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Oregano oil use in broiler diet increases accumulation of carvacrol and thymol in breast meat

El uso de aceite de orégano en la dieta del pollo de engorde incrementa la acumulación de timol y carvacrol en carne de pechuga

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ABSTRACT

Oregano oil is an aromatic used in poultry industry as a natural additive. This research was designed to quantify thymol and carvacrol in chicken breast meat. In total, 320 Broilers were fed on basal corn soybean meal diet contained crude soybean oil (CSO) or acidulated soybean oil (ASO) as a resource of energy with or without oregano oil (OO). Identification and measurement of compounds in meat was performed by gas chromatography (GC) and mass spectrometry (MS). Data obtained were transformed and analyzed parametrically, a significant increase of thymol and carvacrol was found in chicken breast meat supplemented with oregano oil ($p < 0.05$). Breast meat of broilers fed with oregano oil and CSO accumulated more thymol and carvacrol: 333% and 366%, respectively. Breast meat of broilers fed with oregano oil and ASO accumulated thymol, but more carvacrol: 552% and 648% respectively, compared with control. In conclusion, oregano oil supplementation in diet increased deposition of thymol and carvacrol in broiler meat.

RESUMEN

El aceite de orégano es un aceite aromático usado en la industria avícola como un aditivo natural. Esta investigación fue diseñada para cuantificar el timol y carvacrol en carne de pechuga de pollo. En total, 320 pollos fueron alimentados con una dieta a base de maíz-pasta de soya, utilizando aceite de soya crudo (CSO, por sus siglas en inglés) o aceite de soya acidulado (ASO , por sus siglas en inglés) como fuente concentrada de energía, con o sin aceite de orégano (OO, por sus siglas en inglés). La identificación y cuantificación de los compuestos en la carne fueron realizadas con cromatografía de gases y espectrometría de masas. Los datos obtenidos fueron transformados y analizados paramétricamente, un incremento significativo de timol y carvacrol fue encontrado en pechuga de pollo suplementada con aceite de orégano ($p < 0.05$). La carne de pollos alimentados con aceite de orégano y CSO acumuló timol, pero más carvacrol: 333% y 366%, respectivamente. La carne de pollos alimentados con aceite de orégano y ASO acumuló timol, pero más carvacrol: 552% y 648%, respectivamente, comparado con el Testigo (sin aceite de orégano). En conclusión, la adición de aceite de orégano a la dieta incrementa la deposición de timol y carvacrol en carne de pollo.

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Origanum vulgare; crude soybean oil; acidulated soybean oil soapstock.

Palabras clave:
Origanum vulgare; aceite crudo de soya; aceite acidulado de soya.

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INTRODUCTION

Addition of additives through feed may decrease lipid oxidation of unsaturated fatty acids in meat, therefore increasing its shelf life (Marcinčák, Cabadaj, Popelka & Šoltýsová, 2008). In the last decade, knowledge about plants and their composition has increased. Many compounds are natural and safe and because that they have good consumer acceptance; therefore, they can be used to increase meat quality (Liu, Qiu, Ding & Yao, 2008).

Oregano is an aromatic plant used to improve organoleptic characteristics of foods. Oregano oil (OO) is obtained from plant leaves and contains high amounts of thymol and carvacrol (Figiel, Szumny, Gutiérrez-Ortiz & Carbonell-Barrachina, 2010; Montoya *et al.*, 2007). These phenolic compounds have

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anti-parasitic, antimicrobial and antioxidant activity (Arcila-Lozano, Loarca-Piña, Lecona-Uribe & González de Mejía, 2004; Fasseas, Mountzouris, Tarantilis, Polissiou & Zervas, 2007). But, chemical composition of oregano oil may vary due to weather, season of year, harvest cycle, process of extraction and crop location (Baydar, Sağdiç, Özkan & Karadoğan, 2004; Ortega-Nieblas *et al.*, 2011).

