Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2019 / v.21 / n.4 / 001-010

http://dx.doi.org/10.1590/1806-9061-2019-1092

Original Article

Influence of Artemisia Annua on Broiler Performance and Intestinal Microflora

■Author(s)

Panaite TD[I] https://orcid.org/0000-0003-4967-6602
Criste RD[I] https://orcid.org/0000-0002-7724-9870
Vlaicu PA[I] https://orcid.org/0000-0003-4156-8244
Saracila M[I] https://orcid.org/0000-0002-3878-8433
Tabuc C[I] https://orcid.org/0000-0003-3493-2167
Olteanu M[I] https://orcid.org/0000-0002-9938-1557
Turcu RP[I] https://orcid.org/0000-0001-6590-0112
Buleandră M[II] https://orcid.org/0000-0001-9288-1842

[I] National Research-Development Institute for Animal Biology and Nutrition (IBNA), Calea Bucuresti, 1, Balotesti, 077015, Ilfov, Romania.
[II] University of Bucharest, Department of Analitycal Chemistry, 4–12 Regina Elisabeta, District 3, Bucharest, Romania.

■Mail Address

Corresponding author e-mail address
Mihaela Saracila
National Research Development Institute for Animal Biology and Nutrition - Department of Chemistry and Nutrition Physiology Calea Bucuresti, no 1 Balotesti, 077015, Romania.
Phone: (+4021)3512082
Email: mihaela.saracila@ibna.ro

■Keywords

Artemisia annua, broilers, performance, microflora.

Submitted: 05/July/2019
Approved: 24/September/2019

ABSTRACT

The present study aimed to investigate the effect of Artemisia (*Artemisia annua*) supplementation as essential oil and powder, in broiler diet on performance and intestinal microflora. One hundred and eighty Cobb 500 broiler chicks assigned to three experimental groups (six replicates with 10 broilers per replicate) were housed in an environment-controlled house. Compared to the control diet, the experimental diets included 0.05 g kg^{-1} Artemisia essential oil (E1), 0.05 g kg^{-1} essential oil plus 0.1 g kg^{-1} powder of Artemisia (E2), respectively. Growth performance was monitored throughout days 14-42. Artemisia supplementation (E1, E2) did not influence growth performance of the chicks. Compared to the C and E1, the chicks from E2 group had a lower count of *Enterobacteriaceae* in the intestinal and caecal content, both at 35 and at 42 days. The Artemisia supplements did not influence the staphylococci populations from the intestinal content of the chicks (42 days), but in the caecal content samples, this count was lower in E2 (8.836 log10 cfu g^{-1}) than in C (8.876 log10 cfu g^{-1}) and E1 (8.870 log10 cfu g^{-1}). The count of lactobacilli increased in the intestinal and caecal contents of chickens fed the diet supplemented with Artemisia at the 35th and 42nd day. Diet supplementation with *A. annua* essential oil and powder could be an effective solution in maintaining the proper microflora balance in the chicks' intestine.

INTRODUCTION

Poultry production is currently the most efficient animal productive system (Clavijo & Florez, 2017). However, bacterial diseases still pose a serious problem in the intensive poultry production (Adaszyńska-Skwirzyńska & Szczerbińska, 2017). The focus of alternative strategies has been to prevent proliferation of pathogenic bacteria so that the health, immune status and performance are improved (Ravindran, 2006). Given the restrictions imposed on poultry production in terms of food safety and ethical aspects of husbandry, it seems appropriate to look for the use of natural substances in animal nutrition (Christaki *et al.,* 2012). In recent years, there has been an increased interest in biologically active plant substances added to broilers diets, especially in the European countries, Japan, and the USA (Jafari *et al.,* 2011). The phytogenic bioactive compounds had the potential to stimulate the proliferation and growth of absorptive cells in the gastrointestinal tract and to influence the production and activity of the digestive enzymes, to improve the growth performance of birds (Vidanarachchi *et al.,* 2005; Jang *et al.,* 2007).

One of these alternatives is *Artemisia annua L.* (*A. annua*), an annual herb, with several medicinal uses in human diseases as powder or essential oils (Pandey & Singh, 2017). The *Artemisia annua L.* essential

oil has antimicrobial components that could substitute the use of antibiotics in poultry production (Fretté *et al.,* 2011). The efficacy of Artemisia essential oils may be due to the presence of chemical components with effective antimicrobial and antioxidant properties (Dadasoglu *et al.,* 2015). In this regard, Lopez-Lutz *et al.* (2008) reported that the essential oil of Artemisia has inhibitory effects on the growth of some microorganisms, such as *Escherichia coli*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.

Previous studies showed that the supplementation of *A. annua* powder to broiler diets could also enhance growth performance (Dragan *et al.,* 2010; Gholamrezaie *et al.,* 2013). Moreover, Khalaji *et al.* (2011) pointed out that the inclusion of *Artemisia sieberi* leaves had a positive effect on broiler gut health. Based on this background, and because the literature has no studies on the effects of using a combination of Artemisia essential oil and powder in broiler chicks, we designed a study that uses a combination of *A. annua* essential oil and powder, to assess whether it could enhance the growth performance and balance of the intestinal microflora.

