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# *Artemisia Annua as Phytogenic Feed Additive in the Diet of Broilers (14-35 Days) Reared under Heat Stress (32 ºC)*

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## ABSTRACT

The 21 days feeding trial was conducted on 90, Cobb 500 broilers (aged 14 days), assigned to 3 groups (C, E1 and E2) housed in an experimental hall at 32° C constant temperature and 23 h light regimen. During the growth period (14-35 days), the conventional diet (C) had corn and soybean meal as basic ingredients. Unlike the conventional diet formulation (C), the diet formulations for the experimental groups also included 0.005% *Artemisia annua* oil (E1) and 0.005% *Artemisia annua* oil plus 1% *Artemisia annua* powder (E2). Six broilers per group were slaughtered at 35 days of age in order to measure the weight of the carcass and internal organs of broilers, and samples of intestinal and caecal content were collected for bacteriological assessment (*Enterobacteriaceae, E. coli, staphylococci, Lactobacilli, Salmonella spp.*).The following parameters were monitored during the experimental period: bodyweight (g); average daily feed intake (g feed/broiler/day); average daily weight gain (g/broiler/day); feed conversion ratio (g feed/g gain). Under heat stress (32 °C), E2 broilers (mixture of *A. annua* oil and powder) had a significantly ($p<0.05$) higher average of daily feed intake than the broilers receiving the C diet or the diet supplemented just with *A. annua* oil (E1). Both samples of intestinal and caecal content, showed the lowest count ($p<0.05$) of *Enterobacteriaceae, E. coli* and staphylococcus colony forming units in E2 broilers. Diet with *A. annua* oil and powder provided proper conditions for lactic acid bacteria proliferation in the intestine and caecum of heat stressed broilers.

## INTRODUCTION

Their higher production performance and feed conversion efficiency make today's chickens more susceptible to heat stress than ever before (Lin *et al.* 2006). Global warming increases the frequency and intensity and aggravates the negative impacts of heat stress. The main consequence of heat exposure is a reduction in feed intake in order to reduce metabolic heat production. This reduction is approximately 17 % per 10 °C increase in ambient temperature above 20 °C (Austic, 1985). This decreased feed intake leads to growth depression (Geraert *et al.* 1996). The gastrointestinal tract is considered as one of the main target organs affected by heat stress (Song *et al.* 2017). Robust and balanced gut microbiota are required to support health and growth. Overgrowth of gut microbial or pathogens can change ecosystem balance and compromise gut integrity to initiate gastrointestinal complications (Helieh S. Oz, 2017). Health and nutrition are interdependent and the interaction between the two occurs largely in the gut (Choct, 2009).

The ban on antibiotic growth promoters compounds from poultry diets in Europe (Castanon *et al.* 2007) have put pressure on the poultry industry to look for viable alternatives that can improve performance, protect animal health, and maintain profit margins (Yegani & Korver,

2008). Phytogenic feed additives containing phenolics can improve the resistance of broilers to heat stress (Song *et al.* 2017).At the same time, several studies have reported effects on intestinal microflora when herbs and essential oils have been included in broiler diets (Acamovic & Cross, 2007). Herbs, spices, and various other plant extracts are being evaluated as alternatives to antibiotics and some do have growth promoting effects, antimicrobial properties, and other health-related benefits (Diaz-Sanchez *et al.* 2015).

Akbarian *et al.* (2016) consider that the diet supplemented with phytoadditives, which contain abundant phytochemicals and can be used as growth promoters and antioxidants, is a satisfactorily feasible approach that has been developed to ameliorate the detrimental effects of animals under challenging conditions. Phytochemicals are bioactive compounds beneficial for growth and health (Tuorkey, 2015), especially, phenolic compounds in plants are thought to be the major antioxidative compounds (Shahidi *et al.* 1992; Pietta, 2000).

Because of their wide application in the fields of poultry nutrition, pharmacology and agricultural industries, there is a great interest in the study of plant polyphenols for improvement of health benefits, where *Artemisia annua* could be a target plant (Carvalho *et al.* 2011).

