



Archivos de Medicina Veterinaria

ISSN: 0301-732X

archmv@uach.cl

Universidad Austral de Chile

Chile

Escalera-Valente, F; Loya-Olguín, JL; Carmona-Gasca, CA; Martínez-González, S; Avila-Ramos, F

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Archivos de Medicina Veterinaria, vol. 48, núm. 2, 2016, pp. 209-214

Universidad Austral de Chile

Valdivia, Chile

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## Effect of oregano oil supplementation in diets formed using either crude soybean oil or acidulated soybean oil soapstock as source of energy on the growth performance parameters of broilers

Efecto de la suplementación con aceite de orégano en la dieta utilizando aceite de soya crudo o aceite de soya acidulado como fuente de energía sobre los parámetros productivos de crecimiento del pollo de engorda

F Escalera-Valente<sup>a</sup>, JL Loya-Olguín<sup>a</sup>, CA Carmona-Gasca<sup>a</sup>,  
S Martínez-González<sup>a</sup>, F Avila-Ramos<sup>b\*</sup>

**ABSTRACT.** The type of oil used in the broiler diet is mainly based on its price; in the case of soybean oil (CSO), acidulated soybean oil soapstock (ASS) is cheaper. The aim of the present study was to evaluate the effect of oregano oil dietary supplementation when the diet contains either CSO or ASS as source of energy. 320 broilers Ross 308 were assigned to four groups: (1) CSO containing diet without oregano supplementation, (2) ASS containing diet without oregano supplementation, (3) CSO containing diet with oregano supplementation (100 mg/kg), and (4) ASS containing diet with oregano supplementation (100 mg/kg). The growth performance parameters were weekly recorded during the experiment. Data were analysed with a completely randomised design with 2 × 2 factorial arrangement and the mortality rates were adjusted using the arc-sine transformation to fulfill the analysis requirements. The results showed no effect of oregano oil (containing 30.7 and 9.7 % of thymol and carvacrol, respectively) on feed intake, weight gain and feed conversion rate. A significant interaction of energy source by oregano oil supplementation was found in mortality rates ( $P < 0.05$ ); mortality was higher when the diet did not contain oregano oil and was formed using ASS. It can be concluded that the addition of 100 mg of oregano oil per kg of feed did not have a positive effect on the growth performance parameters of broilers when the diet contained either crude soybean oil or acidulated soybean oil soapstock as sources of energy.

**Key words:** acidulated soybean oil soapstock, oregano oil, broiler, growth performance.

**RESUMEN.** El tipo de aceite utilizado en la alimentación de los pollos de engorda se basa principalmente en su precio; en el caso del aceite de soya crudo (CSO) el aceite acidificado de soya (ASS) es más económico. El objetivo del estudio fue evaluar el efecto del aceite de orégano adicionado en la dieta cuando contiene CSO o ASS como fuente de energía. 320 pollos de engorda Ross fueron asignados a cuatro grupos: (1) CSO dieta sin adición de aceite de orégano, (2) ASS dieta sin adición de aceite de orégano, (3) CSO dieta adicionada con aceite de orégano (100 mg/kg), y (4) ASS dieta adicionada con aceite de orégano (100 mg/kg). Se registraron los parámetros de rendimiento productivo y los datos fueron analizados con un diseño completamente al azar con arreglo factorial 2 × 2 y los índices de mortalidad fueron ajustados utilizando la transformación arco-seno para cumplir las bases para su análisis. Los resultados no mostraron efecto del aceite de orégano (su contenido 30,7% y 9,7% de timol y carvacrol, respectivamente) en consumo de alimento, ganancia de peso e índice de conversión alimenticia. Hubo interacción significativa del recurso de energía y la adición del aceite de orégano a la dieta en los índices de mortalidad ( $P < 0,05$ ); la mortalidad fue mayor cuando la dieta no contenía aceite de orégano y fue elaborada con ASS. Se concluye que la adición de 100 mg de aceite de orégano por kg de alimento no tuvo un efecto positivo sobre los parámetros de rendimiento productivo de los pollos de engorda cuando la dieta es formulada con aceite de soya crudo o aceite acidificado de soya como fuente de energía.