Oregano oil is used in broilers to promote growth and reduce conventional antibiotics use (Giannenas, Florou-Paneri, Botsoglou, Christaki, & Spais, 2005; Symeon, Zintilas, Demiris, Bizelis & Deligeorgis, 2010). This oil added to broilers' feed act as an antioxidant and it can be maintained meat lipid oxidative stability. Thymol and carvacrol as analytical reagents have antioxidant effects in meat similar to oregano oil (Botsoglou, Christaki, Fletouris, Florou-Paneri & Spais, 2002a; Luna, Labaque, Zygadlo & Marin, 2010). However, these antioxidants have different effects caused by composition of oil or fat in broiler diet that can modify their activity (Yanishlieva, Marinova, Gordon & Raneva, 1999).

Acidulated soybean oil (ASO) is a concentrated source of energy, compared to soybean oil it contains high levels of free fatty acids (~50%) and a lot of impurities. This oil has a low market price because it is a refining crude soybean oil (CSO) industry's byproduct. ASO not affect productive response of broilers, but its inclusion in diet could decreased oxidative stability of meat when is used as a source of energy in diet (Ávila-Ramos *et al.*, 2012).

Use of natural compounds as alternative to a broiler feed could improve meat and their meat products quality (Marcinčák *et al.*, 2008; Young, Stagsted, Jensen, Karlsson & Henckel, 2003). However, many alternative products can be lost by interaction with different ingredients into diet. Therefore, the aim of the study was to quantify amount of thymol and carvacrol deposited in chicken breast meat when broilers received ASO or CSO as a concentrated feed source of energy.

MATERIAL AND METHODS

Broiler production

Three hundred and twenty Ross 308 broilers were raised from 1 to 42 days of age, given feed supplemented with ASO or CSO and 100 mg kg^{-1} oregano oil (80 broilers by treatment) and without oregano oil (80 broilers). Broilers were randomly distributed into four treatments with four replications of 20 birds (20 birds × 4 replications × 4 treatments = 320 birds). Diet was formulated according National Research Council (1994); Starter ASO as % (corn 65.55, soybean meal 29.10, ASO 1.17, L-Lysine HCL 0.30) and starter CSO as % (corn 65.61, soybean meal 29.22, CSO 1, L-Lysine HCL 0.29), for both: calcium bicarbonate (38%) 1.64, dicalcium phosphate (18/21) 1.49, salt 0.30, mineral and vitamin 0.11, DL Methionine 0.30 and coccidiostate 0.05. Grower-finisher ASO as % (corn 71.36, soybean meal 22.19, ASO 2.22, calcium bicarbonate (38%) 1.51, L-Lysine HCL 0.18) and CSO as % (corn 71.79, soybean meal 22.11, CSO 1.85, calcium bicarbonate (38%) 1.52, L-Lysine HCL 0.19, for both: dicalcium phosphate (18/21) 1.30, salt 0.30, mineral and vitamin 0.11, xantophyls 0.60 and coccidiostate 0.05. Experimental diets and water, were provided *ad libitum* during entire grow-out period. Data of broiler production variables were not reported.

Broiler processing

At the end of growing period, 42 d, four broilers per replication were randomly selected for slaughter according to Mexican Official Standard NOM-033-ZOO-1995 (Diario Oficial de la Federación [DOF], 2012 (Humane slaughter of domestic and wild animals). Broilers were not fed for a period of 8 h and slaughtered by severing jugular vein and carotid artery, bleeding for 3 min., scalding during 45 s in hot water (45 s, ~64 °C), defeathering, and manually eviscerating them. Carcasses were chilled in ice-water for 1 h, kept them in coolers with ice, and at 6 h *postmortem* breast meat was deboned. Skin and visible fat were removed from *Longissimus dorsi* and then meat was vacuum-packed and stored frozen (-20 °C) for ~1 month. One samples of eight chicken breast meat were obtained from each treatment. Meat was thawed (4 °C) for 24 h, then mixed and ground it (Mill, Model DPA 139, Moulinex, Mexico).

