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Effects of dietary essential oils on productive performance, blood lipid profile, enzyme activity and immunological response of broiler chickens

Bewertung des Zusatzes von essentiellen Ölen zum Futter auf die Leistung, auf die Lipidspiegel und die Enzymaktivität im Blut sowie auf die Immunantwort von Broilern

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Introduction

In recent years, the use of herbal growth promoters (HGP) as natural alternative to antibiotic growth promoters (AGP) has taken a very important place in livestock and poultry nutrition (Mountzouris et al., 2011). After the feeding ban of antibiotic growth promoters in European Union (EU) countries in 2006 (Puvača et al., 2015a), the search for safe and healthy alternatives has become a very hot research topic. Therefore, utilised parts of medical plants and spices, or their extracts, also known as phytogenic feed additives, found their place in promotion of production performance and improvement of the quality of food of animal origin (Goodarzi et al., 2014). Likewise, numerous studies reported positive effects regarding the improvement of animal health and wellbeing after the administration of medicinal plants or spices in animal nutrition (Ghasemi et al., 2014; Puvača et al., 2015b; Kostadinović et al., 2015; Mountzouris et al., 2011; Hernandes et al., 2004; Ertas et al., 2005; Zhang et al., 2005). Phytogenic additives may show antimicrobial activity, stimulating effects on animal digestive systems, antioxidative and anticoccidial activity, may increase production of digestive enzymes and improve utilisation of digestive products by enhancing liver functions (Fotea et al., 2015). Chemical compounds synthesised by medical plants, also known as phytochemicals, are responsible for the influences prescribed to these plants. Oregano (*Origanum vulgare*), thyme (*Thymus vulagris*) and rosemary (*Rosmarinus officinalis)* more recently has been used as alternative growth promoters in swine and poultry nutrition as environmental friendly alternatives to the antibiotic growth promoters commonly used in animal and poultry feed.

Recently, much effort was been made to investigate the cholesterol lowering activity of essential oils or plant extracts (Cross et al., 2003) as well as investigate their immunostimulatory effects (El-Faham et al., 2015). Furthermore, some studies reported that medical plants may have stimulatory effects on pancreatic secretions such as digestive enzymes which help to digest and absorb more amino acids, monosaccharaides and fatty acids from the digestive tract (Lji et al., 2001; Lewis et al., 2003; Demir et al., 2008; Mansoub, 2011). Respectively, protease digests protein into amino acids, while amylase is responsible for digestion of carbohydrates, breaking them down into smaller units such as disaccharides, which are later converted into monosaccharaides like glucose and fructose. Finally, lipase breaks down triglycerides into glycerol and fatty acids which are major sources of energy in the organism as well as precursors of essential substances in the body. Different studies report of medicinal herbs having immunomodulatory activity and some of them have been recognised as modulators of the immune system (Saleh et al., 2014). The active constituents of these herbs have been stimulating the immune responses by interacting with various cells of the immune system.

The aim of the present study was to investigate the effect of dietary thyme, rosemary and oregano essential oils in broiler nutrition on productive performance, blood lipid profile, serum enzyme activity and serum levels of immunoglobulins in broiler chickens.

Materials and methods

Animal trials

Experimental trial with chickens was carried out under production conditions at the *Poultry research Center* in Crvenka in the property of the Patent Co., Serbia. At the beginning of the experiment, a total of 1120 day-old Ross 308 strain broilers, of both sexes, were equally distributed into 4 dietary treatments. Every dietary treatment included 280 chickens, which were divided in 8 pens with 35 chicken per each pen. Dietary treatments in the experiments were as follows: T1 (Basal diet Patent Co.), T2 (Basal diet Patent Co. + Herbal PHP 0.05%), T3 (Basal diet Patent Co. + Herbal PHP 0.1%) and T4 (Basal diet Patent Co. + 10% Oxytetracycline 0.1%). The commercial product Herbal PHP consists of thyme, rosemary and oregano essential oils which are present in the product in the following concentrations: 4000, 3000 and 3000 mg/kg. For the first 21 days, chickens were fed with starter mixture. Afterwards, chickens were fed with grower dietary feed mixtures for the next 14 days, and for the last 7 days of the fattening period with finisher mixtures according to the experimental design given in Table 1. The experiment lasted for 42 days. Structural and chemical composition of used mixtures is given in Table 2. During the whole trial, feed and water were provided *ad libitum*. Rearing and housing conditions were in accordance with specific strain requirements, while the microclimatic condition was regularly monitored with SKOV climate system (Glyngøre, Denmark) for poultry houses.

