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Influence of some dry herbs as a dietary supplement on productivity, natural humoral immunity and oxidative status in broiler turkeys

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Abstract

Oblakova, M., Nikolova, G., Hristakieva, P., Mincheva, N., Ivanova, I., Karamalakova, Y. & Gadjeva, V. (2022). Influence of some dry herbs as a dietary supplement on productivity, natural humoral immunity and oxidative status in broiler turkeys. *Bulg. J. Agric. Sci., 28 (4)*, 752–763

The aim of the present work was to study the influence of dried herbs addition to feed: E1 – 1% *Matricaria chamomilla*; E2 – 1% *Rosmarinus officinalis*; E3 – 1% *Lavandula angustifolia*; E4 – 1% *Origanum vulgare*; E5 – 1% *Thymus vulgaris*; E6 – 1% *Hypericum perforatum* on productivity, health status and natural immunity in turkeys. A total of 105 one-day-old female broiler turkeys were weighed, marked, and divided into 7 groups of 15 turkeys (each of which included 3 replicates of 5 turkeys) – one control (C) and six experimental (E1, E2, E3, E4, E5, E6). The addition of these dried herbs to broiler turkey feed did not affect the final live weight at 126 days of age, the average daily gain, and the conversion of feed for the entire rearing period. This affects the values of the biochemical parameters of the blood, but the differences are not statistically significant. The studied parameters are variable and with insignificant differences between the different groups. Cholesterol and triglyceride levels decreased in the group with added 1% rosemary in the feed ($P<0.01$). The differences in the groups of the three liver enzymes ASAT (U/l), ALAT (U/l), GGT (U/l) were not statistically different. The alternative pathway for complement activation (APAC) had the highest activity in the group treated with thyme (E5) 718.94 ($P<0.001$). The activity of betalysine is the highest in the group E3- 17.04%, E4- 16.39%, and E6- 18.05% ($P <0.001$), and the lowest is in the group E1 -7.02% and C- 8.94%.

Keywords: turkeys; herbs; lysozyme; complement activity; betalysine; oxidative status

Introduction

In recent years, following the ban on the use of antibiotics in livestock and especially poultry, there has been increased interest in biotech and natural products and their application to improve the productivity, health status, quality, and safety of their products (Liu et al., 2011). Numerous studies have been conducted worldwide on the effect of the addition of herbs and medicinal plants on various productivity indicators in broiler chickens (Ocak et al., 2008; Moorthy et al., 2009; Ali, 2014; Mohamed, 2015). Steiner (2009) and Wallace et al. (2010) report that plant extracts and various phytobiotics derived from leaves, roots, tubers, or fruits of herbs, spices, and other plants are excellent growth enhancers in poultry farming. Research on the use of herbal mixtures as substitutes for antibiotics in broiler diets has indicated in-consistent results. Some authors reported positive effects on performance (Ertas et al., 2005; Peric et al., 2008). Also, others established that there is no consequence on body weight gain (BWG), feed intake (FI), or feed conversion ratio (FCR) (Ocak et al., 2008; Mikaili et al., 2010).

Chamomile (*M. Chamomilla*) can be used as a natural antimicrobial agent for infectious diseases. Jakubcova et al. (2014) conducted an experiment with broilers hybrid combination Ross 308 with Chamomile extract in concentrations of 0.3%, 0.6%, and 1.2% and found that it did not significantly affect the productivity of chickens. While Skomorucha et al. (2013) reported that chamomile extract or St. John's wort extract added to the water of chickens increases their weight. Schleicher et al. (1998) reported that chamomile or dandelion added to the diet of broiler chickens had a lower mortality rate. Chamomile flowers have an antimicrobial and anti-inflammatory effect on broiler chickens (Abaza, 2003). In addition, Chamomile flowers reduced total cholesterol and GOT levels in serum (Abaza, 2004). Moreover, chamomile flowers have improved the growth and conversion of feed-in broilers (Al-Kaisse, 2011; Onwurah, 2011). Other studies suggest that thyme may be an alternative natural growth promoter in poultry instead of antibiotics (Mc Devitt et al., 2007).

Poultry has become a major source of animal protein, so maintaining quality is important. The high content of polyunsaturated fatty acids and the low level of natural antioxidants in poultry meat can lead to deterioration of quality by oxidation of lipids during storage. Synthetic antioxidants have been widely used, but some of them have shown several carcinogenic effects (Avila-Ramos et al., 2013; El Abed et al., 2014; Marzoni et al., 2014). Therefore, the use of synthetic antioxidants has been abandoned by many countries due to their toxic effects and putative carcinogenic potential (Spigno & De Faveri, 2007). Plant extracts that contain some bioactive compounds have very effective antioxidant activity compared to synthetic antioxidants (Zeković et al., 2014).

The increased antioxidant status of farm animals, and hence the increased oxidative stability of the raw meat product, are considered to be beneficial for both consumers and the processing industry. Research has focused mainly on the effects of medicinal and aromatic plants on mortality, stress hormone levels, blood and muscle metabolism, meat quality, and even the function of the immune system in domestic animals. It has been found that plants such as tea (Tang et al., 2000), rosemary, sage (Lopez-Bote et al., 1998), and lavender in various *in vitro* models of systems (Dorman et al., 2000) containing high concentrations of antioxidants, reduce the lipid peroxidation in the chickens' muscles.

