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Original paper

Dietary *Origanum vulgare* supplements for broilers

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Abstract

The purpose of the study was to assess the effect of dietary oregano (*Origanum vulgare*) oil and powder on the performance, development of internal organs and balance of intestinal microflora in broilers (14-42 days) reared on the floor. A number of 150 chicks of Cobb 500 broilers hybrid, aged 14 days were assigned to three groups (C, E1 and E2) housed in an experimental hall under controlled microclimate conditions. Diet C was conventional, with corn, gluten and soybean meal, while diets E1 and E2 were supplemented with 0.01% oregano oil (E1), and concomitant with 0.005% oregano oil and 1% oregano powder (E2). Six broilers per group were slaughtered at the end of the feeding trial and measurements were performed on the carcass and internal organs, while the intestinal content was sampled for subsequent bacteriological determinations. The overall (14-42 days) performance shows that both experimental groups (E1 and E2) had a significantly ($P \leq 0.05$) higher daily weight gain compared to C group, while the daily feed intake was not significantly different between the three groups. The *Enterobacteriaceae* and *Escherichia coli* count was significantly ($P \leq 0.05$) lower in the intestine of the experimental groups than in group C, while the *Lactobacillus* spp. count was significantly ($P \leq 0.05$) higher in the intestine of the experimental groups than in group C.

Keywords

Chicks, oregano oil and powder, performance, organ weight, gut microflora.

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Introduction

In recent years, there has been a growing interest in isolating antioxidants from plant ingredients and using them in animal nutrition with the intention of replacing antibiotics (J.H. PARK & al [1]). Antibiotics have been much used for a long time in animal diets for three main purposes: curing sick animals, preventing diseases among the animals susceptible to infections and growth promotion. However, by using the antibiotics in animal diets to prevent different disease, it has been promoted the spread of bacteria resistant to antibiotics (T. STEINER [2], W. WINDISCH & al [3]). After the 2006 ban on antibiotics, the European Union intensified the research to identify natural alternatives having similar beneficial effects. Many studies have been exploring the additive effects of herb materials such as oregano, rosemary, thyme, sage, basil, and mint as growth promoters, antimicrobial agents, or natural antioxidants in poultry production (N.A. BOTSOGLOU & al [4], F.C. GUO & al [5]). Due to their antibacterial, antifungal, antiviral and antioxidant properties, several authors (D.H. GILLING & al [6]) showed that the use of phyto-genic additives has many advantages over the antibiotics commonly used in animal feeding as growth promoters. S.L. CUPPETT & HALL [7] consider that plant extracts, such as the oregano essential oil, have strong antioxidant effects due to their large amounts of monoterpenes timol and carvacrol. The antimicrobial effect of the essential oil could be important against pathogens, fungi included, has been properly ascertained *in vitro* (K.A. HAMMER & al [8], A. SMITH-PALMER & al [9]) and *in vivo* against *Escherichia coli* and *Clostridium perfringens* in broilers (P. MITSCH & al [10]). The antioxidant activity of oregano (*Origanum vulgare*) is known from the *in vitro* studies or from its direct feeding to animals in various forms, such as the oil extract of dry plant (E. HERNÁNDEZ-HERNÁNDEZ & al [11], A. SANCHEZ-ESCALANTE & al [12], TEIXEIRA & al [13]). Oregano is an aromatic plant, containing more than 30 mainly phenolic antioxidants constituents, with antimicrobial and anti-inflammatory activity (M.H. ALMA & al [14]). It has been shown that oregano herb has a beneficial effect on productivity, mortality, modulation of gastrointestinal microflora, pathogen inhibition, and immune system stimulation in poultry (J.H. PARK & al [1]). No references were found on the effect of simultaneous use of the oregano oil extract and of the plant in broiler diets on organ development and on the balance of the intestinal microflora.

Our study evaluated a conventional diet and a diet with oregano oil in comparison with a diet supplemented concomitant with both, oregano oil and oregano powder, given to broilers (14-42 days), assessing broiler performance, development of internal organs and the intestinal microbiota populations.

