Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECT OF THE DIETARY OREGANO (*Origanum vulgare* L.) POWDER AND OIL ON THE BALANCE OF THE INTESTINAL MICROFLORA OF BROILERS REARED UNDER HEAT STRESS (32°C)

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Abstract

An experiment on 90, COBB 500 broiler chicks (14-42 days), reared under heat stress, evaluated the effect of the dietary oregano (Origanum vulgare L.) powder and oil, on the balance of the intestinal microflora of broilers. The broilers, assigned to three groups (C, E1, E2), were housed in an experimental hall with 32°C constant temperature, humidity 36% and 23 h light regimen. The conventional diet C included monensin in the premix for the grower phase (14-35 days). Unlike the control group, the diet for the experimental groups included 0.01% oregano oil (E1), or 0.005% oregano oil plus 1% oregano powder (E2). Six broilers/ group were slaughtered in the end of the experiment (42 days), and samples of cecal and intestinal content were collected for bacteriological examination. The experimental results showed that the total Enterobacteriaceae, E. coli and Staphylococcus count was significantly (P≤ 0.05) lower both in the cecal microbiota and in the intestinal microbiota of the experimental groups than in group C, while the lactobacilli count was significantly (P≤ 0.05) higher in groups E1 and E2 than in group C.

Key words*: oregano, heat stress, broiler, intestinal microflora.*

INTRODUCTION

Along with the prohibition of antibiotics as feed growth promoters, there has been a growing interest in phytoadditives, recognized as safe substances that can replace synthetic substances. This new approach to monogastric nutrition has been imposed by the emergence of antibiotic resistance in the animal body as a result of their use as growth promoters (Windisch et al., 2008). Thus, there is a great interest in the development of innovative feeding strategies to stimulate the development of the digestive system and digestive tract health, especially in young animals, in order to improve the bioproductive performance even with the elimination of the presence of antibiotics. Considering the advances made in understanding how to use nutrients in the intestine and metabolism, a goal of nutrition may be to formulate monogastric diets with precise targets such as optimizing growth by maintaining healthy digestive tract to ensure good the development of physiological functions (Choct, 2009).

Fuller (1999) shows that young animals under stressful conditions suffer from changes in the composition and activity of the gut microbiota. Suzuki et al. (1983) demonstrated that heat stress resulted in a marked change of bacterial composition in chicken intestine, which was subsequently associated with depression of body-weight gain. The gastro intestinal tract is particularly responsive to stressors like heat stress, which modify the normal and protective microbiota (Bailey et al., 2004) and decreased integrity of the intestinal epithelium (Lambert, 2009) which, in turn, can affect its barrier function and the absorption of nutrients, impairing productive performance of animals (Liu et al., 2009).

Many natural compounds used as alternatives to antibiotics in animal feed have shown positive effects on growth performance and on

different health parameters (Jamroz et al., 2005; Steiner, 2009; Windisch et al., 2008). Globally, herbs play an important role in nutrition because they are important ingredients in numerous products (Black et al., 2010). This has caused plants and essential oils extracted from plants to attract attention as sources of natural compounds and be studied because of the potential they own. They can be used as alternative remedies for the treatment of many infectious and oxidative diseases and for the preservation of food against the toxic effects of bacteria and oxidants (Sepahvand et al., 2014). Several types of herbal products and their oils have improved the performance of broilers by growth promoting.

Oregano (*Origanum vulgare* L.) is an aromatic herb which, due to its very strong chemical nature, has been used primarily for the preservation of food quality, for the inhibition of microbial proliferation of poultry meat and recently as an alternative growth promoter in poultry feed (Halle, 2001; Modeva et al., 2003; Bampidis et al., 2005; Çabuk et al., 2006; LiHua et al., 2007). Various studies that investigated the influence of oregano (dried leaf and oil) on the performance of broilers have led to the conclusion that oregano contributes to improving the performance of broilers by promoting their growth (Giannenas et al., 2003; Halle, 2001; Modeva et al., 2003; LiHua et al., 2007) and can reduce bacterial gastrointestinal tract populations such as *Clostridium perfringens* and *Escherichia coli* (Giannenas et al., 2004; Fukayama et al., 2007). However, the prediction of the oregano broiler's response is not very simple, because it is influenced by the variety of the plant (Fritz et al., 1992; Lee et al., 2003; Demir et al., 2003; Hassan et al., 2004; Halle et al., 2004), the level of inclusion in diets (Giannenas et al., 2004; Alçiçek, et al., 2003; Alçiçek et al., 2004; Ertas et al., 2005), sanitary and environmental conditions, nutritional composition of diet (Fritz et al., 1992; Fritz et al., 1991; Jamroz et al., 2006) and possible interaction with other additives.