The effect of rosemary on the antioxidant parameters of serum superoxide dismutase activity (SOD) and concentrations of GSH and MDA in birds in particular in chickens has been demonstrated. Serum SOD activity increased in the rosemary groups and reached a maximum (290 U/ml) at 6 g/kg. Hashemipour et al. (2013) noted that the intake of herbs or their content leads to an increase in serum antioxidant enzyme activities such as SOD and GSH and a decrease in MDA concentration.

In the scientific literature data on the influence of dried herbs Chamomile (*Matricaria chamomilla*), Rosemary (*Rosmarinus officinalis*), Lavender (*Lavandula angustifolia*), Oregano (*Origanum vulgare*), Thyme (*Thymus vulgaris*) and St. John's wort (*Hypericum perforatum*) on productivity, health status, natural immunity in turkeys are very limited. The aim of the present study is to explore the influence the dried herbs on growth ability, biochemical parameters, important factors of humoral natural immunity, and the oxidant/ antioxidant status in broiler turkeys.

Materials and Methods

Experimental Design

The experimental part of this study was conducted in the production experimental base of the Agricultural Institute – Stara Zagora in the period May-September 2019. A total of 105 one-day-old female broiler turkeys were weighed, marked, and divided into 7 groups of 15 turkeys (each of which included 3 replicates of 5 turkeys). The turkeys were reared in boxes, flooring on sawdust from 1 day to 126 days of age in controlled conditions (12 h light/dark cycles), the temperature of 18 -23ºC, and humidity of 40-70%, with free access to tap water and standard laboratory chow. In the first week, temperature control circles and corrugated cardboard were used to limit sawdust bites. Experiments were carried out in accordance with European directive 86/609/EEC of 24.11.1986 for the protection of animals used for scientific and experimental purposes.

The groups were divided into seven: one control (C) and six experimental (E1, E2, E3, E4, E5, E6). The control turkeys group received the feed that met the age requirements without the addition of dried herbs. The experimental groups together with age-appropriate feed received at E1- 1% Chamomile *(Matricaria chamomilla)*; E2- 1% *Rosemary (Rosmarinus officinalis)*; E3- 1% *Lavender (Lavandula angustifolia)*; E4 – 1% *Oregano (Origanum vulgare)*; E5- 1% *Thyme (Thymus vulgaris)*; E6 – 1% St. John's wort(*Hypericum perforatum)*.

The main feed received by all groups of turkeys was prepared depending on the category and age.

Starter- (Starter 1 from 1 to 21 days of age- Crude protein- 28% and Metabolic energy- 2800 kcal/kg; Starter 2- from 22 to 42 days of age – Crude protein- 26% and Metabolic energy- 2900 kcal/kg; Starter 3- from 43 to 63 days of age – Crude protein – 24% and Metabolic energy – 3000 kcal/kg).

Grower (Grower 1 from 64 to 84 days of age: Crude protein – 22% and Metabolic energy – 3100 kcal/kg; Grower 2- from 85 to 100 days of age: Crude protein – 19% and Metabolic energy – 3250 kcal/kg).

Finisher (finisher from 101 to 126 days of age: Crude protein – 17% and Metabolic energy – 3350 kcal/kg). The feed was in powder form. Bird weight and feed consumption were monitored in each group.

At the end of the experimental period, body weight (BW) was reported, for the whole period the feed conversion (FCR), the average daily feed consumption (AFC), and the average daily gain (ADG) for the whole growing period (126 days) were calculated.

Humoral immunity

At the end of the fattening at 126 days of age, blood was collected from *v. subcutanea ulnaris* from 6 turkeys from each group to assay some parameters of humoral innate immunity. Serum lysozyme concentrations were determined by the method of Lie (1985), the alternative pathway of complement activation (APCA) was evaluated by the method of Sotirov (1986) and beta-lysine activity was assessed by the method of Buharin et al. (1977).

Biochemical blood test

To determine the blood parameters, blood was taken at the end of the fattening period 126 days from the axillary vein. The biochemical parameters of the blood were determined in the biochemical laboratory of the Veterinary Medicine Faculty at the Trakia University. Blood samples were used to determine the following chemical parameters: blood sugar (mmol/l), Total protein (g/l), Creatinine (mmol/l), Picric acid (mmol/l), Cholesterol (mmol/l), TG (mmol/l), ASAT (U/l), ALAT (U/l), GGT (U/l). The blood was collected in sterile containers. The study was performed using an automated biochemical analyzer. Within 30 minutes after receiving the blood, all blood samples were centrifuged for 10 min at 1500 rpm. Immediately thereafter, the plasmas were separated and stored at -20 °C until analysis. For the determination we used an automatic biochemical analyzer, using reagents from the company "Biomed": glucose-GOD-PAP-; cholesterol-Peg 6000; total protein PLUS. The birds were examined according to the stipulations Regulation № 20 of 1.11.2012 on the minimum requirements for protection and welfare of experimental animals and the requirements for sites for use, breeding, and/or delivery.