**Palabras clave:** aceite acidificado de soya, aceite de orégano, pollo de engorda, rendimiento productivo.

## INTRODUCTION

Plants contain an extensive variety of phytochemical compounds that can have beneficial effects on broilers' growth (Zakeri and Kashefi 2011). At present, they serve as an alternative to synthetic products in some European countries (Castañón 2007), since the use of antibiotics as

animal growth promoters was banned in 2006 by the EU (Marković *et al* 2009). An increase in the use of natural agents is possible in the future in other countries too, due to food safety issues related with general health risks and bacterial resistance (Cross *et al* 2007, Theuretzbacher 2013).

The use of various plant parts as dietary supplements, including culinary herbs may positively influence poultry health and productivity (Lee *et al* 2004, Freitas *et al* 2013, Mohiti-Asli and Ghanaatparast-Rashti 2015). Oregano oil is a natural additive obtained from leaves of *Origanum vulgare*, an aromatic plant well-known for its use as a food spice (Arcila *et al* 2004). The concentration of thymol and carvacrol in the oregano oil mainly influence its biological activity (Lee *et al* 2004). The levels of these compounds can be different varying according to their geographic

Accepted: 16.09.2015.

<sup>a</sup>Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nayarit, Nayarit, México.

<sup>b</sup>División de Ciencias de la Vida, Programa de Medicina Veterinaria y Zootecnia, Universidad de Guanajuato, Guanajuato, México.

\*Corresponding author: F Avila-Ramos; Ex Hacienda El Copal Km. 9 Carretera Irapuato-Silao, C.P. 36500, Irapuato, Guanajuato, México; ledifar@hotmail.com

region of origin, harvesting time, the extraction method used, season and altitude (Huyghebaert *et al* 2011).

Oregano oil dietary supplementation appears to improve weight gain and feed conversion rate, due to thymol and carvacrol, compounds that can cause an improvement of digestive enzymes' action and therefore of nutrient absorption in broilers (Jang *et al* 2007). However, these results vary, since the ingredients of the diet can affect the action of oregano oil (Abdel *et al* 2012). Increasing oregano oil from 50 to 300 mg per kg of feed does not increase broiler productivity (Kirkpınar *et al* 2011), and this controversy with the previous results possibly is an impact of the source of energy used in the diet that can alter oregano oil dietary supplementation effect (Avila *et al* 2012).

In poultry various fats and essential fatty acids are used as a source of energy. Two of the most common fats used in poultry diets are animal/vegetable blends and crude oils, respectively (Blanch *et al* 1995). Acidulated soybean oil soapstock is one of the oil sources with the lowest cost for poultry diets, mainly because it is a by product from soybean oil refining (Hammond *et al* 2005). Feed intake, weight gain and feed conversion rate of broilers are not affected when this oil is used as source of energy (Thacker *et al* 1994, Baião and Lara 2005, Irandoust *et al* 2012). Compared to soybean oil, acidulated soybean oil soapstock contains high levels of free fatty acids (50%), unsaponifiable matter and oxidized fatty acids (Bartov *et al* 1974, Pardio *et al*, 2001, Kessler *et al* 2009). The nature of used oils can affect the efficacy of natural antioxidants, therefore, the objective of this research was to evaluate the effect of oregano oil dietary supplementation either with a diet containing acidulated soybean oil soapstock or crude soybean oil as a source of energy on broiler growth performance parameters.

## MATERIAL AND METHODS

### DETERMINATION OF THYMOL AND CARVACROL IN OREGANO OIL

To identify and determine the amount of thymol and carvacrol, a gas chromatograph (Hewlett Packard P-6890, USA., California) coupled a mass spectrometer (ME; Hewlett Packard 7953., USA, California) containing a capillary column Hewlett Packard 5ms® (30 m length, 0.25 mm internal diameter and 0.25 µm film thickness, USA., California) was used. The temperature of the GC injection port was 150 °C, the initial temperature of the oven was 60 °C during 5 min, and was increased 20 °C per min to reach 200 °C. Helium was used as a carrier gas (Helio, Infra S.A. de C.V., State of Mexico, Mexico). The ME was operated in scan mode (range m/z: 30-550) electron ionization (70 eV). The temperatures of the quadrupole, ion source and interfase were 150, 230 and 220 °C, respectively. 1 µL of the sample was injected into the GC using the splitless mode, and the mass spectra obtained

was compared with the NIST (Manion *et al* 2002) 2.0 database. Ions were identified, as well as their retention times and their relative abundance.