The literature data show distinct biological functions of the essential oils derived from aromatic plants, such as antibacterial, antimicrobial properties (Chen et al., 2016), antifungal (Hossain et al., 2016), antiviral (Gavanji et al., 2015), antioxidant (Shaaban et al., 2012) and antiproliferative properties (Park et al., 2014). Essential oils have positive effects on animal growth and health (Puvaca et al., 2013). The inclusion of oils in poultry diets had beneficial results on intestinal microflora (Helander et al., 1998) and digestive enzymes (Jang et al., 2004; Lee et al., 2003).

Criste et al. (2017) conducted a study on Cobb 500 broilers to investigate the effect of inclusion of 2% oregano and 3% rosehip powder on the health of intestinal microflora. The chicks were reared under in heat stress (32^{o}C). These researchers reported a beneficial action for maintaining the intestinal health of phytoadiatives used in the experiment by maintaining the balance of populations of microorganisms wich colonizing the intestine. In another study on maintaining the health of cecal microflora, Roofchaee et al. (2011) evaluated the effect of 300, 600 and 1200 mg/kg oregano oil (*Origanum vulgare* L.) inclusion in Ross 308 broilers diet. The chicks were housed at 34°C temperature, which gradually decreased to 28°C towards the end of the experiment. Although there were no significant differences ($P \geq 0.05$) for the *Lactobacillus* populations, the *Escherichia coli* populations were significantly decreased ($P \leq 0.05$) on broilers that received 300 and 600 mg/kg oregano oil in diets, compared to chicks from the control group and those who received 1200 mg/kg oregano oil. Peng et al. (2016) evaluated the effect of oregano oil inclusion on the intestinal morphology of chicks over a 42-day period. The broilers used in the experiment were from the Arbor Acres hybrid, and the control group received avilamycin in addition in diets. The results showed that oregano oil has a significant effect on the intestinal health of broilers, so it may be an alternative to antibiotics.

Within this context, the present paper aims to evaluate the effects of Oregano (*Origanum vulgare* L.), oil and powder, on the balance of intestinal microflora of broilers reared under heat stress (32°C).

MATERIALS AND METHODS

The trial was conducted within the experimental halls of the National Research-

Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania), according to the provisions of the protocol approved by the Ethics commission of the IBNA-Balotesti. 90, Cobb 500 broiler chicks (day-old) were purchased for the experiment. During the starter stage (1-14 days) all chicks received a conventional diet formulation with corn and soybean meal as basic ingredients. At 14 days, the broilers were weighed and assigned to three groups (C, E1, E2). The chicks were house in an experimental hall with 32°C constant temperature, humidity 36% and 23 h light regimen. They had free access to the feed and water. During the growing (14-35 days) and finishing (35-42 days) phase, the chicks from the control group (C) received a conventional diet based on corn and soybean meal (Table 1). Compared to the control diet (C), the experimental diets differed by the addition of 0.01% oregano oil (E1), or 0.005% oregano oil plus 1% oregano powder (E2). During the growth stage (14-35 days), only the conventional diet C included monensin in the premix. The oregano essential oil studied in this paper was purchased from China from Jiangxi Xuesong Natural Medicinal Oil Co. Ltd., and the oregano powder from an SME from Livezeni, Mureș County (46.55°N, 24.63°E).

Table 1. Diet formulation

Ingredients	Grower phase (14 - 35 days)			Finisher phase (35 - 42 days)		
	C	E1	E2	C	E1	E2
Corn, %	62	62	61	60.45	60.45	59.45
Soybean meal, %	26.58	26.57	26.575	25.54	25.53	25.545
Oil %	2.5	2.5	2.5	3.72	3.72	3.72
Oregano oil, %	-	*0.01*	*0.005*	-	*0.01*	*0.005*
Oregano powder, %	-	-	*1*	-	-	*1*
Gluten %	4	4	4	6	6	6
Methionine, %	0.26	0.26	0.26	0.25	0.25	0.25
Lysine, %	0.48	0.48	0.48	0.2	0.2	0.2
Carbonate, %	1.4	1.4	1.4	1.33	1.33	1.32
Monocalcium phosphate, %	1.36	1.36	1.36	1.13	1.13	1.13
Salt, %	0.37	0.37	0.37	0.33	0.33	0.33
Choline, %	0.05	0.05	0.05	0.05	0.05	0.05
Vitamino-mineral premix*with coccidiostatic, %	1	-	-	-	-	-
Vitamino-mineral premix without coccidiostatic, %	-	1	1	1	1	1
Total	100	100	100	100	100	100
Chemical composition determinated						
Dry matter, %	87.70	87.92	88.81	89.60	90.53	90.04
Organic matter, %	82.17	82.86	83.18	84.81	85.50	84.09
Crude protein, %	20.51	21.77	21.44	19.40	19.07	20.18
Ether extractives, %	4.13	4.30	4.27	5.59	5.65	5.69
Fibre, %	3.49	3.81	3.90	3.53	3.98	3.44
Ash, %	5.53	5.06	5.63	4.79	5.03	5.95
Nitrogen-free extractives, %	54.04	52.98	53.57	56.29	56.80	54.78
Calcium, mg/kg DM	0.84	0.85	0.86	0.85	0.84	0.84
Phosphorus, mg/kg DM	0.84	0.75	0.89	0.85	0.88	0.73

