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Oregano essential oil in the diet of laying hens in winter reduces lipid peroxidation in yolks and increases shelf life in eggs

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ABSTRACT. We evaluated the effects of oregano essential oil (OEO) added to the feed of semi-heavy laying hens during winter. We measured performance as well as physical and chemical quality of fresh and 21-day stored eggs. A total of 240 semi-heavy laying hens were distributed into six treatments and five replicates (n = 8 each). Treatments consisted of five groups of hens fed diets supplemented with 0, 50, 100, 150 and 200 mg OEO/kg. We measured the average of three productive cycles (1^{st}: week 1–4, 2^{nd}: week 5–8 and 3^{rd}: week 9–12), and found that feed consumption increased in hens in the control group and those in the group treated with 200 mg OEO/kg; other zootechnical variables did not differ between treatments. When we analyzed each individual production cycle individually, we did not observe differences between treatments for the performance variables in the first or second cycles. However, in the third cycle, when the animals were exposed to a greater number of days to cold stress, we recorded improved conversion rate/dozen eggs, conversion rate/daily feed consumed and egg production at T50 (50 mg OEO/kg). Stored eggs from hens supplemented with 50 mg OEO/kg showed lower eggshell percentages and higher yolk pHs. The intensity of the yellow was higher in yolks of the control group and in those from hens supplemented with 200 mg OEO/kg. Lipid peroxidation was lower in fresh egg yolks from hens that received 200 mg OEO/kg and stored eggs of T150. The reduction of lipid peroxidation in egg yolk is beneficial to consumer health by reducing levels of free radicals consumed. Reduction of lipid peroxidation associated with 150 mg OEO/kg in laying hens in winter might be useful for maintaining egg quality and for prolonging shelf life; productive efficiency was improving even at 39.8 mg OEO/kg if we consider feed conversion (kg/kg).

Keywords: herbal extract; *Origanum vulgare;* shelf life; egg quality; thermal stress.

1. INTRODUCTION

In Brazil, eggs are usually stored without refrigeration. This practice affects egg quality because the yolk is highly susceptible to lipid oxidation (Hayat et al., 2010). The diet composition of laying hens directly affects oxidative stability of the egg. Dietary supplementation with antioxidants might improve egg oxidative stability (Yesilbag et al., 2013). In this regard, the use of natural products such as plant extracts have been considered a valuable alternative to antibiotics used as feed additives (Akyurek and Yel, 2011).

Essential oils are natural volatile compounds formed as secondary metabolites by aromatic plants (Bakkali and Idaomar, 2008). These compounds improve food products derived from animals because of antioxidant effects related to hydroxyl groups (Yesilbag et al., 2013). The reaction of free radicals with fatty acids initiates a process known as peroxidation in living systems and oxidative rancidity in foods (Silva et al., 1999). Particularly in eggs, these processes are often accelerated by variations of room temperature. Malondialdehyde (MDA) is widely used to evaluate lipid oxidation in foods, in particular oxidative stress in biological samples (Lima and Abdalla, 2001).

Various combinations of essential oils of oregano, thyme, rosemary and *Curcuma longa* have been studied as supplements in diets of laying hens, aiming to improve their performance and oxidative stability of their eggs (Nadia et al., 2008). Among these compounds, oregano (*Origanum vulgare*) is considered an important source of antimicrobial and antioxidant molecules (Arpášová et al., 2014), with desirable biological properties that improve the health of laying hens.

Recently, researchers found that oregano essential oil (OEO) showed protective effects against heat stress in broilers (Tekce and Gül, 2017). In 2010, a study reported that aromatic herbal extract alone or its blend with organic acids could be used in diets of chicks to improve their production parameters, physiological performance and immune responses in conditions of cold temperatures (Tollba and Shabaan, 2010). Therefore, there is strong evidence that feed

supplementation with oils and herbaceous extracts such as those derived from oregano can improve performance and health in thermal stress conditions. OEO would then be a desirable ingredient in laying hens under cold stress. According to the literature, because hens have reduced feed intake capacity, they are not able to access the metabolic energy required to maintain body temperature and egg production, resulting in economic losses (Alves et al., 2012). It is important to identify agents that will minimize the negative effects of cold, because most poultry house do not have air conditioning and birds suffer from cold stress. Therefore, the objective of this study was to determine whether OEO in experimental diets of laying hens during winter would improve performance as well as physical, and chemical quality of fresh eggs and eggs stored for 21 days at room temperature.

2. MATERIAL AND METHODS

2.1. Animals

A total of 240 commercial semi-heavy laying hens (59-week-old) were allocated in galvanized wire cages (50 x 50 x 40 cm) of an experimental poultry house without air conditioning and received water and feed *ad libitum*. The experiment lasted 84 days, subdivided into three cycles of 28 days each. Each cycle productive was composed of four weeks (1st cycle: week 1–4, 2nd cycle: week 5–8, and 3rd cycle: week 9–12).