Materials and Methods

The feeding trial was conducted in the experimental halls of the National Research-Development of Animal Biology and Nutrition (IBNA), Romania, according to

a protocol approved by the Commission of Ethics of the IBNA. The feeding trial was conducted on 150 Cobb 500 broiler chicks (14-42 days) in an experimental hall with floor rearing, under $27.02 \pm 2.79^\circ\text{C}$ air temperature, $61.05 \pm 13.55\%$ humidity and $33.71 \pm 27.38\%$ ventilation. The hall was split into 3 experimental compartments (3.5 sq. m/rearing area), each experimental compartment having a capacity of 16 chicks/ sq. m. The broiler chicks were reared on permanent litter of wood shaves (10-12 cm thick). The chicks had free access to the feed and water. The one-day old chicks were fed during the starter period (1-14 days) with a compound feed with corn, soybean meal and gluten, having 20.56% crude protein and 3140.03 kcal/kg metabolizable energy (ME). When the actual feeding trial started (14 days) the chicks were assigned to 3 groups (C, E1, E2) with 50 chicks/group. Table 1 shows that the grower (14-35 days) and finisher (35-42 days) diets included, unlike the control (C) diet, oregano oil (E1), and oregano oil and oregano powder (E2). The oregano oil was purchased from Jiangxi Xuesong Natural Medicinal Oil Co., Ltd, China. The oregano plant material used was harvested when plants were in the late vegetative stage in Livezeni, Targu-Mures (46.55°N , 24.63°E). Plants were dried for three weeks under shade at ambient temperature (20°C) and ground finely to obtain dried oregano powder. Diets formulations were calculated in agreement with the feeding requirements (NRC [15]) and the nutritional requirements of Cobb 500 hybrid.

Throughout the experimental period were monitored the following parameters: bodyweight (g); daily feed consumption (g feed/broiler/day); daily weight gain (g/broiler/day) and feed conversion ratio (g feed/g gain). According to the experimental protocol, on the 35th and 42nd day of age, 6 broiler chickens per group were slaughtered by sectioning the jugular vein and carotid artery, after which they were let to bled for 2 min, scalded in hot water and manually defeathered. Kern scales (0.001% precision, Germany) were used to measure the weight of the carcass, gizzard, liver, spleen, heart, bile, full and empty intestine. The length of the intestine and the length of the caecum were also measured. The pH value of the intestinal and caecal contents was determined with a Mettler Toledo pH meter. Samples of intestinal and caecal contents were collected for subsequent bacteriological determinations (*Enterobacteriaceae*, *Escherichia coli*, *Lactobacillus* spp., *Staphylococcus* spp., *Salmonella* spp.). A classical medium of isolation, G.E.A.M. or Levine, was used to determine the *Enterobacteriaceae* and the *Escherichia coli* in the samples of intestinal and caecal content. The samples were first immersed into a medium with lauryl sulphate (enrichment medium), properly homogenized, and left for 20-30 minutes at room temperature ($23-24^\circ\text{C}$). Decimal dilutions in physiological serum sterile were performed, and 1 ml from 10^{-3} , 10^{-4} , 10^{-5} dilutions, from every sample was inoculated on three Petri dishes with Merck Levine medium. The Petri dishes were incubated for 48 h at 37°C , and the colonies which developed in the plates were cultural examined. *Escherichia coli* developed characteristic colonies (dark violet with metallic shining). The other *Enterobacteriaceae* formed either intense red, opaque colonies (lactose-positive species),

or pale pink or colourless, semi-transparent colonies (lactose-negative species). The colony forming units (CFU/ml) from *Enterobacteriaceae*, *Escherichia coli* and *Lactobacillus* spp. was determined by a colony counter (Scan 300, INTERSCIENCE France). The effects of

treatments were analyzed using one-way analysis variance (ANOVA) with STATVIEW for Windows (SAS, version 6.0). The experimental results were expressed as mean values, SEM, the differences being considered statistically significant for P <0.001.