*Artemisia annua* (*Sweet wormwood*) belongs to the plant family of *Asteraceae* and has been applied to poultry production as anticoccidial and antiparasitic agents due to the artemisinin composition in this medicinal plant (Song *et al.* 2017). Important constituents of *A. annua* are essential oil components like camphor, 1,8 – cineole and artemisa ketone, which are present in leaves in concentrations between 0.04 and 1.9% on a dry matter basis (Franz *et al.* 2010; Tzenkova *et al.* 2010). The essential oils are useful in the maintenance of a favourable microflora balance, suppression of protozoa, increasing nitrogen uptake and reducing methane production (Brisibe *et al.* 2008). Due to the content of antimicrobial components, it was expected that the dietary addition of *A. annua* would influence the composition of the intestinal microbiota of the ileum and caeca (Engberg *et al*. 2012). It is speculated that Artemisia leaves powder and extract in poultry rations have the potential to enhance daily weight gain and better feed conversion ratio (Gholamrezaie *et al.* 2013). Among the various bioactive compounds, such as flavonoids, coumarine, steroids, phenols and purines (Carra *et al.* 2014), the polyphenols account for several biological activities, among which anti-inflammatory, antipyretic, anti-cancer, anti-fungal, antiparasitic, and cytotoxic activities (Ćavar *et al.* 2012).

Although the inclusion of essential oils in broiler diets is intensely studied because of the many benefits, the use of essential oils involves higher poultry feeding costs than the use of plant powders.

The objective of this study was to investigate whether dietary *Artemisia annua* (oil or oil and powder) would alleviate the negative effects of heat stress (32 °C), applied between 14 and 35 days of age, on performance, relative carcass and organ weights, and gut microbiota of broiler chickens. The temperature of 32 ºC occurs often during daytime in the summer months in southern Romania (Latitude 44–44.5º; Longitude 23-28º).

## MATERIALS AND METHODS

The feeding trial was conducted in the experimental halls of the Laboratory of Chemistry and Nutrition Physiology within the National Research-Development Institute for Animal Biology and Nutrition (IBNA–Balotesti, Romania) according to an experimental protocol approved by the Ethics Commission of the Institute. The veterinary conditions regarding the protection of the animals used in this research complied with all national and EU standards and legislation. A total of 90 day – old Cobb 500 broiler chicks were purchased and received a conventional starter (1-14 days) diet (3000 kcal/kg metabolizable energy, 22 % crude protein). At the age of 14 days, the broilers were weighted and assigned to three groups (C, E1, E2). The chicks were housed in an experimental hall at 32 ºC constant temperature, humidity 36% and 23 h light regimen, with 0.38% ventilation/broiler, and 899 ppm $CO_2$ emission. The broilers had free access to the feed and water.

During the grower period (14-35 days), the conventional diet (group C) had corn and soybean meal as basic ingredients (Table 1), while the diet formulations for the experimental groups also included 0.005% *Artemisia annua* oil (E1) or 0.005% *Artemisia annua* oil plus 1% *Artemisia annua* powder (E2).

The dietary Artemisia oil used in the experimental formulations (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 (46.55º N, 24.63º E). Plants were dried for three weeks under shade at ambient temperature (20˚C) and ground finely to obtain *A. annua* powder.

**Table 1 –** Diet formulation.

| Ingredients | *Grower diet* (14-35 days) | | |
|---|---|---|---|
| | C | E1 | E2 |
| | % | | |
| Corn | 62 | 62 | 62 |
| Soybean meal | 26.58 | 26.58 | 26.58 |
| *Artemisia annua* oil | - | 0.005 | 0.005 |
| *Artemisia annua* powder | - | - | 1 |
| Gluten | 4 | 4 | 4 |
| Vegetable oil | 2.5 | 2.5 | 2.5 |
| Lysine | 0.47 | 0.48 | 0.48 |
| Methionine | 0.26 | 0.26 | 0.26 |
| Choline | 0.05 | 0.05 | 0.05 |
| Calcium carbonate | 1.4 | 1.4 | 1.4 |
| Monocalcium phosphate | 1.36 | 1.36 | 1.36 |
| Salt | 0.37 | 0.37 | 0.37 |
| Vitamin-mineral premix* | 1 | 1 | 1 |
| Total | 100 | 100 | 100 |
| Calculated composition | | | |
| Metabolisable energy, kcal/kg | 3,140.03 | 3,140.03 | 3,140.03 |
| Crude protein, % | 20.00 | 20.00 | 20.00 |
| Ether extractives, % | 4.46 | 4.46 | 4.46 |
| Crude fibre, % | 3.54 | 3.54 | 3.54 |
| Lysine, % | 1.35 | 1.35 | 1.35 |
| Methionine, % | 0.58 | 0.58 | 0.58 |
| Calcium, % | 0.84 | 0.84 | 0.84 |
| Phosphorus, % | 0.75 | 0.75 | 0.75 |
| Available Phosphorus, % | 0.42 | 0.42 | 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.