Serum and caecal lysozyme concentrations were determined by the method of Lie et al. (1985). The alternative pathway of complement activation (APCA) was studied by the method of Sotirov (1986). Optical density was measured by the "Sumal-PE2" ELISA reader (Karl Zeiss, Germany) at 540 nm. Lysozyme content, APAC activity, and statistical analysis were calculated using special computer programs developed at Trakia University. Betalysines were assessed by the method of Buharin et al. (1977). Briefly in a flat bottomed plate (Flow Laboratories, UK) dropped 80 μL serum + 80 μL suspensions of *Bacillus subtilis* (Merck, cat. № 1.10649) and optical density was measured at 600 nm using 'Sumal-PE2' ELISA reader (Karl Zeiss, Germany).

Immuno-enzymatic methods

Standard ELISA kits were used to determine the levels of the antioxidant enzyme Superoxide Dismutase (SOD), Reduced Glutathione (GSH), and Malondialdehyde (MDA). All enzyme-linked immunosorbent assays were performed according to the procedure described in the respective kit.

Electron paramagnetic resonance (EPR) spectroscopy

EPR measurements of all tested samples were conducted at room temperature (18-23ºC) on an X-band EMXmicro, spectrometer Bruker, Germany, equipped with a standard Resonator. Quartz capillaries were used as sample tubes. The sample tube was sealed and placed in a standard EPR quartz tube (i.d. 3 mm) which was fixed in the EPR cavity. All EPR experiments were carried out in triplicate and repeated. Spectral processing was performed using Bruker WIN-EPR and SimFonia software.

EPR ex vivo evaluation of ascorbate radicals

The Asc• measurement was according to Bailey et al. (2004) with some modification. Briefly, the sera were mixed with DMSO at 1:3 ratios, and centrifuged for 10 min at 4000 rpm. Then, the supernatant was immediately transferred and measured. The EPR settings were 3505.00 G center field, 20.00 mW microwave power, 1.00 G modulation amplitude, 15 G sweep width, 1×105 gain, 40.96 ms time constant, 60.42 s sweep time, 10 scans per sample.

Ex vivo ROS evaluation

The ROS level measurement was according to Shi et al. (2005) and modified by Zheleva et al. (2012). The real-time formation of ROS in the serum was investigated by mixing the samples with PBN spin trapping. The EPR settings were 3503.73 G center field, 20.00 mW microwave power, 5 G modulation amplitude, 50 G sweep width, 1 x 105 gain, 81.92 ms time constant, 125.95 s sweep time, 5 scans per sample.

Ex vivo EPR evaluation the levels of •NO radicals

Based on the previous methods (Yoshioka et al., 1996; Yokoyama et al., 2004) we developed and adapted the EPR method for evaluating the levels of •NO radicals in serum. Briefly, the solution of Carboxy PTIO.K (50 mM) was prepared after dissolving in a mixture of Tris buffer (50 mM, pH 7.5) and DMSO in a ratio of 9:1. To 100 ml sera were added 900 ml Tris buffer plus DMSO (9:1) and centrifuged at 4000 rpm for 10 min at 4ºC. The tested sample (100 mL) and 100 mL 50 mM solution of Carboxy PTIO were mixed. The EPR spectrums of the spin adduct formed between the spin trap Carboxy. PTIO and generated •NO radicals were recorded. The levels of •NO radicals were calculated as double integrated plots of EPR spectra and results were expressed in arbitrary units. The EPR settings were: 3505 G centerfield, 6.42 mW microwave power, 5 G modulation amplitude, 75 G sweep width, 2.5 x 102 gain, 40.96 ms time constant, 60.42 s sweep time, 1 scan per sample.

Statistical analysis

Statistical analyses were conducted with the STATISTICA program, ver. 10 (StatSoft, Inc., 2011). One-way analysis of variance (ANOVA) tests was performed to compare the means of all data. Dependent on the variance homogeneity (evaluated by Levene's test), identification of significant differences (P<0.05) was carried out making use of the LSD post-hoc test, while for nonparametric analysis was used the Kruskal-Wallis test.

Results and Discussion

Table 1 shows the results for body weight at 126 days of age, average daily growth, average daily feed consumption and feed conversion up to 126 days of age.

The addition of dried herbs to broiler turkey feed did not affect the final body weight at 126 days of age (Table 1). The average daily growth and conversion of feed for the whole rearing period was not affected by the addition of dried herbs to the diet in the studied groups of turkeys (P> 0.05). This contradicts the research of El-Ghousein and Al-Beitawi (2009). They reported that the addition of 2% *T. vulgaris* to chicken feed increased FI, BWG, and FCE as well as the dressing percentage and the weights of liver, heart, and gizzard, and those treatments decreased abdominal fat. While the average daily consumption of fodder throughout the growing period showed the highest values of this indicator in groups E3, E4, E5 and E6 compared to the control group (C), which has the lowest average daily consumption – 0.312 kg/day at P <0.05, followed by the group with addition to chamomile feed (E1) with an average daily feed consumption of 0.320 kg/day. Manafi et al. (2014) added 500 ppm rosemary extract to broiler feed, and the bodyweight in the rosemary group was higher compared to the control group at 42 days of age. The addition of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) at 15 or 20 g/kg^{-1} feed may increase the feed conversion ratio, body weight gain, feed intake, and performance of broilers (Abdel – Wareth et al., 2012). Limited information is available in the literature on the effects of lavender on broiler performance. Nasiri- Moghaddam et al. (2012) reported that dietary supplementation of lavender essential oil (at 350 mg/kg) increased body weight gain (BWG) and decreased feed conversion ratio (FCR) at the period of 22 to 42 d age. Kheiri et al. (2014) found that the addition of *Hypericum persicum* extract of 100, 150, and 200 mg/L can improve body performance. Landy et al. (2012) states that the addition of *Hypericum perforatum* powder does not have a positive effect on growth. They found that broilers receiving *Hypericum perforatum* in a feed (10 g/kg) had a higher feed intake than other groups during the start-up period.