Table 1. Composition of the diets

Ingredient	Stage II – Grower (14-35 days)			Stage III - Finisher (35-42 days)		
	C	E1	E2	C	E1	E2
Corn, %	62.00	62.00	61.095	60.45	60.45	59.455
Soybean meal, %	26.58	26.57	26.58	25.54	25.54	25.54
Oregano oil, %	-	0.01	0.005	-	0.01	0.005
Oregano powder, %	-	-	1.00	-	-	1.00
Gluten, %	4.00	4.00	4.00	6.00	6.00	6.00
Plant oil, %	2.50	2.50	2.50	3.72	3.72	3.72
Lysine, %	0.48	0.48	0.48	0.20	0.20	0.20
Methionine, %	0.26	0.26	0.16	0.25	0.25	0.25
Choline, %	0.05	0.05	0.05	0.05	0.05	0.05
Calcium carbonate, %	1.40	1.40	1.40	1.33	1.32	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
* Vitamin-mineral premix %	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated						
Metabolizable Energy, kcal/kg	3.250,00	3.250,00	3.250,00	3.108,48	3.108,48	3.108,48
Dry matter, %	88.07	88.17	87.80	89.71	89.85	89.75
Crude protein, %	21.78	21.30	21.42	20.40	20.50	20.10
Ether extractives, %	5.08	5.04	5.10	5.60	5.64	5.72
Crude fibre, %	3.76	3.68	3.95	4.47	4.14	4.18
Ash, %	5.60	5.40	5.95	5.27	5.13	5.72

*1kg premix for group C contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg vit. B1; 400 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; 2000 mg/kg vit. C; 8000 mg manganese /kg; 8000 mg iron / kg; 500 mg copper /kg; 6000 mg/ zinc kg; 37 mg/ cobalt kg; 152 mg/ iodine kg; 18 mg selenium /kg

**Where: C= basal diet; E1= basal diet +0.01% *Origanum vulgare* oil; E2= basal diet + 0.005% *Origanum vulgare* oil +1% *Origanum vulgare* powder.

Results and Discussions

The final (42 days) body weight of E1 broilers (0.001% oregano oil) was significantly (P<0.05) higher than that of C group broilers (Table 2). The overall (14-42 days) performance shows that both experimental groups (E1 and E2) had a significantly (P<0.05) higher daily weight gain than group C, while the daily feed consumption was not significantly different between the three groups (Table 2). F. HERNANDEZ & al [16] consider that the positive results of the dietary essential oils on broiler

performance are due to their antimicrobial properties established by *in vitro* experiments and to their seeming appetizing effects. These results agree with the reports of the literature on the potential of oregano to enhance broiler performance (I. GIANNENAS & al [17], T. MODEVA & Y. PROFIROV, [18], C. LI HUA & al [19]). I. GIANNENAS & al [20] have shown that the body weight, daily weight gain and feed conversion ratio were improved in the broilers treated with oregano powder (5 g/kg). S. MARCINČÁK & al [21] reported that the 0.05% dietary oregano increased slightly the final body weight of broilers.

Table 2. Broiler performance 14-42 days (average values/group)

Item	Days	C	E1	E2	SEM	P value
Body weight gain (g)	14	461.90 ^a	467.06 ^a	469.34 ^a	4.5150	0.7898
	35	2165.51 ^a	2267.94 ^b	2203.16 ^{ab}	16.556	0.0305
	42	2749.18 ^a	2861.09 ^b	2828.99 ^{ab}	24.562	0.0694
Daily weight gain (g/day/bird)	14-35	80.90 ^a	85.52 ^b	82.56 ^{ab}	0.738	0.039
	35-42	83.38 ^a	84.30 ^a	89.40 ^a	3.526	0.5633
	14-42	77.42 ^a	81.70 ^b	81.33 ^b	0.891	0.0883
Daily feed consumption (g CF/bird/day)	14-35	131.27 ^a	132.64 ^a	135.34 ^a	4.012	0.9173
	35-42	165.40 ^a	170.30 ^a	166.70 ^a	2.513	0.7299
	14-42	139.50 ^a	141.70 ^a	142.90 ^a	3.479	0.9225

*Where: a-b 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; **CF= compound feed.