*1 kg premix vitamino-mineral contains: = 1.350.000 IU/kg vit. A; 300.000 IU/kg vit. D3; 2700 IU/kg vit. E; 200 mg/kg Vit. K; 200 mg/kg Vit. B1; 480 mg/kg Vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg Vit. B6; 4 mg/kg Vit. B7; 100 mg/kg Vit. B9; 1.8 mg/kg Vit. B12; 2500 mg/kg Vit. C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium; 50 g sodium monensin /kg.

Throughout the experimental period (14-42 days) the following parameters were monitored: average daily feed intake (kg feed/chick/day), average daily weight gain (kg/chick/day), feed conversion ratio (kg feed/kg gain) and final weight (kg). In the end of the feeding trial (42 days broilers), six broilers per group were slaughtered, according to the working protocol. Samples of intestinal content were collected, in sterile tubes, from

the slaughtered chicks, for microbiological examination (determination of the *Enterobacteriaceae*, *E. coli, Salmonella*, and lactobacilli).

Gas chromatography coupled with a mass spectrometer was used to determinate the profile of volatile compounds of oregano oil and powder of the whole plant. For headspace analysis, 1.0 g of sample was placed in a 20 mL headspace vial sealed with silicone rubber septum and aluminum cap. The vial was heated to 80°C for 10 min before the injection. The essential oil and oregano herb samples diluted in hexane (1:100) (1 µL injection) and headspace gas (500 µL) were analyzed using a Thermo Electron system - Focus GC chromatograph coupled with a Polaris Q ion trap mass detector, both controlled with Xcalibur® software. A DB-5MS capillary column (25 m length, 0.25 mm i.d., and 0.25 µm of film thickness) was used. Both headspace and liquid samples were analyzed under the same chromatographic conditions. The GC oven temperature program was: initial temperature 60°C (3 min) followed by an increase of 10°C/min up to 200°C (2 min) and then 12°C/min to the final temperature of 240°C (2 min). The carrier gas (helium) flow rate was 1 mL/min. The source and interface temperature were 200°C and 250°C, respectively. Detector operated in electron impact mode (70 eV). Detection was performed in the range of m/z 35-300.The retention indices were determined using an alkane standard solution for GC (C8-C20 in hexane) (Sigma Aldrich Co., St. Louis, USA). Relative percent of individual components was calculated based on GC peak areas. All compounds were identified according to their retention indices and based on mass spectrum provided by electronic libraries (Wiley, NIST).

To determinate the basic chemical composition of feed, standardized methods were used in accordance with Regulation (EC) No. 152/2009 (Methods of sampling and analysis for official inspection of feeds).

The *Enterobacteriaceae* and *E. coli* were determined using a classical isolation medium, G.E.A.M. or Levine. The samples were first soaked in medium with lauryl-sulphate (enrichment medium), homogenized and left for 20-30 minutes are room temperature (23-24°C). Decimal dilutions were made up to 10^{-5} in the medium with lauryl-sulphate. The dilutions of 10^{-2} - 10^{-5} were used to seed 2 Petri dishes each per dilution, on Levine medium. The Petri dishes were incubated for 48 h at 37°C and the colonies were count. *E. coli* formed characteristic colonies on this medium (dark violet with metallic shine). The other *Enterobacteriaceae* formed either dark red opaque colonies (lactic-positive species) or pale pink semi-transparent or colourless colonies (lactic-negative species). The lactobacilli were determined on selective mediums (MRS broth and MRS agar), characteristic for the isolation and counting of these bacteria. The colony counter Scan 300, INTERSCIENCE (France) was used to determine the colony count of *Enterobacteriaceae*, *E. coli* and lactobacilli.

The experimental results are expressed as mean values ± standard error; StatView software and the analysis of variance (ANOVA and t test) were used for statistical processing of the data, the differences being considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Table 2 shows the profile of the volatile compounds identified in oregano oil and powder of the whole plant. As can be seen, the major constituent of oregano oil is carvacrol, which, which according to Yanishlieva and Marinova (1995); Yanishlieva et al. (1999) accounts for about 78% to 82% of the total oil, with timol, being responsible for its antioxidant activity. Bampidis et al. (2005) studied oregano oil and recorded 85.49% carvacrol concentration. The oil used in this study showed a lower concentration by 59.08%.

