. The offered and rejected feed weights were recorded.

Blood sampling and blood characterization were performed according to the method of Hong et al. (2012) with modifications. Blood samples of one randomly selected broiler per pen (n = 6 broilers per treatment) were obtained from the wing vein at 42 days. Immediately after collection, blood was set at 4 °C, then serum was separated by centrifugation at 1,500 × g for 15 min. Cholesterol (CHOL), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were measured with a biochemical analyzer (KONTROLab® EKEM, Roma, Italy) using commercial reagents (Stanbio Laboratory, Boerne, TX, USA). Complete blood cell count was determined according the methods of Medway et al. (1969) and Maxine (1984).

Table 1 - Essential oil composition of Mexican oregano

Component (vol%) <sup>1</sup>	Mexican oregano	
	LBS	PLG
Carvacrol	60.02	13.89
Thymol	3.96	28.49
Cineole 1,8	23.63	0.56
p-cymene	9.57	5.53
γ-terpinene	0.11	17.85
Menthol	ND	0.80
Eugenol	ND	0.62
Others	2.71	32.26

LBS - *Lippia berlandieri* Schauer; PLG - *Poliomintha longiflora* Gray; ND - not detected.

<sup>1</sup> Components were analyzed by GC/MS Clarus SQ 8 (PerkinElmer®).

The slaughter process was carried out according to the Official Mexican Standard (NOM-033-SAG/ZOO, 2014) and the method of Méndez-Zamora et al. (2015a). Twenty-four randomly selected broilers from each treatment (four broilers per pen), were slaughtered at 42 days (electrically stunned at 70 V, killed by cervical dislocation). Slaughter weight (SW) and hot carcass weight (HCW) were recorded, and hot carcass yield [HCY; (HCW/SW) × 100] was calculated. The cold carcass weight (CCW) was taken 24 h *post mortem* to determine the cold carcass yield [CCY; (CCW/SW) × 100].

Twelve carcasses were randomly selected from each treatment (two carcasses per replicate). Cut pieces of each carcass were made to record the weights of legs, thighs, hips, backs, wings, and breasts for each broiler. These weights were expressed as a percentage of SW and were considered as piece variables [% piece = (piece weight/SW) × 100]. Similarly, cooking loss (CL) was evaluated for twelve carcass pieces according to the method applied by López et al. (2011) and was determined through the formula %CL = [(raw weight piece – cooked weight piece)/raw weight piece] × 100.

The chemical composition analysis of the breasts (n = 12) and legs (n = 12) was performed in triplicate 24 h *post mortem* according to methods of the AOAC (1998). Moisture was measured by weight loss after 12-h drying at 100 °C in a forced-air oven (code 950.46). Protein contents were determined by the Kjeldahl method (code 992.15). Fat content was measured by the Soxhlet extraction solvent method (code 985.15). Ash weight was measured after exposing the muscle for 4 h at 400 °C in a muffle furnace (code 920.153).

Linear, quadratic, and cubic effects were analyzed to examine model fits of dependent variables, and the GLM multivariate ANOVA (MANOVA) procedure of SAS (Statistical Analysis System, version 9) was used to determine global effects of observed differences for all performance measures, fitting the treatments and days as the fixed effects, as well as their interactions. Production variables were analyzed using the MIXED procedure of SAS and the following statistical model (1) (Wang and Goonewardene, 2004):

$$y_{ijk} = \mu + T_i + \delta_j + (T\delta)_{ij} + \Phi_{k(ij)} + \lambda + \varepsilon_{ijk} \quad (1)$$

in which  $y_{ijk}$  = production variables measured during the experiment,  $\mu$  = general mean,  $T_i$  = effect of the  $i$ -th treatment (control, LBS, PLG, and LBS+PLG),  $\delta_j$  = effect of the  $j$ -th day of fattening (7, 14, 21, 28, 35, and 42 days),  $(T\delta)_{ij}$  = fixed effect of the interaction between  $i$ -th treatment and  $j$ -th day of fattening,  $\Phi_{k(ij)}$  = nested effect of the  $i$ -th treatment