Reagents and standard solutions

Thymol (99.5%) and carvacrol (98%) standards (Catalog T0501 and W224502, Sigma-Aldrich, Mexico, Toluca); methylene chloride 99.9% (Catalog PQ06235, CH_2Cl_2, Fermont, Mexico), sodium chloride (Catalog 3624-01, NaCl, J. T. Baker, State of México, Toluca) and magnesium sulfate (Catalog M7506, $MgSO_4$, Sigma-Aldrich, Japan).

Two solutions, one of thymol and one of carvacrol were prepared (965 µg mL^{-1} and 976 µg mL^{-1}, respectively). Thymol was weighed on an analytical balance (Model AP210S, Ohaus®, Switzerland), and carvacrol was measured with a micro pipette (Hamilton®, USA,

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Reno Nevada). From these solutions, 100 µL were taken to 1 mL with CH_2Cl_2 (Kimax®, USA) and from them, 80 µL, 50 µL, 20 µL, 10 µL, 5 µL and 2 µL were also taken with CH_2Cl_2 to 100 µL, these solutions were used to obtain standard curves.

Meat thymol and carvacrol extraction

Meat was ground in a mill (Model DPA139, Moulinex, GSEB Mexican, State of Mexico) for a period of 20 s and mixed by gloved hands for 5 min; 10 g of meat were placed in a polypropylene tube with 10 mL of CH_2Cl_2. Solution was mixed for 2 min and allowed to stand for 30 min. Then, 1.5 g of $MgSO_4$ and 1 g of NaCl were added. Sample was shaken vigorously and centrifuged (Mod. 5804, Eppendorf®, USA, New York) at 4083 relative centrifugal force (rcf), for a period of 5 min. From the upper layer of sample, 6 mL were taken, then 0.9 g of $MgSO_4$ was added and solution was again centrifuged (4083 rcf). One mL of the upper layer was concentrated to 100 µL by nitrogen flow, and finally 1 µL was injected into gas chromatograph (GC).

Determination of thymol and carvacrol

To identify and determine thymol and carvacrol amount, a gas chromatograph was used (GC, Hewlett Packard P-6890, USA, California) coupled with a mass spectrometer (MS; 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 as well. Temperature of GC injection port was 150 °C, initial temperature of oven was 60 °C during 5 min, and was increased 20 °C per m to reach 200 °C. Helium was used as a carrier gas (Helio, Infra S.A. de C.V., and State of Mexico, Mexico). MS was operated in scan mode (range m/z: 30 – 550) electron ionization (70 eV). Temperatures of quadrupole, ion source and interface were 150 °C, 230 °C y 220 °C, respectively. One µL of sample was injected into GC by using splitless mode, and mass spectrum obtained were compared to NIST 2.0 (National Institute of Standards and Technology, USA) database. Ions were identified, as well as their retention times and their relative abundance.

Linearity and analytic interval

Four standard curves were obtained with injection of 1 µL of thymol and carvacrol at different concentrations. Two standard curves to determine amount of thymol (1.93 µg mL^{-1}, 4.82 µg mL^{-1}, 9.65 µg mL^{-1}, 19.30 µg mL^{-1}, 48.25 µg mL^{-1} and 77.20 µg mL^{-1}) and carvacrol (1.95 µg mL^{-1}, 4.88 µg mL^{-1}, 9.76 µg mL^{-1}, 19.52 µg mL^{-1}, 48.80 µg mL^{-1} y 78.08 µg mL^{-1}) in oregano oil, and two standard curves to determine amount of thymol (from 0.0193 µg mL^{-1} to 19.3000 µg mL^{-1}) and carvacrol (from 0.0195 µg mL^{-1} to 19.5200 µg mL^{-1}) in chicken breast meat were used.