A. ROOFCHAEI & al [22] investigated the effects of the oregano oil on broiler performance and reported a significant ($P < 0.05$) increase of broiler body weight compared to the C group. The 600 mg and 1200 mg/kg supplemental oregano oil improved significantly the feed conversion ratio (FCR) compared to the C group. Figure 1 shows the FCR (kg feed/kg gain) of group E1 was lowest for the overall experimental period (14-42 days), but the difference from experimental groups E2 and C was not statistically significant. M. MOHITI-ASLI & M. GHANA-ATPARAST-RASHTI [23], reported that the dietary oregano oil given to broiler chicks (22-28 days) increased the body weight and improved the FCR compared to the C group. Other authors too (M.S.R. BARRETO & al [24], S. MARCINCAK & al [21]) studied the effect of the supplemental oregano to broiler diets but, reported no

difference in the final body weight at the end of the feeding trials. N.A. BOTSOGLOU & al [25], who studied the effect of the essential oregano oil (50 and 100 mg/kg feed), also didn't report any change in broiler performance. They concluded that the dietary oregano oil didn't have any influence on broiler growth. Moreover, E. DEMIR & al [26] reported that the dietary oregano depressed body weight and feed consumption. R.D. CRISTE & al [27] studied the effect of the dietary oregano powder on broilers (14-35 days) reared under heat stress and didn't notice significant differences in broilers performances, concluding that the oregano powder didn't have any effect on broiler growth. D.E. CROSS & al [28] reported similar effects, from a feeding trial (2-28 days) with oregano plant and oregano essential oil, on broiler performance and diet digestibility.

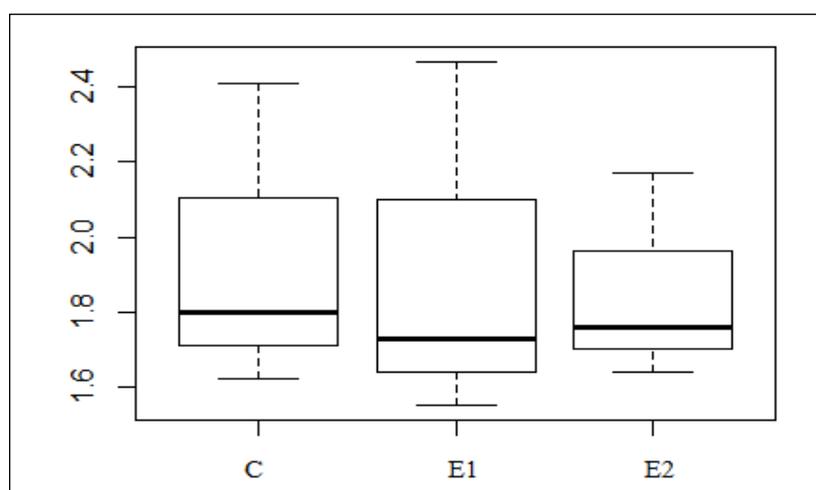


Figure 1. Feed conversion ratio (kg feed/kg gain) 14-42 days

Table 3 shows the results of the post-slaughter measurements on broilers at 35 and 42 days. Table data show that the only significant ($P \leq 0.05$) difference in organ weight was for the gizzard, at 42 days, which was significantly ($P \leq 0.05$) higher in E1 broilers compared to C broilers. These results are somehow similar with those reported by P.A. VLAICU & al [29] who showed that the dietary oregano powder and oil given to broilers (14-42 days), reared under heat stress, produced no differences in organ weight, compared to the C group, except for the liver and gizzard. M. CABUK & al [30] reported that the use of plants as powder or oil didn't influence the relative organ weights of the broilers. A. TEKELI [31], too, reported negative influence of the dietary *Origanum vulgare*, on gizzard, heart and liver development and on the weight and length of other organs.