in each pen where the broilers remained for the  $j$ -th day of fattening,  $\lambda$  = effect of the covariate IW, and  $\varepsilon_{ijk}$  = random error normally distributed with mean zero and variance  $\sigma^2$  [ $\varepsilon_{ijk} \sim N(0, \sigma^2)$ ]. Slaughter, carcass cut pieces, cooking loss, and dissected and chemical composition (moisture, protein, fat, and ash) results were analyzed with the GLM procedure and the following statistical model (2):

$$y_{ij} = \mu + T_i + \lambda + \varepsilon_{ij} \quad (2)$$

in which  $y_{ij}$  = variables evaluated,  $\mu$  = general mean,  $T_i$  = effect of the  $i$ -th treatment (control, LBS, PLG and LBS+PLG);  $\lambda$  = effect of the covariate IW, and  $\varepsilon_{ij}$  = random error normally distributed with mean zero and variance  $\sigma^2$  [ $\varepsilon_{ij} \sim N(0, \sigma^2)$ ]. A significance level of  $P < 0.05$  was used to detect significant statistical difference, and when the  $P$ -value was less than 0.05, the treatment means were compared and analyzed using the instruction Adjust = Tukey.

## Results

Linear effects of treatments were found for BW and FI (Table 2). Quadratic and cubic effects were not seen in BW, FI, WI, ADG, and FCR. Linear, quadratic, and cubic effects of days were significant for broiler performance variables. In global effects (MANOVA), the fixed effects and interaction showed differences ( $P < 0.05$ ) for all performance variables.

Body weight was different by day (7-42 days) for each treatment, with BW being higher ( $P < 0.05$ ) at 42 days (Table 3). Broilers given the control diet presented the highest ( $P < 0.05$ ) weight at 42 days while those given LBS presented the lowest ( $P < 0.05$ ). At 14, 21, 28, 35, and 42 days, weights for groups fed PLG and LBS+PLG were not different from the control groups, although BW was 0.15 kg less for these groups compared with the control. The effect of treatments on FI was different ( $P < 0.05$ ) at 7, 14, 21, and 35 days; in general, FI was higher in the control and lower in LBS-fed group. Feed intake for groups fed PLG and LBS+PLG was not different ( $P > 0.05$ ) from control at 14 and 21 days, while groups fed LBS and PLG were not different ( $P > 0.05$ ) from control at 35 days. Throughout the experiment (0-42 days), FI was different ( $P < 0.05$ ) between treatments with FI highest in control and lowest in LBS-fed broilers, although groups fed PLG and LBS+PLG were not different ( $P > 0.05$ ) from control and LBS groups. The effect of treatment on water intake (WI) was significant ( $P < 0.05$ ) at 14, 21, and 28 days, but there were no significant differences ( $P > 0.05$ ) by treatment at 7, 35, and 42 days. Specifically, WI for LBS-fed group at 14, 21, and 28 days exhibited the lowest values ( $P < 0.05$ ). From

0 to 42 days, control, PLG, and LBS+PLG broilers had the highest ( $P = 0.045$ ) total water intake, while LBS-fed broilers had the lowest intake.

Production efficiency was affected ( $P < 0.05$ ) over time and between treatments (Table 4). Average daily gain (ADG) was different ( $P < 0.05$ ) among treatments at 7, 14, 21, and 28 days, but was not different ( $P > 0.05$ ) among treatments at 35 and 42 days. Specifically, treatments affected ( $P < 0.05$ )

ADG in the period from 7 to 28 days, in which LBS was generally significantly ( $P < 0.05$ ) lower and ADG for control group was slightly higher than that for PLG and LBS+PLG groups. Total ADG from 0 to 42 days for control broilers was higher ( $P < 0.05$ ) than that for LBS broilers and showed improvement over broilers given PLG and LBS+PLG. Within treatment, FCR changed ( $P < 0.05$ ) over time with FCR at 7 days being the lowest and highest at 42 days.