Table 1. Effects of dietary treatments on broilers performance up to 126 days of age

Groups	Parameters			
	BW	ADG	AFC	FCR
C	9873.33	0.097	0.312[b]	3.333
E1	9638.46	0.098	0.320[ab]	3.241
E2	9560.00	0.094	0.325[a]	3.500
E3	9623.08	0.100	0.329[a]	3.303
E4	9807.69	0.103	0.330[a]	3.214
E5	9671.43	0.097	0.333[a]	3.478
E6	9969.23	0.105	0.336[a]	3.212
SEM	50.47	0.001	0.003	0.973
p-value	0.267	0.800	0.045	0.917

The means within the same column with at least one common letter, do not have a statistically significant difference (P>0.05); SEM: standard error of the means; BW(кg): bodyweight at 126 days of age; ADG- Average Daily Growth(kg); AFC -Average Feed Consumption (kg/day); FCR: feed conversion ratio (kg/kg)

Usually, the biochemical parameters of the blood reflect the health and are vital indicators of the nutritional and physiological status of the birds (Abd El-Hack & Alagawany, 2015). Table 2 shows the results of the biochemical parameters of the blood in turkeys fed with the addition of 1% dry herbs in the feed. It can be seen that the plasma glucose level varies (P> 0.05) between the groups receiving in the ration a rosemary (E2) (16.05 mmol/l) and lavender (E3) (17.49

mmol/l). The total protein 38.28 g/l and the uric acid 237.67 mol/l is the lowest in the birds' consumed rosemary in the feed. The differences in the levels in the other groups on these indicators are reliable (P> 0.05). The studied parameters, including cholesterol, were variable and without a statistically significant difference between the different groups. Cholesterol levels of 2.42 mmol/l and triglycerides TG 0.91 mmol/l (P <0.05) were reduced in the same group E2 with rosemary added to the feed.

Alagawany & Abd El-Hack (2015) studied the effect of dried rosemary on the productivity of laying hens, found that biochemical parameters were not significantly (P> 0.05) affected by the addition of rosemary, except for urea and total cholesterol. According to Hashemipour et al. (2013) a diet enriched with rosemary reduced numerically serum triglycerides and total cholesterol, as well as concentrations of LDL-cholesterol but not HDL-cholesterol. Bölükbaşi et al. (2008) reported that the addition of rosemary to the laying hen diet significantly suppressed serum triglyceride and total cholesterol levels.

Alagawany et al. (2015a, b) reported that medicinal plants or their products often affect blood lipid parameters in opposite ways. In study with addition of three parts dry rosemary and three parts rosemary essential oil is found that total cholesterol levels were significantly different between dry rosemary and rosemary oil (Polat et al., 2011). Hence adding the dry rosemary leaves to the diet decrease the total cholesterol levels.

The reduced total cholesterol content may reflect the hypocholesterolemic properties attributed to the defatted portion of the leaves, which have a fibrous (25.24%) content and may block the absorption of intestinal cholesterol (Lansky et al., 1993). This finding is consistent with a study of total cholesterol conducted by Ghazalah & Ali (2008), broiler chickens that they studied were on a diet supplemented with 0.5, 1.0 and 2.0% dried rosemary. The results showed that the addition of rosemary leaves lowers the plasma content of total cholesterol levels. In another study, the addition of Anise seeds to broiler feed showed a slight decrease in serum cholesterol levels (Soltan et al., 2008).

On the other hand, according to Ali (2014) by adding dried thyme powder in diets did not establish a significant effect (P>0.05) on body weight at 21 days of age, feed intake (FI) during all periods, the conversion rate of feed (FCR) from 0-3 weeks of age, hematocrit HCT, hemoglobin, total protein, albumin, globulin, albumin/globulin ratio, LDL cholesterol, heterophiles (H), lymphocytes (L), monocytes, H/L ratio, mean titers of serum hemagglutinin antibodies (Ab).