At high concentrations, p-cymene (20.75%) and γ-terpinene (4.45%), were also identified, two monoterpenic hydrocarbons, which confirm the strong antioxidant properties of the oregano oil (Botsoglou et al., 2002) and which represents about 5%-7% of the total oil (Adam et al., 1998). These results are consistent with those reported by Kokkini et al. (2004), which identified high concentrations of p-cymene and γ-terpinene in oregano oil. Licina et al. (2013) also analysed the chemical composition of oregano oil, recording a concentration of 0.9% α-pinene, 1.2% β-pinene, 0.1% α-phellandrene,

4.0% p-cymene, 1.5% limonene, 5.6% γ-terpinene, 1.5% linalool, 0.1% camphor and 5.4% caryophyllene oxide.

Other volatile compounds were also identified in the oregano oil content used in experimental diets, as follows: 1.33% α-pinene, 0.21% α-phellandrene, 1.10% limonene, 1.19% caryophyllene oxide. Bampidis et al., (2005) analyzed oregano oil and obtained a concentration of 0.22% α-pinene, compared to the data presented in this paper, 0.08% alpha-phellandrene, 0.19% limonene and 0.29% caryophyllene oxide.

Table 2. Volatile compounds identified in oregano (essential oil and herb)

Compounds	CAS number	Esential oil (%)	Herb (%)
α-Pinene	80-56-8	1.33	3.44
Camphene	79-92-5		1.48
Sabinene	3387-41-5		56.57
β-Pinene	127-91-3	1.79	2.20
β-Myrcene	123-35-3		3.75
α-Phellandrene	99-83-2	0.21	
p-Cymene	99-87-6	20.75	3.98
Limonene	138-86-3	1.10	1.75
Eucalyptol (1,8-Cineole)	470-82-6		12.27
E-β-Ocimene	3779-61-1		3.26
γ-terpinene	99-85-4	4.45	1.75
cis-Sabinene hydrate	15826-82-1		0.64
α-Terpinolene	586-62-9	0.98	
Linalool	78-70-6	1.19	0.62
Camphor	76-22-2	0.14	3.90
Estragole	140-67-0	0.85	
Carvacrol	499-75-2	59.08	
Caryophyllene	87-44-5	6.95	2.74
Germacrene D	23986-74-5		1.46
Caryophyllene oxide	1139-30-6	1.19	0.21

Unlike oregano oil, the major compounds in the oregano powder were represented by sabinene and eucalyptol (1,8-cineole). Research done by Kokkini et al. (2004) and Nurzyńska-Wierdak (2009) also reported a 20.13% high concentration of sabine in oregano. In the same study, in which Nurzyńska-Wierdak (2009) assessed the chemical composition of oregano according to the stage of plant development, the researcher concluded that the developmental stage of plant in herb harvesting period have a significantly importance for their chemical composition, the best term for herb harvesting being on the full flowering phase. This also influences the chemical composition of essential oil of oregano, which depends by the development stage of plant at the extraction time. The researcher claimed that the oil obtained from plant on the flowering phase does not contain carvacrol. This statement may be an explanation for the fact that in this study carvacrol was not identified in the entire oregano plant analysed.

The parameters on feed conversion ratio (kg feed/kg gain) and final weight (kg) are shown in Figure 1. There were no significant differences ($P \geq 0.05$) between groups in terms of broilers feed conversion ratio (kg feed/kg gain) and final weight (kg). The results obtained are different from the Cobb Broiler Management Guide which indicates a final weight at 42 days by 2.857 kg and feed conversion ratio by 1.675 (kg feed/kg gain). Thus, in this study the final weight gains were lower than those established in the guide with 32.90% in the control group, with 25.51% in the E1 group, respectively with 36.61% in the E2 group. Regarding on feed conversion ratio of broilers for the entire experimental period, the chicks from the control group had 17.48% higher consumption to that mentioned in the guide, 28.11% in E1 group and 29.91%. in E2 group Contrary to the data obtained in this study, Roofchaee et al. (2011), which evaluated the effect of the oregano oil (*Origanum vulgare* L.) inclusion in Ross 308 broilers reared under heat stress conditions (34^{o}C), reported a significant improvement ($P \leq 0.05$) of feed conversion ratio (kg feed/kg gain). They also concluded that oregano oil used in broilers diet has beneficial effects on chicks growth parameters. Florou-Paneri et al. (2005) evaluated the effect of oregano essential oil and herb inclusion in turkeys' diet, reared under normal temperature conditions. The dietary supplementation was performed with 5 g/kg and 10 g/kg oregano herb, respectively 100/kg and 200 mg/kg oregano oil. The results showed that the inclusion of oregano oil and herb in

turkeys diets had no effect on average daily fed intake (kg/broiler/day) and average daily weight gain (g/broiler/day). Another study regarding the effect of oregano oil inclusion on the performance of broilers during the 42 days was undertaken by Peng et al. (2016). The recorded data revealed an improvement of final body weight (kg) and average daily weight gain (kg/broiler/day) In fact, feed conversion ratio (kg feed/kg gain) was significantly reduced ($P \leq 0.05$).