The experiment was carried out in southern Brazil, and during the experiment, the temperature oscillated throughout the day, reaching negative temperatures (lower than 0 ºC) on some days of experiment. The maximum and minimum temperature as well as relative humidity of the experimental period are presented in Figure 1.

2.2. Experimental design

The hens were distributed in a completely randomized design of six treatments and five repetitions with eight birds per cage. The basal diet (Table 1) was formulated based on the

nutritional values and in accordance with the requirements established by the Brazilian Poultry and Pork Tables (Rostagno and Becker 2017), with the inclusion of a commercial vitamin and mineral blend (2%). The treatments consisted of a control treatment (CT) consisting of basal diet with a performance improver (30 mg of zinc bacitracin/kg of feed) and five treatments with basal diet without bacitracin, supplemented with five levels of OEO (0, 50, 100, 150 and 200 mg/kg, respectively). OEO was diluted in soy oil and subsequently the mixture was mixed with ground corn in a vertical mixer (500 kg). The animals received 16 h of light daily throughout the experiment.

2.3. Production and characterization of oregano oil

Oregano leaves were purchased from a wholesaler located in São Paulo, Brazil. The OEO was extracted from dehydrated *O. vulgare* using steam distillation methodology. The material was placed in an extraction flask and the distillation was maintained for 2 hours. The average yield of the extraction was 0.8%. OEO characterization was performed using gas chromatograph Varian Star model 3400 CX (CA, EUA) equipped with a flame ionization detection (GC-FID), in addition to qualitative analyses of the compounds by gas chromatograph Shimadzu model QP2010 Plus coupled with a mass spectrometer (GC/MS, Shimadzu Corporation, Kyoto, Japan). The analyses revealed the existence of 35 compounds present in the OEO, with five representing the majority (54.56%), as shown in Table 2.

2.4. Zootechnical performance

During each period of the experiment (28 days), daily feed average consumption for each hen was monitored (g/bird/day). The daily number of eggs was also registered and the average performance of the hens after each period was estimated (%). Feed conversion was evaluated as kg of feed per dozen eggs and as kg of feed per kg of egg. Eggs were weighed in the last three

130 days of each period and the daily average egg mass was estimated (g/bird/day). The number of
131 dead hens was used to evaluate viability (%).
132
133 **2.5. Egg quality**
134 To evaluate egg quality, a sample composed of four eggs for each group was used. Two
135 of these eggs were used immediately after collection (fresh eggs) and two eggs were stored in a
136 cellulose tray at room temperature (25 ± 2 °C) for 21 days. Specific gravity of the eggs was
137 determined according to Freitas et al. (2004). Eggshell strength (kgf) was measured with a
138 texture analyzer (TA.XT plus) coupled to a specific probe (SMS P 75). Albumen height was
139 measured using a tripod Micrometer. Haugh units (HU) were calculated from albumen height
140 and egg weight according to the following equation (Haugh, 1937): $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$, where H is albumen height (mm) and W is egg weight (g).
142 The yolk index (YI) was calculated as the ratio between yolk height (mm) and diameter
143 (mm), measured using a digital pachymeter. Yolk color index was determined using a DSM
144 color fan and a colorimeter (Minolta CR-400) that provided the parameters of luminosity (L*),
145 red intensity (a*) and yellow intensity (b*). Yolks were separated from the albumen. The
146 eggshells were washed and dried at room temperature for 48 hours. After drying, they were
147 weighed and yolk, albumen and eggshell percentages were determined. Yolk and albumen pH
148 were measured using a digital pHmeter (Testo 205).
149
150 **2.6. Lipid peroxidation**
151 Lipid peroxidation was determined using a spectrophotometer (Giampietro et al. 2008) by
152 measuring thiobarbituric acid reactive substances (TBARS) at 532 nm, formed during the
153 decomposition of lipid peroxides. The compound 1,1,3,3 tetramethoxypropane (TMP) was used
154 as a TBARS standard. Results were expressed as mg TMP/kg of yolk.
155

2.7. Statistical analysis

The data showed normal distribution. Data were subjected to analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) using SAS (Statistical Analysis System). Then, significant data were subjected to regression analysis to identify the best OEO concentration for laying hens (0, 50, 100, 150 and 200 mg/kg); the control treatment (TC: 30 mg of zinc bacitracin in diet) was not included these regression analyses.

3. RESULTS

3.1. Zootechnical performance

We considered the average of three productive cycles, and found that laying hens in the control treatment (TC) with zinc bacitracin and those fed 200 mg/kg of OEO (T200) had higher feed intake than those fed treatments T0 and T150 (Table 3). Nevertheless, when the regression analysis was performed, we did not find effect of OEO levels on feed consumption in the three productive cycles ($P = 0.095$). Significant differences were not observed between treatments for the other performance parameters (conversion rate/dozen eggs, conversion rate/daily feed consumed, egg production and mass). There were no significant differences between the treatments in terms of viability.