The most likely cause of elevated serum glucose may be due to abdominal lipid catabolism during gluconeogenesis (El-Ghousein & Al-Beitawi, 2009). Toghyani et al. (2011) found that the addition of thyme at levels of 5 and 10 g / kg had no effect on total protein, albumin and A / G ratio. Other authors also reported a generally significant reduction in cholesterol and an increase in HDL cholesterol compared to controls (Ademola et al., 2009; Toghyani et al., 2011; Al Mashhadani et al., 2011; Mohamed et al., 2012). According to Tekeli et al. (2006) cholesterol levels were not affected (P> 0.05) by the addition of medicinal herbs (*Z. Officina-*

Table 2. Biochemical parameters of blood in turkeys after inclusion in the ration of 1% dried herbs

Indicators	Groups							SEM	P-value
	C	E1	E2	E3	E4	E5	E6		
Glucose mmol/l	16.75	16.96	16.05	17.18	17.49	16.99	21.16	0.57	0.288
Total protein g/l	43.33	38.98	38.28	47.22	42.47	41.38	38.93	0.86	0.052
Creatinine Mmol/l	37.33	35.83	37.17	35.33	33.17	35.67	35.17	0.43	0.159
Uric acid Mmol/l	320	259.83	237.67	266.67	310.00	305.50	258.67	11.91	0.437
Cholesterol mmol/l	2.97	2.72	2.42	2.63	2.83	3.13	2.51	0.07	0.081
TG mmol/l	$1.25^{b,c}$	1.36^{b}	$0.91^{c,d}$	1.33^{b}	1.56^{b}	2.04^{a}	$1.18^{b,d}$	0.07	0.000
ASAT U/l	532.33	534.67	448.17	442.17	543.67	517.5	512.5	13.05	0.177
ALAT U/l	12.33	10.83	13.33	10.67	12.5	12.67	11.33	0.37	0.398
GGT U/l	4.17	4.00	4.00	5.83	4.50	3.83	4.00	0.25	0.369

a, b, c, d – the different letters in the line mark statistically significant differences at P <0.05

le and *S. aromaticum*) while glucose and triglyceride concentrations increased (P <0.05).

Creatinine is a chemical waste molecule that is generated by muscle metabolism. The kidneys keep the creatinine in the blood within normal limits. The results obtained in our study (Table 2) show that creatinine levels are not significantly affected by the intake of herbs during the fattening period. Higher creatinine values were observed in the control group (37.17 mmol/l) and rosemary (E2) (37.33 mmol/l) respectively. The lowest ones were reported for oregano group (33.17 mmol/l) the other groups had intermediate results, and no statistically significant difference (P> 0.05).

The uric acid values are most influenced by the herbs rosemary (237.67mmol/l), St. John's wort (258.67 mmol/l) and lavender (266.67 mmol/l). In the other groups the uric acid values were increased, but without statistical significance, 320 mmol/l in the control group compared to 300.50 mmol/l in thyme.

The creatinine in the blood plasma of Arbor Acres broiler chickens, after added different levels (0.5%, 1.0% and 2.0%) of dry ground rosemary in the ration were statistically significantly reduced (1.12, 1.14 and 1.22 mg/dl), compared to the control group 2.54 mg/dl (Ghazalah & Ali, 2008). Creatinine levels were reduced when taking rosemary leaves compared to control groups, reported in a study by Ghazalah & Ali (2008). Another study with the addition of anise in a 0.75 and 1.0 g/kg to the diet significantly increased creatinine levels compared to the control.

Aspartate aminotransferase (ASAT) in turkeys ranged from 442.17 U/l at E3 to 543.67 U/l at E4. Alanine aminotransferase (ALAT) is 10.67 U/l in group E3, with E2 reaching the highest measured values 13.33 U/l. Gamma-glutamyl transferase (GGT) ranged from 3.83U/l at E5 to 5.83 U/ l at E3 with no group differences in the three enzymes. In a study by Abo Ghanima et al. (2020) alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), urea, immunity and antioxidant parameters were significantly (P<0.05 or 0.01) better in groups receiving rosemary and cinnamon than in the control group.

The results presented in Table 3 show that the highest concentration of lysozyme was in the control group (C) 4.29 mg and the groups treated with rosemary (E2) 4.29 mg, oregano (E4) 4.38 mg and thyme (E5) 4.29 mg. In lavender (E3) 3.44 mg and chamomile (E1) 3.77 mg, the levels were lower, but there was no statistical significance in the differences between the groups. The alternative complement activation pathway (ACAP) had a statistically significant increase in the thyme-treated group (E5) 718.94 (P <0.001), compared to the control group (C) 716.05. The values for oregano E4 (P <0.01) and rosemary E2 (P <0.001) are relatively lower, respectively 661.42 and 634.47. These results show that the studied herbs significantly affect the activity of this important factor of natural immunity in turkey.

Table 3. Influence of dry herbs on some indicators of humoral natural immunity in turkeys

Groups	Indicators		
	Lysozyme (mg/L)	ACAP (CH50)	Betalysine, %
C	4.29 ± 0.46	716.05 ± 8.84	8.94 ± 1.2[a]
E1	3.77 ± 0.28	712.35 ± 40.41	7.02 ± 0.9
E2	4.29 ± 1.09	634.47± 19.53[a]	11.79 ± 1.2
E3	3.44 ± 0.89	707.41 ± 11.63	17.04 ± 1.7[a]
E4	4.38 ± 0.62	661.42 ± 28.06[b]	16.39 ± 0.5[a]
E5	4.29 ± 0.46	718.94 ± 20.07[a]	15.42 ± 0.3[a]
E6	3.46 ± 0.44	573.6 ± 12.19[a]	18.05 ± 1.5[a]

b – P <0.05; a – P <0.001, a-b- the different letters in the column mark statistically significant differences between the groups.

