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Oregano essential oil (*Origanum vulgare*) to feed laying hens and its effects on animal health

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Abstract: This study evaluated the effect of oregano essential oil added to the feed of commercial laying hens. This research was focused on the analysis of biochemical changes linked to hepatic function, and protein and lipid metabolism. It was used 240 laying hens (59 weeks-old) distributed in a completely randomized design of six treatments (five repetitions with eight birds each). The experiments were constituted by a control treatment (CT) with the inclusion of zinc bacitracin and five treatments of oregano essential oil (OEO: 0, 50, 100, 150, and 200 mg.kg-1), respectively. After 28 days of feeding, an increase on serum levels of total proteins and globulins was observed on groups T150 and T200, as well as an increase on albumin levels on group CT. After 84 days of feeding, a significant reduction on total proteins and albumin was observed on group T200, as well as an increase in serum triglycerides. OEO at 200 mg.kg-1 increased globulin levels on day 28, which may be considered an effect in the inflammatory response, which increases serum immunoglobulins and proteins.

Key words: albumin, cholesterol, globulin, total proteins, triglycerides, health.

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INTRODUCTION

The search of new alternatives for feed supplementation in poultry production in order to improve animal health, and quantitative and qualitative productivity is considered one of the main priorities (Gerzilov et al. 2015). Essential oils are a mixture of several compounds of herbal origin, that can be used for animal feeding to improve its performance and health (Roofchaee et al. 2011). Oregano essential oil when used in the diet may exert antioxidant property since it has two important phenol compounds, corresponding to 78-85% of the oil composition, i.e., carvacrol (2-methyl-5-isopropyiphenol) and thymol (2-isopropyl-5-methylphenol) (Basmacioglu Malayoğlu et al. 2010). According to Botsoglou et al. (2002), these compounds enter the circulatory system, being distributed to muscles and other tissues. Moreover, this oil possesses antimicrobial activity, acting in the reduction of undesirable intestinal microflora, which favors the absorption of nutrients (Bampidis et al. 2005).

Essential oils can act as stimulant agents of the immune system during acute or chronic inflammatory processes that can be characterized by an increase on the levels of serum globulins (Rosa Neto and Carvalho 2009), which can express the metabolic and nutritional status of the animal (Zhu et al. 2014). Moreover, essential oils may improve nutrient digestion and absorption by enzymatic stimulation and they also may exert positive effects when used in laying hens. It is important to emphasize that the response of essential oils as supplements added in the feed depends on the level, composition, and the 44 combination of their compounds (Zhang et al. 2005).

It is known that the addition of thymol in the diet of chickens reduces the serum levels of cholesterol (Case et al. 1995), without any effect on laying hens (Abdel-Wareth 2016). The action of this compound on cholesterol synthesis is linked with the inhibition on the production of 3-hydroxy-3- methylglutaryl coenzyme A (HMG-CoA) reductase, a regulatory enzyme involved in the synthesis of cholesterol (Bampidis et al. 2005). The absence or presence of this effect depends on several factors, such as race, lineage, age and feed composition (Lee et al. 2003a). In addition, evidences suggest that thymol protects the intestinal microvilli responsible for the absorption of nutrients, influencing positively the secretion of endogenous digestible enzymes and influencing blood constituents (Hashemipour et al. 2013). A study revealed that carvacrol was more efficient to reduce plasmatic triglycerides compared to thymol (Lee et al. 2003b). Moreover, high supplementation of 56 oregano essential oil causes an increase on the serum levels of cholesterol in chickens (Basmacioglu Malayoğlu et al. 2010), suggesting that optimal concentrations of these lipids may have the same effect in laying hens, which is the main objective of this study. Based on these evidences, the aim of this study was to evaluate whether the addition of oregano essential oil in different concentrations in the feed of laying hens causes a positive effect on hepatic function, as well as on lipid and protein metabolism of commercial laying hens.

MATERIALS AND METHODS

All procedures were carried out according to the international practices for animal use and care, approved by the Committee of Ethics on Animal Experimentation, from the Santa Catarina State University, protocol no. 1.39.15.

