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Performance, blood parameters, and carcass yield of broiler chickens supplemented with Mexican oregano oil

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ABSTRACT - The objective of the current study was to evaluate performance, blood parameters, and carcass yield of broilers supplemented with Mexican oregano oil. A total of 162 one-day-old broilers were randomly distributed into three dietary treatments: control diet (without oregano oil or antibiotic); control diet + 0.25 g kg^{-1} of oxytetracycline; and control diet + 0.4 g kg^{-1} of Mexican oregano oil. Treatment with organ oil had positive effects on body weight at 35 and 42 days. Feed intake was significantly different at 21 and at 1-42 days; control treatment presented the highest feed intake at 28 and 35 days, while treatment with oxytetracycline had the lowest feed intake from 21 to 42 days. Weekly body weight gain was different at 42 days, in which treatment with oregano oil was the highest and control the lowest. Feed efficiency rate was significantly different at 42 and at 1-42 days; treatment with oxytetracycline and treatment with oregano oil had the best values over control treatment. Blood parameters were found different among treatments, in which treatment with oregano oil was the highest in cholesterol and high-density lipoprotein at 42 days. Carcass and blood biometric variables were not different among treatments. The amount of 0.4 g kg^{-1} of Mexican oregano oil in diets improves high-density lipoprotein, body weight, and feed efficiency rate of broiler chickens.

Key Words: blood profile, broilers, Mexican oregano

Introduction

Antibiotics have been used prophylactically and in disease control. However, the European Union has banned antibiotic use in animal feed production out of concerns for bacterial resistance and hazards to public health (Hong et al., 2012). The ban has prompted researchers to study natural additives for growth promoter, antibiotic, and antioxidant properties in animal production (Adil et al., 2011; Hong et al., 2012; Cho et al., 2014; Park et al., 2014; Mohiti-Asli and Ghanaatparast-Rashti, 2015; Hashemipour et al., 2016). Hippenstiel et al. (2011) stated that oregano essential oil (OEO) has antimicrobial and antioxidant properties and stimulate the secretion of digestive enzymes. Lee et al. (2003) and Zhang et al. (2005) described OEO as having antifungal and antiviral properties. These researchers also demonstrated that OEO stimulate appetite and improve digestion and respiratory disorders. In addition, OEO is generally recognized as safe (GRAS; Silva and Dunford, 2005).

The main active components of OEO are thymol and carvacrol, which can improve feed digestibility, due to positive effects on nutrient digestibility (Jamroz et al., 2005), and induce a higher secretion of bile acids (Hashemipour et al., 2016). Oil extracts of *Origanum vulgare* L. tested in broiler diets improve body weight and feed intake at 1,000 ppm (Bozkurt et al., 2009), 500 ppm (Mohiti-Asli and Ghanaatparast-Rashti, 2015), 100 and 250 ppm (Symeon et al., 2009), 150 and 300 ppm (Kırkpınar et al., 2011), while 200 ppm of thymol and carvacrol (Lee et al., 2003; Hassanpour et al., 2013; Silva Vázquez et al., 2015; Hashemipour et al., 2016) also result in improvements in chicken performance.

Most studies involving oregano oil extracts have used OEO from *Origanum vulgare* L. and some results have led to several controversies regarding efficiency in broilers (Kirkpinar et al., 2011; Hong et al., 2012; Cho et al., 2014;

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Park et al., 2014; Mohiti-Asli and Ghanaatparast-Rashti, 2015; Hashemipour et al., 2016). Moreover, little attention has been given to Mexican oregano oil (MOO), prepared from *Lippia berlandieri* Schauer, in broiler production, and its use in broilers in Mexico and other countries has not been documented extensively. Therefore, the hypothesis is that MOO has potential in the intensive production of broilers as supplement in diets and as a nutraceutical additive (Gopi et al., 2014). The objective of the current study was to evaluate performance, blood parameters, and carcass yield of broilers supplemented with Mexican oregano oil. The data obtained will benefit oregano and broiler producers.