Statistical analysis

Because of lack of normality, concentration of antioxidants in meat was expressed as percentage of maximum value obtained and these data were transformed with arcsine to be analyzed with Kolmogorov-Smirnov test (Statistical Analysis Systems, 2000). Results indicated a trend of normality for thymol and carvacrol (p = 0.064 and p = 0.078, respectively). The main factor oregano oil was significant (p < 0.05). Then, an analysis of variance was performed to test effects of oregano oil: implemented and no; thymol and carvacrol as response variables in meat. Additionally, four treatments (CSO and ASO with and without oregano oil supplementation) were compared using test of Tukey.

RESULTS

Major ions in thymol (91, 135 and 150) and carvacrol coincide with sample and standard at NIST library ions (figure 1 y 2). Retention time was 9.93 min for thymol and 10.07 min in carvacrol, principal ion was 135 and qualifier ions were 150, 91 y 115 for thymol and 150, 91 y 136 for carvacrol (table 1).

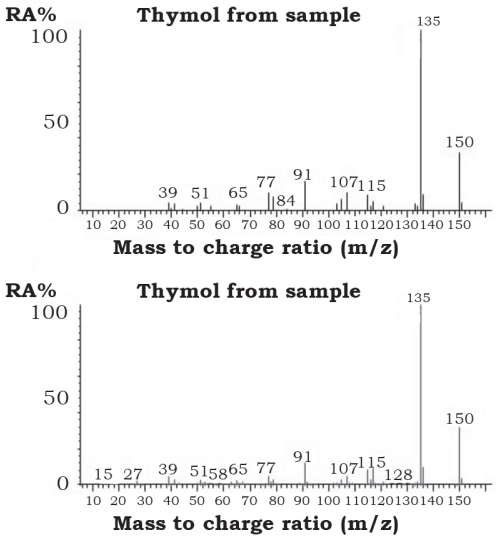

Figure 1. Thymol characteristic ions #22610, adapted from the library (National Institute of Standards and Technology, 2002). There was coincidence of at least three ions. RA: Relative abundance.
Source: Author's own elaboration.

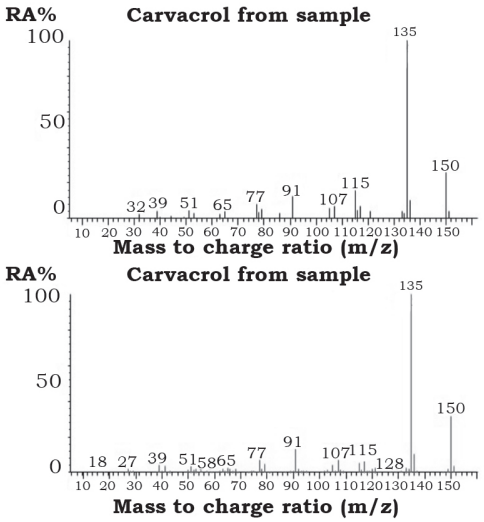

Figure 2. Carvacrol characteristic ions #22723, adapted from the library (National Institute of Standards and Technology, 2002). There was coincidence of at least three ions. RA: Relative abundance.
Source: Author's own elaboration.

Table 1
Characteristic ions used for quantification of thymol and carvacrol in standards and in chicken breast meat.

	RT (min)	Mass to Charge Ratio (m/z)	
Compound	**[SD, min]**	**Quantifier ion** (m/z) (RA, %)	**Qualifier ions** (m/z) (RA, %)
Thymol	9.93 [0.02]	135 (100)	150 (24), 91 (15.7), 115 (9.1)
Carvacrol	10.07 [0.03]	135 (100)	150 (31.4), 91 (13.1), 136 (10.2)

RT = Retention time in the gas chromatograph; SD = Standard deviation; RA = Relative abundance.
Source: Author's own elaboration.