BIRD HUSBANDRY

A total of 240 commercial Hy-Line Brown laying hens, 59 weeks-old with average body weight of 1890 ± 5g were used in this study. The birds were allocated in an experimental house type Californian equipped with galvanized wire cages (100x40x45 cm). The birds received water ad libitum by nipple drinkers and 16 h of light. The experiment lasted 84 days, divided into three cycles of 28 days.

EXPERIMENTAL DESIGN AND ANIMAL DIETS

The hens were randomly assigned to six treatment groups with five replicates of eight hens. The basal diet (Table I) was formulated in accordance with Rostagno et al. (2011), with the inclusion (2%) of a mineral and vitamin commercial blend. The control treatment (CT) consisted of a basal diet with a performance improver (30 mg of zinc bacitracin per kg of feed). Five treatment groups named (T0, T50, T100, T150 and T200) were used without bacitracin but supplemented with five concentrations of OEO (0, 50, 100, 150 and 200 $mg.kg^{-1}$), respectively. The OEO was diluted in soy oil and subsequently the mixture was mixed with ground corn in a vertical mixer.

OEO EXTRACTION AND CHARACTERIZATION

The OEO was extracted from a dehydrated *Origanum vulgare* of Chilean origin by the steam distillation method. The herbal sample was placed in an extraction flask and the distillation was kept for 2 hours. The average of the extraction yield was 0.8%. The OEO characterization was performed using a gas chromatographer Varian Star 3400CX (CA, EUA) equipped with a flame ionization detection (GC-FID). The qualitative analyses of oil compounds were performed using a gas chromatograph Shimadzu QP2010 Plus coupled to a mass spectrometer (GC/MS, Shimadzu 91 Corporation, Kyoto, Japan). The analyses revealed different compounds where five of them represented 54.56% (Figure 1).

SAMPLING

At the end of each cycle (days 28, 56 and 84 of the experiment) two hens per cage (10 hens/treatment) were randomly selected for blood sampling. Blood was collected from the branchial vein into non-heparinized tubes and immediately centrifuged (3500 rpm x 10 min) to obtain serum, which was stored at -20 ºC for further analyses.

The serum levels of total proteins (g/dL), albumin (g/dL), glolubins (g/dL), triglycerides (mg/dL), cholesterol (mg/dL), uric acid (mg/dL), alanine aminotransferase (ALT) (U/L) and alkaline phosphatase (ALP) (U/L) were evaluated in a semi-automated biochemical analyzer (BioPlus Bio-2000) using commercial kits. Globulins values were calculated by the difference between total serum proteins and albumin.

TABLE I
Percentual and calculated composition of experimental ration used to the treatment of laying hens.

Ingredients	Composition (%)
Maize	65.95
Soybean meal	21.75
Soy oil	1.10
Limestone	9.20
Vitamin and Mineral Core *	2.00
Total	100
Calculated composition	
Crude protein (%)	15.60
Metabolic energy(kcal/kg)	2.850
Available phosphorus (%)	0.275
Calcium (%)	3.85
Sodium (%)	0.211
Digestible lysine (%)	0.684
Digestible methionine + cysteine (%)	0.622
Digestible methionine (%)	0.342
Digestible threonine (%)	0.520

* Product composition (kg): folic acid 54,00 mg, nicotinic acid 1.000,00 mg, pantothenic acid 680,00 mg, biotin 2,70 mg, calcium 80,00/160,00 g, cobalt 27,0 mg, cooper 6.000,00 mg, choline 10,00 g, iron 5.000,00 mg, phytase 20,00 ftu, fluorine 650,00 mg, phosphorus 42,00 g, iodine 40,00 mg, manganese 2.500,00 mg, mineral matter 900,00 g, methionine 38,00 g, selenium 10,00 mg, sodium 95,00 g, humidity 120,00 g, vitamin A 374.000,00 UI, vitamin B1 40,00 mg, vitamin B12 1.000,00 mcg, vitamin B2 200,00 mg, vitamin B6 54,00 mg, vitamin D3 75.000,00 UI, vitamin E 1.500,00 UI, vitamin K 100,00 mg and zinc 4.000,00 mg.