Table 1. Experimental design

Versuchsdesign

	Concentration of additives in chicken diets			
		Starter, %	Grower, %	Finisher, %
Experimental treatments	Additive	1 – 21 days	22 – 35 days	36 – 42 days
T1	Control	0.000	0.000	0.000
T2	Herbal PHP®	0.050	0.050	0.050
T3	Herbal PHP®	0.100	0.100	0.100
T4	Antibiotic	0.100	0.000	0.000

Table 2. Composition and nutrient contents of the starter, grower and finisher diets (basal diets excluding the additives)

Zusammensetzung und Nährstoffgehalte der Starter-, Grower- und Finisher-Rationen ohne Futterzusätze

	Diet mixtures*		
Ingredients, %	**Starter**	**Grower**	**Finisher**
Maize	30.2	38.5	42.4
Wheat	25.0	25.0	25.0
Vegetable oil	0.950	1.25	1.85
Soybean cake	21.6	16.1	13.5
Soybean meal	18.0	15.0	9.00
Sunflower meal	–	–	4.00
Monocalcium phosphate	0.100	0.100	0.200
Limestone	0.020	0.030	–
Methionine DL	0.060	0.040	–
Ronazyme WX	0.010	0.010	0.010
Premix	4.00	4.00	4.00
Nutrients, % of as-fed basis			
Dry matter	89.6	89.2	88.9
Moisture	10.4	10.8	11.1
Crude protein	22.0	19.0	17.0
Crude fat	5.10	5.10	5.60
Crude fibre	3.50	3.20	3.60
Crude ash	6.30	6.00	5.50
Ca	1.00	1.00	0.900
P	0.800	0.800	0.700
Metabolisable energy, MJ/kg	12.5	12.8	13.0

* Additives were added on top of the basal diet.

Body weight gain, feed consumption and feed conversion ratio

During the 42 day experimental period, body weight (BW) was monitored at an individual level during the entire experimental period every 7 days, while the feed consumption (FC) and feed conversion ratio (feed to gain, FCR) were monitored at the pen level also every 7 days.

European broiler index (EBI)

The European broiler index (EBI) was calculated for the entire feeding period according to the equation (KORELESKI et al., 2010):

EBI = [average body weight (kg) × survival rate (%)/age (days)/feed conversion ratio (kg feed/kg body weight gain)] ×100

Blood lipids

At the end of 6th week, 12 chickens were randomly chosen from each treatment and bled via wing vein puncture to obtain blood samples. Commercially available kits (Randox Laboratories Limited – United Kingdom) were used to analyse the serum for triglycerides, total cholesterol, HDL, LDL and VLDL on an biochemical autoanalyser Cobas Mira Plus (Roche Diagnostics). Values were expressed as mg/dl.

Enzyme activity and IgG and IgA titration

Amylase and lipase activity were measured in serum by Pars'Azmoon standard kit based on colorimetric analysis. Protease activity was assayed according to modified method of ANSON (1938) and FOLIN and CIOCALTEAU (1929).

The chicken serum IgG and IgA values was determined by a commercially available ELISA kit (Cat. No. E33–104. Bethyl Laboratories, Inc., USA) and microwell reader instrument (Thermolabsystem, Thermo, Finland). The procedures for sample assays were performed according to the manufacturers instructions, and the colorimetric reactions were read at 450 nm wavelength. All samples were assayed three times.

Statistical analyses

Statistical analyses were conducted using the statistical software program Statistica 12 for Windows (StatSoft, Inc., Tulsa, OK, USA). Significant effects were explored using analysis of variance (ANOVA), with repeated measurements, least square means (LSM) and standard errors of least square means (SE_{LSM}). Fisher's l.s.d. post-hoc multiple-range test was used to ascertain differences among treatments. A significance level of $P<0.05$ was used.