Table 2 - Global effects on performance in broilers fed diets supplemented with dietary Mexican oregano essential oil extracts

Statistic effect	Dependent variable				
	BW	FI	WI	ADG	FCR
	Treatments				
Linear	0.0364	0.0187	0.9852	0.1775	0.7726
Quadratic	0.0886	0.2380	0.8800	0.4031	0.6224
Cubic	0.4626	0.4395	0.8552	0.5678	0.6053
	Days				
Linear	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cubic	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Coefficient of determination ( $R^2$ )				
Linear	0.9763	0.9584	0.8823	0.7823	0.8372
Quadratic	0.9783	0.8746	0.7579	0.6469	0.8964
Cubic	0.9246	0.7708	0.6295	0.5192	0.8795
	Multivariate ANOVA (MANOVA; Wilks lambda)				
Treatments ( $T_i$ )		<0.0001			
Days ( $\delta_j$ )		<0.0001			
( $T\delta$ ) <sub>ij</sub>		<0.0001			

BW - body weight; FI - feed intake; WI - water intake; ADG - average daily gain; FCR - feed conversion ratio.

Table 3 - Performance parameters of broilers fed diets supplemented with dietary Mexican oregano essential oil extracts

Trait/Treatment <sup>1</sup>	Days						
	7	14	21	28	35	42	
	BW (g)						
Control	181.50aF	465.33aE	891.50aD	1,478.17aC	2,067.17aB	2,641.50aA	
LBS	170.00bF	384.83bE	737.16bD	1,286.83bC	1,878.50bB	2,423.17bA	
PLG	159.94bF	438.17aE	879.83aD	1,437.17aC	1,983.50abB	2,500.50abA	
LBS+PLG	159.83bF	429.50abE	851.50aD	1,422.17aC	1,952.50abB	2,501.83abA	
SEM	4.48	13.33	17.65	31.39	35.26	53.44	
P-value	0.0014	0.0086	<0.0001	0.0055	0.0145	0.0237	
	7	14	21	28	35	42	
	FI (g)						
Control	150.00aE	406.67aD	678.33aC	980.00B	1,033.33aB	1,225.00A	4,471.95a
LBS	140.00bF	308.33bE	550.00bD	896.67C	1,023.33abB	1,176.67A	4,082.51b
PLG	126.67bF	381.67aE	631.67aD	918.33C	1,025.00abB	1,205.00A	4,288.03ab
LBS+PLG	133.33bE	393.33aD	631.67aC	930.00B	936.67bB	1,170.00A	4,190.18ab
SEM	4.15	11.94	14.50	26.50	24.26	28.51	74.99
P-value	0.0031	0.0003	<0.0001	0.1864	0.0286	0.4480	0.0103
	WI (g)						
Control	403.33D	971.67aC	1,695.00aB	2,253.33aA	2,398.33A	2,395.00A	10,115.43a
LBS	420.33E	703.33bD	1,318.33bC	1,955.00bB	2,361.67A	2,288.33A	9,220.73b
PLG	383.33D	923.33aC	1,658.33abB	2,285.00aA	2,435.00A	2,476.67A	10,162.45a
LBS+PLG	375.00D	881.67aC	1,518.33abB	2,333.33aA	2,390.00A	2,461.67A	9,958.11a
SEM	12.66	26.54	94.11	55.25	74.79	67.24	244.07
P-value	0.2180	<0.0001	0.0371	0.0017	0.8984	0.5794	0.0450

BW - body weight; FI - feed intake; WI - water intake; SEM - standard error of the mean.

<sup>1</sup> Control diet: without Mexican oregano essential oils; LBS: control + 0.40 g of *Lippia berlandieri* Schauer (LBS)/kg of feed; PLG: control + 0.40 g of *Poliomntha longiflora* Gray (PLG)/kg; LBS+PLG: control + 0.40 g of LBS/kg + 0.40 g of PLG/kg.

a, b - Means ( $n = 6$  replicate) in columns and with different letters are significantly different ( $P < 0.05$ ).

A-F - Means ( $n = 6$  replicate) in rows and with different letters are significantly different ( $P < 0.05$ ).



The FCR between treatments was different ( $P<0.05$ ) at 14, 28, and 35 days, but did not differ ( $P>0.05$ ) at days 7, 21, and 42 (Table 4). Broilers given LBS presented maximum efficiency at 14 days and the lowest at 28 and 35 days, while PLG-fed broilers had the highest ( $P<0.05$ ) FCR at 28 days and the lowest ( $P<0.05$ ) at 14 days, and the LBS+PLG group was the best ( $P<0.05$ ) at 35 days. Throughout the experiment (0-42 days), FCR was not different ( $P>0.05$ ) among treatments.