Throughout the experimental period (14-35 days, broiler age) the following parameters were monitored: bodyweight (g); average daily feed intake (g feed/broiler/day); average daily weight gain (g/broiler/day) and feed conversion ratio (g feed/g gain). Mortality was recorded throughout the experimental period. At 35 days, according to the approved working protocol, 6 broilers/group were slaughtered by cervical dislocation and immediately bled. Carcasses were eviscerated manually and the gastrointestinal tract was excised. The weights of the carcass and internal organs (gizzard, heart, liver, spleen, bile and bursa of Fabricius) and the lengths of the small intestine and of the caeca were measured. The results were expressed as organ weight (g) and length of intestinal tract (cm). Small intestinal (duodenum, jejunum, ileum) and caecal contents (2 caeca per bird) were collected aseptically in sterilized plastic tubes and preserved at -20 ºC until the bacteriological analyses (*Enterobacteriaceae, E. coli*, lactobacilli, staphylococci, *Salmonella spp*). The intestinal digesta pH measurements were performed with a portable pH meter Five Go F2-Food kit with LE 427IP67, Sensor Metler Tolledo, by inserting the pH meter electrode into the tubes with intestinal and caecal content.

A classical medium of isolation, G.E.A.M. or Levine, was used to determine the *Enterobacteriaceae* and the *E. coli* in the samples of intestinal (duodenum, jejunum and ileum) 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 ºC). Decimal solutions up to $10^{-5}$ in medium with lauryl sulphate were prepared. Dilutions $10^{-2}$-$10^{-5}$ were used to seed 2 Petri dishes/dilution, on Levine medium. The Petri dishes were incubated for 48h at 37 ºC, and the colonies which developed in the dishes were thereafter counted. *E. 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 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, according to the model $Yi = Ti + ei$, where $Yi$ was the dependent variable, $Ti$ is the treatment and $ei$ is the error. When overall F-test was significant, differences between means were declared significant at $p<0.05$ using the test of Tukey.

## RESULTS AND DISCUSSIONS

According to Table 2 data, the body weight at 35 days of E2 broilers (treated with a mixture of *A. annua* oil and powder) was significantly ($p<0.05$) higher than that of the broilers treated with Artemisia oil only (E1).

Broilers fed E2 diet also had a significantly ($p<0.05$) higher average daily feed intake than those fed the conventional diet or those treated with *A. annua* oil (Table 2). Also working under heat stress, Wan *et al.* (2017) reported a higher body weight and average daily feed intake in Arbor Acres (22-42 days) broilers fed enzymatically treated Artemisia diets than the control group. On the contrary, Engberg *et al*. (2012)

**Table 2 –** The effect of *Artemisia annua* (oil/oil and powder) on broiler performance (14 – 35 days).

| Performance | Period | C *Conventional diet* | E1 0.005% *Artemisia annua* oil | E2 *Artemisia annua*: 0.005% oil +1% powder | Reference values* | SEM | *p*-value |
|---|---|---|---|---|---|---|---|
| Body weight (g/broiler) | 14 days | 318.96[a] | 298.34[a] | 304.97[a] | 465 | 5.829 | 0.343 |
| | 35 days | 1523.05[ab] | 1477.22[a] | 1680.26[b] | 2191 | 40.836 | 0.098 |
| Average daily feed intake (g/broiler/day) | 14 – 35 days | 88.39[a] | 84.04[a] | 97.94[b] | 133.81 | 1.378 | <0.0001 |
| Average daily weight gain (g/broiler/day) | 14 – 35 days | 54.001[ab] | 51.267[a] | 59.957[b] | 62.6 | 1.554 | 0.0603 |
| Feed conversion ratio (g feed/g gain) | 14 – 35 days | 1.657[a] | 1.667[a] | 1.629[a] | 1.53 | 0.067 | 0.9717 |

[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.