MATERIAL AND METHODS

The feeding trial was conducted in the experimental house of a research institute from Balotesti, Ilfov, Romania (44º36'46"N 26º4'43"E). The experiment was conducted according to the guidelines of the Commission of Ethics of the institute (case no. 3620/31.05.2017). A total of 180, day-old Cobb 500 broiler chicks, (average weight 39.31± 2.99 g) were obtained from a commercial hatchery and housed in an environment-controlled house (16 broilers/m^2 capacity). The broilers were reared on permanent wood shaves litter (10-12 cm thick). During the first 14 days, all broilers received a control diet (C) with corn, soybean meal, gluten and sunflower oil. After 14 days, the chicks were individually weighed and assigned to 3 groups (C, E1, E2) homogenous in terms of bodyweight: C (461.899 ± 8.15 g), E1 (469.265 ± 6.60 g), E2 (454.42 ± 7.46 g). Each group consisted of six replicates (10 chick/ replicate). The environmental conditions in the house were 27.02±2.79ºC air temperature, 61.05±13.55% humidity; 33.71±27.38% ventilation; 0.857±0.91 ppm NH_3 level and 0.641±0.20 ppm CO_2 level. The light regimen was adequate to broiler age, i.e. 23h light/1h darkness.

The broilers had free access to the feeds and water. Compared to the control diet C (Table 1), during the grower (14-35 days) and finisher (35-42 days) stages, the experimental diets (E1 and E2) included 0.05 g kg^{-1} *Artemisia annua* (*A. annua*) essential oil (E1) or 0.05 g kg^{-1}essential oil plus 0.1 g kg^{-1} *A. annua* (E2) powder. Diet formulations were calculated to meet or exceed the minimal requirements for broiler chicks, in agreement with The Management guide of Cobb 500 hybrid (2015). The following parameters were monitored throughout the experimental period (14-42 days, broiler age): bodyweight (g); average daily feed intake (g feed/broiler/day); average daily weight gain (g/broiler/day); feed conversion ratio (g feed/g gain). Mortality was recorded throughout the experimental period.

The dietary Artemisia essential oil (obtained by solid-liquid extraction) used in the experimental diets (E1, E2) was purchased from Jiangxi Xuesong Natural Medicinal Oil Co., Ltd. *A. annua* plant material used in the E2 diet was harvested when plants were in the late vegetative stage from Livezeni, Târgu-Mureş, Romania (46.55° N, 24.63° E). Whole plants were dried for three weeks under shade at ambient temperature (20°C) and ground finely to obtain *A. annua* powder. Samples were taken from both *A. annua* essential oil and powder and assayed for volatile compounds. Gas chromatography (GC) coupled with a mass spectrometer was used to determinate the profile of volatile compounds of Artemisia oil and whole plant powder. Each sample was prepared and analysed as described previously by Crisan *et al.* (2015) using a Thermo Electron system - Focus GC chromatograph coupled with a Polaris Q ion trap mass detector, both controlled with Xcalibur® software. DB-5MS capillary column (25 m length, 0.25 mm i.d., and 0.25 µm of film thickness) was used.

Samples were taken from each batch of compound feeds and assayed for the basic chemical composition (dry matter, crude protein, ether extractives, crude fibre, ash) and for calcium and phosphorus. These assays observed standardized methods according to Regulation (CE) no. 152/ 2009 (Methods of sampling and analysis for the official inspection of feeds): dry matter (DM) was determined by the gravimetric method, according to SR ISO 6496:2001; crude protein (CP) was determined by the Kjeldahl method, according to SR EN ISO 5983-2:2009; ether extract (EE) was determined by extraction in organic solvents, according to SR ISO 6492:2001; crude fibre (CF) was determined by successive hydrolysis in alkali and acid environment, according to SR EN ISO 6865:2002; ash (Ash) was determined by the gravimetric method,

Table 1 – Composition (g kg $^{-1}$ as fed) and chemical composition of the experimental diets.

Ingredients	Grower stage (14-35 days)			Finisher stage (35-42 days)		
	C	E1	E2	C	E1	E2
	g kg^{-1}			g kg^{-1}		
Corn	620	620	610	604.5	604.5	594.6
Soybean meal	265.9	265.8	265.8	255.4	255.4	255.4
Gluten	40	40	40	60	60	60
Sunflower oil	25	25	25	32	37.2	37.2
Artemisia essential oil	-	0.05	0. 05	-	0.05	0.05
Artemisia powder	-	-	0.1	-	-	0.1
Calcium carbonate	14	14	14	13.3	13.2	13.2
Monocalcium phosphate	13.6	13.6	13.6	11.3	11.3	11.3
Salt	3.7	3.7	3.7	3.3	3.3	3.3
Methionine	2.6	2.6	2.6	2.5	2.5	2.5
Lysine	4.8	4.8	4.8	2.0	2.0	2.0
Choline	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin-mineral premix*	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000
Calculated						
Metabolisable energy, MJ**	13.598	13.598	13.598	13.003	13.003	13.003
Chemical composition						
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, %	4.80	5.04	5.10	5.60	5.64	5.72
Crude fibre, %	3.76	3.68	3.95	3.47	4.14	4.18
Ash, %	5.60	5.40	6.95	4.97	5.13	5.72
Calcium, %	0.93	0.93	0.93	0.90	0.91	0.90
Phosphorus, %	0.72	0.70	0.80	0.88	0.99	0.82
Available phosphorus, %	0.45	0.45	0.44	0.43	0.47	0.42