Hematological biometry (Table 5) for leukocytes, lymphocytes, hematocrit, and hemoglobin concentrations

were influenced by treatments ( $P<0.05$ ). Leukocytes and lymphocytes were higher ( $P<0.05$ ) in broilers fed LBS+PLG and PLG than in the control group. The hematocrit volume percentage (%vol) and hemoglobin content in control broilers were the highest ( $P<0.05$ ) and lowest ( $P<0.05$ ) in the LBS group. Of the lipid components, only LDL was affected by the treatment, with PLG and LBS+PLG broilers presenting the highest values ( $P<0.05$ ) and control the lowest ( $P<0.05$ ). There were no differences ( $P>0.05$ ) among treatment groups for the remaining blood components.

Table 4 - Production efficiency of broilers fed diets supplemented with dietary Mexican oregano essential oil extracts

Trait/Treatment <sup>1</sup>	Days						
	7	14	21	28	35	42	0-42
	ADG (g)						
Control	19.98aD	34.59aC	54.95aB	77.86aA	78.21A	76.12A	61.88a
LBS	16.98bC	28.11bC	46.52bB	62.05bA	79.38A	74.18A	56.69b
PLG	16.40bD	33.00abC	56.48aB	73.00aA	71.48A	67.23A	58.50ab
LBS+PLG	16.04bD	31.72abC	53.51aB	74.71aA	69.02A	71.66A	58.54ab
SEM	0.63	1.59	1.86	3.12	2.55	5.69	1.37
P-value	0.0018	0.0231	0.0041	0.0074	0.0634	0.5215	0.0496
	FCR						
Control	1.21ED	1.14abE	1.32D	1.51abC	2.01abB	2.16A	1.69
LBS	1.22E	1.26aDE	1.35CD	1.44bC	1.84bB	2.07A	1.69
PLG	1.27E	1.14bE	1.39D	1.56aC	1.94abB	2.08A	1.72
LBS+PLG	1.21CD	1.09abD	1.35C	1.53abB	2.09aA	2.14A	1.68
SEM	0.02	0.03	0.02	0.03	0.05	0.05	0.04
P-value	0.2295	0.0119	0.1490	0.0383	0.0283	0.4859	0.8352

ADG - average daily gain; FCR - feed conversion ratio; SEM - standard error of the mean.

<sup>1</sup> Control diet: without Mexican oregano essential oils; LBS: control + 0.40 g of *Lippia berlandieri* Schauer (LBS)/kg of feed; PLG: control + 0.40 g of *Poliomintha longiflora* Gray (PLG)/kg; LBS+PLG: control + 0.40 g of LBS/kg + 0.40 g of PLG/kg.

a,b - Means (n = 6 replicate) in columns and with different letters are significantly different ( $P<0.05$ ).

A-E - Means (n = 6 replicate) in rows and with different letters are significantly different ( $P<0.05$ ).

Table 5 - Blood profile of broilers fed diets supplemented with dietary Mexican oregano essential oil extracts at 42 days

Parameter	Treatment <sup>1</sup>				SEM	P-value
	Control	LBS	PLG	LBS+PLG		
Hematological biometry						
Leukocytes ( $10^3/\mu\text{L}$ )	17.7b	21.00ab	22.88a	25.32a	1.18	0.001
MCV (fl)	152.45	156.67	157.66	157.74	4.91	0.854
MCH (pg)	50.82	52.22	52.55	52.58	1.64	0.854
Heterophile ( $10^3/\mu\text{L}$ )	5.80	6.32	7.02	6.34	0.86	0.800
Lymphocytes ( $10^3/\mu\text{L}$ )	10.64b	13.75ab	14.83a	16.91a	0.91	0.001
Monocytes ( $10^3/\mu\text{L}$ )	0.84	0.52	0.66	0.87	0.12	0.150
Eosinophils ( $10^3/\mu\text{L}$ )	0.42	0.45	0.38	0.53	0.09	0.653
Erythrocytes ( $10^6/\mu\text{L}$ )	2.78	2.39	2.48	2.64	0.11	0.114
Hematocrit (vol%)	41.83a	37.33b	38.83ab	41.50ab	1.06	0.019
Hemoglobin (g/dL)	13.94a	12.45b	12.95ab	13.83ab	0.35	0.019
Lipids and lipoprotein profile (mg/dL)						
Cholesterol	126.17	121.50	126.33	136.17	5.93	0.378
Triglycerides	27.17	29.00	29.67	28.00	2.50	0.899
HDL	91.83	83.67	78.67	88.33	4.12	0.158
LDL	28.90b	32.03ab	41.73a	42.23a	3.45	0.025
VLDL	5.43	5.80	5.93	5.60	0.50	0.899

MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; HDL - high-density lipoprotein; LDL - low-density lipoprotein; VLDL - very low-density lipoprotein; SEM - standard error of the mean.

<sup>1</sup> Control diet: without Mexican oregano essential oils; LBS: control + 0.40 g of *Lippia berlandieri* Schauer (LBS)/kg of feed; PLG: control + 0.40 g of *Poliomintha longiflora* Gray (PLG)/kg; LBS+PLG: control + 0.40 g of LBS/kg + 0.40 g of PLG/kg.

a,b - Means (n = 6 birds) in rows and with different letters are significantly different ( $P<0.05$ ).

Slaughter variables, carcass piece yields, and cooking loss were influenced by treatment (Table 6). The LBS-fed group had the lowest ( $P<0.05$ ) HCY (Table 6). While HCY of control was only slightly higher, it was not different ( $P>0.05$ ) from that for broilers fed PLG and LBS+PLG. Cold carcass yields were not affected ( $P>0.05$ ) by treatment. In carcass piece yields, leg and hip pieces were influenced ( $P<0.05$ ) by treatment with LBS presenting the best leg yield, while LBS+PLG and control presenting the lowest. Hips from control broilers had the highest yield ( $P<0.05$ ), without differing ( $P>0.05$ ) from yields of broilers fed LBS and LBS+PLG, and PLG-fed broilers had the lowest ( $P<0.05$ ). Thighs, backs, wings, and breasts were not

influenced ( $P>0.05$ ) by the treatment. On the other hand, hip and back cooking loss (Table 6) were affected ( $P<0.05$ ) by treatment. Hip CL was highest ( $P<0.05$ ) in LBS-fed broilers, while back CL was highest ( $P<0.05$ ) for control and LBS-fed broilers. Legs, thighs, wings, and breasts were not influenced ( $P>0.05$ ) by treatment with Mexican oregano oil.

Protein and fat composition of breast meat were influenced ( $P<0.05$ ) by treatment (Table 7), but leg composition was not affected ( $P>0.05$ ). Protein content in breast meat of control broilers was highest ( $P<0.05$ ) and the lowest ( $P<0.05$ ) in LB-fed broilers. However, values of PLG and LBS+PLG treatments were not different from

Table 6 - Influence of Mexican oregano essential oil extracts on slaughter variables, carcass piece yields, and cooking loss of broilers at 42 days

Variable	Treatment <sup>1</sup>				SEM	P-value
	Control	LBS	PLG	LBS+PLG		
SW (kg)	2.83a	2.43c	2.72ab	2.62b	0.04	0.001
HCY (%)	73.41a	70.16b	72.98a	72.42a	0.40	0.001
CCY (%)	72.67	72.45	73.25	73.50	0.41	0.234
Piece yield (%) <sup>2</sup>						
Leg	11.32b	12.26a	11.69ab	11.34b	0.26	0.050
Thigh	10.26	10.42	10.12	9.81	0.23	0.292
Hip	8.40a	7.99ab	7.55b	7.82ab	0.22	0.060
Back	6.07	5.61	5.53	5.81	0.16	0.101
Wings	8.00	8.11	7.90	7.84	0.22	0.830
Breast	29.48	29.32	29.59	30.34	0.59	0.622
Cooking loss (%)						
Leg	10.30	10.83	11.71	10.84	1.35	0.905
Thigh	7.80	6.73	6.69	8.96	1.53	0.690
Hip	8.59b	15.35a	10.66b	10.80b	1.14	0.003
Back	13.60a	13.60a	9.34b	9.31b	1.34	0.038
Wings	6.42	5.62	4.03	5.80	1.19	0.544
Breast	11.65	11.61	10.23	9.60	0.91	0.314

SW - slaughter weight; HCY - hot carcass yield; CCY - cold carcass yield; SEM - standard error of the mean.