When we analyzed each individual production cycle, we did not observe differences between treatments for the performance variables of the first and second cycles (Table 3). However, in the third cycle, when the animals were exposed to a greater number of days of low temperature, there were differences between treatments in terms of conversion rate/dozen eggs, conversion rate/daily feed consumed and egg production (Table 3). The T50 treatment (50 mg/kg OEO had the best zootechnical indexes, that is, higher egg production and lower feed conversion (Table 3).

In the regression analysis, we did not observe a quadratic effect of OEO levels on feed consumption (FC) in the 3rd production cycle (week 9–12) ($R^2 = 0.22$; Figure 3); a square root

effect was verified for feed conversion (FCV, kg/kg) also in the 3rd production cycle (week 9–12) (R^2 = 0.23; Figure 3). Regression analysis showed that OEO levels for HR and VCF were 54.6 and 39.8 mg/kg, respectively. Feed conversion (FCV, kg/dozen; P = 0.19) and egg production (EP, %; P = 0.23) were not significant in the regression analysis.

3.2. Egg quality

There were no significant differences (P > 0.05) in the quality of fresh eggs (Table 4), with exception of the TBARS values, that showed decreases that were linear with respect to OEO concentration. Yolk pH of eggs stored for 21 days was significantly different (P < 0.05) for the T50 treatment (Table 5). T0 and T200 resulted in higher values for eggshell percentage compared to T50. Nevertheless, there were no statistical differences between these two treatments and TC, T100 and T50. Variables yolk pH (P = 0.051) and eggshell percentage (EP, P = 0.216) were not significant in the regression analysis. In terms of yolk color, no differences were observed for lightness, yellowness or redness (Table 5).

TBARS levels in fresh eggs were not significantly different (P < 0.05) between T200, T50 and T150, but were significantly higher in the control group, T0 and T100. For stored eggs, T50 and T100 gave higher TBARS levels than in other groups (Figure 2).

4. DISCUSSION

OEO in laying hen feed did not affect egg production. The only treatment that caused an increase in feed consumption was the T200, similar the result observed in quails (Yesilbag et al., 2013). Herbs and extracts from various plants may exert positive effects on chicken appetite and digestion in addition to antimicrobial properties (Al-Kassie, 2009). Nevertheless, the addition of oregano leaves in the feed of turkeys resulted in lower feed intake (Bampidis et al., 2005). A number of factors may affect feed intake, including animal species, age and the amount of OEO. For example, when 150 mg/kg was added to the diet of broiler chickens, the effect was positive;

however, the same was not seen using 300 mg/kg (Kırkpınar et al., 2011). A mixture of 1.0 and 2.0 g/kg of volatile oils used to supplement the basal diet of cockerels increased their feed consumption due to poor palatability and changes in odor (Tollba and Shabaan, 2010).

With respect to feed conversion, egg production and egg mass, there were no significant differences. Similar results were obtained for laying hens supplemented with 5 g/kg of oregano for 56 days (Botsoglou et al., 2005). According to these authors, the effects could be explained by diet composition, the use of healthy hens, clean environment and moderate animal density. Furthermore, variations in the effects of OEO supplementation can be a result of differences in composition and on the concentration of components with specific biological activity (Amad et al., 2011).

Internal quality of fresh eggs did not change after OEO treatment. Regarding stored eggs, we observed increased pH of the yolk for T50, possibly due to the increase of lipid peroxidation after egg storage (Botsoglou et al., 1997). The alkaline ions from albumen migrate and are replaced by hydrogen ions in the yolk, increasing the pH of the yolk and lowering albumen pH (Shang et al., 2004). The eggshell percentage was reduced by T50. This may enable gas exchange with the environment, with higher losses of carbonic gas to the environment during storage. This result may be explained by the influence of OEO on the metabolic activity of beneficial intestinal bacteria. This influence affects the efficiency of calcium (Ca) and magnesium (Mg) absorption (M Bozkurt et al., 2012). Phenolic compounds, such as carvacrol and thymol, exhibit antibacterial activity, affecting both beneficial and pathogenic bacteria (Fukayama et al., 2005). Other investigators observed increased weight, thickness and strength of the eggshell associated with higher retention and availability of nutrients in the intestinal tract of hens during the eggshell formation (Mehmet Bozkurt et al., 2012). Nevertheless, these findings might be age-dependent, as suggested by Bozkurt et al. (2016).

Some authors reported changes in yolk color when diets were supplemented with rosemary, oregano or saffron. They suggested that some herbal compounds migrate to the yolk

(Botsoglou et al., 2005). The yellow color of the yolk is related to the amount of xanthophyll in the diet and to the antioxidant activity of the pigments, including carotene and xanthophyll, that protect lipids against oxidation (An et al., 2010; Gül et al., 2012).