### BROILER PRODUCTION

320 one-day old Ross 308 broilers were raised till the age of 42 days. Broilers were fed with basal corn-soybean meal diets containing either CSO or ASS (starter and grower-finisher) (Table 1). Broilers were randomly allocated to four treatments with four replications; each replication consisted of 20 birds, which were assigned to the control diet (CON) without supplementation containing either CSO or ASS or to the oregano supplemented diet containing either CSO or ASS (100 mg/kg) (ORE). Oregano oil was added in CSO or ASS before mixing them with the ingredients of the diet. Feed and water were provided *ad libitum*. Feed intake, weight gain and feed conversion rate were weekly recorded for each replication (Advance RH®, 150 kg, digital scale, Mexico). Mortality rates were also daily recorded and presented as total mortality.

### STATISTICAL ANALYSIS

The examined parameters were feed intake, weight gain, feed conversion rate and mortality rates. These results were analyzed using a completely randomized design with factorial arrangement (2 x 2) with oil type and oregano oil as factors. The GLM procedure of SAS for Windows 9.0 was used (SAS 2000). The mortality rates were adjusted using the arc-sine transformation and presented as total mortality (Figure 1). Mean comparisons were conducted using the Tukey's test.

The statistical model used was:  $Y_{ijk} = \mu + T_i + \beta_j + (T\beta)_{ij} + \varepsilon_{ijk}$  Where:  $Y_{ijk}$  feed intake, weight gain, feed conversion rate and mortality rates,  $\mu_i$  is the overall mean,  $T_i$  is the oil type effect,  $\beta_i$  is the oregano oil effect,  $(T\beta)_{ij}$  effect of interaction between oil type and oregano oil,  $\varepsilon_{ijk}$  is the random error.

## RESULTS

According to the analysis of the oregano oil used in the present study, it contained 30.7 of thymol and 9.7 of carvacrol with ion qualifiers: 9.93 min, 150 (24), 91 (15.7), 136 (10.2) and 10.07 min, 150 (31.4), 91 (13.1), 136 (10.2), respectively. Moreover, feed intake, weight gain and feed conversion rate were not affected by oregano oil dietary supplementation at 7, 14, 21, 28, 35 and 42 days of age (tables 2, 3 and 4). In mortality rates, there was an interaction of source of energy by oregano oil supplementation (figure 1;  $P < 0.05$ ); in case of ASS containing diet, oregano supplementation led to a significant decrease of mortality rates (10 vs 2.5 %). On the other hand, no effect of oregano supplementation in CSO containing diet was observed.

**Table 1.** Chemical composition (%) of crude soybean oil or soapstock containing diets.  
Composición química (%) de las dietas con aceite crudo de soya o aceite acidulado de soya.

Ingredient	Starter		Grower-Finisher	
	CSO <sup>1</sup>	ASS <sup>2</sup>	CSO	ASS
Corn	65.61	65.55	71.79	71.36
Soybean meal	29.22	29.10	22.11	22.19
CSO	1.00	0.00	1.86	0.00
ASS	0.00	1.17	0.00	2.21
Calcium bicarbonate (38%)	1.64	1.64	1.52	1.51
Dicalcium phosphate (18/21)	1.49	1.49	1.30	1.30
Salt	0.30	0.30	0.30	0.30
Mineral premix <sup>3</sup>	0.06	0.06	0.06	0.06
Vitamin premix <sup>4</sup>	0.05	0.05	0.05	0.05
DL-Methionine	0.30	0.30	0.18	0.18
L-lysine HCl	0.29	0.30	0.19	0.18
Xantophylls <sup>5</sup>	0.00	0.00	0.60	0.60
Cocciostate	0.05	0.05	0.05	0.05
Nutrient composition				
Metabolizable energy (Mcal/kg)	3.00	3.00	3.10	3.10
Crude Protein	20.06	20.00	17.00	17.00
Calcium	1.00	1.00	0.90	0.90
Linoleic acid	1.90	1.72	2.46	2.13
Lysine	1.30	1.30	1.00	1.00
Methionine + Cystine	0.95	0.95	0.75	0.75
Available phosphorous	0.45	0.45	0.45	0.45
Hystidine	0.51	0.51	0.43	0.43
Tryptophane	0.27	0.27	0.23	0.23
Threonine	0.84	0.84	0.73	0.73
Arginine	1.31	1.31	1.08	1.08