Karimi et al. (2010) investigated the effect of including different levels of dried oregano leaves in the starter phase of Cobb 500, aged one. The chicks were divided into 2 control groups, one with 55 mg/kg penicillin, and 8 experimental groups with an inclusion level of 2.5 to 20 g/kg of oregano leaves. The recorded results showed that diets supplemented with oregano leaves did not influence the final weight (kg), the feed conversion ratio (kg feed/kg gain) or the mortality rate. The researchers concluded that it is necessary to include a higher level of oregano in diets for obtaining strong positive results from chicks to this.

In another study on the effect of the inclusion of oregano in broilers diet, Halle et al. (2004) reported that the gradual introduction of the oregano plant and its oil in diets reduced the average daily fed intake and significantly improved the feed conversion ratio of chicks compared to the control group that received a conventional diet formulation. This finding is very similar to that of Amad et al. (2011) who investigated the effect of phytoaditives, respectively of oregano on the growth performance and ileal digestibility of nutrients of Cobb 500 broilers. Chicks were reared under heat stress conditions of 35°C, the temperature being gradually reduced to 25°C towards the end of the experimental period. The data obtained revealed a decrease in the average daily fed intake and an improvement in both the feed conversion ratio and final weight. Other studies have shown significant improvements on the bioproductive parameters for broilers along with the inclusion of oregano oil in diets (Bozkurt et al., 2009).

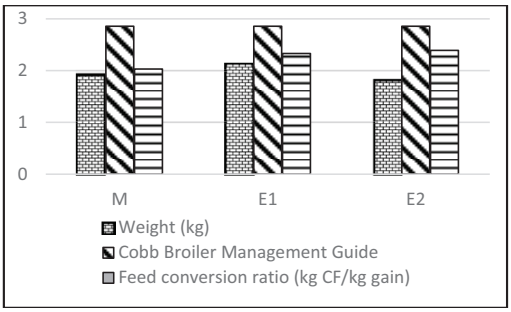

Figure 1. Broiler performance (average values/group)

The results recorded in this study on the inclusion of oregano (*Origanum vulgare* L.) (Table 3) oil (E1), respectively oil and powder (E2) in broilers diet on the intestinal microbiota revealed that the total number of *Enterobacteriaceae, E. coli* and staphylococci was significantly reduced ($P \leq 0.05$) in the experimental groups compared to the control group. Differences were also observed with regarding to the total number of Lactobacilli, which increased significantly ($P \leq 0.05$) in experimental diets, unlike the control diet. The determinations performed did not report the presence of *Salmonella* spp. in intestinal microflora. The results obtained in this study are in agreement with those obtained by Criste et al. (2017), who also reported a significant decrease ($P \leq 0.05$) of *E. coli* populations in a Cobb 500 broiler study, reared under heat stress (32^{o}C) and fed with diets that included 2% oregano powder. Mohiti-Asli and Ghanaatparast-Rashti (2017) investigated the essential oregano oil inclusion on intestinal properties, the number of *Escherichia coli* and lactobacilli in Ross 308 broilers diet. The supplementation of experimental diets with oregano oil resulted in a significant decrease ($P \leq 0.05$) of the *E. coli* populations compared to the control diet, but for the total number of Lactobacilli was not different between the treatments.

Other studies too, determined the inhibitory effects of essential oils against pathogens such as *C. perfringens* or *E. coli* (Zeng et al., 2015). The reduction of the number of pathogenic bacteria in the intestine increases the intestinal absorption capacity.

Table 3. The effect of *Origanum vulgare* L. (oil/ oil and powder) in the diet of broilers (14-42 d) on intestinal microbiota composition (log10 CFU*/g wet intestinal digesta)

Specification	C	E1	E2	SEM	Significance of treatment effect (p<)
Enterobacteriacea, lg10	7.349[a]	7.337[b]	7.273[c] 2	0.009	<0.0001
E. coli, lg 10	6.070[a]	5.994[b]	5.942[c]	0.014	<0.0001
Stafilococci, lg10	5.886[a]	5.863[b]	5.831[c]	0.006	<0.0001
Lactobacilli, lg 10	6.406[a]	6.978[b]	7.120[c]	0.082	<0.0001
Salmonella spp.	Absent	Absent	Absent	-	-
Where: *CFU-colony forming units; [a-c] Mean values within a row having different superscripts are significantly different by least significant difference test (P≤0.05); SEM: standard error of the mean; means in the same row no common superscript significantly different (P≤0.05).					