Material and Methods

Procedures used in this study were approved by the local Care and Animal Welfare Committee in Chihuahua, Chihuahua, Mexico. The city is located between the 28º38' N and 106º04' W parallels at an altitude of 1,440 m, with a mean annual temperature 18 °C, annual precipitation between 200-600 mm, and with a tempered dry climate (INEGI, 2013). Broilers were handled according to guidelines established in the Norma Oficial Mexicana (NOM-062-ZOO-1999) on care and animal welfare.

A total of 162 one-day-old Ross commercial genetic line broilers were used in this study. The birds were not vaccinated against diseases. Feed and water were offered *ad libitum*. Broilers were managed according the methodology of Cho et al. (2014): room temperature began at 33 °C from day 0 to 3 and was reduced gradually to 24 °C until the end of the experiment. Relative humidity fluctuated by 50%, while the lighting regimen consisted of 23 h of light and 1 h of dark.

The supplied diet was isoproteic and isoenergetic and ingredients were based according to NRC (1994). Starter and finisher diets provided from 0 to 21 and 22 to 42 days of age (Table 1) were formulated according to Silva Vázquez et al. (2015). Mexican oregano essential oil, prepared by steam distillation of *Lippia berlandieri* Schauer, was purchased from Natural Solutions S.M.I. (Jimenez, Chihuahua, Mexico). The main components of MOO (24.63% 1,8-cineole, 10.57% p-cymene, 0.11% γ-terpinene, 4.06% thymol, and 60.62% carvacrol) were determined by gas chromatography (Clarus 600 and MS Clarus SQ8 Perkin Elmer USA company) according to Dunford and Silva (2005).

Three treatments were evaluated: the control diet with no additives; control diet + 0.25 g kg^{-1} of oxytetracycline (Bayer Pfizer Inc, New York City, USA); and control diet + 0.4 g kg^{-1} of Mexican oregano oil. A completely randomized design was used for the distribution of treatments in metabolic cages (30 × 33 × 44 cm), with two chickens in each cage and 54 chickens in each treatment group.

The initial weight (g) of each broiler was determined on the first day of the experiment. The productive variables evaluated were body weight (BW; g) and feed intake (FI; g) at 7, 14, 21, 28, 35, and 42 days. These measurements were used to estimate the weekly body weight gain (WBWG; ($BW_{current} - BW_{previous}$)/week) and feed efficiency (FI/WBWG). Blood variables were measured (n = 10 blood samples): cholesterol (CHOL), triglycerides, high-density lipoproteins (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and blood biometrics (erythrocytes, hemoglobin, heterophils, eosinophil mean corpuscular volume, lymphocytes, monocytes, and mean corpuscular hemoglobin) according to Cohn et al. (1988), Medway et al. (1969) and Benjamin (1984). Blood samples were obtained by puncture of the left vein of broilers at 42 days of age according to Hong et al. (2012) with some modifications. Blood samples were immediately centrifuged for 15 min at 1,938 × *g* to obtain serum and were stored in Eppendorf tubes at –20 °C until analysis. Cholesterol and triglycerides were determined according to Cohn et al. (1988) in serum samples and were measured in duplicate by spectrophotometry; a commercial kit (Spinreact; Stanbio Laboratory, Boerne, Texas, USA) was used for this analysis. At 42 days, carcass yield was determined in twenty chickens per treatment for slaughter weight (SW),

Table 1 - Ingredients of the starter and finisher diets for broilers

Ingredient (g kg^{-1})[1]	Diet[2]	
	Starter (0-21 days)	Finisher (22-42 days)
Corn	467.2	556.4
Soybean (48% crude protein)	392.2	312.9
Corn gluten	53.3	44.4
Vitamin and mineral premix	11.7	13.3
Calcium carbonate	14.4	21.4
Dicalcium phosphate	21.3	22.2
Sodium chloride	6.0	6.4
DL-methionine	1.9	0.8
Canola oil®	32.0	22.2
Calculated contents		
Metabolizable energy (ME; kcal kg^{-1})[3]	3,200.0	3,200.0
Crude protein (%)	23.0	19.0
Lysine (%)	1.1	0.9
Methionine + cystine (%)	0.9	0.62
Calcium (%)	1.0	0.8
Available phosphorus (%)	0.5	0.3

[1] Ingredients were incorporated per kg of the experimental diet (expressed on a dry matter basis).
[2] Diets were formulated according to the nutrient requirements for broilers as recommended by NRC (1994).
[3] Calculated contents according to typical values for broiler well-balanced diets providing 3,200 kcal ME kg^{-1}.

hot carcass weight (HCW), cold carcass weight (CCW), hot carcass yield [(HCW/SW)×100], and cold carcass yield [(CCW/SW)×100].