<sup>1</sup>CSO = crude soybean oil.

<sup>2</sup>ASS = acidulated soybean oil soapstock.

<sup>3</sup>Amount in mg per kg of feed: Se 0.27, I 2, Cu 8, Fe 50, Zn 80, Mn 80, and Co 0.2 (Trouw Nutrition, Nuevo León, México).

<sup>4</sup>Amount per kg of feed: A, 12000 UI, D<sub>3</sub>, 3100 UI<sub>3</sub>, K<sub>3</sub>, 5 mg, thiamine, 2 mg, riboflavin, 12 mg, pantothenic acid, 21 mg, pyridoxine, 2.6 mg, folic acid, 1.5 mg, B<sub>12</sub>, 0.018 mg, and biotin, 0.15 mg (Ajinomoto, DF, México).

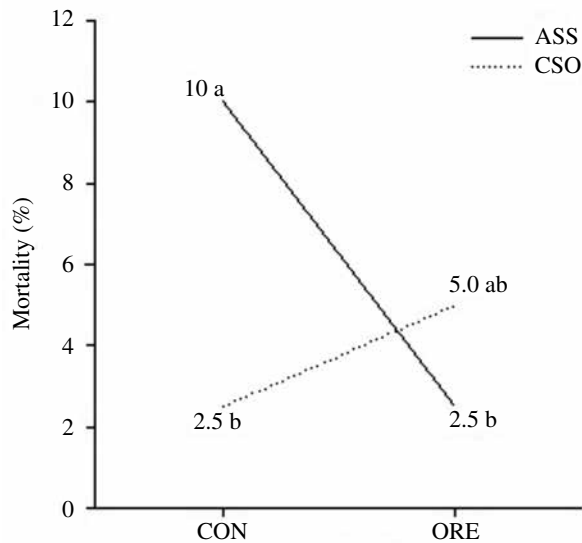
<sup>5</sup>Amount per kg of feed: yellow pigment 15 g (Alcos, DF, México).

## DISCUSSION

The oregano oil used in the present study contained 30.7 and 9.7 % of thymol and carvacrol, respectively, levels that are already reported in oils obtained in the region of Jalisco in Mexico (Arcila *et al* 2004, Arana *et al* 2010). However, these levels are lower compared to the oregano oil harvested from Europe that can contain until 80% of thymol and carvacrol (Abu-Lafi *et al* 2008). In the majority of oregano oils, the amount of thymol is higher compared to that of carvacrol. The two compounds have a negative correlation with each other, so when carvacrol increases, thymol decreases. This variation can cause different effects on broilers, since high levels of carvacrol are related with a higher weight gain and an increase of gastric secretions, therefore, the dietary nutrients are efficiently absorbed (Russo *et al* 1998). No effects were observed in the present study on the examined growth performance

parameters, partially explained by this variation in thymol and carvacrol rates.