The data for betalysine show that the highest activity of the indicator is in the groups treated with lavender 1 E3- 17.04%, oregano E4- 16.39% and St. John's wort E6- 18.05% (P <0.001), and the lowest value of the indicator in the group treated with chamomile E1- 7.02% and C- 8.94%. Obviously, this indicator is also significantly affected by the tested herbs.

Matouskova et al. (2016) reported that encapsulated extract of various herbs including chamomile and rosemary has a pronounced antibacterial effect, which is increased in the presence of lysozyme. Other authors report the effect of chamomile on the specific immunity of broiler chickens. As has been explained, the alternative complement activation pathway (ACAP) is primarily humoral means of combating viruses, virus infected cells, gram negative bacteria, cancer cells and the like. Based on the obtained data and analyzing literature, we can conclude that chamomile has immune-stimulating effect on concentration and lysozyme activity ACAP and betalysine, which in combination with antibacterial and antioxidant activity can increase overall resistance of turkeys. Dehkordi et al. (2009) found a better antimicrobial effect of a natural rosemary extract against Listeria monocytogenes if it is combined with unheated or even better with heated lysozyme at low pH = 5. If we analyze the results obtained by us for the effect of rosemary E2, we will notice that in lysozyme its average value is 4.29 mg/L, but the difference with other herbs is insignificant. In betalysine the result is 11.79%, as the advantage is in favor of those treated with g. St. John's wort, lavender and oregano (P <0.05). From the results obtained by us it is evident that oregano E4 does not have a significant effect on the activity of APAC and betalysine (P <0.05). The established immune modulation is in agreement with the results obtained by other authors described above.

Chun et al. (2001) found an anticomplementary (suppressing the activity of the complement system) action of a polysaccharide isolated from thyme leaves (*Thymus vulgaris* L.). The isolated polysaccharide inactivated both complement and alternative pathway activation pathways (human sera were probably tested because the source of the sera was not identified in the publication). Table 3 shows that the activity of ACAP in the treatment of turkeys with dried thyme herb is the highest (718.94 CH50), and the difference between the compared arithmetic means is statistically significant (P <0.05). The influence of thyme (*Thymus vulgaris*), fenugreek (*Trigonela foenum graecum*) and neem (*Azadirachta indica*) on the concentration of serum lysozyme, which is also an important factor of natural immunity, in tilapia reported Antache et al. (2014). In this study, the lysozyme concentration is increased in the group treated with dried herb thyme (E5) 4.29 mg/L. Our results are consistent with the studies of Chun et al. (2001) and Antache et al. (2014), and suggested a likely immunosuppressive effect of thyme on lysozyme. The results for beta-lysine show that in the thyme treated group with dry herb its activity is low (15.42%). Given these results, we can say that thyme in general has an immunostimulatory effect on the humoral factors of natural immunity in turkeys.

Jiang et al. (2012) examining the effect of St. John's wort methanol extract on the specific humoral immune response in chickens vaccinated against various strains of avian influenza found that the use of this extract as a dietary supplement during the immunization period increases the effect of avian influenza vaccination. Landy et al. (2012) make an interesting experiment to study the possibilities of using dry aerial parts of St. John's wort (ground) as a substitute for nutritional antibiotics in poultry. It was found that this herb improves the digestibility of feed, increases the titer of antibodies against avian influenza, and reduces cholesterol levels compared to chickens treated with a nutritional antibiotic (flavophospholipol). Therefore, the authors allow themselves to recommend the use of St. John's wort as a substitute for nutritional antibiotics. Analyzing our results presented in Table 3 shows that the concentration of lysozyme is 3.46 mg/L in turkeys treated with dry herb St. John's wort -E6. The highest activity of betalysine is 18.05% significantly compared to the control, chamomile and rosemary, respectively with 8.94%, 7.02%, and 11.79%. With the other groups the differences are unproven.

These results show that St. John's wort has an immunomodulatory effect not only on the specific but also on the humoral natural immune response. Taking into account the results obtained by us and taking into account the results obtained by the above authors, we can conclude that the studied herbs have serious immunomodulatory potential in different species of animals, which if used in animal husbandry can contribute to improving their health status and thereby increase their productive qualities.

Herbs are rich source of phenolic compounds, for example, rosemary is dominated by the amount of carnosol, carnose and rosemary acids and related effective compounds having strong antioxidant, anti-cancer and anti-inflammatory effects (Chun et al., 2014). Hashemipour et al. (2013) reported that intake of a mixture of thymol and carvacrol in 4 levels (0, 60, 100 and 200 mg/kg diet) added to broiler chickens linearly increased (P <0.05) superoxide dismutase SOD and glutathione peroxidase activity GSH and decreased (P <0.05) the level of malondialdehyde MDA in the thigh muscles on day 42 and serum and liver on days 24 and 42. Thus, feed additives of herbs rich in thymol + carvacrol improve efficiency, increase antioxidant enzyme activity, slow down lipid peroxidation, improve the activity of digestive enzymes and improve the immune response of broilers chicken. According to several studies, herbal supplements can act with more than one mechanism affecting the uptake and conversion of feed, stimulating the secretion of digestive enzymes and gastrointestinal motility, as well as the immune and endocrine systems and function in addition to their antioxidants, antimicrobials, antivirals anti-inflammatory, anthelmintic and coccidiostatic activities (Hernández-Hernández et al., 2009; Basmacioğlu Malayoğlu et al., 2010). For example, Alagawany & Abd El-Hackaim (2015) studied the effect of dried rosemary on egg productivity, immune function and antioxidant status in laying hens. The authors found that adding rosemary to broilers' diets generally led to improved antioxidant status.

