Original research paper

INFLUENCE OF MEDICINAL PLANTS MIXTURES (*Artemisia absinthium, Thymus vulgaris, Menthae piperitae* and *Thymus serpyllum*) IN BROILERS NUTRITION ON BIOCHEMICAL BLOOD STATUS

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ABSTRACT: Aim of the trial was to investigate the influence of *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum* mixtures in broilers nutrition on productive characteristics and biochemical blood status. Trial involved two treatments with the total of 80 broilers within four replicates. Control treatment (C) of chickens was fed with basic commercial diet mixture, while the experimental treatment (E) was fed with the same basic mixture only with addition of 2.0 g/100g medicinal plants mixture. Addition of *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum* has led to the higher body weight of chickens at the end of the sixth week of the trail (2087.3±230,22 g), followed by control treatment (2059.8±210.63 g) without statistically significant differences ($p>0.05$). Feed conversion ratio was uniform for the entire trial period, without significant differences. European broiler index was higher in E (226.4±4.21 %) with the lower mortality rate (2.6±0.7 %). In recorded amounts of triglycerides and total cholesterol significant differences was absent, while the low density lipoprotein (LDL) and non-high density lipoprotein (non HDL) in broilers on E treatment recorder statistically significant ($p<0.05$) differences compared to a control treatment. The highest amount of high density lipoprotein (HDL) with statistical significance (42.8±1.28 mg/dl) was determined in E treatment. Having in mind aforementioned and on the base on the gained results, it can be concluded that the addition of *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum* in broilers nutrition has positive influence on production characteristics and biochemical blood status of chickens.

Key words: *medicinal plants, nutrition, chickens, cholesterol, lipids*

INTRODUCTION

Over the years, the past few decades, the use of antibiotic as growth promoters in poultry nutrition has been associated with fast growing nature of broiler chickens and their short generation interval in order to improve the quality of the final product

(Schwarz et al., 2001; Sarica et al., 2005; Puvača et al., 2013; Kostadinović et al., 2015). To improve chicken healthiness and to fulfill consumer expectations in relation to food quality, poultry producers nowadays commonly apply natural dietary supplements mainly medical plants (Onibi et al., 2009). Many authors investigated alternatives to antibiotics (Mellor, 2000; Ocak et al., 2008). Medicinal plants have recently reported as alternatives to antibiotics in animal production and are claimed to be "digestive enhancers" (Williams and Losa, 2001). They are very complex mixtures of compounds, such as tannins, terpenoids, alkaloids and flavonoids. Many *in vitro* studies (Demirel et al., 2011; Lević et al., 2011) reported antimicrobial properties of medicinal plants. In addition to their antimicrobial activity, medicinal plants possess various biological activities, one of them is antioxidant activity (Aliyu et al., 2012; Radivojević et al., 2012). Introduction of essential oils in animal feed may have promising potential as a growth and health promoter without adverse effects. Serbia has a wide range of medicinal plants which possess a number of chemical substances for the use in poultry. *Artemisia absinthium* (Asteraceae) contains β-thujone, (Z)-6,7-epoxyocimene and sabinyl acetate (Juteau et al., 2003). *Thymus vulgaris* is characterized by the presence of thymol, carvacrol, p-cymene and γ-terpinene. Menthol and menthone are the main compounds of *Menthae piperitae*. In *Thymus serpyllum* numerous compounds were identified, where the main components are (E)-nerolidol, caryophyllene oxide, myrcene, (E)-β-caryophyllene and germacrene D (Daferera et al., 2000).

The aim of the trial was to investigate the influence of *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum* mixtures in broilers nutrition on productive characteristics and biochemical blood status.

MATERIAL AND METHODS

Collection and processing of medicinal plants

The plants used for this study were *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum*. The stem barks of the plants were collected in April and May time period from Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade. The stem barks of the plants were dried under shade for 10 days at 8h per day and then ground into powder.

Animals and housing

Trial was carried out under experimental conditions at the experimental poultry farm. Trial involved two treatments with the total of 80 broilers within four replicates. Control treatment (C) of chickens was fed with basic commercial diet mixture, while the experimental treatment (E) was fed with the same basic mixture only with addition of 2.0 g/100g medicinal plants mixture. Chickens were reared on floor holding system with the chopped straw as litter material. Chickens were provided with the light regime of 23h of day per entire experimental period of 42 days. Heating of chickens was provided locally with the infrared light heaters and the whole house was supplied with 2 thermometers linked to the heather ventilation controls. House temperature zones were preheated to the temperature between 31-33°C before delivery and receiving of chickens, and the temperature was maintained during the first week. Every next week temperature was decreased for about 2°C, reaching 20-22°C at the end 42nd day of the

experiment. Chickens were fed through pan feeders and watered through the nipple water system. Environmental house conditions during the 42 days of the experiment were regularly monitored. Body weight was monitored at an individual level during the entire experimental period every seven days, while the feed consumption and feed conversion ratio were monitored at the pen level also every seven days.