Small intestine length (Table 3) was lowest in E1 group at 35 days, but highest, also in E1 group, at 42 days; however, the difference was not statistically significant. These results are in contradiction with those reported by A. TEKELI [31], who reported that the dietary oregano didn't influence small intestine length and weight. The pH of the intestinal and caecal contents (Table 3) showed no significant differences at any of the two slaughters (35 and 42 days). However, in absolute values, the caecal pH from E2 broilers was lower than in the other two groups. Plant extracts have been reported to reduce ileal pH value, while increasing the number of lactic acid bacteria in the ileum and caecal contents of broiler chickens, the caecal coliform and *Clostridium perfringens* counts significantly decreased according to some studies (J.K. VIDANARACHCHI & al [32], B. DALKILIÇ [33]).

Table 3. Measurements on broilers slaughtered at 35 and 42 days

Specification	Day	C	E1	E2	SEM	P value
<i>Weight (grams)</i>						
Carcass	35	1728 ^a	1732 ^a	1752 ^a	11.189	0.2006
	42	2103 ^a	2029 ^a	2085 ^a	17.084	0.4932
Gizzard	35	39.34 ^a	41.50 ^a	43.84 ^a	1.768	0.792
	42	37.76 ^a	52.54 ^b	41.68 ^{ab}	2.592	0.0599
Heart	35	8.90 ^a	9.00 ^a	9.72 ^a	0.323	0.2593
	42	11.00 ^a	10.66 ^a	10.20 ^a	0.232	0.1940
Liver	35	46.56 ^a	49.50 ^a	48.34 ^a	1.035	0.3351
	42	51.84 ^a	47.88 ^a	52.62 ^a	1.292	0.4736
Full digestive tract	35	149.40 ^a	148.12 ^a	152.40 ^a	3.899	0.1711
	42	172.92 ^a	172.72 ^a	165.44 ^a	3.985	0.2729
Empty digestive tract	35	60.50 ^a	53.44 ^a	60.02 ^a	1.688	0.1748
	42	76.88 ^a	83.08 ^a	77.10 ^a	1.809	0.4466
<i>Length (cm)</i>						
Small intestine	35	183.80 ^a	167.40 ^a	180.00 ^a	4.189	0.3510
	42	181.40 ^a	198.60 ^a	197.80 ^a	4.361	0.3190
Caecum	35	34.10 ^a	35.40 ^a	31.80 ^a	0.380	0.3872
	42	34.80 ^a	37.00 ^a	35.00 ^a	0.340	0.7216
<i>pH value</i>						
Small intestine	35	6.300 ^a	5.910 ^a	6.280 ^a	0.086	0.0686
	42	5.816 ^a	6.184 ^a	5.958 ^a	0.093	0.5775
Caecum	35	6.116 ^a	5.926 ^a	5.878 ^a	0.183	0.5154
	42	6.780 ^a	6.980 ^a	6.289 ^a	0.132	0.2999

*Where: a-b 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.

Tables 4 and 5 show the results of the bacteriological determinations on ileal and caecal contents collected after slaughter at 35 and 42 days. The concentration of the analysed microorganisms *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus* spp. and *Lactobacillus* spp. is within normal limits (N. GOURNIER-CHATEAU & al [34]). As it can be noticed, the number of *Enterobacteriaceae* colony-forming units (CFU) in the ileal content at 35 days broilers (Table 4) was significantly (P≤0.05) lower in both experimental groups compared to group C. A lower count of populations was also noticed for *Escherichia coli* in the experimental groups compared to group C, but only in E2 the CFU of populations was significantly (P≤0.05) lower compared to group C. The *Staphylococcus* spp. count increased significantly (P≤0.05) in the experimental groups compared to group C. Similar results were published by H.G. PREUSS & al [35] and A.C. OUWEHAND & al [36], a possible explanation could be that is needed a higher content of oregano in the diets, in order to obtain a significant response of thymol and carvacrol action against bacterial development. In our study, the concentrations of main antimicrobial components were below (R.P. TURCU & al [37]) the values reported by the scientific literature (A. IVROPOULOU & al [38]), proving that the oregano chemical composition depends on harvested area, weather, precipitation etc. Same as at 35 days, at 42 days (Table 4)