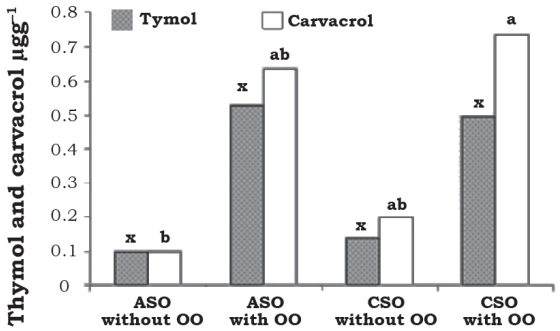

Figure 3. Means of thymol and carvacrol concentrations (µg g^{-1}) in chicken breast meat fed on a diet based on ASO or CSO supplemented or not with OO. There was no difference in thymol (x), a, b Means with different letter on the bar graphs are statistically different ($p < 0.05$).
Source: Author's own elaboration.

Equations used to analyze carvacrol and thymol concentration in oregano oil showed linearity (areas under curve as a function of µg mL^{-1} of thymol and carvacrol), R^2 coefficients were 0.999 and 0.994 respectively, their equations were $Y = 2.16 \times 10^6 X + 2.17 \times 10^7$ for carvacrol and $Y = 2.92 \times 10^6 X + 1.29 \times 10^7$ for thymol. Equations used to analyze oregano oil in chicken breast meat showed R2 of 0.987 for carvacrol and 0.970 for thymol, and their corresponding formula were $Y = 1.31 \times 10^7 X + 5.44 \times 10^6$ and $Y = 1.51 \times 10^7 X + 1.04 \times 10^7$, respectively. Content of thymol and carvacrol in oregano oil was 30.70 % and 9.36 % ($n = 6$).

Intervals of concentrations of thymol and carvacrol in chicken breast meat that received CSO were 0 µg g^{-1} to 0.9526 µg g^{-1} and 0 µg g^{-1} to 2.0122 µg g^{-1}, respectively. Oregano oil supplemented to CSO diet increased thymol and carvacrol levels in chicken breast meat ($p < 0.05$ and $p < 0.075$). Thymol and carvacrol increased from 0.1465^a to 0.4891^b and from 0.2002^a to 0.7340^b µg g^{-1}, which represent an increment of 333% and 366% respectively for each metabolite.

Intervals of thymol and carvacrol concentrations in chicken breast meat that received ASO were 0 µg g^{-1} to 1.8388 µg g^{-1} and 0 µg g^{-1} to 1.8867 µg g^{-1}, respectively. Oregano oil supplemented to ASO diet increased thymol and carvacrol levels in chicken breast meat ($p < 0.096$ and $p < 0.05$). Thymol and carvacrol increased from 0.0953a to 0.5263b and from 0.0979^a µg g^{-1} to 0.6345^b µg g^{-1}, which represent an increment of 552% and 648% respectively for each metabolite.

Diets with ASO showed low accumulation of thymol and carvacrol, however this accumulation increased when feed was supplemented with CSO and oregano oil ($p < 0.05$), respectively, compared with ASO oil without oregano oil (figure 3). Considering four diets (CSO and ASO with and without oregano oil) a significant effect of CSO with oregano oil on carvacrol concentrations was observed ($p < 0.05$) compared ASO without oregano oil (figure 3). However, neither effect of soybean oil or interaction of soybean oil with oregano oil were detected.

Amount of thymol in broiler meat fed with CSO and oregano oil in diet was slightly lower respect to those broilers fed with ASO and oregano oil (0.4591 µg g^{-1} *vs.*, 0.5263 µg g^{-1}). Amount of thymol in broiler meat fed with CSO without oregano oil was higher compared with those broilers fed with ASO without oregano oil (0.1765 µg g^{-1} *vs.*, 0.0953 µg g^{-1}). Amount of carvacrol in broiler meat fed with CSO and oregano oil in diet also was higher compared with those broilers with ASO and oregano oil (0.1765 µg g^{-1}, 0.3002 µg g^{-1} *vs.*,

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0.0953 μg g^{-1}, 0.0979 μg g^{-1}). In general, thymol and carvacrol increase in broilers meat when they fed with oregano oil.