Diet and feeding

At the beginning of the experiment two dietary treatments in four replicates were formed. Every dietary treatment included 40 chickens, which were divided in four pens with 10 chickens per each pen. For nutrition of chicks three mixtures were used, starter, grower and finisher. For the first 14 days, during the preparatory period, chicks were fed with starter mixture. Following the preparation period, chicks were fed with grower mixtures for the next 21 days, and then for the last 7 days of fattening period with finisher mixtures of composition and nutritive value which is given in Table 1. Experimental dietary mixture in the experiment was combined of *Artemisia absinthium, Thymus vulgaris, Menthae piperitae* and *Thymus serpyllum* (powder of selected medicinal plants was in ratio of 1:1:1:1 in total amount of 2.0 g/100g). During the experiment chicks were fed and watered *ad libitum*.

Table 1. Composition and nutritive value of dietary mixtures, g/100g

Indices	Diet mixtures		
	Starter	Grower	Finisher
Ingredients			
Corn	55.0	54.2	57.1
Wheat	5.0	8.0	12.0
Soybean meal, 44%	19.5	19.0	9.9
Soy protein concentrate	8.8	5.7	7.8
Sunflower meal, 42%	2.0	4.0	6.0
Corn gluten	2.0	2.0	-
Yeast	1.5	-	-
Chalk	1.75	1.70	1.50
Monocalcium phosphate	1.38	1.22	0.98
Premix	2.57	2.58	2.32
Chemical composition			
Dry matter	89.4	89.3	89.4
Moisture	10.5	10.7	10.5
Crude protein	21.1	20.7	17.3
Crude fat	3.9	3.9	4.7
Crude fibre	3.5	3.5	3.6
Crude ash	5.0	4.8	5.6
Ca	0.8	0.9	1.1
P	0.6	0.6	0.5
Metabolisable Energy, MJ/kg	12.5	12.8	13.3

Sample preparation and biochemical blood status

At the end of 42 days of the experiment, six broiler chicken, three male and three female of an average body weight of each treatment was selected and bled via wing vein puncture to obtain blood samples. Serum samples from blood were separated by centrifugation (4000 rpm for 5 min at 20°C). Commercially available kits (Randox Laboratories Limited – United KIngdom) were used to analyse the serum for triglycerides, total cholesterol, HDL and LDL on an biochemical autoanalyzer Cobas Mira Plus (Roche Diagnostics). Values were expressed as mg/dl.

Statistical analysis

The results given in tables are reported as the mean ± standard deviations (SD) of a number (n) of independent determinations. The one way ANOVA analysis was performed to assess data differences between various groups using STATISTICA Version 12 (2013). The data means were considered significantly different at $p<0.05$.

RESULTS AND DISCUSSION

Broiler chickens did not show any visible clinical changes during the whole experiment. The effect of dietary mixture of *Artemisia absinthium, Thymus vulgaris, Menthae piperitae* and *Thymus serpyllum* powder on production performance is presented in Table 2. During the whole experimental period addition of medicinal plants didn't show any significant difference regarding the body weight of chickens. Overall final body weight was lower for broilers in the control treatment C (2059.8 g) without statistically significant differences ($p>0.05$) compared to experimental treatment E (2087.3 g). Feed efficiency, calculated from day 1 to 42 of growth was equal for the control and experimental treatment. The results of this study regarding feed conversion ratio and body weight are in close agreement, but also with some differences with the findings of Kim et al. (2002), Brisibe et al. (2008) and Kostadinović et al. (2015) who stated that dietary addition of *Artemisia* sp. levels showed low feed conversion ratio which may be due to the low digestibility and high viscosity of jejunal digesta.