The data obtained from the performance variables were analyzed using the general lineal model (GLM) of SAS (Statistical Analysis System, version 9.0), considering fixed effects of treatment, and day of fattening, considering initial weight as covariate in the statistical model (Wang and Goonewardene, 2004). Performance of blood variables and meat yield were analyzed through the analysis of variance obtained with the GLM procedure of SAS, establishing the treatments as fixed effects in the statistical model. A significance level of P<0.05 was used to detect significant statistical differences among treatments and when the P-value was less than 0.05, the treatment means were compared with the Tukey test.

Results and Discussion

Mexican oregano oil (60.62% carvacrol and 4.06% thymol) had significant effect (P<0.01) on BW at 35 and 42 days and not at the other days tested (Table 2). The influence of MOO was notable at 35 and 42 days, at which times, BW in the group treated with Mexican oregano was higher than in the antibiotic treatment group and the control group. Similar effects were found by Cho et al. (2014) when phytogenic additives were included in the diet of chickens. Other studies showed improvements in BW at 21 and 42 days when the chickens were fed peppermint, thyme leaves, and thymol with cinnamaldehyde or carvacrol (Ocak et al., 2008; Tiihonen et al., 2010; Mohiti-Asli and Ghanaatparast-Rashti, 2015; Hashemipour et al., 2016). In the present study, feed intake was different at days 21 and 1-42 (P<0.01), as well as at 28 and 35 days (P<0.05), in which the oxytetracycline treatment group consumed less feed, followed by MOO treatment group, while control group had the highest FI throughout the study (1-42 days) (Table 2). Feed intake decreased in the antibiotic treatment group. Similar results were obtained by Cabuk et al. (2006) and Küçükyılmaz et al. (2014).

In the present study, WBWG showed significance at 42 days, in which the MOO treatment group had the highest and control the lowest value (P = 0.05) (Table 3). Performance efficiency results obtained by Basmacıoğlu et al. (2004) with oregano oil were lower than results found in the current investigation with MOO. Silva Vázquez et al. (2015) found improvements in productive efficiency when evaluating 400 and 800 mg kg^{-1} of MOO. Karimi et al. (2010) showed differences in composition between Mediterranean and Mexican oregano oils, finding different responses in performance of broiler chickens.

Feed efficiency was significantly (P<0.05) affected at 42 and 1-42 days (Table 3). Feed efficiency values of antibiotic and oregano oil treatment groups were not statistically different, which were different from control. These results coincide partially with Cho et al. (2014), who found improved feed efficiency with the addition of phytogenic additives (250 mg kg^{-1}; oregano, cinnamaldehyde, carvacrol, yucca extract, and a herb mix) compared with a commercial antibiotic (10 mg kg^{-1} of avilamycin) and the control with no additives. Hong et al. (2012) found similar results when an essential oil mix (125 ppm including essential oil from oregano, anise, and citrus peel) was included in the diet. These authors

Table 2 - Effect of Mexican oregano oil on broiler body weight and feed intake

Treatment[1]	Day						
	Initial weight	7	14	21	28	35	42
				Body weight (g)			
T1	35.40	151.29	427.05	854.84	1,454.39	2,064.63b	2,604.31b
T2	35.04	152.92	424.70	842.37	1,431.71	2,049.84b	2,651.73b
T3	33.65	151.57	426.74	899.09	1,494.16	2,138.01a	2,763.42a
SEM	0.28	3.85	8.94	8.45	27.59	25.16	39.08
P-value		0.9496	0.9800	0.0611	0.3546	0.0123	0.0109
	7	14	21	28	35	42	1-42
				Feed intake (g)			
T1	197.34	244.05	563.25a	996.10a	1,257.48a	1,048.45	4,287.40a
T2	180.63	240.82	528.52b	930.73b	1,176.32b	1,040.03	4,103.43b
T3	182.78	232.91	532.51b	934.47b	1,182.13b	1,043.21	4,109.41b
SEM	4.08	1.88	6.82	17.87	14.26	10.22	44.22
P-value	0.0520	0.1015	0.0005	0.0117	0.0484	0.9276	0.0082