Results and Discussion

Addition of the new product Herbal PHP based on thyme, rosemary and oregano essential oils in the broiler diets led to statistically significant ($P<0.05$) differences in body weight (Table 3). At the beginning of the experiment, after 7 days, significant ($P<0.05$) differences in body weight of chickens from treatment T2 and T3, compared to treatments T1 and T4, were found. At the end of the starter period, chickens in treatment T2 had achieved the highest body weight (724 g) with significant differences compared to treatments T1 and T4, while no difference between treatments T2 and T3 were seen. The same was observed at the end of the grower phase, where the highest body weight was recorded in treatments with addition of essential oils in different concentrations (T2 and T3) with significant differences compared to the T1 and T4 treatments. After the completion of the experimental period, treatments with addition of thyme, rosemary and oregano essential oils (T2, T3) achieved a final body weight of 2088 and 2096 g, respectively, significantly ($P<0.05$) higher than body weights of chickens in treatments T1 (2004 g) and T4 (1991 g). Cross et al. (2007) reported active principles of essential oils having digestion-stimulating properties, influencing the balance of gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry. Based on this fact, it seems probable that the added compounds improved the digestibility of the feed leading to an increased body weight of broilers. Al-Kassie et al. (2009) showed that addition of thymus extract in broiler chicken nutrition had positive effects on body weight gain, feed intake and feed conversion ratio. Investigations of El-Ghousein and Al-Beitawi (2009) also showed that dietary addition of crushed thyme (2.0%) in broiler diets led to the highest body weight, where the authors also prescribed this to active substances of thyme. Moreover, Al-Mashhadani et al. (2011) reported that broiler diets supplemented with thyme oil significantly increased body weight of broiler chickens.

Table 3. Chicken body weight (g)

Lebendgewichte (g)

Age of chickens		Experimental treatments			
		T1	T2	T3	T4
0 days	LSM	33.8^{a}	34.3^{a}	33.8^{a}	34.2^{a}
	SE_{LSM}	0.170	0.170	0.170	0.170
7 days	LSM	132^{b}	136^{a}	136^{a}	133^{b}
	SE_{LSM}	1.01	1.01	1.02	1.01
14 days	LSM	328^{c}	339^{b}	351^{a}	341^{b}
	SE_{LSM}	1.63	1.62	1.64	1.62
21 days	LSM	682^{c}	723^{a}	720^{a}	695^{b}
	SE_{LSM}	3.32	3.33	3.33	3.30
28 days	LSM	1037^{c}	1132^{a}	1125^{a}	1069^{b}
	SE_{LSM}	6.54	6.48	6.52	6.47
35 days	LSM	1456^{b}	1543^{a}	1564^{a}	1471^{b}
	SE_{LSM}	8.20	8.03	8.05	7.92
42 days	LSM	2003^{b}	2087^{a}	2095^{a}	1990^{b}
	SE_{LSM}	20.5	20.5	20.5	19.9

Values in a row with no common superscript letter differ significantly ($P< 0.05$).

Table 4 gives an overview on feed consumption. From reported results, it could be noticed that during the starter period feed consumption was uniform without significant differences ($P>0.05$) among dietary treatments. At the end of the grower fattening period, the lowest feed consumption was documented in the T4 treatment (919 g) with significant difference ($P<0.05$) compared to treatment T1. At the end of the experiment, the highest feed consumption was recorded in T3 treatment (676 g) while significant differences compared to other treatments were absent ($P>0.05$). Furthermore, significantly ($P<0.05$) higher feed consumption in treatment T2 (659 g) compared to treatment T4 (626 g) was seen. This absence of substantial influence of added essential oils on feed consumption could be related to the composition of diets, as well to environmental conditions of the experiment. BARRETO et al. (2008) presumed that lack of effect of thyme may be a consequence of bioactive compound purity. These are usually found as complexes, which enhances their action on the body.

Table 4. Feed consumption (g)

Futterverbrauch (g)

Age of chickens		Experimental treatments			
		T1	T2	T3	T4
7 days	LSM	208[a]	201[a]	202[a]	201[a]
	SE_{LSM}	8.19	8.19	8.19	8.19
14 days	LSM	272[a]	268[a]	279[a]	263[a]
	SE_{LSM}	7.39	7.39	7.39	7.39
21 days	LSM	530[a]	549[a]	558[a]	505[a]
	SE_{LSM}	18.6	18.6	18.6	18.6
28 days	LSM	793[a]	750[ab]	772[a]	723[b]
	SE_{LSM}	15.9	15.9	15.9	15.9
35 days	LSM	987[a]	950[ab]	941[ab]	918[b]
	SE_{LSM}	23.7	23.7	23.7	23.7
42 days	LSM	648[a]	659[a]	675[ab]	626[ab]
	SE_{LSM}	12.5	12.5	12.5	12.5

Values in a row with no common superscript letter differ significantly ($P< 0.05$).