*= The Management guide of Cobb 500 hybrid

reported that Ross 308 broilers (1-35 days) reared under optimal temperature conditions and treated with 10 g and 20 g Artemisia /kg diet, had a lower body weight, but a better feed conversion ratio than the broilers receiving the conventional diet. Table 2 data did not show a significant ($p>0.05$) difference between the three groups regarding the feed conversion ratio. Pop *et al*. (2017) has shown that Ross 308 broiler chicks reared under normal conditions of temperature and treated with 50 ppm artemisinin showed gains and feed conversion ratio comparable with those of the broiler receiving the conventional diet formulation. The different results of these studies may be accounted by the different chemical composition of the used plant, or by the concentrations, active matter and biological activity of the plant (Amad *et al.* 2011).

The broilers treated with a mixture of *A. annua* oil and powder (E2) had a significantly ($p<0.05$) higher average daily weight gain (Table 2) than those treated with *A. annua* oil. Gholamrezaie *et al*. (2013) reported higher daily weight gains in Cobb 500 broiler chicks reared under optimal temperature conditions, and treated with 2000 and 4000 ppm *A. annua* leaves powder. Kostadinovic *et al*. (2015) reported similar results using *A. absinthium* powder (100, 150, 200 g/kg) in Arbor Acress broiler diet (1-42 days).

It must be noticed, that the heat (32 °C) depressed broiler body weight compared to the reference values from the *Management guide of Cobb 500 hybrid* for the broilers reared under normal temperature conditions. Compared to the body weight from the *Management guide of Cobb 500 hybrid*, the final body weight of the broilers was 30.48 % lower in group C, 32.58 % lower in group E1, and 23.31 % lower in group E2. Compared to the data from the *Management guide of Cobb 500 hybrid*, the daily feed intake of the broilers was 33.94 % lower in group C, 37.19 % lower in group E1 and 26.81 % lower in group E2. Throughout the experimental weeks under heat stress no mortalities were recorded in any of the three groups.

Table 3 shows the measurements performed after slaughter (35 days). The results showed that the carcass and organ weight of Cobb 500 broilers was not significantly ($p>0.05$) different between groups.

Several researchers studied the influence of the dietary *A. annua* on the weight of broiler internal organs. Habibi *et al.* (2016) has shown that the dietary Artemisia oil (100, 200, 300 ppm) given to Ross 308 broilers reared under thermoneutral conditions did not influence spleen weight, but increased the relative weight of the lymphoid organs, such as the bursa of Fabricius. Similarly, Gholamrezaie *et al.* (2013) reported that *A. annua* treatment increased the weight of the thymus and bursa of Fabricius in Cobb 500 broilers reared under thermoneutral conditions of temperature. The weights of bursa of Fabricius of chicks from this experiment (Table 3) were lower than those reported by Gholamrezaie *et al.* (2013). This consequence is due to heat stress. Quinteiro Filho *et al.* (2010) noticed that the broilers exposed to heat stress (35-42 days) at 31 and 36 °C had the weight of the bursa of Fabricius significantly ($p<0.05$) lower than broilers reared under thermoneutral conditions. These statements were also supported by the results of Niu *et al.* (2009) in a study conducted on Arbor Acress (1-42 days) under heat stress conditions. The bursa of Fabricius plays an important role in the enzymatic maturation and acquiring of the immunologic competence of T and B lymphocytes (Rudrappa & Humphrey, 2007), so that the change in the development of this organ due to stress seriously hampers the immune system of the chickens (Oznurlu *et al.* 2010).

At 35 days, the length of the small intestine and of the caeca was not altered by any of the dietary treatments (Table 3). Bento *et al.* (2013) affirms

**Table 3 –** The effect of dietary *Artemisia annua* (oil/oil and powder) on digestive organ weight, length of the intestinal tract and intestinal digesta pH of broilers at 35 days of age.