*1kg premix 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/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.
** Metabolisable energy of the diet was calculated using optimization program HybriminFutter 5.

according to SR EN ISO 2171:2010; calcium was determined by the titrimetric method according to SR ISO 6490-1/1996, phosphorus was determined by the spectrophotometric method according to SR ISO 6491:1983.

According to the protocol approved by the Commission of Ethics of the institute, at 35 days (grower stage) and 42 days (finisher stage), six broilers (with live weight similar to the average bodyweight of the group) from each group were slaughtered by cervical dislocation and immediately bled. Carcasses were eviscerated manually and the gastrointestinal tract was excised. Small intestinal (duodenum, jejunum, ileum) and caecal contents (2 caeca per bird) were collected aseptically in sterilized plastic tubes and preserved at -20°C until the bacteriological analyses (Enterobacteriaceae, E. coli, lactobacilli, staphylococci, Salmonella spp). The samples were prepared and analysed as described previously by Criste *et al.* (2017). The results were expressed as log base 10 colony-forming units (cfu) per gram of intestinal/caecal contents. The colony forming units from Enterobacteriaceae, E. coli, staphylococci and lactobacilli was determined by a colony counter (Scan 300, Interscience France).

The effects of treatments were tested by analysis of variance using the GLM procedure of the Minitab software (version 17, Minitab® Statistical Software), with treatment as fixed effect. When overall F-test was significant, the differences between means were declared significant at $p<0.05$ using the test of Tukey.

RESULTS

Table 2 shows the composition of volatile compounds in the *A. annua* essential oil and powder. The main volatile compounds in Artemisia oil are the monoterpenes, dominated by eucalyptol (29.17%) and α-pinene (11.32%). Ketones were the second major class of compounds observed in *A. annua* essential oil, including camphor (9.38%) and Artemisia ketone (7.38%).

Table 2 – Volatile compounds (%) determined in the *Artemisia annua* essential oil and powder used in broiler diets.

Compound	CAS	Essential oil, (%)	Powder, (%)
α-Pinene	80-56-8	11.32	11.95
Camphene	79-92-5	2.83	22.52
Sabinene	3387-41-5	3.04	0.88
β-Pinene	127-91-3	4.52	3.55
Yomogi alcohol	30458-12-9	1.45	ND
α-Terpinene	99-86-5	1.18	ND
p-Cymene	99-87-6	1.28	3.07
Eucalyptol	470-82-6	29.17	9.28
Artemisia ketone	546-49-6	7.38	1.33
Trans- sabinene hydrate	17699-16-0	ND	0.53
Artemisia alcohol	27644-04-8	1.19	ND
α-Terpinolene	586-62-9	0.57	ND
trans-Pinocarveol	547-61-5	3.16	ND
Camphor	76-22-2	9.75	46.21
Borneol	507-70-7	ND	0.68
Pinocarvone	34-41-3	2.98	ND
Terpinen-4-ol	562-74-3	3.63	ND
α-Terpineol	98-55-5	1.13	ND
Myrtenal	564-94-3	1.11	ND
Pulegone	89-82-7	0.45	ND
Thymol	89-83-8	0.56	ND
α-Terpineol acetate	80-26-2	0.28	ND
Eugenol	97-53-0	0.35	ND
Butyl benzoate	136-60-7	0.20	ND
α-Copaene	3856-25-5	1.15	ND
Caryophyllene<Z>	118-65-0	4.37	ND
Caryophyllene<E>	87-44-5	0.80	ND
Humulene	6753-98-6	0.26	ND
γ-Muurolene	30021-74-0	3.38	ND
α-Selinene	473-13-2	1.23	ND
α-Muurolene	31983-22-9	0.72	ND
δ-Cadinene	483-76-1	0.31	ND
Caryophyllene oxide	1139-30-6	0.24	ND
Total		100	100

Where: CAS: Chemical Abstract Service; ND: Not detected.

Table 3 shows the effects of Artemisia oil and combination of Artemisia essential oil and powder on broiler's performance. There were no significant differences ($p>0.05$) throughout the experimental period among the three groups in terms of broiler body weight.

Table 3 – Effect of *Artemisia annua* (essential oil/ essential oil and powder) on broiler performance (14-42 days).