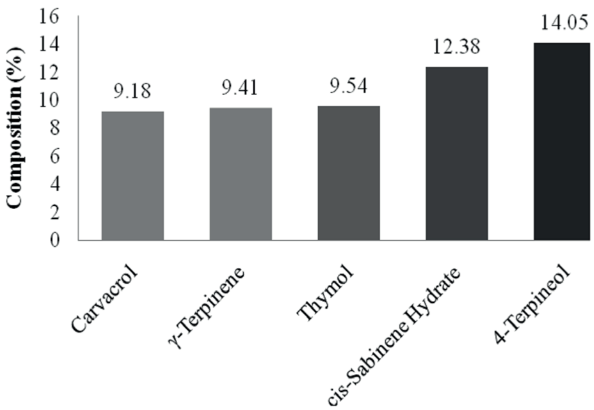

Figure 1 - Composition of *Origanum vulgare* essential oil (oregano) obtained by gas chromatograph.

STATISTICAL ANALYSIS

At first, the experimental data were analyzed descriptively, and it was calculated the measure of central tendency (mean) and data of dispersion (standard deviation). Moreover, all variables were submitted to the Shapiro Wilk test to verify if the data were normally distributed. The data was log transformed to meet the assumptions of normality before the application of analyses of variance (ANOVA). The comparison of the means between groups on each observation (days 28, 56 and 84) for all evaluated biochemical variables, followed by Tukey post hoc test ($p< 0.05$) were carried out with the statistical software R, v.2.15.2 Development Core Team, 2012.

RESULTS

The percentage of carvacrol, γ-terpinene, thymol, cis-sabinene hydrate and 4-terpineol, which together add up to approximately 45% of the volatile compounds (54.56%) in the *Origanum vulgare* essential oil (Figure 1). The values are close to those recommended by Teixeira et al. (2013) and it is important to mention that the composition of the oil depends on many factors such as soil fertility, region and variety of the plant, among others.

The results showed significant differences on total proteins, albumin, globulins and triglyceride levels for the treatments as shown in Tables II and III, in which total protein level had a significant difference for all three sampling days. A significant increase ($p=0.002$) on total protein was observed on groups T150 and T200 compared to other groups (Table II) on day 28, while on day 56 only the group T50 showed an increase on total proteins compared to groups CT and T0. On day 84, the animals from group T100 showed increased levels of serum total proteins, while the group T200 showed a reduction when compared to CT, T0, T50 and T150 groups.

Regarding the levels of serum albumin (Table II), there were significant differences on days 28 and 84. On day 28, the hens of group CT showed higher ($p=0.02$) levels than those of the group T150, while on day 84 the group T0 showed higher ($p=0.04$) levels than the group T100. The group T200 showed an increase on globulin concentrations compared to groups CT, T0 and T50, as well as groups T100 and 150 showed an increase when compared to groups CT and T50.

The serum triglyceride levels (Table III) differ ($p<0.001$) for all studied groups only on day 84, wherein the group T200 showed an increase compared to groups CT, T50, and T100. No differences were observed regarding cholesterol, uric acid, ALT and ALP levels ($p>0.05$) (Tables III and IV).

DISCUSSION

The serum biochemical analyses of hens revealed that higher doses of OEO caused changes in the metabolism of proteins by increasing serum levels of globulins, in accordance to results reported by Alp et al. (2012). These authors detected an increase on globulin levels of broilers fed with 300 $mg.kg^{-1}$ of OEO.

Albumin and globulin represent the most important serum proteins, exerting important physiological functions, such as transportation

TABLE II
Seric levels of total proteins, albumin and globulin on days 28, 56 and 84 post-treatment.