Moreover, the controlled pathogenic load contributes to the establishment of healthy microbial metabolites, the improvement of intestinal integrity and the protection against enteric diseases (Placha et al., 2013; Tiihonen et al., 2010; Oviedo-Rondón et al., 2006; Baker et al., 2010.

With regard to the cecal microbiota, the results obtained (Table 4) revealed that the inclusion of oregano (*Origanum vulgare* L.) oil (E1) and oil and powder (E2) in broilers diet reduced the total number of *Enterobacteriaceae, E. coli* and staphylococci, this being significant (P≤0.05) lower in the experimental groups compared to the control group. Concerning the total number of Lactobacilli, it was significantly (P≤0.05) higher in groups E1 and E2, compared to the control group. Horošová et al. (2006) pointed out the potential negative effects induced by essential oils included in diets on the healthy intestinal bacteria. They reported that the inclusion of oregano oil in broilers diet showed a strong bactericidal effect against isolated Lactobacilli in manure samples.

As with intestinal microflora, no populations of *Salmonella* spp. have been identified in cecum microflora. Roofchaee et al. (2011), which evaluated the effect of the inclusion of oregano oil (*Origanum vulgare* L.) in Ross 308 broilers diet, reared under heat stress (34°C), reported strong antibacterial effects of oregano oil against cecal *E. coli*.

Table 4. The effect of *Origanum vulgare* L. (oil/ oil and powder) in the diet of broilers (14-42d) on cecal microbiota composition (log10 CFU*/g wet cecal digesta)

Specification	C	E1	E2	SEM	Significance of treatment effect (p<)
Enterobacteriaceae, lg10	11.360[a]	11.331[b]	11.282[c]	0.009	<0.0001
E. coli, lg 10	10.106[a]	10.086[b]	10.044[c]	0.007	<0.0001
Stafilococci, lg10	8.804[a]	8.775[b]	8.734[c]	0.008	<0.0001
Lactobacilli, lg 10	11.257[a]	11.284[b]	11.365[c]	0.012	<0.0001
Salmonella spp.	Absent	Absent	Absent	-	-
Where: *CFU-colony forming units; [a-c] Mean values within a row having different superscripts are significantly different by least significant difference test (P≤0.05); SEM: standard error of the mean; means in the same row no common superscript significantly different (P≤0.05).					

CONCLUSIONS

The data obtained in this study confirm the inhibitory, antibacterial effect of oil and oregano powder against pathogens and for chicks reared under heat stress. The bio productive parameters of broilers, such as feed conversion ratio (kg feed/kg gain) and final weight (kg), did not differ between groups but were affected by heat stress.

ACKNOWLEDGEMENTS

The article presents part of the results obtained within project PN 1641_04.01, Contract 24 N / 2016, Nucleus Program.

REFERENCES

Alçiçek A., Bozkurt M., Çabuk M., 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. South African Journal of Animal Science, 33, 89–94.

Alçiçek A., Bozkurt M., Çabuk M., 2004. The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. South African Journal of Animal Science, 34, 217–222.

Amad A.A., Männer K., Wendler K.R., Neumann K., Zentek J., 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Journal of Poultry Sci, 90 (12), 2811-2816.

Bailey M.T., Lubach G.R., Coe C.L., 2004. Prenatal stress alters bacterial colonization of the gut in infant monkeys. Journal of Pediatric Gastroenterology and Nutrition, 38, 414–421.

Baker J., Brown K., Rajendiran E., Yip A., Decoffe D., Dai C., 2012. Medicinal Lavender Modulates the Enteric Microbiota to Protect Against *Citrobacter rodentium*-Induced Colitis. G825–36.

Bampidis, V.A., Christodoulou V., Florou-Paneri P., Christaki E., ChatzopoulouP.S., Tsiligianni T., Spais A.B., 2005. Effect of dietary dried oregano leaves on growth performance, carcase characteristics and serum cholesterol of female early maturing turkeys. British Poultry Science, 46, 595–601.

Black C., Haughey S.A., Chevallier O.P., Galvin-King P., Elliott C.T., 2016. A comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach. Food Chemistry, 210, 551–557.

Bozkurt M., Küçükyılmaz K.,ÇatlıA.U., Çınar M., 2009. The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. South African Journal of Animal Science, 39, 3.

Çabuk, M., Bozkurt M., Alçiçek A., Akbaş Y., Küçükyilmaz K., 2006. Effect of an herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks. South African Journal of Animal Science, 36, 135–141.

Chen Z., He B., Zhou J., He D., Deng J., Zeng R., 2016. Chemical compositions and antibacterial activities of essential oils extracted from *Alpini aguilinensis* against selected foodborne pathogens. Industrial Crops and Products, 83, 607-613.

Choct M., 2009. Managing gut health through nutrition. British Poultry Science, 50(1), 9-15.