The obtained results indicated that the addition of oregano oil at the level of 100 mg per kg of feed did not increase feed intake, weight gain and feed conversion rate of chickens either when the diet contained crude soybean oil or acidulated soybean oil soapstock as source of energy (Kessler *et al* 2009). These results also confirmed that oregano essential oil can be fed to broilers without detrimental effects on their productivity. No negative effects on productivity variables were also observed in other studies conducted with different levels of oregano oil (per kg of feed); from 50 to 1000 ml (Botsoglou *et al* 2002, Hernández *et al* 2004, Marčincák *et al* 2008), 200 mg (Jang *et al* 2007), 300, 500 and 1000 mg (Cross *et al* 2007, Freitas *et al* 2013). In general, oregano oil appears to increase the stimulation of digestive enzymes in the intestinal mucosa, therefore nutrient absorption in the



**Figure 1.** Mortality rates in the different groups of broilers.  
Tasa de mortalidad en diferentes grupos de pollos de engorda.  
ASS = acidulated soybean oil soapstock, CSO = crude soybean oil.  
CON = control, ARE = oregano oil at 100 mg/kg.  
<sup>a,b</sup>Means with different superscripts are statistically different ( $P < 0.05$ ).

digestive system of broilers can be improved (Bhat *et al* 1984, Sambaiah and Srinivasan 1991, Platel and Srinivasan 1996). However, the observation of insignificant effects of oregano oil might be a result of the conditions of the present study; well-nourished birds and disinfected environment. It has also been also well documented that dietary antibiotics such penicillin do not promote growth

performance when animals were raised in germ free conditions (Coates *et al* 1963). Similar to antibiotics, dietary oregano oil as a growth promoter could not give beneficial results when birds are kept at optimal conditions such as highly digestible diets and clean conditions (Botsoglou *et al* 2002). Therefore, extensive research is necessary to highlight the factors and the metabolic processes that influence feed intake, weight gain and feed conversion rate when the diet contains different oils as ingredients.

Oregano oil dietary supplementation could be associated with an improvement in feed efficiency (Lee *et al* 2004). However, the impact of essential oils on the performance parameters has generally been inconsistent, at least partially due to their hydrophobic nature, which makes their delivery to the intestine ineffective (Kohlert *et al* 2000, Michiels *et al* 2008). The ASS is a byproduct of the soybean oil and it is obtained through the alkaline neutralization of the CSO. ASS contains higher levels of free fatty acids (50%) compared to CSO, a fact that influences the nutritive value of the ingredients in the diet (Blanch *et al* 1995). The inclusion of ASS as a source of energy in the diet could be associated with a lower absorption of thymol and carvacrol by broilers compared to CSO containing diets (Wiseman and Salvador 1991). This process has been already observed with vitamin E (Drevon 1991) and the compounds of oregano oil may be similarly influenced. ASS does not have enough monoglycerides to be combined with all free fatty acids when they are offered as the only fat source (Blanch *et al* 1995, Baião and Lara 2005). Besides, broilers can not absorb the same amount of nutrients when a fat of lower quality is added in the diet (Baião and Lara 2005).

**Table 2.** Feed intake (kg) of broilers fed with crude soybean oil or acidulated soybean oil soapstock as a source of energy, further supplemented or not with oregano oil (means  $\pm$  SE).

Consumo de alimento (kg) de los pollos alimentados con aceite crudo de soja o aceite acidulado de soja como fuente de energía, adicionada o no con aceite de orégano (medias  $\pm$  DE).

Treatment		Starter			Grower-finisher		
		7	14	21	28	35	42
CSO <sup>1</sup>	CON <sup>3</sup>	0.155	0.414	0.596	0.843	1.070	1.253
	ORE <sup>4</sup>	0.133	0.435	0.605	0.867	1.097	1.252
ASS <sup>2</sup>	CON	0.151	0.406	0.579	0.808	1.040	1.214
	ORE	0.153	0.407	0.584	0.834	1.063	1.223
P > F		0.604	0.239	0.413	0.651	0.511	0.292
SEM		0.006	0.027	0.034	0.034	0.029	0.017
Parameters		Probabilities ( <i>P</i> values)					
Source of energy (S)		0.525	0.118	0.118	0.248	0.238	0.067
Oregano Oil (O)		0.455	0.320	0.320	0.663	0.364	0.788
S x O		0.362	0.353	0.353	0.912	0.939	0.809

<sup>1</sup>CSO = crude soybean oil.