[1] T1: control diet; T2: control diet + 0.25 g kg^{-1} of oxytetracycline (Bayer); and T3: control diet + 0.4 g kg^{-1} Mexican oregano oil (4.06% thymol and 60.62% carvacrol).
Means (n = 54) of body weight (g) and feed intake in the same column with different letters are significantly different (P<0.05).
SEM - standard error of the mean.

concluded that the oil mix acted as a growth promoter, positively affecting production parameters. The current results with Mexican oregano oil were similar to those of Jamroz et al. (2005), Hong et al. (2012), and Silva Vázquez et al. (2015). Mountzouris et al. (2011) indicated that incorporating 125 and 250 mg kg^{-1} of a commercial phytogenic feed additive strengthened broiler production and improved efficiency and FI at the final stage. These results could be due to the properties of essential oils to enhance stimulation of digestive secretion, circulation, antioxidant and antimicrobial properties, and the immune status (Brenes and Roura, 2010; Sugiharto, 2014). Results of other studies demonstrated that phytobiotics improves the growth performance of broiler chickens (Windisch and Kroismayr, 2007; El-Ghany and Ismail, 2013). Thereby, the results obtained by Hong et al. (2012) and the current study with Mexican oregano oil suggest that Mexican oregano oil can be considered a natural growth promoter.

The effect of Mexican oregano essential oils on lipoproteins has not been widely reported. In the current study, at 42 days, significant statistical differences were found in CHOL, HDL, and LDL (P<0.05) but not in triglycerides and VLDL (P>0.05) (Table 4). The oregano oil treatment group had the highest CHOL value, while

Table 3 - Effect of Mexican oregano oil on broiler feed efficiency and weekly body weight gain

Treatment	Day						
	7	14	21	28	35	42	1-42
				Weekly body weight gain			
T1	116.20	277.09	428.85	599.09	611.30	561.92b	437.03
T2	117.84	281.69	431.84	588.66	620.13	603.75ab	441.95
T3	116.49	269.02	477.54	594.78	640.98	698.31a	460.57
SEM	3.97	9.39	15.08	14.76	19.93	17.09	9.25
P-value	0.9496	0.4419	0.0638	0.8900	0.6112	0.0501	0.1661
				Feed efficiency			
T1	1.75	0.94	1.32	1.67	2.08	2.13a	2.10a
T2	1.67	0.88	1.29	1.62	1.94	1.90ab	1.85ab
T3	1.60	0.90	1.20	1.59	1.88	1.60b	1.66b
SEM	0.08	0.03	0.05	0.05	0.07	0.12	0.12
P-value	0.3894	0.2371	0.2269	0.4984	0.1072	0.0184	0.03

T1: control diet; T2: control diet + 0.25 g kg^{-1} of oxytetracycline (Bayer); and T3: control diet + 0.4 g kg^{-1} Mexican oregano oil (4.06% thymol and 60.62% carvacrol).
Mean (n = 54) of weekly body weight gain [WBWG = $(BW_{current} - BW_{previous})$/week] and feed efficiency [FE = FI/WBWG] in the same column with different letters are significantly different (P<0.05).
SEM - standard error of means.

Table 4 - Effect of Mexican oregano oil and oxytetracycline on broiler blood profiles at 42 days