<sup>1</sup> Control diet: without Mexican oregano essential oils; LBS: control + 0.40 g of *Lippia berlandieri* Schauer (LBS)/kg of feed; PLG: control + 0.40 g of *Poliomintha longiflora* Gray (PLG)/kg; LBS+PLG: control + 0.40 g of LBS/kg + 0.40 g of PLG/kg.

<sup>2</sup> % = (piece or carcass weight/SW)×100.

a-c - Means (n = 24 carcasses to slaughter; n = 12 carcass to piece yield and cooking loss) in rows and with different letters are significantly different ( $P<0.05$ ).

Table 7 - Influence of Mexican oregano oils on chemical composition of breast and leg meat

Piece composition	Treatment <sup>1</sup>				SEM	P-value
	Control	LBS	PLG	LBS+PLG		
Breast (%) <sup>2</sup>						
Moisture	74.13	73.65	73.82	73.94	0.24	0.895
Protein	23.06a	22.33b	22.85ab	22.85ab	0.14	0.006
Fat	1.00b	1.06ab	1.04ab	1.13a	0.03	0.050
Ash	1.15	1.19	1.20	1.15	0.03	0.408
Leg (%)						
Moisture	76.02	76.08	76.15	75.82	0.12	0.265
Protein	20.26	20.17	20.06	20.33	0.12	0.439
Fat	1.62	1.67	1.65	1.71	0.03	0.338
Ash	1.07	1.09	1.10	1.06	0.02	0.501

SEM - standard error of the mean; SW - slaughter weight.

<sup>1</sup> Control diet: without Mexican oregano essential oils; LBS: control + 0.40 g of *Lippia berlandieri* Schauer (LBS)/kg of feed; PLG: control + 0.40 g of *Poliomintha longiflora* Gray (PLG)/kg; LBS+PLG: control + 0.40 g of LBS/kg + 0.40 g of PLG/kg.

<sup>2</sup> % = (piece or carcass weight/SW)×100.

a-b - Means (n = 12; two breasts per replicate) in rows and with different letters are significantly different ( $P<0.05$ ).

control and from LBS. Fat content was highest ( $P < 0.05$ ) in breast meat from broilers fed LBS+PLG and lowest ( $P < 0.05$ ) in meat from control broilers. Breast meat fat content in LBS- and PLG-fed broilers was not different ( $P > 0.05$ ) from control and LBS+PLG broilers.

## Discussion

In the current study, linear effects of treatments for broiler BW and FI were similar to those reported by Hashemipour et al. (2013), Giannenas et al. (2014), Hossain et al. (2014), and Alba et al. (2015), using natural compounds to enhance broiler performance. The  $R^2$  values showed that the best analysis for treatments is the linear model, while the linear, quadratic, and cubic models were useful for indicating broiler performance over time.