the number of *Enterobacteriaceae* CFU decreased significantly (P≤0.05) in E2 compared to E1 and C. Significant differences were also reported for the *Escherichia coli*, which decreased significantly (P≤0.05) in both experimental groups compared to group C. The *Staphylococcus* spp. count at 42 days was not statistically different between groups, but it was slightly lower in E2 compared to C. *Lactobacillus* spp. CFU was not significantly different in the samples collected from all three groups at 35 days. These results are similar to those reported by F. KIRKPINAR & al [39], however at 42 days the samples collected from E1 and E2 were significantly (P≤0.05) higher compared with C group. R.P. TURCU & al [37] and P.A. VLAICU & al [29] obtained similar results in an experiment on broilers reared under heat stress, fed with similar diets. *Salmonella* spp. was absent in all cases.

Table 4 data agree with other literature data (P. MITSCH & al [10], M. SARACILA & al [40]) from *in vivo* feeding trials on broiler chicks, which showed the efficacy of the dietary oils against *Escherichia coli* (I. HALLE & al [41], P.A. VLAICU & al [42]). However, D.E. CROSS & al [28] who studied the effect of oregano plant and oregano oil given to broiler chicks (7-28 days), reported no influence of these supplements on the intestinal microflora.

Table 4. Microbiological determinations in the ileum content samples collected at slaughter on days 35 and 42

Item	C1	E1	E2	SEM	P value
CFU/g intestinal content					
<i>Intestinal content at 35 days</i>					
<i>Enterobacteriaceae</i> lg. 10	7.26 ^a	7.25 ^a	7.24 ^b	0.003	<0.0052
<i>Escherichia coli</i> lg. 10	5.89 ^a	5.88 ^{ab}	5.87 ^b	0.004	0.0401
<i>Staphylococcus</i> spp. lg. 10	5.61 ^a	5.94 ^b	5.70 ^c	0.037	<0.0001
<i>Lactobacillus</i> spp. lg. 10	6.42 ^a	6.65 ^a	6.51 ^a	0.067	0.3359
<i>Salmonella</i> spp. lg. 10	Absent	Absent	Absent	n/a	n/a
<i>Intestinal content at 42 days</i>					
<i>Enterobacteriaceae</i> lg. 10	7.34 ^a	7.34 ^b	7.32 ^c	0.002	<0.0001
<i>Escherichia coli</i> lg. 10	6.01 ^a	5.99 ^b	5.98 ^c	0.004	<0.001
<i>Staphylococcus</i> spp. lg. 10	5.93 ^a	6.22 ^a	5.92 ^a	0.071	0.1623
<i>Lactobacillus</i> spp. lg. 10	7.02 ^a	7.06 ^b	7.11 ^c	0.010	<0.001
<i>Salmonella</i> spp. lg. 10	Absent	Absent	Absent	n/a	n/a

*Where: a-c means values within a row having different superscripts are significantly different by least significant difference test (P<0.05); SEM-standard error of the mean; n/a= not applicable.

Table 5. Microbiological determinations in the caecal content samples collected at slaughter on days 35 and 42

Item	C1	E1	E2	SEM	P value
CFU/g caecal content					
<i>Caecum content at 35 days</i>					
<i>Enterobacteriaceae</i> lg. 10	11.34 ^a	11.34 ^a	11.05 ^b	0.036	<0.0001
<i>Escherichia coli</i> lg. 10	9.93 ^a	9.97 ^b	9.92 ^a	0.007	0.0028
<i>Staphylococcus</i> spp. lg. 10	8.64 ^a	8.59 ^b	8.58 ^b	0.076	0.0007
<i>Lactobacillus</i> spp. lg. 10	11.40 ^a	11.39 ^b	11.40 ^a	0.002	0.0043
<i>Salmonella</i> spp. lg. 10	Absent	Absent	Absent	n/a	n/a
<i>Caecum content at 42 days</i>					
<i>Enterobacteriaceae</i> lg. 10	11.32 ^a	11.28 ^b	11.24 ^c	0.008	<0.0001
<i>Escherichia coli</i> lg. 10	10.02 ^a	9.99 ^b	9.97 ^c	0.006	<0.0001
<i>Staphylococcus</i> spp. lg. 10	8.87 ^a	8.87 ^a	8.85 ^b	0.040	0.0010
<i>Lactobacillus</i> spp. lg. 10	11.30 ^a	11.31 ^b	12.33 ^c	0.129	<0.0001
<i>Salmonella</i> spp. lg. 10	Absent	Absent	Absent	n/a	n/a