Table 2. Body weight of chickens, g

	Treatments			
	C		E	
	$\overline{x}$	SD	$\overline{x}$	SD
0 day	42.6[a]	3.62	43.0[a]	3.42
7 day	163.8[a]	14.96	162.2[a]	26.26
14 day	385.2[a]	46.68	385.6[a]	70.11
21 day	757.1[a]	80.94	760.8[a]	80.68
28 day	1222.6[a]	170.15	1203.8[a]	119.4
35 day	1617.6[a]	144.17	1674.2[a]	194.8
42 day	2059.8[a]	210.63	2087.3[a]	230.22

Treatments with different letter indexes in the same row are significantly different ($p < 0.05$)

From the results given in Table 3 it can be seen that feed conversion ratio in the starter phase was uniform in experimental treatment and control treatment as well as during the total experimental period which have ranging between 2.05 and 2.19 kg of feed per kg of gain without statistically significant differences ($p>0.05$). Al-Kassie (2009) found that *Thymus vulgaris* improved feed conversion ratio value, which is related to the greater efficiency in the utilization of feed due to addition of phytochemicals. On the other side, research of Ocak et al. (2008) based on the use of dry thyme in broiler nutrition showed contradictory results, since addition of this plant did not affect body weight gain, feed intake or feed conversion ratio in broilers which partially is in agreement with this research.

Table 3. Feed conversion ratio, kg feed/kg gain

	Treatments			
	C		E	
	$\overline{x}$	SD	$\overline{x}$	SD
1-14 day	1.39[a]	0.00	1.38[a]	0.00
15-35 day	1.83[a]	0.05	1.94[a]	0.07
36-42 day	2.94[a]	0.23	3.25[a]	0.02
1-42 day	2.05[a]	0.79	2.19[a]	0.95

Treatments with different letter indexes in the same row are significantly different ($p < 0.05$)

The highest mortality rate of 5.2% was recorded in treatment C, which was significantly ($p<0.05$) higher than in treatment E (Table 4). The highest recorded European broiler index (EBI) values ranged between 214.5% in treatment C and 226.4% in E treatment with significant ($p<0.05$) differences amongst dietary treatments.

Table 4. European broiler index and mortality rate of chickens, %

	Treatments			
	C		E	
	$\overline{x}$	SD	$\overline{x}$	SD
EBI	214.5[b]	16.22	226.4[a]	4.21
Mortality rate	5.2[a]	0.8	2.6[b]	0.7

Treatments with different letter indexes in the same row are significantly different ($p < 0.05$)

A result in Table 5 summarizes data obtained on serum biochemical parameters. Addition of *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum* powder mixture did not result in any decrease in triglycerides concentration compared to control treatment. Also, no significant influence of medicinal plants diet addition on total cholesterol was observed ($p>0.05$). The feeding of the broilers with 2.0 g/100g of *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum* powder mixture tended ($p>0.05$) to increase in HDL-cholesterol concentration compared to control treatment, while the differences in LDL-cholesterol concentration also reached statistically significant difference between treatments. Previous research are similar to those reported by Toghyani et al. (2010) where addition of *Thymus vulgaris* in broiler diets in concentrations of 5 g/kg and 10 g/kg did not affect the

triglyceride, total and LDL-cholesterol concentration. Results obtained in the current study are not in agreement with results reported by Ali et al. (2007) who showed that adding *Thymus vulgaris* to hen diets significantly decreased plasma HDL. Positive effects of medicinal plants on blood lipid status and antioxidative capacity was also reported in research of Kostadinović et al. (2015a) in broilers nutrition and Popović et al. (2017) in rabbit's diet.

Table 5. Biochemical blood profile, mg/dl

	Treatments			
	C		E	
	$\overline{x}$	SD	$\overline{x}$	SD
Triglycerides	63.2[a]	0.21	61.7[a]	1.23
Total cholesterol	98.6[a]	0.98	96.5[a]	0.47
HDL	17.9[b]	1.47	42.8[a]	1.28
LDL	38.4[a]	2.04	2.7[b]	1.76
HDL/LDL	0.47[b]	1.75	15.85[a]	1.52

Treatments with different letter indexes in the same row are significantly different ($p < 0.05$)

CONCLUSIONS

Based on the gain results, it can be concluded that the addition of *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum* powder mixture in broiler chicken nutrition had positive effect on production performances without significant difference compared to control treatment. Addition of medicinal plant mixture in the amount of 2.0 g/100 g has led to the higher final body weights with the higher percentage of European broiler index and significantly lower mortality rate. Also it can be concluded that significant lowering of blood cholesterol, triglycerides, LDL and increase of HDL by these medicinal plants supplementation in broiler diet indicate *Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum* are effective in regulation of lipid metabolism, but further investigation of their mode of action is still necessary.

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