Lipid peroxidation is one of the main causes of food spoilage (Ruben et al., 2014). In the current study, we observed that lipid peroxidation was reduced in the yolk of fresh eggs for the T200 and in eggs after 21 day of storage for T150. This possible transfer of antioxidant compounds in OEO to the yolk resulted in eggs with higher antioxidant properties involved in the reduction of the amount of malondialdehyde (Botsoglou et al., 2005). The antioxidant effect was related to carvacrol and thymol in another study involving oregano and sage leaf oils, associated with reduction in the concentration of malondialdehyde in the yolk of stored eggs (Mehmet Bozkurt et al., 2012). These phenolic substances in OEO added to laying hen diets are subsequently transferred to the yolk where they reduce lipid peroxidation via antioxidant activity (Table 2). The antioxidant mechanisms of OEO are based on both their ability to donate a hydrogen or an electron to free radicals and their ability to delocalize the unpaired electron within the aromatic structure of the phenolic substances, exerting a protective effect against lipid oxidation (Fernandez-Panchon et al., 2008). Supplementation of turkey diets with OEO at 200 mg/kg decreased lipid oxidation of cooked and fresh meat during refrigerated storage for 1 week. OEO also preserved the quality of chicken meat under refrigeration or frozen storage alone or in combination with extracts from other aromatic plants of the same family, showing strong antioxidant activity (Botsoglou et al., 2003, 2002). According to the literature, lipid peroxidation was lower in eggs stored at refrigeration temperatures; however, the reduction was not affected by the duration of the storage. This behavior suggests that there is transfer of antioxidant compounds from OEO (Florou-Paneri et al., 2006). Furthermore, the concentration of malondialdehyde was reduced in yolks of refrigerated eggs stored for 30 days in hens fed with rosemary and oregano (Yesilbag et al., 2013). The influence exerted by essential oils on eggs may be related to the presence of phenolic compounds that consist of a hydroxyl group acting as

hydrogen donors to peroxide radicals. This action retards the formation of hydrogen peroxides (Farag et al., 1989).

Taken together, our results suggest that OEO exerted an antioxidant effect by reducing lipid peroxidation in both fresh eggs and eggs stored for 21 days. OEO produced no increase in production performance in the hens in winter when considering the three productive cycles evaluated; we observed only an increase in feed consumption in the T200 group. Analyzing the third production cycle (week 9 to 12) individually, we found that the recommended OEO dose for feed intake was 54.6 mg/kg. On the other hand, if we consider feed conversion (kg/kg), the recommended OEO dose would be 39.8 mg/kg when laying hens were exposed to colder temperatures. A global analysis led to the conclusion that OEO feed supplementation of 150 mg/kg might be a useful alternative to maintain egg quality and to increase egg shelf life. Therefore, we conclude that the OEO dose in laying hen diets should be chosen according to the production purpose, i.e., production efficiency (lower doses) or egg quality (higher doses).

Conflict of interest

The authors declare no conflict of interest.

Ethics committee

This study was approved by the Ethics Committee in Animal Research at the University of Santa Catarina State (UDESC), under protocol number 1.39.15.

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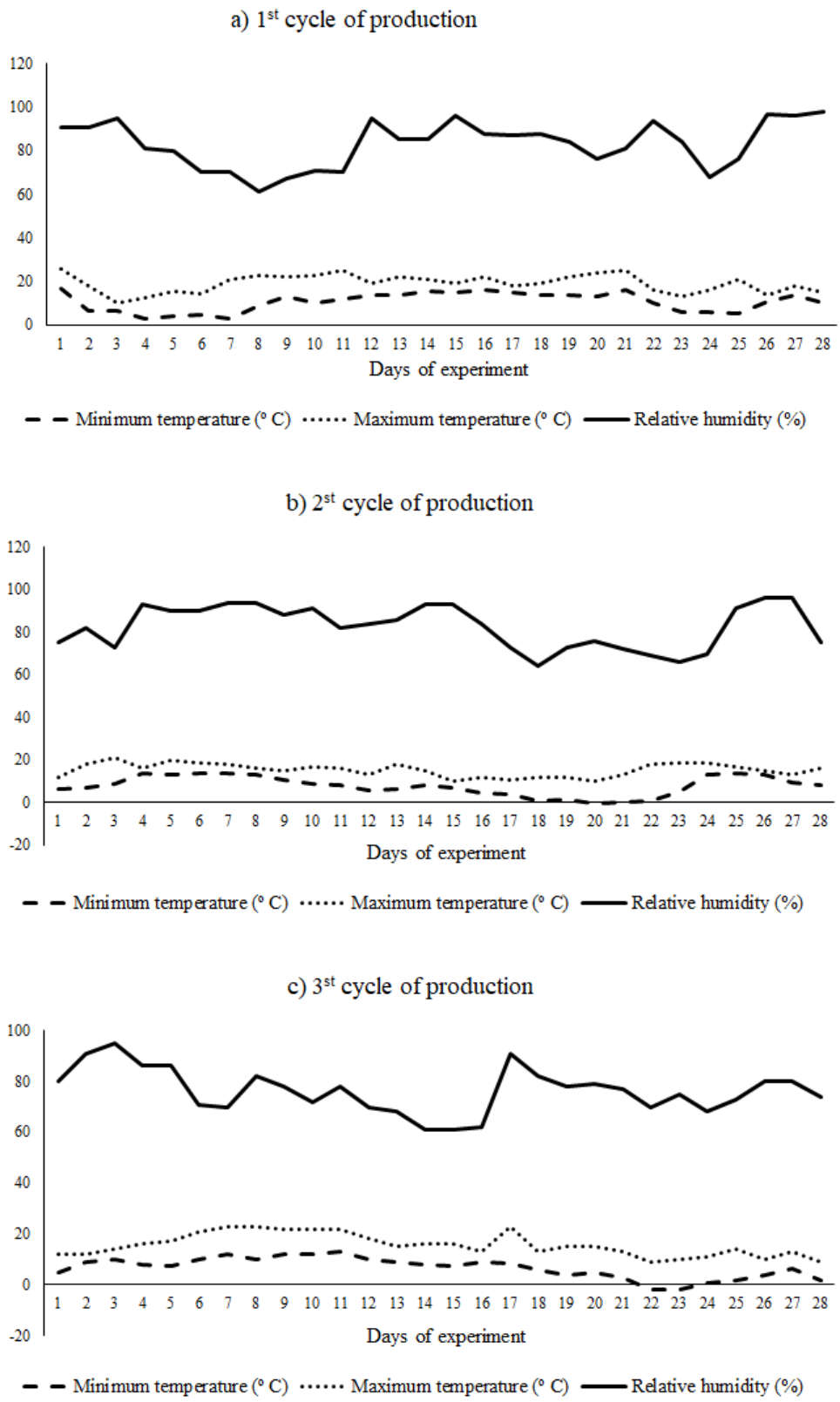