*Where: a-c means values within a row having different superscripts are significantly different by least significant difference test (P<0.05); SEM-standard error of the mean, n/a= not applicable.

Table 5 shows the results regarding the caecal content of the broilers slaughtered at 35 and 42 days. The number of *Enterobacteriaceae* CFU at 35 and 42 days were significantly lower (P<0.05) in E2 compared to E1 and C group. *Escherichia coli* count was also lower in E2 at 35 days, but the difference was not significantly (P≥0.05) different from C, however at 42 days, the differences were significantly (P<0.05) lower in both experimental groups compared with C. The caecal counts of *Staphylococcus* spp. and *Lactobacillus* spp. were not different between the three groups. However, at 42 days, the caecal microbiota of the broilers showed a significant (P≤0.05) decrease of the *Enterobacteriaceae* and *Escherichia coli* populations in both experimental groups, compared to the C group. The *Staphylococcus* spp. count was also lower, in experimental groups compared with C. The *Lactobacillus* spp. counts, the beneficial bacteria from the caecum, were significantly (P<0.05) different between the three groups at 42 days too. The number of *Lactobacillus* spp. in E2 group was with 9.11% higher than C group and with 9.01% higher than E1 group. These results are partially in accord with

those reported by L. PERIĆ & al [43] who investigated the effects of the dietary probiotics and plant additives on gut morphology and on the caecal microbial populations. *Escherichia coli* was also significantly (P<0.05) lower in both E1 and E2 group compared with C group at 42 days slaughter. A. ROOFCHAE & al [22] reported that broiler diet supplementation with 300 and 600 mg/kg oregano oil reduced significantly the *Escherichia coli* count from the caecal microbiota, both compared to the C group and E groups treated with 1200 mg / kg. VLAICU & al [44], sustained that generally oil blends have beneficial effects on the intestinal microflora population of broilers, because they act as potential additives. PENALVER & al [45] after *in vitro* investigations, showed that the oregano oil displayed an incredible antibacterial effect on *Escherichia coli* populations. According to the data from Table 5, in this study, the most potent antibacterial effect on the *Escherichia coli* population had the diet supplemented concomitant with oregano oil and oregano powder. *Salmonella* spp, was also absent in all cases.

Conclusions

The final (42 days) weight of E1 broilers (oregano oil treatment) was significantly ($P \leq 0.05$) higher than that of the C group. In both experimental groups (E1, E2) the daily weight gain was significantly ($P \leq 0.05$) higher than that of C group, while the feed conversion ratio was not different between the three groups. The microbiologic determinations on the ileal content collected at 35 and 42 days (upon slaughter) showed that both the *Enterobacteriaceae* and *Escherichia coli* counts decreased significantly in the group treated with the mix of oregano oil and powder, compared to the C group, while the ileal *Lactobacilli* count at 42 days, was significantly higher in E1 and E2 groups compared with C. The number of *Enterobacteriaceae* CFU was significantly ($P \leq 0.05$) lower in the group treated with the mix of oregano oil and powder, both at 35 and 42 days in caecum. At 42 days, the caecal *Escherichia coli* count was significantly ($P \leq 0.05$) lower in the experimental groups compared to the C group, while the number of *Lactobacillus* spp. in E2 group was with 9.11% higher than C group and with 9.01% higher than E1 group.

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