Item	Period (days)	C	E1	E2	SEM	*p*-value
Body weight (g/broiler)	14	461.9	469.26	454.42	4.288	0.371
	35	2165.51	2200.2	2124.87	17.922	0.231
	42	2634.77	2727.89	2667.5	23.553	0.256
Average daily feed intake (g/broiler/day)	14-35	131.42	138.47	128.86	4.213	0.617
	35-42	165.58	173.38	158.14	3.253	0.230
	14-42	139.68	146.81	135.95	0.360	0.465
Average daily weight gain (g/broiler/day)	14-35	81.12	82.42	79.54	0.793	0.264
	35-42	67.04	75.38	77.52	3.615	0.505
	14-42	77.60	80.66	79.04	0.859	0.309
Feed conversion ratio (kg CF/kg gain)	14-35	1.62	1.68	1.63	0.140	0.109
	35-42	2.46	2.30	2.19	0.127	0.525
	14-42	1.80	1.82	1.73	0.022	0.223

Where**:** SEM: standard error of the mean.

Nevertheless, it can be noticed that both at 35 days, and at 42 days, the broilers treated with *A. annua* essential oil (E1) had a higher body weight (p>0.05) than the other two groups (C and E2). Also, according to Table 3 data, during the period 14-42 days, the average of daily feed intake and the average of daily weight gain of E1 broilers were higher than in groups C and E2 (p>0.05), but the difference was not statistically significant. The broilers treated with the mixture of *A. annua* essential oil and powder (E2) had a better feed conversion ratio (Table 3) than those which received the conventional diet and E1 diet, but not statistically significant (p>0.05).

The lowest number of *E. coli* colony-forming units was detected in the intestinal content of E2 broilers, lower (p<0.05) than in C and E1 (Table 4). The *Enterobacteriaceae* count in the intestinal content, both at 35 and 42 days (Table 5) was lower (p<0.05) in E1 broilers than in C broilers. Similarly, to the data concerning the intestinal content, the *Enterobacteriaceae* count was lower (p< 0.05), at 35 days, in groupE2 compared to C.

At 35 days, the caecal count of *E. coli* decreased (p<0.05) in E2 compared to E1, but it was not significantly (p>0.05) different from C.

The number of staphylococci colony-forming units was higher (p<0.05) in the intestinal content of the experimental groups (E1 and E2) compared to the control group (Table 4). On the contrary, a lower (p>0.05) staphylococci count was determined in the caecal content of E2 broilers (Table 4). Both in the samples of intestinal and caecal content collected at 35 days, the lactobacilli count was higher (p<0.05) in E2 broilers than in E1 and C broilers. Moreover, the lactobacilli populations from the intestinal content of E2 broilers were 10% higher than in C and 1.9% higher than in E1.

Table 4 – Effect of *Artemisia annua* supplements (essential oil/ essential oil and powder) to broiler diets on the intestinal and caecal microflora at 35 days (log_{10}cfug^{-1}intestinal or caecal content).

Item	C	E1	E2	SEM	p-value
Small intestine content					
Enterobacteriaceae, log_{10}	7.26^{a}	7.25^{b}	7.22^{c}	0.01	<0.001
Escherichia coli, log_{10}	5.89^{a}	5.89^{a}	5.82^{b}	0.01	<0.001
Staphylococci, log_{10}	5.61^{a}	5.86^{b}	5.76^{c}	0.03	<0.001
Lactobacilli, log_{10}	6.42^{a}	6.93^{b}	7.06^{c}	0.07	<0.001
Salmonella spp.	ND	ND	ND	-	-
Caecal content					
Enterobacteriaceae, log_{10}	11.35^{a}	11.16^{b}	11.06^{c}	0.03	<0.001
Escherichia coli, log_{10}	9.93^{a}	9.96^{b}	9.92^{a}	0.01	0.002
Staphylococci, log_{10}	8.64^{a}	8.78^{a}	8.54^{a}	0.07	0.367
Lactobacilli, log_{10}	11.41^{a}	11.40^{b}	11.43^{c}	0.04	<0.001
Salmonella spp.	ND	ND	ND	-	-

Where: cfu=colony-forming units; SEM: standard error of the mean; ND: Not detected; n=6;

^{a,b,c} Mean values within a row having different superscripts are significantly different by least significant difference test (p<0.05).

At 42 days, the *Enterobacteriaceae* and *E. coli* counts were lower (p<0.05) in the intestinal and caecal contents of E1 and E2 broilers compared to C broilers (Table 5).

While no significant differences (p>0.05) were recorded in terms of the staphylococci count from the small intestine among groups, the caecal staphylococci populations were lower (p<0.05) in E2 broilers compared to C and E1 broilers.

The *A. annua* supplements to broiler diets had a positive influence on the lactobacilli count from the intestinal content. Thus, the lactobacilli count was higher (p<0.05) in E1 (7.098 log10 cfug^{-1} intestinal content) and E2 (7.372 log10 cfug^{-1} intestinal content) than in C (7.028 log10 cfug^{-1} intestinal content). Similar results were reported for the samples of caecal content. *Salmonella spp.* were absent in all samples of intestinal and caecal content.