Figure 1. Temperature (minimum and maximum) and relative humidity during the experimental period of 84 days, divided into three productive cycles of 28 days each. a) 1st cycle productive: week 1–4 (1 to 28 days); b) 2nd cycle productive: week 5–8 (28 to 56 days); c) 3rd cycle productive: week 9–12 (56 to 84 days).

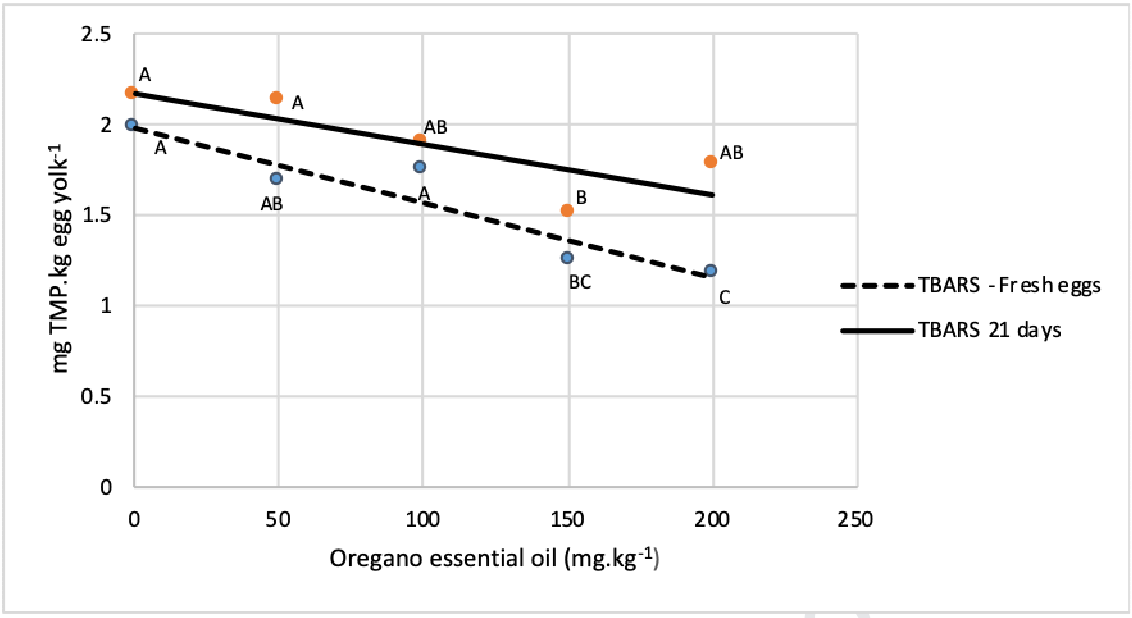

Figure 2. TBARS levels in egg yolk (fresh and stored at 21 days) of hens fed diets supplemented with oregano oil levels: T0: 0 mg/kg of oregano essential oil (OEO); T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg; *p <0.05. Fresh eggs: $Y = 1.964 – 0.00342X$ ($R^2 = 0.57$); storage by 21 days: $Y = 2.24 – 0.00538X$ ($R^2 = 0.34$). TC: the control treatment with 30 mg of zinc bacitracin was not included in the regression analysis to TBARS. The results of TBARS were presented as mean of the three production cycles (1st: week 1–4, 2nd: week 5–8 and 3rd: week 9–12). Different letters in the same line shows the difference between oil concentrations tested at two moments: fresh eggs and stocked eggs (21 days).