Variables		Mean ± DP						p value
		CT	T0	T50	T100	T150	T200	
Total proteins (g/dl)	Day 28	7.18^{b} (±0.77)	7.52^{b} (±1.43)	6.98^{b} (±0.61)	7.78^{b} (±1.63)	10.78^{a} (±3.64)	11.32^{a} (±2.89)	0.002*
	Day 56	6.22^{b} (±0.53)	6.38^{b} (±0.78)	7.68^{a} (±0.92)	7.17^{ab} (±0.89)	7.02^{ab} (±0.25)	7.07^{ab} (±0.86)	0.020*
	Day 84	6.33^{b} (±0.63)	6.60^{b} (±0.64)	5.35^{b} (±0.34)	6.67^{a} (±1.33)	5.65^{b} (±0.32)	5.30^{c} (±0.60)	0.003*
Albumin (g/dl)	Day 28	2.70^{a} (±0.43)	2.35^{ab} (±0.87)	1.97^{ab} (±0.14)	2.02^{ab} (±0.18)	1.82^{b} (±0.38)	2.05^{ab} (0.45)	0.020*
	Day 56	1.92 (±0.54)	1.90 (±0.51)	2.13 (±0.24)	1.62 (±0.59)	2.18 (±0.50)	2.15 (±0.14)	0.250^{ns}
	Day 84	1.87^{ab} (±0.10)	2.07^{a} (±0.12)	1.87^{ab} (±0.36)	1.65^{b} (±0.28)	1.73^{ab} (±0.16)	1.87^{ab} (±0.12)	0.040*
Globulin (g/dl)	Day 28	4.32^{c} (±0.51)	5.17^{b} (±1.54)	5.02^{c} (±0.69)	5.77^{ab} (±1.70)	8.97^{ab} (±3.72)	9.27^{a} (±3.26)	0.001*
	Day 56	4.30 (±0.99)	4.48 (±1.14)	5.55 (±1.04)	5.55 (±1.16)	4.83 (±0.54)	4.92 (±0.79)	0.150^{ns}
	Day 84	4.47 (±0.59)	4.53 (±0.67)	3.48 (±0.65)	5.02 (±1.37)	3.92 (±0.34)	3.43 (±0.55)	0.050^{ns}

$^{a, b, c}$ Different letters in the same line differs statistically between groups by Tukey post hoc test (5%). Standard deviation (DP), non-significative (^{NS}); Group CT: control treated with 30 mg of zinc bacitracin; Group T0: 0% of oregano essential oil (OEO); group T50: 50 mg/kg^{-1} of OEO; group T100: 100 mg/kg^{-1} of OEO; Group T150: 150 mg/kg^{-1} of OEO; Group T200: 200 mg/kg^{-1} of OEO. * $p<0.05$.

of substances (hormones, vitamins, minerals and lipids), maintenance of plasmatic osmotic pressure and immunity (Polat et al. 2011). The increase on total protein levels, as well as on globulin levels in hens treated with OEO may be attributed to a direct influence on proteic metabolism linked to the immune system (Zhu et al. 2014).

According to Moomivand et al. (2015), the compounds present in essential oils can stimulate the synthesis of proteins and the immune system, protecting the cells against oxidation. These effects were observed by Tollba et al. (2010) using a mixture of 1.0 and 2.0 g/kg of oils (thyme, oregano, cinnamon and pepper), with a simultaneous increase on albumin and globulin levels and a reduction of lipid content in chickens. According to these authors, increased levels of globulins is linked to the immunostimulant effects, important to hens immunity (Ghazalah and Ali 2008), i.e., this improvement is linked to a possible response to inflammatory process.

The basal serum levels of total protein in hens varies from 3.0 to 6.0 mg/dL, and values above 6.0 mg/dL, such as observed in this present study, may occur due to dehydration or increased levels of total globulins, hyperglobulinemia associated with diseases or chronic bacterial infections. However, in female hens, the concentration of total proteins increases before the oviposition, which may be attributed to estrogen induction in the ovary, raising globulin levels (Hasegawa et al. 2002). In this study, the increase on serum levels of total proteins

TABLE III
Seric levels of triglycerides, cholesterols and uric acid (mg/dL) on days 28, 56 and 84 post-treatment.