DISCUSSION

The characterisation of *A. annua* essential oil (Table 2) shows a high content in volatile compounds. As table 2 data shows, a higher number of compounds (31 compounds) were detected in the *A. annua* essential oil than in the powder (10 compounds). A higher number of monoterpenes were detected in *A. annua* powder, especially: camphor (46.21%), camphene (22.52%), α-pinene (11.95%), eucalyptol (9.28%). These monoterpenes are well-known chemicals for their pronounced antimicrobial properties. This profile

Table 5 – Effect of *Artemisia annua* supplements (essential oil/ essential oil and powder) to broiler diets on the intestinal and caecal microflora at 42 days ($log_{10}cfug^{-1}$ intestinal or caecal content).

Item	C	E1	E2	SEM	*P*-value
Small intestine content					
Enterobacteriaceae, log10	7.35[a]	7.33[b]	7.32[c]	0.01	<0.001
Escherichia coli, log 10	6.02[a]	5.99[b]	5.95[c]	0.04	<0.001
Staphylococci, log10	5.94[a]	5.95[a]	5.95[a]	0.04	0.121
Lactobacilli, log 10	7.03[a]	7.01[b]	7.37[c]	0.02	<0.001
Salmonella spp.	ND	ND	ND	-	-
Caecal content					
Enterobacteriaceae, log_{10}	11.32[a]	11.27[b]	11.21[c]	0.01	<0.001
Escherichia coli, log_{10}	10.02[a]	10.00[b]	9.96[c]	0.01	<0.001
Staphylococci, log_{10}	8.88[a]	8.87[a]	8.84[b]	0.04	<0.001
Lactobacilli, log_{10}	11.30[a]	11.47[b]	12.41[c]	0.09	<0.001
Salmonella spp.	ND	ND	ND	-	-

Where: cfu=colony-forming units; SEM: standard error of the mean; ND: Not detected; n=6;

[a,b,c] Mean values within a row having different superscripts are significantly different by least significant difference test ($p< 0.05$).

of volatile compounds detected in the plant powder supports its use as phytoadditive with effect on broiler growth performance and gut health assurance. Perazzo *et al.* (2003) reported that *A. annua* plant included camphor, β-cubebene and trans-caryophyllene. On the other hand, Nezhadali & Parsa (2010) detected 72 compounds in *Artemisia absinthium* leaves, including camphor (14.83%), p-cymene (10.35%), isoledene (8.52%), caryophyllene (6.92%). The concentration of volatile compounds in Artemisia depends on the plant material (cultivar, variety), age, growing conditions, as well as on the seasonal and geographical variations (Olsson *et al.,* 2009). Factors such as temperature changes and stress treatments can be applied to affect the content of secondary metabolites of plants (Malik *et al.,* 2009). However, as Marinas *et al.* (2015) shows, the essential oil from *A. annua* plant grows in Romania, had contained camphor (17.74%), α-pinene (9.66%), germacrene D (7.55%), 1,8-cineole (7.24%), α-caryophyllene (7.02%), artemisia ketone (6.26%) as main compounds. In Bosnia, Artemisia ketone (30.7%) and camphor (15.8%) are the major constituents in the essential oil of *A. annua* (Cavar *et al.,* 2012). Radulovic *et al.* (2013) in a characterization study of *A. annua* essential oil from Serbia, have shown that it contains Artemisia ketone (35.7%), alpha-pinene (16.5%), 1,8-cineole (5.5%). Different from it, Kazemi (2015) identified in an oil from Iran, α-Pinene (7.33%), camphene (5.68%), sabinene (4.78%), α-myrcene (22.41%), 1,8-cineole (17.17%), camphor (20.41%). The composition of essential oils from the same species varied depending on the different geographical origin (Tzenkova *et al.,* 2010; Verdian-Rizi Mohammadreza, 2008; Pandey & Singh, 2017). Variation in the volatile components of these plants may occur during plant ontogeny or growth at different altitudes (Pandey & Singh, 2017). The strong antimicrobial activity of *A. annua* (Atta-Ur-Rahman, 2008) is due to the high content in monoterpene hydrocarbons.

The supplement of *A. annua* did not influence broiler performance, as seen from Table 3. Neither the plant, nor the essential oil of *A. annua* did not improve broiler appetite, although feed intake was higher in the group treated with *A. annua* essential oil (E1) than in the other two groups, but the difference was not statistically significant. It is noteworthy that the performance of E2 broilers (mixture of *A. annua* essential oil and powder) was comparable with that of E1 broilers, which could be due to the rather low dietary essential oil level (0.05 g kg^{-1}) knowing that the action of this phytoadditive depends on the level of inclusion in the diet. However, the combination of Artemisia essential oil and powder (group E2), generally produced lower values of the average daily feed intake both in groups C and E1, but the differences are not statistically significant ($p>0.05$). In the present work, the higher average of daily feed intake and the higher average of daily weight gain of E1 broilers substantiate the statements of Windisch *et al.* (2008) and Grashorn (2010), that phytobiotics, especially those from the group of essential oils, have been reported to improve the flavour and palatability of the feed. The data from table 3 were consistent with those reported by Khalaji *et al.* (2011). They showed that the supplementation of 0.1 g kg^{-1}*Artemisia sieberi* leaves in Ross 308 chicken diets did not affect the body weight and feed conversion ratio of broilers during the entire experimental period (1-42 days). Broiler weight (42 days) reported by Habibi *et al.* (2016) who used Ross 308 broilers treated with diets which included 100, 200, 300 mg/kg *A. absinthium* oil were 14.06%,