DISCUSSION

The number of characteristic ions (quantifier and qualifier), their relative abundance, retention times and ratios of four mass to charge (m/z) signals indicated in table 1, figures 1 and 2, agrees with Méndez-Antolín, Balcinde-Quiñónez, González-Canavaciolo & Rodríguez-Cruz (2008). To validate characteristics of compounds assessed by GC-MS: (1) presence of at least three characteristic ions of mass spectrum, (2) chromatographic peak with the same retention time as standard of reference, (3) relative abundances of characteristic ions matching standard of reference, (4) more than three m/z signals and (5) blank should not present interference or peaks with same retention time as compounds of interest (Maštovská & Lehotay, 2003). In general, ions are alike in carvacrol and thymol because these are isomers and have the same mass.

Oregano oil used to contain 30.70 % of thymol and 9.36% of carvacrol that was similar to different Mexican oils analyzed by Ortega-Nieblas *et al.* (2011). Oregano oil reports in European countries indicate 67% of both compounds (Kulisic, Radonic, Katalinic & Milos, 2004). This abundance could be due to phenols are secondary metabolites, essentials for protection of plants, but their content varies depending on harvesting time and of the place they have been grown (Ortega-Nieblas *et al.*, 2011). For this reason, one objective of this research was to quantify these compounds that contain a hydroxyl group on their aromatic chemical structure (Windholz, Budavari & Blumetti, 1983), which gives to them their antioxidant capacity (Giannenas *et al.*, 2005; Kulisic *et al.*, 2004).

Thymol and carvacrol were detected in chicken breast meat samples without receiving oregano oil in the diet. Their presence in meat was produced by yellow corn used to produce feed (Cabrera-Soto, Salinas-Moreno, Velázquez-Cardelas, & Espinosa-Trujillo, 2009; Dykes, Rooney, Waniska & Rooney, 2005). Phenols in broilers diet are absorbed and deposited in cell membranes of meat. This process has been evaluated indirectly as oxidative stability tests in meat of broilers that were fed both compounds and compared with meat of broilers that did not receive them through feed (Botsoglou *et al.*, 2002a; Luna *et al.*, 2010; Marcinčák *et al.*, 2008). Amount of thymol and carvacrol in meat increased with oregano oil supplementation into feed (100 mg kg^{-1}). In this research, only breast meat was used to measure both compounds, but their accumulation could be higher in liver, brain, kidney, thigh, and abdominal fat or skin, because these body tissues contain more fatty acids (Bjørneboe, Bjørneboe & Drevon, 1990; Botsoglou, Florou-Paneri, Christaki, Fletouris & Spais, 2002b; Luna *et al.*, 2010).

It has been reported that free fatty acids amount contained in acidulated soybean oil (ASO) can explain poor accumulation of thymol in muscle fibers of meat. Mixed micelles formed in broilers' duodenum can be affected amount of free fatty acids in medium, decreasing intestinal absorption of various compounds added to diet, an example can be oregano oil (Bjørneboe *et al.*, 1990; Wiseman & Salvador, 1991).

A higher concentration of thymol and carvacrol was observed by indirectly method in meat of broilers fed with CSO, when they were compared to those broilers fed with ASO. Probably, decreasing in antioxidants effect was caused by low accumulation of antioxidants in meat, ASO is very variable and it can decrease meat quality (Ávila-Ramos *et al.*, 2012; Tavárez *et al.*, 2011; Yanishlieva *et al.*, 1999). This research indicated that oregano oil can be accumulated in high concentration in form of thymol and carvacrol in meat, increasing more than 250% compared to control diets without oregano oil supplementation ($p < 0.05$).

CONCLUSIONS

Thymol and carvacrol appear in broilers breast meat but in large quantities when diet is supplemented with oregano oil. Meat accumulates more carvacrol when diet is supplemented with crude soybean oil compared with acidulated soybean oil without oregano oil.

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