From the results given in Table 5 it can be seen that FCR in the starter phase was uniform in experimental treatments ranging between 1.42 and 1.43 kg of feed per kg of gain, while the highest feed conversion ratio was documented in the T1 treatment (1.50 kg/kg). Feed conversion ratio in the grower phase was the highest in the control treatment T1 (1.87 kg/kg) with significant ($P<0.05$) differences compared to other dietary treatments. In the finisher phase the lowest achieved feed conversion ratio was in treatments T2 and T4 (1.54 kg/kg), while the highest FCR was in the T1 (1.68 kg/kg) treatment. AL-KASSIE et al. (2009) found that extract oil from thyme improved FCR value, which is related to the greater efficiency in the utilisation of feed due to addition of phytochemicals. Likewise, LEE et al. (2003) explained that better feed utilisation and nutrient availability is a consequence of increased activity of pancreatic digestive enzymes including amylase, lipase, trypsin and chymotrypsin in broilers which is caused by bioactive components present in essential oils. 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 FCR in broilers.

Table 5. Chicken feed conversion ratio (kg feed/kg gain)

Futterverwertung (kg/kg)

Age of chickens		Experimental treatments			
		T1	T2	T3	T4
7 days	LSM	1.57[a]	1.48[a]	1.49[a]	1.51[a]
	SE_{LSM}	0.070	0.070	0.070	0.070
14 days	LSM	1.46[a]	1.38[b]	1.37[b]	1.36[b]
	SE_{LSM}	0.030	0.030	0.030	0.030
21 days	LSM	1.49[a]	1.42[a]	1.44[a]	1.39[a]
	SE_{LSM}	0.050	0.050	0.050	0.050
Starter period 1–21 day	LSM	1.50[a]	1.43[b]	1.43[b]	1.42[b]
	SE_{LSM}	0.030	0.030	0.030	0.030
28 days	LSM	1.76[a]	1.57[b]	1.61[b]	1.59[b]
	SE_{LSM}	0.050	0.050	0.050	0.050
35 days	LSM	1.98[a]	1.77[b]	1.76[b]	1.76[b]
	SE_{LSM}	0.040	0.040	0.040	0.040
Grower period 22–35 days	LSM	1.87[a]	1.67[b]	1.69[b]	1.68[b]
	SE_{LSM}	0.040	0.040	0.040	0.040
Finisher period 36–42 days	LSM	1.72[a]	1.65[b]	1.66[b]	1.61[b]
	SE_{LSM}	0.030	0.030	0.030	0.030
Entire period 1–42 days	LSM	1.68[a]	1.54[b]	1.56[b]	1.54[b]
	SE_{LSM}	0.030	0.030	0.030	0.030

Values in a row with no common superscript letter differ significantly ($P< 0.05$).

The highest mortality rate of 4.1% was recorded in treatment T3, which was significantly ($P<0.05$) higher than in treatments T2 and T4 (Table 6). On the other side, the lowest mortality rate was in treatment T4 (1.0%), while there was no significant ($P>0.05$) differences compared to treatments T1 (1.7%) and T2 (1.35%). The highest survival rate was found in the T4 treatment (99.0%) without statistically significant ($P>0.05$) differences compared to treatments T1 and T2. The survival rate of 96.0% was recorded in treatment with higher dose of essential oils mixture, which is significantly ($P<0.05$) lower survival rate compared to treatment T2 and similar to the control treatment. The highest recorded EBI values ranged between 273% in treatment T1 and 298% in T2 treatment without significant ($P>0.05$) differences amongst all dietary treatments.