12.88%, and 14.70% lower, respectively, compared to the results of our study, while the expected difference among the two hybrids is about 1.71% (according to Management Guide of Cobb 500 Hybrid and Management Guide of Ross 308 Hybrid). Pop *et al.* (2017) noticed that the use of lower concentrations of artemisinin (5 ppm) in Ross 308 broilers (1-28 days) increased the average daily feed intake, the average daily weight gain and improved the feed conversion ratio, while the higher concentration of artemisinin (500 ppm) caused reduced weight gain, inefficient feed conversion, and a lower feed intake.

Several researchers have shown that the essential oils act against Gram-negative bacteria, such as Campylobacter jejuni, Escherichia coli, Mycoplasma gallisepticum, Mycoplasma synoviae, Pseudomonas aeruginosa, Salmonella enteridis, or Klebsiella sp. (Solorzano-Santos & Miranda-Novales, 2012; Kurekci *et al.*, 2013; Nimbarte *et al.*, 2013; Alali *et al.*, 2013; Cerisuelo *et al.*, 2014; Zengin & Baysal, 2014). The results of the present work (Tables 4 and 5) were in agreement with those. The dietary *A. annua* essential oil decreased (p<0.05) the Enterobacteriaceae and E. coli populations in the intestinal content, both at 35 days and at 42 days. This beneficial action could be due to the higher concentration of terpenoids, including monoterpenes in the *A. annua* essential oil (Table 2). These compounds are well-known to cause membrane disruption in microorganisms by the action of lipophilic compounds in the oils, thereby impairing the antibacterial activity (Sahoo *et al.*, 2012). Khalaji *et al.* (2011) and Ghazanfari *et al.* (2015) reported that Artemisia sieberi leaves (1%), and oil (300 mgkg^{-1}) given to Ross 308 broilers (1-42 days) reduced (p<0.05) the caecal E. coli populations. On the contrary, Saracila *et al.* (2018) showed that broilers (14-35 days) treated with Artemisia oil (0.05 g kg^{-1}) had a significantly higher number of Enterobacteriaceae and E. coli in the caecal content than those treated with mix of Artemisia oil and powder. In addition, there are studies that highlight the action of essential oils against Gram-positive bacteria: Bacillus cereus, Bacillus subtilis, Clostridium colinum, Clostridium septicum, Listeria monocytogenes, Staphylococcus aureus, or Streptococcus gallolyticus (Jerzsele *et al.*, 2012; Muthayian *et al.*, 2012; Solorzano-Santos & Miranda-Novales, 2012; Nimbarte & Kulkarni, 2013; Zengin & Baysal, 2014).

The balance between the count of intestinal beneficial and "bad" bacteria (at least 85% of the total bacterial count should be beneficial) is vital for the host, and the impact on gut health often comes from bacterial imbalance in broiler gut (Choct, 2009). Lactobacilli produce a wide range of short-chain fatty acids (SCFAs), which have bacteriostatic activity on some bacterial strains, either directly, or by reducing the intestinal pH; they produce bacteriocins which have microbiocid or microbiostatic properties and thereby contribute to the resistance to pathogens by modifying the receptors they use (Adil & Magray, 2012; Rinttila & Apajalahti, 2013). This microbial imbalance is maximized when antibiotics are withdrawn from the feed (Choct, 2009). It is possible to manipulate nutritionally the intestinal microbial population, concomitantly with the increase of the number of beneficial bacteria in broiler gut (Adil & Magray, 2012).

The addition of *A. annua* (essential oil and powder) to broiler diets determined the multiplication of lactobacilli populations in the intestinal and caecal contents at 35 and 42 days. Similar results were reported by Ghazanfari *et al.* (2015), using *A. sieberi* oil (300 mgkg^{-1}) given to Ross 308 broilers (1-42 days). On the contrary, Khalaji *et al.* (2011) showed that *Artemisia sieberi* leaves (1%) given to Ross 308 broilers had no effect on the caecal lactobacilli populations. The increase of the lactobacilli populations in the intestine of broilers treated with *A. annua* (essential oil and powder) diet maintained the balance between the bacteria colonizing the gastrointestinal tract of the broilers.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Education and Research (Project PN 18 20 0102).

CONCLUSIONS

The dietary supplementation of *A. annua* (essential oil or combination of essential oil and powder) did not influence broiler performance (14-42 days).

Supplementing *A. annua* as a combination of essential oil and powder to broiler diets can improve the balance of gut microflora (as measured by changes in populations of *Escherichia coli* and *Lactobacillus*).

Both at 35 and at 42 days, the broilers treated with the combination of *A. annua* essential oil and powder had the lowest count of *Enterobacteriaceae* in the intestinal and caecal content from all groups. The number of lactobacilli colony-forming units was higher, in the intestinal and caecal contents of chickens (on days 35 and 42), fed the diet supplemented with *A. annua* (essential oil and powder).