The effects found in the current study with 0.4 g of MOO/kg of feed coincided with results from recent studies with plant extracts in diets on broiler performance and feed efficiency (Cho et al., 2014; Khattak et al., 2014; Park et al., 2014; Ghazi et al., 2015; Sun et al., 2015; Hashemipour et al., 2016; Peng et al., 2016; Chowdhury et al., 2018). Specifically, Reyer et al. (2017) revealed improved growth performance in all groups fed the phytogetic additives [25.0 mg of an essential oil blend (star anise, rosemary, thyme, and oregano)/kg of feed, 46.0 mg of a *Quillaja* saponin blend/kg, or a combination of both preparations (essential oils + saponins)] compared with control broilers from 8 to 21 days; our results at 21 days with PLG and LBS+PLG in feed was not different from the control group. At 42 days of the current study, body weight was maintained by MOO treatments, results similar to those of Kırkpınar et al. (2011), Cho et al. (2014), and Mohiti-Asli and Ghanaatparast-Rashti (2015), who found that 300 and 500 ppm of OEO and 250 mg/kg of feed of phytogetic (oregano, carvacrol, cinnamaldehyde) supplements maintained broiler weight over 22-35 days and 42 days, respectively. These changes may be correlated with the levels of feed additives used in those diets in association with their chemical compositions. Contrary to our results, Hashemipour et al. (2016) obtained improved growth performance in broilers using 300 and 600 mg of OEO/kg of feed. For our study, results indicated that MOO supplementation did not change FI and WI throughout the experiment. In the current study, essential oil extract from *Poliomintha longiflora* (PLG) presented FI similar to the control group, and similar to results of Khattak et al. (2014), who demonstrated no change in broiler FI when evaluating mixtures of essential oils. Similar results were obtained by Küçükyılmaz et al. (2014) in BW and FI in the period of

0-42 days when evaluating 48 mg of a dietary OEO mixture (oregano, sage, myrtle, fennel, and citrus peel)/kg of feed.

Throughout the experimental period in the current study, ADG was influenced by supplementation of MOO, but FCR was not different among treatment groups. It may be that MOO odors prepare the gastrointestinal tract for feed reception and stimulate digestive secretions (saliva, salivary amylase, lipase, amylase, and proteases) and gut motility (Brenes and Roura, 2010), and, as a consequence, improve ADG. These effects agree with Ghazi et al. (2015), Sun et al. (2015), Hashemipour et al. (2016), and Peng et al. (2016) when evaluating 60, 250, 300, and 600 mg of OEO/kg of feed, and 100 and 200 mg of a mixture of thymol plus carvacrol/kg, respectively. Similarly, these results on production efficiency and variations in the results could be due to the composition and inclusion level of extracts. Furthermore, it is important to consider that diets formulated with OEO improve digestibility, regulate the microbiota of the intestinal ecosystem, and stimulate the secretion of endogenous digestive enzymes (Ghazi et al., 2015), and thymol+carvacrol could have positive effects on broiler performance (Hashemipour et al., 2016). The maximum efficiency at 14 days exhibited in LBS-fed broilers and 28 days in the PLG-fed broilers could be explained by results demonstrated in the study carried out by Reyer et al. (2017), who indicated that differences in the relative expression of intestinal transporters such as PepT1 (SLC15A1), GLUT2, and EAAT3 (SLC1A1) affect body weight and feed conversion efficiency in broilers. Analysis by Reyer et al. (2017) at the transcriptional level in Caco-2 cells validated these findings. Furthermore, those authors observed significant increases in membrane recruitment of SGLT1 and PEPT1 after the addition of essential oils and saponins and postulated that increases in the apparent ileal digestibility of nutrients could be explained by the interaction of tested feed additives with the epithelial function of the intestine. These mechanisms of action may have occurred with MOO tested in the current study. Such results may be overall benefits of phytogetic feed additives in terms of the metabolic usage of macronutrients provided by evidence at the transcriptional level by consistent alterations of lipid and carbohydrate metabolism (Reyer et al., 2017).

Few studies have reported results on blood and lipid profiles in broilers given OEO (Hong et al., 2012; Ghazi et al., 2015; Méndez-Zamora et al., 2017). However, effects on blood parameters in the current study with MOO could be of interest to other researchers, in which LDL was highest in PLG- and LBPL-treated broilers. Compared with results from the current study, Ghazi et al. (2015) found increased blood triglyceride and cholesterol levels