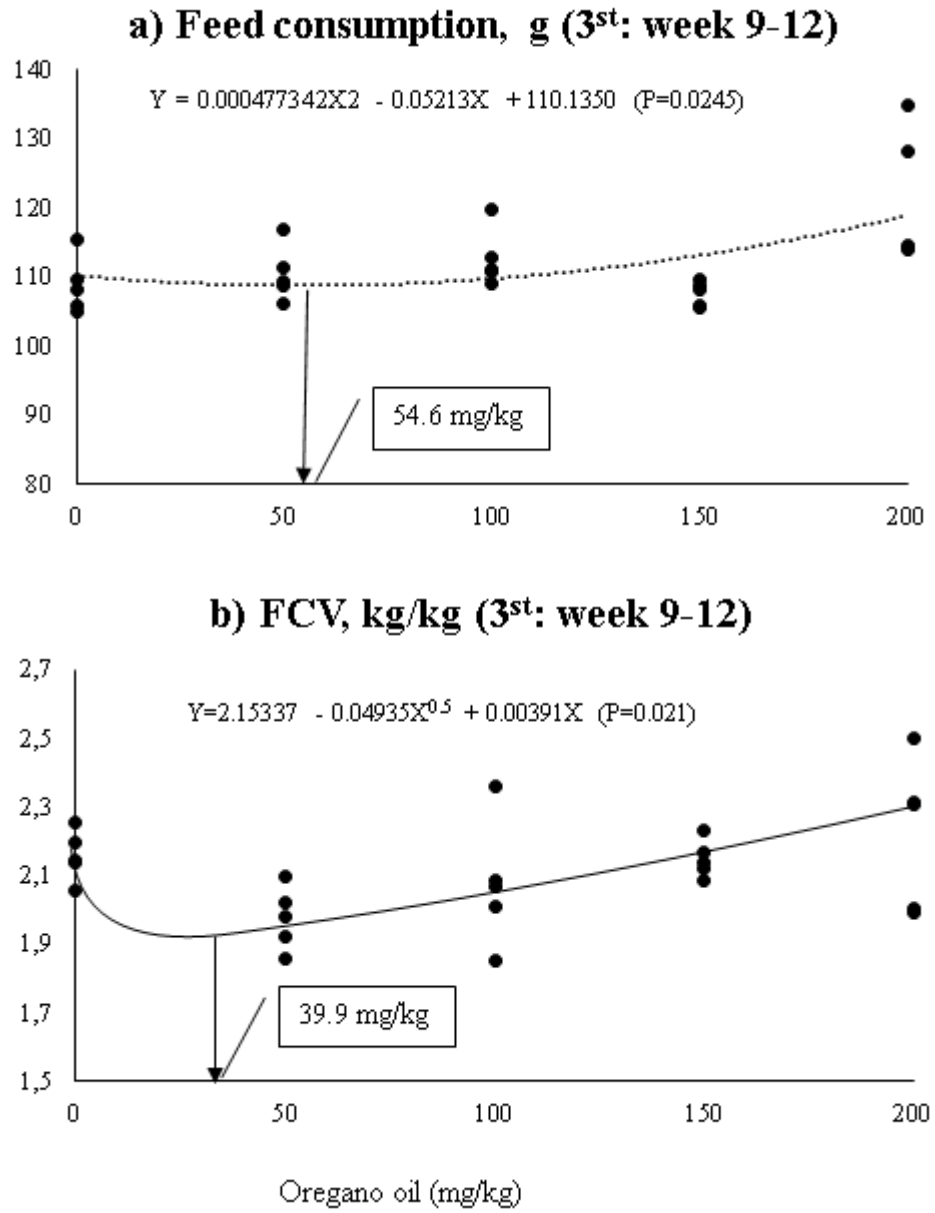

Figure 3: Feed consumption (FC, g/hen/day) and feed conversion (FCV, kg/kg) of hens fed diets supplemented with oregano oil levels in the 3rd production cycle (week 9–12): T0: 0 mg/kg of oregano essential oil (OEO); T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg; *p <0.05. TC: the control treatment with 30 mg of zinc bacitracin was not included in the regression analysis to FC and FCV.

Table 1 Percentage and calculated composition of the experimental diets used to treat laying hens.

Ingredients	**Composition (%)**
Corn	63.34
Soybean meal (45 % CP)	22.16
Soy oil	1.30
Limestone	11.20
Vitamin and Mineral Blend *	2.00
Total	100.00
Calculated composition	
Crude protein (%)	15.00
Metabolizable energy (kcal/kg)	2.85
Available phosphorus (%)	0.28
Calcium (%)	4.00
Digestible methionine + cysteine (%)	0.69
Digestible methionine (%)	0.35
Digestible lysine (%)	0.68

* Product composition (kg): folic acid 54 mg, nicotinic acid 1.000 mg, pantothenic acid 680 mg, biotin 2.70 mg, calcium 80/160 g, cobalt 27 mg, copper 6.000 mg, choline 10 g, iron 5.000 mg, phytase 20 FTU, phosphorus 42 g, iodine 40 mg, manganese 2.500 mg, mineral matter 900 g, methionine 38 g, selenium 10 mg, sodium 95 g, vitamin A 374.000 IU, vitamin B1 40 mg, vitamin B12 1.000 mcg, vitamin B2 200 mg, vitamin B6 54 mg, vitamin D3 75.000 IU, vitamin E 1.500 IU, vitamin K 100 mg, and zinc 4.000 mg. CP = Crude protein.