<sup>2</sup>ASS = acidulated soybean oil soapstock.

<sup>3</sup>CON = control.

<sup>4</sup>ORE = oregano essential oil at 100 mg/kg.

**Table 3.** Weight gain (kg) of broilers fed with crude soybean oil or acidulated soybean oil soapstock as a source of energy, further supplemented or not with oregano oil (means  $\pm$  SE).

Ganancia de peso (kg) de pollos alimentados con aceite de soya crudo o aceite acidulado de soya como fuente de energía, adicionada o no con aceite de orégano (medias  $\pm$  DE).

Treatment		Starter			Grower-finisher		
		7	14	21	28	35	42
CSO <sup>1</sup>	CON <sup>3</sup>	0.116	0.274	0.376	0.431	0.563	0.568
	ORE <sup>4</sup>	0.115	0.272	0.393	0.427	0.567	0.609
ASS <sup>2</sup>	CON	0.107	0.25	0.357	0.429	0.566	0.674
	ORE	0.118	0.266	0.363	0.411	0.579	0.612
P > F		0.500	0.399	0.775	0.914	0.922	0.379
SEM		0.017	0.030	0.034	0.033	0.027	0.005
Parameters		Probabilities ( <i>P values</i> )					
Source of energy (S)		0.548	0.177	0.371	0.691	0.692	0.212
Oregano Oil (O)		0.345	0.550	0.654	0.622	0.624	0.804
S x O		0.303	0.396	0.844	0.771	0.816	0.235

<sup>1</sup>CSO = crude soybean oil.

<sup>2</sup>ASS = acidulated soybean oil soapstock.

<sup>3</sup>CON = control.

<sup>4</sup>ORE = oregano essential oil at 100 mg/kg.

**Table 4.** Feed conversion rate of broilers fed with crude soybean oil or acidulated soybean oil soapstock as a source of energy, further supplemented or not with oregano oil (means  $\pm$  SE).

Tasa de conversión alimenticia (kg) de pollos alimentados con aceite de soya crudo o aceite acidulado de soya como fuente de energía, adicionada o no con aceite de orégano (medias  $\pm$  DE).

Treatments		Starter			Grower-finisher		
		7	14	21	28	35	42
CSO <sup>1</sup>	CON <sup>3</sup>	1.135	1.507	1.592	1.960	1.905	2.205
	ORE <sup>4</sup>	1.338	1.605	1.540	2.042	1.932	2.062
ASS <sup>2</sup>	CON	1.450	1.645	1.555	1.882	1.845	2.000
	ORE	1.310	1.532	1.542	2.062	1.835	1.990
Pr > F		0.226	0.570	0.814	0.440	0.336	0.219
SEM5		0.023	0.032	0.034	0.031	0.023	0.014
Parameters		Probabilities ( <i>P values</i> )					
Source of energy (S)		0.175	0.676	0.385	0.738	0.086	0.091
Oregano Oil (O)		0.117	0.923	0.818	0.144	0.838	0.918
S x O		0.771	0.192	0.781	0.572	0.664	0.214

<sup>1</sup>CSO = crude soybean oil.

<sup>2</sup>ASS = acidulated soybean oil soapstock.

<sup>3</sup>CON = control.

<sup>4</sup>ORE = oregano essential oil at 100 mg/kg.

As it is shown in figure 1, birds fed with the ASS containing diet without oregano essential oil supplementation had the higher mortality rates compared to the other groups. However, the addition of oregano oil appeared to diminish this negative effect of ASS. Therefore, although the use of ASS causes increased mortality rates, the addition of oregano at the level of 100 mg per kg of feed could serve as an alternative when ASS is used as source of energy.

It can be concluded that the addition of oregano oil as a natural additive in diets containing either crude soybean oil or acidulated soybean oil soapstock as a source of energy did not affect the growth performance parameters in broilers. Therefore, acidulated soybean oil soapstock can be used as an inexpensive source of energy in broilers' diets. The results concerning mortality rates suggest that extensive research is necessary to explain the interaction

of oregano oil supplementation by the source of oil added in the diets and their effects on health status of broilers.

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