REFERENCES

Adaszyńska-Skwirzyńska M, Szczerbińska D. Use of essential oils in broiler chicken production – a review. Annals of Animal Science 2017;17:317-335.

Adil S, Magray SN. Impact and manipulation of gut microflora in poultry: a Review. Journal of Animal and Veterinary Advances 2012;11(6):873-877.

Alali WQ, Hofacre CL, Mathis GF, Faltys G. Effect of essential oil compound on shedding and colonization of *Salmonella enterica serovar* Heidelberg in broilers. Poultry Science 2013;92(3):836-841.

Atta-Ur-Rahman FRS, editor. Studies in natural products chemistry. Amsterdam: Elsevier Science; 2008.

Cavar S, Maksimovic M, Vidic D, Paric A. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua L*. from Bosnia. Industrial Crops and Production 2012;37:479-485.

Cerisuelo A, Marín C, Sánchez -Vizcaíno F, Gómez EA, De La Fuente JM, Durán R, *et al.* The impact of a specific blend of essential oil components and sodium butyrate in feed on growth performance and *Salmonella* counts in experimentally challenged broilers. Poultry Science 2014;93(3):599-606.

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

Christaki E, Bonos E, Giannenas I, Florou-Paneri P. Aromatic plants as a source of bioactive compounds. Agriculture 2012;2:228-243.

Clavijo V, FlorezVives JM. The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: s review. Poultry Science 2017;97:1006-1021.

Criste RD, Panaite TD, Tabuc C, Saracila M, Soica C, Olteanu M. Effect of oregano and rosehip supplements on broiler (14-35 days) performance, carcass and internal organs development and gut health. AgroLife Scientific Journal 2017;6(1):75-83.

Crisan CC, Buleandra M, Calinescu I, Zalaru C, David IG, Badea IA. Chemical composition of the aerial part and fruits of *Careopsistinctoria.* Chemistry of Natural Compounds 2015;51(3):571- 572.

Dadasoglu F, Kotan R, Cakir A, Cakmakci R, Kordali S, Ozer H, *et al.* Antibacterial activities of essential oils, extracts and some of their major components of *Artemisia spp. L.* against seed-borne plant pathogenic bacteria. Fresenius Environmental Bulletin 2015;24(9):2715-2724.

Dragan L, Titilincu A, Dan I, Dunca I, Dragan M, Mircean V. Effects of *Artemisia annua* and *Pimpinella anisum* on *Eimeria tenella* (*Phylum Apicomplexa*) low infection in chickens. Scientia Parasitologica 2010;11(2):77-82.

Fretté XC, Engberg RM, Kjær A, Ivarsen E, Christensen KB, Grevsen K, *et al.* Production and use of *Artemisia annua* (sweet wormwood) against bacterial diseases in poultry stocks and its effect on food quality. Planta Medica 2011;77-SL69.

Ghazanfari S, Moradi MA, Niat FR. Effects of different levels of *Artemisia sieberi* essential oil on intestinal morphology characteristics, microflora population and immune system in broiler chickens. Journal of Veterinary Research 2015;70(2):195-202.

GholamrezaieSani L, Mohammadi M, JalaliSendi J, Abolghasemi SA, Roostaie Ali Mehr M. Extract and leaf powder effect of *Artemisia annua* on performance, cellular and humoral immunity in broilers. Iranian Journal of Veterinary Research, Shiraz University 2013;14(1):15-20.

Grashorn MA. Use of phytobiotics in broiler nutrition – an alternative to in feed antibiotics? Journal of Animan Feed Science 2010;19(3):338-347.

Habibi R, Jalilvand G, Samadi S, Azizpour A. Effect of different levels of essential oils of wormwood (*Artemisia absinthium*) and cumin (*Cuminumcyminum*) on growth performance carcass characteristics and immune system in broiler chicks. Iranian Journal of Applied Animal Science 2016;6(2):395-400.

Jafari M, Ebrahimnezhad Y, Janmohammadi H, Nazeradl K, Nemati M. Evaluation of protein and energy quality of poultry by-product meal using poultry assays. African Journal of Agriccultural Research 2011;6:1407-1412.

Jang IS, Ko YH, Kang SY, Lee CY. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Animal Feed Science Technology 2007;134(3):304-315.

Jerzsele A, Szeker K, Csizinszky R, Gere E, Jakab C, Mallo JJ, Galfi P. Efficacy of protected sodium butyrate, a protected blend of essential oils, their combination, and *Bacillus amyloliquefaciens* spore suspension against artificially induced necrotic enteritis in broilers. Poultry Science 2012;4:837-843.

Kazemi M. Essential oil of the aerial parts of *Artemisia annua* (*Asteraceae*) from Iran. Journal of Essential Oil Bearing Plants 2015;18(4):1003-1005.

Khalaji S, Zaghari M, Hatami KH, Hedari-Dastjerdi S, Lotfi L, Nazarian H. Black cumin seeds, *Artemisia leaves* (*Artemisia sieberi*), and *Camellia L.* plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. Poultry Science 2011;90(11):2500-2510.