Table 2 Composition of oregano essential oil (*Origanum vulgare).*

Components	**Composition (%)**
Sabinene	3.09
β-Myrcene	1.13
α-Terpinene	4.99
p-Cymene	3.73
β-Phellandrene	1.11
γ-Terpinene	9.41
trans-Sabinene Hydrate	2.97
Terpinolene	1.67
Linalool	1.32
cis-Sabinene Hydrate	12.38
Cis-menth-2-en-1-ol	1.40
4-Terpineol	14.05
α-Terpineol	3.31
Carvacrol methyl ether	1.54
Linalyl acetate	4.18
Thymol	9.54
Carvacrol	9.18
trans-Caryophyllene	3.22
Bicyclogermacrene	1.85
Others*	8.18

*Percentage composition (α-thujene, α-pinene, β-pinene, cyclobutanol, α-phellandrene, limonene, α-methyl adamantane methyl amine, β-ocymene, trans-menth-2-en-1-ol, endo-borneol, cispiperitol acetate, thymol methyl ether, gamma-terpinene, α-humulene, spathulenol, caryophyllene oxide).

Table 3 Results for feed consumption (FC, g/hen/day), feed conversion (FCV, kg/dozen and kg/kg) for each produced egg, egg production (EP, %) and egg mass (EM, g/hen/day) of laying hens.

Treatment	FC	FCV (kg/dz)	FCV (kg/kg)	EP	EM
Mean of the three cycles (84 days)					
TC	116.0[a]	1.60	2.03	87.46	57.15
T0	109.2[b]	1.64	2.08	80.71	53.04
T50	111.6[ab]	1.55	1.98	86.83	56.57
T100	111.0[ab]	1.58	1.99	84.80	55.88
T150	108.8[b]	1.61	2.02	81.04	53.70
T200	114.8[a]	1.66	2.07	83.77	55.71
p-value	**0.0021***	**0.7826[ns]**	**0.8301[ns]**	**0.4946[ns]**	**0.4270[ns]**
CV (%)	**2.55**	**8.08**	**6.58**	**7.93**	**6.49**
Results 1st cycle: week 1–4					
TC	119.5	1.65	2.09	87.3	57.4
T0	117.2	1.74	2.16	82.0	54.8
T50	118.1	1.64	2.09	87.2	57.0
T100	117.3	1.66	2.07	85.7	57.2
T150	120.3	1.71	2.10	84.7	57.3
T200	118.9	1.66	2.10	86.5	56.9
p-value	**0.652 [ns]**	**0.547 [ns]**	**0.847 [ns]**	**0.796 [ns]**	**0.804 [ns]**
CV (%)	**1.06**	**5.64**	**4.69**	**3.96**	**4.05**
Results 2st cycle: week 5–8					
TC	105.1	1.42	1.81	89.2	58.3
T0	102.2	1.49	1.91	82.7	57.8
T50	106.2	1.46	1.89	87.9	56.6
T100	102.4	1.43	1.84	86.1	55.7
T150	98.0	1.44	1.83	81.7	53.7
T200	104.5	1.52	1.90	83.1	55.3
p-value	**0.074 [ns]**	**0.413 [ns]**	**0.745 [ns]**	**0.203 [ns]**	**0.189 [ns]**
CV (%)	**2.14**	**6.95**	**5.26**	**3.78**	**2.97**
Results 3st cycle: week 9–12					
TC	122.8[a]	1.72[a]	2.21[a]	85.8[a]	55.6
T0	108.8[c]	1.69[a]	2.16[a]	77.2[b]	50.4
T50	110.4[bc]	1.56[b]	1.98[b]	85.2[a]	56.0
T100	112.5[ab]	1.65[ab]	2.07[ab]	82.6[ab]	54.6
T150	107.5[c]	1.69[a]	2.15[a]	76.7[b]	50.0
T200	121.0[a]	1.80[a]	2.22[a]	81.6[ab]	54.7
p-value	**0.001***	**0.048***	**0.031***	**0.050***	**0.068 [ns]**
CV (%)	**1.86**	**3.85**	**4.01**	**3.13**	**3.34**

[A, B] Different letters in the same column differ statistically by Tukey test (5%); Coefficient of variation (CV). No significantly different (ns); TC: the control treatment with 30 mg of zinc bacitracin; T0: 0 mg/kg of oregano essential oil (OEO); T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg. *($p < 0.05$).