Criste R.D., Panaite T.D., Tabuc C., Saracila M., Soica C., Olteanu M., 2017. Effect of oregano and rosehip supplementson broiler (14-35 days) performance, carcass and internal organs development and gut health. AgroLife Scientific Journal, 6(1), 75-83.

Demir, E., Sarica S., Özcan M.A., Suicmez M., 2003. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. British Poultry Science, 44, S44–S45

Ertas O.N., Güler T., Çiftçi M., Dalkihç B., Simsek Ü.G., 2005. The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. International Journal of Poultry Science, 4, 879-884.

Florou-Paneri P., Palatos G., Govaris A., Botsoglou D., Giannenas I., Ambrosiadis I., 2005. Oregano Herb Versus Oregano Essential Oil as Feed Supplements to Increase the Oxidative Stability of Turkey Meat. International Journal of Poultry Science 4(11), 866-871.

Fritz, Z., Schleicher A., Kinal S., Jarosz L., Majdanski F., 1992. Substitution of antibiotics by herbs in feed mixtures for broiler chicks. Roczniki Naukowe Zootechniki, 11, 315–325.

Fuller R., 1999. Probiotics for farm animals. In: Tannock, G. W. (ed), Probiotics: a critical review. Horizon Scientific Press, Wymondham, UK, 15-22.

Gavanji S., Sayedipour S.S., Larki B., Bakhtari A., 2015. Antiviral activity of some plant oils against herpes simplex virus type 1 in Vero cell culture. Journal of Acute Medicine, 5(3), 62-68.

Giannenas, I. A., Florou-Panera P., Papazahariadou M., Christaki E., Botsoglou N.A., Spais V., 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. Archives of Animal Nutrition, 57, 99–106.

Giannenas, I.A., Florou-Paneri P., Papazahariadou M., Botsoglou N.A., Christaki E., Spais A.B., 2004. Effect of diet supplementation with ground oregano on performance of broiler chickens challenged with *Eimeria tenella*. Archiv fur Geflugelkunde, 68, 247–252.

Halle I., 2001. Effects of essential oils and herbal mixtures on growth of broiler chicks. Pages 439–442 in Proc. Symp. Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier, Jena, Thüringen, Germany. Inst. Ernähr. Biol. Pharm. Fak, Friedrich-Schiller-Univ., Jena, Germany.

Halle I., Thomann R., Bauermann U., Henning M., Kohler P., 2004. Effects of a graded supplementation of herbs and essential oils in broiler feed on growth and carcass traits. Landbauforschung Volkenrode, 54, 219-229.

Hassan I.I., Askar A.A., El-Shourbagy V., 2004. Influence of some medicinal plants on performance: Physiological and meat quality traits of broiler chicks. Egyptian Poultry Science Journal, 24, 247–266.

Helander I.M., Alakomi H.L., Latva-Kala K., Mattila-Sandholm T., Pol I., Smid E.J.,1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. Journal of Agricultural and Food Chemistry, 46(9), 3590-3595.

Horošová K., Bujňáková D., Kmet V., 2006. Effect of oregano essential oil on chicken lactobacilli and *E. coli*.Folia Microbiologica, 51, 278–80.

Hossain F., Follett P., Vu K.D., Harich M., Salmieri S., Lacroix M., 2016. Evidence for synergistic activity of plant-derived essential oils against fungal pathogens of food. Journal of Food Microbiology, 53(Part B), 24-30.

Jamroz D., Wiliczkiewicz A., Wertelecki T., Orda J., Skorupinska J., 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. Journal of British Poultry Science, 46, 485–493.

Jamroz, D., T. Wertelecki, M. Houszka, and C. Kamel. 2006. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical 483 characteristics of the stomach and jejunum walls in chicken. Journal of Animal Physiology and Animal Nutrition, 90, 255-268.

Jang I.S., Ko Y.H., Yang H.Y., Ha J.S., Kim J.Y., Kang S.Y., Yoo D.H., Nam D.S., Kim N.D.H., Lee C.Y., 2004. Influence of Essential Oil Components on Growth Performance and the Functional Activity of the Pancreas and Small Intestine in Broiler Chickens. Australian Journal of Plant Physiology, 394-400.

Karimi A., Yan F.,Coto C., Park J. H., Min Y., Lu C., Gidden J. A., Lay Jr J.O., Waldroup P.W., 2010. Effects of level and source of oregano leaf in starter diets for broiler chicks. The Journal of Applied Poultry Research, 19, 137–145.

Kokkini S., Karousou R., Handilou E., Lanaras T., 2004. Essential Oil Composition of Greek (*Origanum vulgare* ssp. *hirtum*) and Turkish (*O. onites*) Oregano: a Tool for Their Distinction. Journal of Essential Oil Research, 16(4), 334-338.

Lambert G.P., 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. Journal of Animal Science 87 (E. Suppl.), E101–E108.