Table 4 shows the results obtained from the measured indicators of oxidative / antioxidant status: the activities of antioxidants SOD and GSH, the levels of MDA, oxygen/nitrogen-containing radicals (ROS, NO) and ascorbate radicals (Asc.) in turkey blood consumed in rations dry herbs 1% herbs. The antioxidant enzyme SOD is a metal-protein enzyme that living cells rely on to neutralize harmful free radicals. Superoxide dismutase (SOD) is the first enzyme that contributes to the body's antioxidant defense system. High concentrations of this enzyme can improve the balance of the antioxidant system of chicken, contributing to increased shelf life. Elevated levels of the antioxidant enzyme SOD lead to improved stability of the antioxidant system in poultry. The results presented in Table 4 show that the levels of superoxide dismutase (SOD) activity were statistically significantly increased in almost all groups consuming dry herbs (P <0.05). Exceptions are the groups consuming 1% *Rosmarinus officinalis* (E2) and 1% *Lavandula angustifolia* (E3), the SOD activity in these two groups is almost the same as the control.

Superoxide is a waste product of cellular metabolism that cells produce in the process of creating energy. In this process, the cells also produce another highly damaging free radical called hydrogen peroxide (H2O2) or a hydroxyl radical (•OH). Superoxide dismutase SOD counteracts these two damaging free radicals, and through the disproportionation process, the body breaks down these radicals, and protects cells from damage. Glutathione peroxidase is also involved in protecting cells against the harmful effects of ROS along with superoxide dismutase. Table 4 reports the effect of natural dry herbs on the concentration of glutathione measured as reduced glutathione (GSH). GSH activity was statistically significantly increased compared to the control 59.80 (U) in the groups with added 1% *Thymus vulgaris* E5 -66.04 (U), 1% *Origanum vulgare* E4 – 62.45 (U), 1% *Lavandula angustifolia* E3 – 60.95 (U) and 1% *Hypericum perforatum* E6 – 60.03 (U) respectively. In the groups treated with 1% *Matricaria chamomilla* – E1 and 1% *Rosmarinus officinalis* - E2 values were close to the control group.

Reactive oxygen species (ROS) can be produced by both enzymes and non-enzymatic chemical reduction of molecular oxygen (O2). ROS are highly reactive and attack a variety of biomolecules, including proteins, DNA, and lipids such as polyunsaturated fatty acids (PUFAs). PUFA arachidonic acid is peroxidized to the final formation of malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE) and other reaction products such as F2-isoprostanes. The specific reaction of ROS with lipids is commonly known as "lipid peroxidation". The MDA is a widely accepted biomarker of oxidative stress, namely lipid peroxidation. Our results show (Table 4) that the level of MDA in almost all groups of turkeys with added dry herb 1% to the diet were almost the same as controls. An exception was observed in the group of turkeys fed with 1% *Lavandula angustifolia* E3, where a statistically significant decrease compared to controls was reported (P <0.05).

In general, from the obtained results we can summarize that the dried herbs added to the diet of turkeys show good antioxidant activity. The best indicators of antioxidant activity are in the groups treated with 1% thyme, 1% oregano, and 1% St. John's wort.

Table 4 shows the results obtained in blood serum measured reactive oxygen species –measured as ROS products and reactive nitrogen species represented as nitrogen NO radicals. These free radicals are compared to the levels of ascorbate radicals (Asc). The results show that the levels of ascorbate radicals were reduced in all experimental groups received 1% dry herbs compared to controls. Moreover, in the groups fed 1% E4 -*Origanum vulgare*; 1% and 1% E5- *Thymus vulgaris*, the decrease was statistically significant compared to the control (P <0.05). The only increase according to controls, but not statistically significant, was reported in the group fed with 1% *Rosmarinus officinalis* E2 (mean 4.61 arb.units vs mean 3.90 arb. units).

NO radical levels were statistically significantly reduced (P <0.05) in all experimental groups, and only the turkeys group fed with E3-1% *Lavandula angustifolia* had values close to the control. Similar results have been reported in the ROS products levels. In all groups the levels of ROS products were statistically significant decrease compared to the control (P <0.05), except for the turkeys fed with addition to the diet the E1-1% *Matricaria chamomilla*, where the ROS products value were identical to the control group.