Table 6. European broiler index and chicken mortality (%)

Europäischer Produktionsfaktor und Mortalität (%)

Parameter		Experimental treatments			
		T1	T2	T3	T4
MR	LSM	1.69^{ab}	1.35^{b}	4.05^{a}	1.01^{b}
	SE_{LSM}	0.840	0.840	0.840	0.840
SR	LSM	98.3^{ab}	98.6^{a}	95.9^{b}	99.0^{a}
	SE_{LSM}	0.840	0.840	0.840	0.840
EBI	LSM	273^{a}	298^{a}	289^{a}	292^{a}
	SE_{LSM}	9.71	9.71	9.71	9.71

MR-Mortality rate; SR-Survival rate; EBI-European broiler index.
Values in a row with no common superscript letter differ significantly ($P< 0.05$).

Table 7 summarises data obtained on serum biochemical parameters. Addition of thyme, rosemary and oregano essential oils did not result in any decrease in triglycerides concentration compared to other treatments. Also, no significant influence of experimental diets on total cholesterol was observed ($P>0.05$). The feeding of the broilers with 0.1 g/kg of HERBAL PHP tended ($P>0.05$) to increase in HDL-cholesterol concentration compared to other treatments. The differences in LDL-cholesterol concentration did not reach statistical significance between treatments T1, T2 and T3, while the lowest LDL concentration was in treatment T4 (1.89 mg/dl). However, previous results are similar to those reported by TOGHYANI et al. (2010) where addition of thyme in broiler diets in concentrations of 5 g/kg and 10 g/kg did not affect the triglyceride, total and LDL-cholesterol concentration. On the other hand, results obtained in the current study are not in agreement with results reported by ALI et al. (2007) who showed that adding thyme to hen diets significantly decreased plasma HDL, total cholesterol, triglycerides and total lipids. KIRKPINAR et al. (2011) showed that broilers fed with diets supplemented with oregano had significantly lower cholesterol and triglycerides compared to the control treatment. Beside the positive effects on lipid profile of broiler serum, the lack of significant effect on these parameters in the present study could be explained by the too low dosage of essential oils used in the trial. Since the World Health Organisation (WHO) pointed to the importance of fat deposition which is undesirable in humans because it might lead to fatal diseases such as atherosclerosis, the favourable lipid profile of chicken meat presents important parameter for consumers. This is achieved in the current study to a great extent by dietary thyme, rosemary and oregano essential oils.

Table 7. Blood lipid status (mg/dl)

Blutlipidspiegel (mg/dl)

Parameter		Experimental treatments			
		T1	T2	T3	T4
Triglycerides	LSM	0.530^{b}	1.00^{b}	1.65^{a}	2.03^{a}
	SE_{LSM}	0.210	0.210	0.210	0.210
Total cholesterol	LSM	2.03^{a}	2.26^{a}	2.53^{a}	2.04^{a}
	SE_{LSM}	0.240	0.240	0.240	0.240
HDL	LSM	1.58^{a}	1.44^{a}	1.68^{a}	1.63^{a}
	SE_{LSM}	0.110	0.110	0.110	0.110
LDL	LSM	2.33^{abc}	2.60^{b}	2.67^{ab}	1.89^{c}
	SE_{LSM}	0.200	0.200	0.200	0.200
VLDL	LSM	0.500^{bc}	0.460^{bc}	0.750^{b}	0.920^{a}
	SE_{LSM}	0.090	0.090	0.090	0.090
LDL/HDL	LSM	1.52^{ab}	1.89^{a}	1.61^{ab}	1.22^{b}
	SE_{LSM}	0.190	0.190	0.190	0.190

Values in a row with no common superscript letter differ significantly ($P< 0.05$).

The effect of Herbal PHP supplementation on enzyme activity and immunological indices is shown in Table 8. The highest recorded amylase activity was recorded in treatment T3 without significant ($P>0.05$) differences to treatments with lower dose of essential oils, whereas the results were significantly higher compared to treatments T1 and T4. Regarding lipase activity, the highest activity was in treatment T3 (10.63 U/l) which was followed by treatments T1 (10.04 U/l) and T2 (10.03 U/l) with significant ($P<0.05$) difference between treatments T2 and T3, as well between treatments T3 and T1. The lowest lipase activity was recorded in treatment T4 with significant ($P<0.05$) difference to all other treatments. Regarding the protease activity, the highest activity (0.423 U/mg) was recorded in treatment T3 followed by treatment T2 (0.395 U/mg). Both treatments showed statistically significant ($P<0.05$) difference to the control treatment.