Lee K.W., Everts H., Kappert H.J., Frehner M., Losa R., Beynen A.C., 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. British Poultry Science, 44(3), 450-457.

Licina B. Z., Stefanovic O.D., Vasic S.M., Radojevic I.D., Dekic M.S., Comic L.R., 2013. Biological activities of the extracts from wild growing *Origanum vulgare* L. Food Control, 33, 498-504.

LiHua, C., Ying Y., Yi Fu L., Lei C., 2007. Effects of oregano oil on growth performance and carcass quality of broilers. China Poultry, 29, 9–11.

Liu Y., Yan G., Chen G., Zhang J., 2009. Efficacy trials of crude extraction from Artemisia Annul L. against newcastle disease virus in vivo in Xinjiang. Modern Applied Science, 3, 176-178.

Modeva T., Profirov Y. 2003. Influence of the oregano etheric oil on weight gain and some blood biochemical indices in chickens. Zhivotnoe Nauki, 40, 59–62.

Mohiti-Asli M., Ghanaatparast-Rashti M., 2017. Comparing the effects of a combined phytogenic feed additive with an individual essential oil of oregano on intestinal morphology and microflora in broilers. Journal of Applied Animal Research, DOI:10.1080/09712119.2017.1284074.

Nurzyńska-Wierdak R., 2009. Herb yield and chemical composition of common oregano (*Origanum vulgare* L.) essential oil according to the plant's developmental stage. Herba Polonica, 55(3), 55-62.

Oviedo-Rondón E.O., Hume M.E., Hernández C., Clemente-Hernández S., 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species, and supplemented with essential oil blends. Poultry Science, 85, 854–60.

Park S.E., Shin W.T., Park C., Hong S.H., Kim G.Y., Kim S.O., 2014. Induction of apoptosis in MDA-MB-231 human breast carcinoma cells with an ethanol extract of *Cyperus rotundus* L. by activating caspases. Oncology Reports, 32(6), 2461-2470.

Peng Q.Y., Li D.J., Li Z., Duan Z.Y., Wu Y.P., 2016. Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. Animal Feed Science and Technology, 214, 148-153.

Placha I., Chrastinova L., Laukova A., Cobanova K., Takacova J., Strompfova V., 2013. Effect of thyme oil on small intestine integrity and antioxidant status, phagocytic activity and gastrointestinal microbiota in rabbits. Acta Vet. Hung., 61, 197–208.

Puvaca N., Stanacev V., Glamocic D., Levic J., Peric L., Milic D., 2013. Beneficial effects of phytoadditives in broiler nutrition. World's Poultry Science Journal, 69(01), 27-34.

Roofchaee A., Irani M., Ebrahimzadeh M.A., Akbari M.R., 2011. Effect of dietary oregano (*Origanum vulgare* L.) essential oil on growth performance, cecalmicroflora and serum antioxidant activity of broiler chickens. African Journal of Biotechnology, 10(32), 6177-6183.

Sepahvand R., Delfan B., Ghanbarzadeh S., Rashidipour M., Veiskarami G.H., Ghasemian-Yadegari J., 2014. Chemical composition, antioxidant activity and antibacterial effect of essential oil of the aerial parts of *Salvia sclareoides*. Asian Pacific Journal of Tropical Medicine, 7(Suppl 1), S491-S496.

Shaaban H.A.E., El-Ghorab A., Shibamoto T., 2012. Bioactivity of essential oils and their volatile aroma components: Review. Journal of Essential Oil Research, Volume 24, Issue 2: Chemistry and Biology of Citrus Essential Oils.

Steiner T., 2009. Phytogenics in Animal Nutrition-Natural Concepts to Optimize Gut Health and Performance. Nottingham University Press, Nottingham, UK.

Suzuki K., Harasawa R., Yoshitake Y., Mitsuoka T., 1983. Effects of crowding and heat stress on

intestinal flora, body weight gain, and feed efficiency of growing rats and chicks. Japanese Journal of Veterinary Science, 45(3), 331-338.

Tiihonen K., Kettunen H., Bento M.H., Saarinen M., Lahtinen S., Ouwehand A.C., 2010. The effect of feeding essential oils on broiler performance and gut microbiota. British Poultry Sciece, 51, 381–92.

Windisch W., Schedle K., Plitzner C., Kroismayr A., 2008. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science, 86, E140–E148.

Yanishlieva N. V., Marinova E. M., 1995. Antioxidant activity of selected species of the family *Lamiaceae* grown in Bulgaria. Die Nahrung, 39, 458–463.

Yanishlieva N.V., Marinova E.M., Gordon M.H., Raneva V.G., 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chemistry, 64, 59–66.

Zeng Z., Zhang S., Wang H. Piao X., 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. Journal of Animal Science and Biotechnology, 6, 7.

***2015. The management guide of Cobb 500 hybrid (http:// www.cobb-vantress.com).