| Specification | C<br>*Conventional diet* | E1<br>0.005%<br>*Artemisia annua* oil | E2<br>*Artemisia annua*:<br>0.005% oil +1% powder | SEM | *p*-value |
|---|---|---|---|---|---|
| *Weight (grams)* | | | | | |
| Carcass | 1192[a] | 1216[a] | 1251[a] | 18.339 | 0.4499 |
| Gizzard | 18.26[a] | 19.12[a] | 19.02[a] | 1.178 | 0.9553 |
| Heart | 5.76[a] | 6.32[a] | 5.62[a] | 0.204 | 0.3608 |
| Liver | 32.26[a] | 33.56[a] | 33.86[a] | 1.027 | 0.8195 |
| Spleen | 1.18[a] | 0.96[a] | 1.22[a] | 0.136 | 0.7317 |
| Bile | 1.14[a] | 1.66[a] | 1.79[a] | 0.202 | 0.4073 |
| Bursa of Fabricius | 0.54[a] | 0.70[a] | 0.88[a] | 0.091 | 0.3334 |
| Full digestive tract | 42.66[a] | 46.42[a] | 45.76[a] | 1.601 | 0.6270 |
| *Length (cm)* | | | | | |
| Small intestine | 174.4[a] | 177.4[a] | 180.8[a] | 4.23 | 0.8476 |
| Caecum 1 | 14.8[a] | 15.4[a] | 16.8[a] | 0.494 | 0.2521 |
| Caecum 2 | 15.4[a] | 15.1[a] | 16.5[a] | 0.611 | 0.6497 |
| *pH value* | | | | | |
| Small intestine | 5.56[a] | 5.4[a] | 5.48[a] | 0.107 | 0.8443 |
| Caeca | 6.27[a] | 6.69[a] | 6.05[a] | 0.153 | 0.2262 |

[a] = Not significant difference ($p$>0.05). SEM: standard error of the mean.

that the pH of the essential oils matrix affects their hydrophobicity, thus influencing the interaction with the bacterial cell membrane and, therefore, will affect the antibacterial action of the essential oils in different segments of the intestine. Caecal pH was lower in E2 broilers than in C and E1 broilers, while the intestinal pH was lower in E1, but the difference between groups was not statistically significant (Table 3).

The number of *Enterobacteriaceae* colony-forming units was significantly ($p$<0.05) lower in the intestinal content of E1 broilers (*A. annua* oil) and E2 (mixture of *A. annua* oil and powder) than in the control group (Table 4). However, E1 broilers had a significantly ($p$<0.05) higher number of *E. coli* and staphylococci colony-forming units than E2. Most studies investigating the antimicrobial activity of different essential oils against different bacteria agree that essential oils are slightly more active against Gram positive bacteria than against Gram-negative bacteria (Brenes & Roura, 2010). Lopes-Lutz *et al*. (2008) reported that oils of different species of Artemisia had varying degrees of growth inhibition against microorganisms such as *Escherichia coli, Staphylococcus epidermidis* and *Staphylococcus aureus.* Although the treatment with Artemisia oil (E1) did not inhibit the proliferation of some pathogens from the intestine, it multiplied the lactobacilli species, resulting a significantly ($p$<0.05) higher number of colony-forming units compared to group C, but lower, however, than in group E2 ($p$<0.05). The broilers treated with the mixture of *A. annua* oil and powder (E2) had a significantly ($p$<0.05) higher lactobacilli count in the intestinal content than groups C and E1 (*A. annua* oil). Therefore, the combination of *A. annua* oil and powder proved, even under heat stress, to have a beneficial effect for the proliferation of lactobacilli species (Table 4). Criste *et al.* (2017) showed that the dietary oregano powder given to Cobb 500 broilers (14-35 days) reared under heat stress, influenced the

**Table 4 –** The effect of *Artemisia annua* (oil/oil and powder) in the diet of broilers (14 – 35 days) on intestinal microbiota composition ($log_{10}$ CFU*/g wet intestinal digesta).

| Specification | C<br>*Conventional diet* | E1<br>0.005%<br>*Artemisia annua* oil | E2<br>*Artemisia annua:*<br>0.005% oil +1% owder | SEM | *p*-value |
|---|---|---|---|---|---|
| *Enterobacteriaceae*, $log_{10}$ | 7.27[c] | 7.25[b] | 7.22[a] | 0.005 | <0.0001 |
| *Escherichia coli*, $log_{10}$ | 5.88[b] | 5.89[b] | 5.83[a] | 0.008 | 0.0002 |
| Staphylococci, $log_{10}$ | 5.60[b] | 5.63[c] | 5.53[a] | 0.012 | <0.0001 |
| Lactobacilli, $log_{10}$ | 6.41[a] | 6.66[b] | 7.00[c] | 0.65 | <0.0001 |
| *Salmonella spp.* | Absent | Absent | Absent | - | - |

Where: * = 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.

count of *Enterobacteriaceae* and lactic acid bacteria in the ileal content, so the *Enterobacteriaceae* count was significantly ($p$<0.05) lower, while the lactic acid bacteria count increased compared to group C broilers.