Table 8. Enzyme activity and serum levels of immunoglobulin in chickens

Enzymaktivitäten und Serum-Immunglobulin-Spiegel

Parameter		Experimental treatments			
		T1	T2	T3	T4
Amylase, U/l	LSM	506^{a}	454^{a}	424^{a}	521^{a}
	SE_{LSM}	46.7	46.7	46.7	46.7
Lypase, U/l	LSM	10.0^{b}	10.0^{b}	10.6^{a}	9.8^{c}
	SE_{LSM}	0.330	0.330	0.330	0.330
Protease, U/mg	LSM	0.186^{c}	0.395^{a}	0.423^{a}	0.251^{b}
	SE_{LSM}	0.020	0.020	0.020	0.020
IgA, U/l	LSM	0.260^{a}	0.250^{a}	0.370^{a}	0.330^{a}
	SE_{LSM}	0.070	0.070	0.070	0.070
IgG, U/l	LSM	1.39^{b}	1.55^{a}	1.65^{a}	1.85^{a}
	SE_{LSM}	0.170	0.170	0.170	0.170

Values in a row with no common superscript letter differ significantly ($P< 0.05$).

Since the purpose of the present study was to further determine the potential of Herbal PHP, the effect of thyme, rosemary and oregano essential oils on immune responses was evaluated by measuring specific antibody titres (IgA and IgG) in blood serum. The results showed that addition of the phytogenic substances to broiler diets increased IgA and IgG concentration in the T2 and T3 treatments significantly ($P<0.05$) compared to treatment T1. However, to the best of our knowledge, there are no reports available on the impact of thyme, rosemary and oregano essential oils on bird immune responses. TOGHYANI et al. (2010) examined the impact of thyme on immune related parameters in broilers and found no significant effects which is not in agreement with results obtained in the current study. The present results imply that dietary thyme, rosemary and oregano essential oils stimulate the humoral immune system in broilers to provide more antibodies, presenting an immunoprotection against invading pathogens in the gastrointestinal tract. Increased amounts of antibodies cover the surface of the intestinal mucosa and can protect villi from damage. Finally, no deleterious impact was detected on immune related parameters measured in broilers in this study.

Conclusions

From reported findings, it could be concluded that the dietary supplementation of thyme, rosemary and oregano essential oils in form of Herbal PHP resulted in an improvement of production performance of broiler chickens, specifically on body weight and feed conversion ratio. Even if addition of essential oils resulted in less improvement of biochemical parameters than expected, this study showed significant effects of selected phytogenic additives on amylase, lipase and protease activity, as well as on IgA and IgG specific immune responses in broiler chickens, improving passive immunoprotection against pathogens in the gastrointestinal tract. Thus, the general conclusion is that a thyme, rosemary and oregano essential oil mixture added in broiler diets has a remarkable impact on different aspects of broiler health, as well as on production parameters. This is of great importance from an economic point of view but also from the standpoint of the consumers who demand healthy meat.

Summary

The experiment was conducted in order to investigate the effect thyme, rosemary and oregano essential oils in broiler chicken nutrition on productive performance and blood lipid profile. Beside this, enzyme activity in blood and serum level of immunoglobulin was investigated. The experiment was carried out under production conditions on a total of 1120 day-old Ross 308 strain broilers which were equally distributed into 4 dietary treatments with 8 replicates each. In the control treatment (T1) chickens were fed with a commercial diet, while experimental treatments were formed by supplementing the commercial feed with thyme, rosemary and oregano essential oils mixture in form of a commercial product, Herbal PHP, as follows: 0.05% (T2) and 0.1% (T3). In treatment T4 chickens were fed with the commercial diet supplemented with antibiotics in a concentration of 0.1% for the first 21 days of fattening.

At the end of the experiment (42 day of age) chickens in experimental treatment T3 had achieved the highest body weight (2096 g) with significant difference compared to treatments T1 and T2, while the lowest feed conversion ratio was recorded in treatments T2 and T4 with significant difference compared to treatment T1. Regarding the European broiler index (EBI) the treatments did not differ significantly. Also, no significant influence of experimental diets on triglycerides and total cholesterol was observed during the experimental period. On the other side, the highest recorded enzyme activities were mainly in treatment T3 with significant difference compared to treatment T1. Immune responses was evaluated by measuring serum antibody concentration, where the IgA and IgG concentrations in T2 and T3 treatments differed significantly to treatments T1 and T4. Based on the obtained results it could be concluded that addition of essential oils in broiler diets positively affects production performance and various parameters of broilers health. Further investigation might show other positive effects of these natural additives.