with their evaluation of 250 mg of oregano oil/kg of diet, while Méndez-Zamora et al. (2017) found that 400 mg of MOO/kg of feed increased broiler blood HDL and LDL levels. In addition, Park et al. (2014), using extracts from three plants, *Saposhnikovia divaricata*, *Lonicera japonica*, and *Chelidonium majus*, found increases in white blood cells, red blood cells, lymphocytes, hemoglobin, and hematocrit. Likewise, other studies found blood parameter effects following treatment with OEO, anise, and powered peel citrus (Hong et al., 2012), extracts from *Mentha spicata* (Nanekarani et al., 2012), medicinal plants (*Cuminum cyminum*, *Mentha piperita*, *Achillea millefolium*, *Teucrium polium*) (Sharifi et al., 2013), and coriander essential oil (Ghazanfari et al., 2015). In contrast, Méndez-Zamora et al. (2017) found no effect on blood biometric parameters with 0.40 g of OEO/kg of diet; however, those studies did obtain slightly increased levels of white blood cells, erythrocytes, and hemoglobin. Similar results were obtained in the current study, in which increased levels of leukocytes, lymphocytes, hematocrit, and hemoglobin were found with MOO as a feed supplement. The results from Méndez-Zamora et al. (2017) and the current study demonstrated that oregano essential oils and Mexican oregano oils, as feed supplements, could be used to improve broiler health; particularly, the MOO plant extracts, PLG-thymol, and LBS-carvacrol could have intrinsic biological effects on broiler physiology and metabolism (Méndez-Zamora et al., 2017).

In slaughter variables, Méndez-Zamora et al. (2015a) found results similar to those of the current study; however, carcass yields were higher in the current study. Méndez-Zamora et al. (2015a) suggested that OEO stimulated digestibility, enhancing nutrient absorption, and broiler body antioxidative stability could be improved by essential oils (Zhai et al., 2018). Khattak et al. (2014) found similar carcass yields to those from the LBS treatment at 400 mg/kg of diet when they used a mixture of essential oils (15, 30, 45, and 60 mg/kg), obtaining differences between the control and 15 mg/kg treatment. Results from those studies and the current study with MOO indicated that the OEO could influence slaughter variables. In addition, diets supplemented with MOO in the current study improved carcass pieces compared with the control group. In contrast, Küçükyılmaz et al. (2014) found no differences in carcass yield, thighs, breasts, and wings when evaluating 28.8 mg of carvacrol/kg of feed over 81 days, but their results were higher than those of the current study. Kırkpınar et al. (2014) found no influence on carcass yield, breasts, and thighs when evaluating 150 and 300 mg of OEO/kg in diets. Few studies have examined the effect of essential oils on cut piece yields and cooking loss. However, Méndez-Zamora

et al. (2015a) stated that components of oregano oil could reduce the bone weight and increase lean meat yields with applications of high levels (0.40 g/kg) of extract in broiler diets. This observation may explain leg and hip piece yields in LBS and PLG treatments. Furthermore, this observation may explain differences in carcass pieces when a thermic treatment was applied, in which hip and back cooking loss were affected by the LBS treatment.

Broiler breast protein and fat content were influenced by MOO. In contrast, Hong et al. (2012) and Kırkpınar et al. (2014) found no treatment differences for breast meat chemical compositions. This discrepancy could indicate that levels above 300 mg of OEO/kg of feed do influence protein and fat content. Likewise, leg composition in the current study was similar to results obtained by Hong et al. (2012) and Kırkpınar et al. (2014). Otherwise, Starčević et al. (2015) obtained effects of breast and leg meat on protein and fat content, with the effects of thymol (200 mg/kg), tannic, and gallic acids (5 g/kg) similar to those of LBS, PLG, and LBPL treatments. It is possible that OEO affects lipid content, deposited as fat in muscle tissue. Méndez-Zamora et al. (2015b) found differences in fat composition of breast meat in broilers given 0.40 and 0.80 g of OEO (60% carvacrol and 40% thymol)/kg of feed, indicating that high doses of OEO could increase fat and protein content. Similar increases in these components were obtained in the LBS+PLG treatment (0.80 g/kg total combination), which indicated that OEO combinations improve the protein and fat content of breast meat.

## Conclusions

The evaluation of two sources of Mexican oregano oils at 0.40 g/kg in the diet exhibit positive effects on broiler performance, blood profiles, carcass traits, and meat composition. Mexican oregano oils improve leukocyte and lymphocyte concentrations without altering hemoglobin and hematocrit. Mexican oregano essential oils may be promising alternatives to synthetic antioxidants and performance enhancers in broiler production and as enhancers of carcass meat quality.

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