Phytogenic feed additives, including rosemary, chamomile, thyme, etc. exercise their antioxidant mechanism by trapping free radicals, forming chelates with metal ions, and preventing or reducing lipid oxidation (Lobo et al., 2010; Carocho et al., 2013). In particular, a lot of research point out that herbal plants or/and their essential oils added to the diet of chickens improve growth and productive results (Cabuk, et al., 2006; Cross et al., 2007; Hashemi & Davoodi, 2011; Alagawany et al., 2015). Moreover, the authors' summaries that supplementing the diet of broilers with dried herbs can be used as an effective feed additive to improve productivity,

Table 4. Oxidative status of blood serum from turkeys with dried herbs included in the ration 1%

Indicators	Groups							SEM	P-value
	C	E1	E2	E3	E4	E5	E6		
SOD(U/mL)	13.28[a]	11.50[a,c]	9.86[b,c]	9.83[b,c]	10.38[a,c]	10.07[b,c]	9.94[b,c]	0.20	0.000
GSH(U)	59.80[b]	55.19 [c]	51.00[d]	60.95[b]	62.45[a,b]	66.04[a]	60.03[b]	1.52	0.000
MDA(Mmol/L)	14.08[c]	14.89[c]	14.21[c]	13.83[c]	15.07[c]	15.14[c]	15.20[c]	0.72	0.000
Asc radicals (Arb Units)	3.90 [b]	3.68[b]	4.61[a]	3.73[b]	2.68[c]	2.78 [c]	3.11 [b,c]	0.17	0.000
NO radicals (Arb Units)	8.11[a]	7.24[b]	5.46[c]	8.15[a]	7.28[b]	7.04 [b]	7.00[b]	0.19	0.000
ROS product (Arb Units)	5.97[a]	5.57[a]	3.38[b,c]	5.28[a,b]	5.36[a]	5.05[a,c]	4.61[c]	0.15	0.000

a, b, c, d, e – the different letters in the row mark statistically significant differences at P < 0.05

immunity and antioxidant status in laying hens.

The herbs obtained by drying contain a large number of different phenolic compounds with biological activities, such as carnosol, carnosic acid, rosmanol, epirosmanol, etc. Carnoic acid is the most active antioxidant present in dry herb or rosemary extract with antioxidant activity approximately three times higher than carnosol and seven times higher than synthetic antioxidants (Angioni et al., 2004).

The balance between free radicals and internal antioxidant protection is crucial for cellular antioxidant activity (Burton & Jauniaux, 2011). Lipid peroxidation leads to the formation of various products such as MDA (Droge, 2002), ascorbate, ROS products and NO radicals. The results of a study by Abdel-Ghaney et al. (2017) show that adding thyme to a broiler diet reduces MDA levels in the chest and thigh muscles. Interestingly, tissue GSH content and SOD and GST activities were significantly increased in the chest and thigh muscles, but the MDA levels in the muscles were significantly reduced with the thyme addition. Hashemipour et al. (2013) suggest that the high biological activity of thyme as a natural antioxidant is due to the presence of phenolic hydroxyl groups, which serve as a hydrogen donor to peroxide radicals produced in the first stage of lipid oxidation, thus inhibiting the formation of hydroxy peroxide.

The results of our study are in agreement with some scientific data showing that extracted and dried herbs from oregano, rosemary and sage significantly reduce oxidative damage to lipid in serum and tissues, (Basmacioğlu et al., 2004; Botsoglou et al., 2004, Yasar et al., 2011; Küçükyilmaz et al., 2017). The antioxidant, antibacterial, anti-inflammatory, antifungal and antimicrobial properties of the phenolic compounds of herbs can affect the growth efficiency of broiler chickens, as well the characteristics of their blood profile, thus improving the quality of poultry meat (Mokhtari et al., 2018).

Conclusion

The inclusion of 1% dried herbs (*Matricaria chamomilla; Rosmarinus officinalis; Lavandula angustifolia; Origanum vulgare; Thymus vulgaris; Hypericum perforatum*) in turkey feed showed that the average daily feed consumption was lowest in the control group (C) – 0.312 kg/day at $P < 0.05$, but did not show an effect on final live weight and feed conversion in all groups. We can conclude that the studied herbs have a serious immunomodulatory potential, which if used in animal husbandry can contribute to improving the health status and increase productivity in broiler turkeys. The addition of dried herbs to turkey feed affects the values of biochemical parameters and the differences are not statistically significant. The studied parameters, including cholesterol, are variable and insignificant between the different groups. Cholesterol levels of 2.42 mol/l and triglycerides TG 0.91 μmol/l ($P < 0.01$) were reduced in the same group E2 with added rosemary in the feed.

It is obvious that the addition of 1% thyme, 1% oregano, 1% St. John's wort in the diet of the studied turkeys effectively improves their antioxidant status through increased antioxidant activities of SOD, GSH as well as through reduced levels of MDA. The production of free radicals is significantly reduced with the use of It is obvious that the addition of 1% thyme, 1% oregano, 1% St. John's wort in the diet of the studied turkeys effectively improves their antioxidant status through increased antioxidant activities as well as through reduced levels of lipid peroxidation. The production of oxygen/nitrogen containing radicals (ROS, NO) and ascorbate radicals ($Asc^{\cdot}$) are also reduced with the use of extracted and dried herbs, which shows an improved oxidative state of the birds.

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