Key words

Broiler, nutrition, essential oils, growth performance, biochemical profile, serum antibody response

Zusammenfassung

Bewertung des Zusatzes von essentiellen Ölen zum Futter auf die Leistung, auf die Lipidspiegel und die Enzymaktivität im Blut sowie auf die Immunantwort von Broilern

Das Ziel der Studie war die Untersuchung des Einflusses von Zusätzen essentieller Öle aus Thymian, Rosmarin und Oregano zum Futter auf die Mastleistung und die Blutprofile von Broilern. Zusätzlich wurde die Aktivität der Enzyme im Blutserum und die Serumspiegel der Immunglobuline bestimmt. Hierzu wurden insgesamt 1120 Ross 308 Eintagsküken auf vier Behandlungsgruppen mit jeweils acht Wiederholungen verteilt und unter kommerziellen Produktionsbedingungen gehalten. Die Tiere der Kontrollgruppe erhielten ein Standardfutter ohne Zusätze (T1). In den Versuchsgruppen wurde das kommerzielle Produkt Herbal PHP, das Thymian, Rosmarin und Oregano enthält, in der Höhe von 0,05% (T2) und 0,1% (T3) zugesetzt. In der vierten Behandlungsgruppe wurde für die ersten 21 Masttage ein Antibiotikum mit 0,1% zugesetzt (T4).

Am Versuchsende (42. Masttag) erreichten die Tiere der Behandlung T3 die höchste Lebendmasse (2096 g), die sich signifikant von den Gruppen T1 und T2 unterschied ($P<0,05$). Die signifikant günstigste Futterverwertung wurde im Vergleich zur Kontrolle in den Gruppen T2 und T4 erzielt ($P<0,05$). Dagegen unterschieden sich die Europäischen Produktionsindizes nicht signifikant zwischen den Behandlungsgruppen. In ähnlicher Weise haben sich die Behandlungen nicht auf die Gehalte an Triglyzeriden und Gesamt-Cholesterol im Blut ausgewirkt ($P>0,05$). Die höchsten Enzymaktivitäten wurde in der Gruppe T3 gemessen, die sich in erster Linie von der Kontrollgruppe unterschieden. In den Behandlungen T2 und T3 wurden im Vergleich zur Kontrolle (T1) und zur Gruppe T4 signifikant höhere IgA- und IgG-Konzentrationen gemessen ($P<0,05$). Es wurde der Schluss gezogen, dass die Zulage von essentiellen Ölen zum Broilerfutter in erster Linie die Leistung positiv beeinflusst. Es konnten aber auch positive Effekte auf die Gesundheit festgestellt werden, die noch näher untersucht werden sollten.

Stichworte

Broiler, Fütterung, essentielle Öle, Wachstum, biochemische Blutparameter, Antikörper, Blutserum

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References

AL-KASSIE, G.A.M., 2009: Influence of two plant extracts derived from thyme and cinnamon on broiler performance. Pakistan Vet. J. **29**, 169-173.

ALI, M.N, M.S. HASSAN, F.A. ABDEL-GHANY, 2007: Effect of strain, type of natural antioxidant and sulphate on productive, physiological and hatching performance of native laying hens. Int. J. Poult. Sci. **6**, 539-554.

AL-MASHHADANI, E.A., K.A. FARAH, Y.M. FARAH, H.E. AL-MASHHADANI, 2011: Effect of anise, thyme essential oils and their mixture on broiler performance and some on physiological traits. Egypt. Poult. Sci. **31**, 481-489.

ANSON, M.L., 1938: The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J. Gen. Physiol. **22**, 79-89.

BARRETO, M.S.R., J.F.M. MENTON, A.M.C. RACANICCI, P.W.Z. PEREIRA, 2008: Plant extract used as growth promoters in broilers. Braz. J. Poult. Sci. **10**, 109-115.

CROSS, D.E., R.M. MCDEVITT, K. HILLMAN, T. ACAMOVIC, 2007: The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Brit. Poult. Sci. **48**, 496-506.