Blood parameter	Treatment			SEM	P-value
	T1	T2	T3		
Lipid and lipoprotein profile (mg dL^{-1})					
Cholesterol	121.42ab	104.16b	138.42a	8.10	0.03
Triglycerides	28.71	39.50	37.00	4.38	0.21
HDL	69.00ab	58.83b	72.14a	4.06	0.05
LDL	46.68a	37.43b	45.51ab	2.77	0.05
VLDL	5.74	7.90	7.40	0.87	0.21
Blood biometrics					
Leukocytes (K μL^{-1})	17.96	18.24	18.59	0.49	0.65
Eosinophils (K μL^{-1})	1.57	2.00	1.71	0.34	0.68
Heterophile (K μL^{-1})	27.83	33.60	30.43	2.58	0.34
Lymphocytes (K μL^{-1})	12,048.55	10,745.00	11,977.71	611.96	0.29
Heterocytes (K μL^{-1})	4,814.64	5,887.00	5,131.60	359.12	0.13
Monocytes (%vol)	4.14	2.80	3.17	0.56	0.24
Eosinophils (%vol)	240.32	261.00	219.40	43.58	0.81
Erythrocytes (10^6 μL^{-1})	2.73	2.50	3.01	0.28	0.49
Hematocrit (%vol)	37.50	36.33	38.29	1.10	0.46
Hemoglobin (g dL^{-1})	12.51	12.11	12.76	0.37	0.46
MCV (fL)	127.81	118.48	113.46	9.34	0.53
MCH (pg)	47.28	47.01	44.96	5.90	0.95

T1: control diet; T2: control diet + 0.25 g kg^{-1} oxytetracycline (Bayer); T3: control diet + 0.4 g kg^{-1} Mexican oregano oil (4.06% thymol and 60.62% carvacrol).
HDL - high-density lipoprotein; LDL - low-density lipoprotein; VLDL - very low-density lipoprotein; K μL^{-1} - thousands per microliter; MCV - corpuscular volume; fL - femtoliters; MCH - mean corpuscular hemoglobin (pg - picogram); SEM - standard error of means.
Means in the same row with different letters are significantly different (P<0.05).

Table 5 - Effect of Mexican oregano oil on broiler slaughter variables at 42 days

Slaughter variable	Treatment			SEM	P-value
	T1	T2	T3		
Slaughter weight	2,552.22	2,718.64	2,771.78	0.08	0.18
Hot carcass weight	2,080.36	2,112.52	2,252.21	0.08	0.33
Cold carcass weight	2,109.48	2,136.48	2,274.62	0.08	0.36
Hot carcass yield	2,163.27	2,188.76	2,332.52	0.09	0.34
Cold carcass yield	2,189.67	2,214.35	2,357.27	0.08	0.35

T1: control diet; T2: control diet + 0.25 g kg^{-1} oxytetracycline (Bayer); T3: control diet + 0.4 g kg^{-1} Mexican oregano oil (4.06% thymol and 60.62% carvacrol).
SEM - standard error of means.

the antibiotic treatment had the lowest CHOL, HDL, and LDL values. These results were similar to those of Cho et al. (2014), although their results on CHOL were lower compared with the results obtained in this study with Mexican oregano oil, in which oregano oil treatment increased the total CHOL ($P<0.05$) as well as HDL ($P<0.05$). These results partially agree with Hong et al. (2012), who found that essential oils reduced total CHOL, decreased VLDL, and increased HDL. Contrasting results were presented by Najafi and Torki (2010), who found no effect on cholesterol and HDL when studying essential oils in the diet. Results in blood biometrics were not different among treatments at 42 days ($P>0.05$) (Table 4). In general, the blood biometrics of the three treatment groups were within normal ranges. Broilers given Mexican oregano oil had slightly increased white blood cells, erythrocytes, and hemoglobin. The control group had high values of mean corpuscular volume, lymphocytes, monocytes, and mean corpuscular hemoglobin, followed by antibiotic and MOO treatment groups. Treatment with oxytetracycline increased, but not significantly, the levels of heterophiles and eosinophils.

No significant statistical differences ($P>0.05$) were found among treatments regarding meat production variables. In the current study, the group treated with MOO had high values among slaughter variables, followed by control and oxytetracycline treatment groups (Table 5). Similar results were observed in hot carcass weight, cold carcass weight, hot carcass yield, and cold carcass yield by Mendez-Zamora et al. (2015) and by Kırkpınar et al. (2014) only on carcass yields.

Conclusions

Supplementation of 0.4 g kg^{-1} of Mexican oregano oil has positive effect on body weight, feed intake, and feed efficiency. Mexican oregano oil can be used as a supplement in the diet of broilers to improve health and slaughter parameters.

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