Variable		Mean ± DP						p value
		CT	T0	T50	T100	T150	T200	
Triglycerides (mg/dl)	Day 28	998.33 (±158.16)	1089.83 (±188.94)	992 (±118.98)	1069.33 (±220.44)	1049 (±56.85)	995.83 (±237.84)	0.870ns
	Day 56	879.17 (±341.68)	878.00 (±210.65)	916.83 (±171.15)	723.17 (±320.22)	874.17 (±153.93)	834.17 (±183.20)	0.790ns
	Day 84	986.17b (±151.42)	1097.67ab (±308.19)	708.17c (±134.56)	684.67c (±119.55)	987ab (±101.89)	1340.83a (±236.47)	0.001*
Cholesterol (mg/dl)	Day 28	97.33 (±14.15)	103.50 (±34.55)	96.83 (±31.75)	102.67 (±29.99)	96.83 (±24.70)	103.33 (±29.62)	0.990ns
	Day 56	89.50 (±21.58)	82.17 (±19.36)	115.67 (±43.29)	102.50 (±40.27)	119.17 (±36.41)	113.83 (±42.07)	0.370ns
	Day 84	85.33 (±35.56)	100.50 (±35.57)	115.17 (±36.50)	116.67 (±36.52)	92.50 (±19.46)	80.00 (±20.67)	0.440ns
Uric acid (mg/dl)	Day 28	5.53 (±1.30)	5.88 (±1.48)	4.93 (±1.01)	4.18 (±1.78)	6.00 (±1.84)	5.38 (±1.11)	0.290ns
	Day 56	5.67 (±1.60)	4.22 (±1.25)	4.97 (±0.96)	5.73 (±1.70)	5.70 (±2.15)	3.85 (±1.35)	0.160ns
	Day 84	8.93 (±2.99)	6.82 (±1.95)	6.87 (±2.21)	7.20 (±1.40)	8.03 (±2.01)	7.18 (±1.61)	0.470ns

[a, b, c] Different letters in the same line differs statistically between groups by Tukey post hoc test (5%). Standard deviation (DP), non-significative ([NS]); Group CT: control treated with 30 mg of zinc bacitracin; Group T0: 0% of oregano essential oil (OEO); group T50: 50 mg/kg^{-1} of OEO; group T100: 100 mg/kg^{-1} of OEO; Group T150: 150 mg/kg^{-1} of OEO; Group T200: 200 mg/kg^{-1} of OEO. * $p<0.05$.

may be partially explained by possible hormonal effects associated to the immunostimulatory effect of OEO, increasing globulin levels.

Albumin is a protein responsible for the transport of fatty acids, minerals, uric acid, vitamins and hormones (Maciel et al. 2007). According to Traesel et al. (2011b), the reduction of serum total proteins (albumin and globulin) associated to increased levels of triglycerides suggest hepatic insufficiency (but not hepatic disease), since albumin synthesis occurs only in the liver.

The increase on serum levels of triglycerides on day 84 was observed in hens that received 200 mg of OEO per kg of feed, similarly to the results observed by Bolukbasi et al. (2006) in broilers supplemented with thyme essential oil. According to these authors, this fact occurs due to an increase on the metabolism of proteins, fat and carbohydrates, associated to an increase on the concentration of triglycerides (Sirvydis et al. 2003). According to Traesel et al. (2011a), the essential oils can produce some toxic effects when administered in higher doses. Thus, it is necessary to establish safety levels in hens in order to avoid health issues.

In a study conducted by Toghyani et al. (2017), it was observed an increase on serum triglyceride levels in hens fed *ad libitum*. This occurs due to an

TABLE IV
Seric levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) on days 28, 56 and 84 post-treatment.

Variable		Mean ± DP						p value
		CT	T0	T50	T100	T150	T200	
ALT(U/L)	Day 28	13.73 (±4.96)	12.33 (±5.82)	14.67 (±6.31)	12.33 (±3.61)	16.17 (±10.85)	13.83 (±7.68)	0.190[ns]
	Day 56	11.83 (±2.56)	10.50 (±0.84)	9.33 (±3.20)	10.17 (±4.12)	8.50 (±3.56)	8.00 (±3.63)	0.340[ns]
	Day 84	8.50 (±3.62)	8.50 (±2.66)	8.33 (±5.32)	8.50 (±3.33)	8.17 (±2.56)	7.83 (±2.71)	0.990[ns]
ALP(U/L)	Day 28	178.50 (±70.94)	255.00 (±79.63)	248.50 (±98.32)	215.00 (±115.49)	247.50 (±115.89)	213.83 (±34.45)	0.670[ns]
	Day 56	193.17 (±72.73)	142.00 (±25.50)	124.00 (±53.58)	179.50 (±78.31)	187.33 (±68.51)	170.00 (±56.75)	0.330[ns]
	Day 84	180.50 (±47.09)	142.00 (±44.69)	165.33 (±71.60)	175.17 (±56.87)	199.83 (±78.01)	203.67 (±78.90)	0.590[ns]