The data on the intestinal microflora are partially different from those determined in the caecal content, particularly for group E1 (Table 5).

Similarly to the data obtained on the intestinal content, E1 broilers had a significantly ($p$<0.05) higher number of *Enterobacteriaceae* and *E coli* colony-forming units than E2 broilers (Table 5). Unlike Table 4 data, the staphylococci count determined in E1 broilers was significantly ($p$< 0.05) lower than in group C, rather comparable with group E2. However, the lactobacilli count determined in E1 broilers was significantly ($p$<0.05) lower than in groups C and E2. The beneficial influence of the mixture of *A. annua* oil and powder on the composition of the caecal microflora can be observed in group E2 broilers (Table 5), with significantly ($p$<0.05) lower counts of *Enterobacteriaceae* and *E. coli*, and significantly ($p$<0.05) higher counts of lactobacilli. According to the data of this study, the mixture of *A. annua* oil and powder had a synergic effect on the caecal microbiota composition, ensuring proper conditions for lactobacilli proliferation, which maintained the balance between the various bacterial species from the gut microflora. *Salmonella spp.* were absent in all samples of intestinal and caecal content (Tables 4 and 5).

There is rather scarce information in the literature about the effect of the mixture of *A. annua* oil and powder on the intestinal microbial profile. However, there are studies which investigated the use of different forms of *A. annua*. Thus, Engberg *et al.* (2012) showed that *A. annua* extract in n-hexane (500 mg/kg), added to the diet of broilers infected with *Clostridium perfringens* reduced the count of anaerobe bacteria in the caecal content of the broilers. They also tested, separately, the dietary effect of dry *A. annua* (10 g/kg), and noticed that the chicken treated with it had a lower count of lactose – negative *Enterobacteriaceae* than the chicken which received the conventional diet.

## CONCLUSIONS

Broilers fed diet with mixture of *A. annua* oil and powder had a significantly ($p$<0.05) higher average daily feed intake compared to the control broilers or to E1 broilers, while their average daily weight gain was significantly higher only compared to E1 broilers. However, the heat stress (32 °C) depressed the body weight, average daily feed intake and average daily weight gain of all three groups, throughout the growth period (14-35 days), as compared to the performance stated in the *Management guide of Cobb 500 hybrid* for broilers reared under normal conditions of temperature.

The *A. annua* oil treatment did not inhibit the development of some pathogens in the intestine, but supported the development of lactobacilli species in the intestine, yielding a significantly ($p$<0.05) higher number of colony-forming units compared to the control group, but still lower than the group treated with the mixture of *A. annua* oil and powder. Both in the samples of intestinal content, and in the samples of caecal content, the lowest ($p$<0.05) counts of *Enterobacteriaceae, E. coli* and staphylococci colony – forming units was determined in the broilers treated with the mixture of *A. annua* oil and powder.

The mixture of *A. annua* oil and powder added to the diet of Cobb 500 broilers reared under heat stress (32°C) had a beneficial effect by supporting the replication of the lactobacilli species throughout the entire segment of the small intestine and in the caeca.

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**Table 5 –** The effect of *Artemisia annua* (oil/ oil and powder) in the diet of broilers (14 – 35 days) on caecal microbiota composition ($log_{10}$ CFU*/g wet *caecal digesta*).

| Specification | C<br>*Conventional diet* | E1<br>0.005%<br>*Artemisia annua* oil | E2<br>*Artemisia annua*:<br>0.005% oil +1% powder | SEM | *p*-value |
|---|---|---|---|---|---|
| *Enterobacteriaceae,* $log_{10}$ | 11.02[b] | 11.01[b] | 10.98[a] | 0.005 | 0.0007 |
| *Escherichia coli,* $log_{10}$ | 9.93[c] | 9.91[b] | 9.84[a] | 0.010 | <0.0001 |
| Staphylococci, $log_{10}$ | 8.65[b] | 8.57[a] | 8.54[a] | 0.013 | <0.0001 |
| Lactobacilli, $log_{10}$ | 11.45[b] | 11.44[a] | 12.27[c] | 0.103 | <0.0001 |
| *Salmonella spp*. | Absent | Absent | Absent | - | - |

Where: *=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.

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