Kurekci C, Padmanabha J, Bishop-Hurley SL, Hassan E, AlJassim RA, Mc Sweeney CS. Antimicrobial activity of essential oils and five terpenoid compounds against *Campylobacter jejuni* in pure and mixed culture experiments. International Journal Food Microbiology 2013;166(3):450-457.

Lopes-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry 2008;69(8):1732-1738.

Malik AA, Ahmad J, Mir SR, Ali M, Abdin MZ. Influence of chemical and biological treatments on volatile oil composition of *Artemisia annua* Linn. Industrial Crops and Products 2009;30:380-383.

Marinas IC, Oprea E, Chifiriuc MC, Badea IA, Buleandra M, Lazar V. Chemical composition and antipathogenic activity of *Artemisia annua* essential oil from Romania. Chemistry & Biodiversity 2015;12(10):1554-1564.

Muthaiyan A, Martin EM, Natesan S, Crandall PG, Wilkinson BJ, Ricke SC. Antimicrobial effect and mode of action of terpeneless cold-pressed Valencia orange essential oil on methicillin-resistant *Staphylococcus aureus.* Journal of Applield Microbiology 2012;5:1020-1033.

Nezhadali A, Parsa M. Study of the volatile compounds in *Artemisia absinthium* from Iran using HS/SPME/GC/MS. Advances in Applied Science Research 2010;1(3):174-179.

Nimbarte S, Kulkarni A. Comparative phytochemical analysis and resilience pattern exhibited by thyme and tea tree oil against selected poultry isolates. Journal of Agriculture and Veterinary Science 2013;4(4):113-117.

Olsson ME, Olofsson LM, Lindahl A, Lundgren A, Brodelius M, Brodelius PE. Localization of enzymes of artemisinin biosynthesis to the apical cells of glandular secretory trichomes of *Artemisia annua L*. Phytochemistry 2009;70:11223-11228.

Pandey K, Singh PA. The genus artemisia: a 2012-2017 literature review on chemical composition, antimicrobial, insecticidal and antioxidant activities of essential oils. Medicines 2017;4(3):68.

Perazzo FF, Carvalho JCT, Carvalho JE, Rehder VLG. Central properties of the essential oil and the crude ethanol extract from aerial parts of *Artemisia annua.* Journal of Pharmacology Research 2003;48(5):497-502.

Pop LM, Ştefănuţ LC, Tăbăran AF, Paştiu AI, Kalmár Z, Magdaş CA, Mircean V, *et al.* Influence of dietary artemisinin supplementation on productive performance and haematological parameters of broiler chickens. Revista Brasileira de Zootecnia 2017;46(2):130-137.

Radulovic NS, Randjelovic PJ, Stojanovic NM, Blagojevic PD, Stojanovic-Radic ZZ, Ilic IR, *et al.* Toxic essential oils. Part II: chemical, toxicological, pharmacological and microbiological profiles of *Artemisia annua L*. volatiles. Food and Chemical Toxicology 2013;58:37-49.

Ravindran V. Broiler nutrition in New Zealand - challenges and strategies. Arkansas Annual Nutrition Conference; 2006; Rogers, Arkansas.USA. p.1-7.

Rinttilä T, Apajalahti J. Intestinal microbiota and metabolites- Implications for broiler chicken health and performance. The Journal of Applied Poultry Research 2013;22(3):647-658.

Sahoo G, Mulla NSS, Ansari ZA, Mohandass C. Antibacterial activity of mangrove leaf extracts against human pathogens. Indian Journal of Pharmaceutical Sciences 2012;74(4):348-35.

Saracila M, Criste RD, Panaite TD, Vlaicu PA, Tabuc C, Turcu RP, *et al. Artemisia annua* as phytogenic feed additive in the diet of broilers (14-35 days) reared under heat stress (32 °C). Brazilian Journal of Poultry Science 2018;20(4):825-832.

Solorzano-Santos F, Miranda-Novales MG. Essential oils from aromatic herbs as antimicrobial agents. Current Opinion in Biotechnology 2012;23:136-141.

Tzenkova R,Kamenarska Z,Draganov A,Atanassov A. Composition of *Artemisia Annua* essential oil obtained from species growing wild in Bulgaria. Biotechnology & Biotechnological Equipment 2010;24(2):1833-1835.

Verdian-Rizi M. Variation in the essential oil composition of *Artemisia annua L*. of different growth stages cultivated in Iran. African Journal of Plant Science 2008;2(2):16-18.

Vidanarachchi JK, Mikkelsen LL, Sims I,Iji PA, Choct M. Phytobiotics: alternatives to antibiotic growth promoters in monogastric animal feeds. Recent Advances in Animal Nutrition in Australia 2005;15:131-144.

Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science 2008;86(14), E140-E148.

Zengin H, Baysal AH. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. Molecules 2014;19(11):17773–17798.

***Regulation (CE) no. 152/ 2009- (Methods of sampling and analysis for the official inspection of feeds).

***(2014)- The Management guide of Ross 308 hybrid (www.en.aviagen.com).

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