Table 4 Specific gravity (SG), Haugh unity (HU), yolk index (YI), yolk pH (YpH), albumen pH (ApH), eggshell strength (ES. kgf), yolk percentage (YP), eggshell percentage (EP), albumen percentage (AP), yolk color index (CI), luminosity (L*), red intensity (a*), and yellow intensity (b*) of fresh eggs.

Parameter	TC	T0	T50	T100	T150	T200	p-value	CV (%)
SG	1.091	1.093	1.090	1.089	1.091	1.090	0.3547^{ns}	0.22
HU	82.13	84.13	83.78	85.17	81.13	84.97	0.0941^{ns}	2.93
YI	0.469	0.476	0.497	0.480	0.471	0.473	0.7383^{ns}	20.04
YpH	6.03	6.05	6.08	6.02	6.06	6.11	0.3559^{ns}	0.67
ApH	8.51	8.48	8.56	8.50	8.51	8.57	0.9356^{ns}	1.78
ES	4701	4956	4971	4940	5566	5183	0.1096^{ns}	9.13
YP	26.35	26.31	27.19	26.48	26.67	26.61	0.5118^{ns}	2.87
EP	10.22	10.30	10.07	10.06	10.41	10.02	0.2958^{ns}	3.02
AP	63.43	63.42	62.69	63.46	62.91	63.48	0.5718^{ns}	1.37
CI	7.2	7.1	7.1	7.0	6.9	7.1	0.8594^{ns}	5.71
L*	57.40	58.39	59.83	59.10	59.72	59.54	0.1390^{ns}	2.61
a*	-1.50	-1.89	-2.31	-1.95	-1.89	-2.14	0.1646^{ns}	23.96
b*	41.88	42.96	44.69	43.77	43.88	42.91	0.2428^{ns}	4.18

Coefficient of variation (CV); Not significantly different (NS). TC: the control treatment with 30 mg of zinc bacitracin; T0: 0 mg/kg of oregano essential oil (OEO); T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg of OEO; *($p < 0.05$). Note: The results were presented as mean of the three production cycles (1^{st}: week 1–4, 2^{nd} : week 5–8 and 3^{rd}: week 9–12).

Table 5 Specific gravity (SG), Haugh unity (HU), yolk index (YI), yolk pH (YpH), albumen pH (ApH), eggshell strength (ES. kgf), yolk percentage (YP), eggshell percentage (EP), albumen percentage (AP), yolk color index (CI) by color fan, luminosity (L*), red intensity (a*), and yellow intensity (b*) in eggs after 21 days of room temperature storage.

Parameter	TC	T0	T50	T100	T150	T200	pvalue	CV (%)
SG	1.047	1.047	1.021	1.038	1.046	1.055	0.0743^{ns}	0.94
HU	23.26	34.08	46.94	28.45	26.17	36.53	0.0509^{ns}	36.52
YI	0.399	0.354	0.35	0.33	0.338	0.346	0.5938^{ns}	6.62
YpH	6.12^{b}	6.23^{b}	6.57^{a}	6.11^{b}	6.19^{b}	6.18^{b}	**0.0005***	2.34
ApH	9.43	9.41	9.23	9.39	9.37	9.37	0.0605^{ns}	0.37
ES	5115	5063	3499	4406	4519	4999	0.0757^{ns}	19.68
YP	28.92	27.8	29.07	29.13	28.04	28.37	0.6760^{ns}	5.54
EP	9.99^{ab}	10.37^{a}	9.13^{b}	9.77^{ab}	9.85^{ab}	10.18^{a}	**0.0204***	5.33
AP	61.09	61.95	61.82	60.92	62.14	61.45	0.8080^{ns}	2.65
CI	6.6	6.9	7.2	6.7	7.3	7.2	0.1765^{ns}	7.22
L*	60.27	58.59	58.43	59.83	62.53	63.19	0.0729^{ns}	4.81
a*	-1.12	-0.48	-0.72	-0.86	-0.69	-0.6	0.7549^{ns}	92.01
b*	57.09	52.61	54.55	57.14	54.70	56.55	0.2031^{ns}	8.35

$^{A, B}$ Different letters in the same column differ statistically by Tukey test (5%); Coefficient of variation (CV); Not significantly different (NS). TC: the control treatment with 30 mg of zinc bacitracin; T0: 0 mg/kg of oregano essential oil (OEO); T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg of OEO *($p < 0.05$). Note: The results were presented as mean of the three production cycles (1^{st}: week 1–4, 2^{nd}: week 5–8 and 3^{rd}: week 9–12).

Highlights

- In the intense cold stress, hens supplemented with oregano oil (50 mg/kg) in the long term had better productive efficiency.
- Oregano essential oil reducing lipid peroxidation in both fresh eggs and eggs store (150 mg oil/kg).
- The antioxidant effect has been related to carvacrol and thymol.
- Lipid peroxidation was reduced in the yolk of fresh eggs and after 21 day of storage.
- Oregano essential oil has improved the egg quality of laying hens under cold stress.