Standard deviation (DP), non-significative ([NS]); Group CT: control treated with 30 mg of zinc bacitracin; Group T0: 0% of oregano essential oil (OEO); group T50: 50 mg/kg^{-1} of OEO; group T100: 100 mg/kg^{-1} of OEO; Group T150: 150 mg/kg^{-1} of OEO; Group T200: 200 mg/kg^{-1} of OEO. $p<0.05$.

increase on very low-density lipoprotein (VLDL) in serum, the main triglyceride transporter. It may be considered an indicator of changes in hepatic secretion of VLDL and hepatic lipogeneses (synthesis of triglycerides and fatty acids). Other explanation might be linked to the catabolism of fatty acids and regulation of energetic metabolism, which affect directly physiological responses.

According to Melo et al. (2016), the addition of new elements to feed may cause an increase on the mobilization of triglycerides from tissues to blood flow. Concentrations of triglycerides and cholesterol in hens that received feed supplementation of natural additives may be linked to antioxidant and antibacterial properties (Gálik et al. 2015). Some factors contribute to these results, such as age, sex, type of hens, nutritional and physiological status, 189 as well as differences on oil composition (Moomivand et al. 2015, Akbari and Mehran 2014). However, this increase on the levels of triglycerides in hens is not considered a negative effect on their health.

Bampidis et al. (2005) observed the absence of differences regarding the plasmatic cholesterol levels in turkeys fed with dried oregano leaves, similarly to the results observed in this study. Moreover, diets used for broilers containing carvacrol and thymol (Lee et al. 2003b) and used for laying hens with a mixture of essential oils (Bozkurt et al. 2012) caused no differences on cholesterols levels. This non-significant effect may be associated to OEO components that were ineffective in the inhibition of the enzyme 3-hidroxi-3-methyl-glutaril198 CoA reductase (HMG-CoA reductase), which is a limitant on cholesterol synthesis (Khattak et al. 2014).

A study conducted by Basmacioglu Malayoğlu et al. (2010) reported that thymol and carvacrol may exhibit hypocholesterolemic effects by the inhibition of HMG-CoA reductase. Moreover, a reduction on lipid content and total cholesterol levels in a diet containing rosemary oil for broilers can be attributed to thymol and carvacrol compounds (Polat et al. 2011). However, despite the presence of thymol and carvacrol on OEO used in this study, decreased levels of serum triglycerides was not observed, probably due to the amount of OEO used in this study.

No differences regarding serum uric acid levels, as well as on ALT and alkaline phosphatase levels were observed for the studied groups. Since uric acid is synthetized in the liver and kidneys, renal changes can increase the serum or plasmatic concentration of uric acid in laying hens (Schmidt et al. 2007), being involved in antioxidants functions, and is the major product of purine catabolism. According to Zhu et al. (2014), hepatic damages or increased permeability of hepatic cells increased the serum ALT levels. This increase was not observed in the present study. The alkaline phosphatase (ALP) activity produced by many organs, such as liver, is an important marker of bone metabolism, and the age of poultry may increase or decrease its activity (Zhu et al. 2014). The increase on serum biomarkers of hepatic function is correlated to hepatic oxidative lesions, and serum ALT and ALP activities are considerable sensible indicators of hepatic cellular lesion (Botsoglou et al. 2008). The intestinal ALP showed important functions, such as regulating the absorption of lipids by the intestine, and a significant reduction on ALP activity in chickens fed with a diet containing oregano was linked with improved lipid digestibility (Levkut et al. 2011). The activity of ALP did not differ for the investigated treatments, therefore, OEO treatment did not cause hepatic injury. One of the main problem linked with the use of essential oils in the diet is the component variation, leading to no effect in some moments (Basmacioglu Malayoğlu et al. 2010).

Based on these results, it is noted that the use of 200 mg of OEO/kg has an immunostimulatory effect on the first phase of the production cycle, but it caused no increased on globulins levels. Later, the use of OEO increased serum levels of triglycerides. In summary, it is concluded that the treatment with OEO exerts beneficial effects on bird health without any side effect. In addition, there is a need of more studies to evaluate wheather OEO may enhance egg quantity and quality.

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