CROSS, D.E., K. SVOBODA, R.M. MCDEVITT, T. ACAMOVIC, 2003: The performance of chickens fed diets with thyme oil and enzymes. Brit. Poult. Sci. **22**, 18-19.

DEMIR, E., K. KILINC, Y. YILDIRIM, F. DINCER, H. ESECELI, 2008: Comparative effects of mint, sage, thyme and flavomycin in wheat based broiler diets. Arch. Zootech. **11**, 54-63.

EL-FAHAM, A.I., A.M.H. AHMED, M.H.S. EL-SANHOURY, 2015: Thyme leaves or its extracted oil for enhancing productive and physiological status of broiler chickens. Egypt. Poult. Sci. **35**, 215-236.

EL-GHOUSEIN, S.S., N.A. AL-BEITAWI, 2009: The effect of feeding of crushed thyme (Thymus Vulgaris L.) on growth, blood constituents, gastrointestinal tract and carcass characteristics of broiler chickens. J. Poult. Sci. **46**, 100-104.

ERATS, O.N., T. GÜLER, M. CIFTICI, B. DALKILIC, 2005: The effect of on essential oil mixed derived from oregano, clove and anise on broiler performance. Int. J. Poult. Sci. **4**, 879-884.

FOLIN, O., V. CIOCALTEAU, 1929: on tyrosine and tryptophane determinations in proteins. J. Biol. Chem. **73**, 627-635.

FOTEA, L., E. COSTĂCHESCU, G. HOHA, D. LEONTE, 2015: The effect of oregano essential oil (Origanum vulgare L) on broiler performance. Lucrări Ştiinţifice - Seria Zootehnie **53**, 253-256.

GHASEMI, H.A., N. KASANI, K. TAHERPOUR, 2014: Effects of black cumin seed (*Nigella sativa* L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers. Livest. Sci. **164**, 128-134.

GOODARZI, M., S. NANEKARANI, N. LANDY, 2014: Effect of dietary supplementation with onion (*Allium cepa* L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. Asian Pacific J. Trop. Dis. **4**, S297-S301.

HERNANDEZ, F., J. MADRID, V. GARCIA, J. ORENGO, M.D. MEGIAS, 2004: Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult. Sci. **83**, 169-174.

KIRKPINAR, F., H.B. ÜNLÜ, G. ÖZDEMIR, 2011: Effects of oregano and garlic essential oils on performance, carcase, organ and blood characteristics and intestinal microflora of broilers. Livest. Sci. **137**, 219-225.

KORELESKI, J., S. ŚWIĄTKIEWICZ, A. ARCZEWSKA, 2010: The effect of dietary potassium and sodium on performance, carcass traits, and nitrogen balance and excreta moisture in broiler chickens. J. Anim. Feed Sci. **19**, 244-256.

KOSTADINOVIĆ, LJ., J. LEVIĆ, S. POPOVIĆ, I. ČABARKAPA, N. PUVAČA, O. ĐURAGIĆ, Š. KORMANJOŠ, 2015: Dietary inclusion of *Artemisia absinthium* for management of growth performance, antioxidative status and quality of poultry meat. Arch. Geflügelk. **79**, 1-10.

LEE, K.W., H. EVERTS, H.J. KAPPERT, 2003: Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Brit. Poult. Sci. **44**, 450-457.

LEWIS, M.R., S.P. ROSE, A.M. MACHENZIE, L.A. TUCKER, 2003: Effects of dietary inclusion of plant extracts on the growth performance of male broiler chickens. Brit. Poult. Sci. **44**, 43-44.

LJI, P.A., H.R.J. CHOCT, D.R. TIVEY, 2001: Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. Asian-Australasian J. Anim. Sci. **14**, 54-60.

MANSOUB, N.H., 2011: Comparison of effects of using Nettle (Utrica dioica) and probiotic on performance and serum composition of broiler chickens. Global Vet. **6**, 247-250.

MOUNTZOURIS, K.C., V. PARASKEVAS, P. TSIRTSIKOS, I. PALAMIDI, T. STEINER, G. SCHATZMAYR, K. FEGEROS, 2011: Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim. Feed Sci. Technol. **168**, 223-231.

OCAK, N., G. ERENER, F. BURAK AK, M. SUNGU, A. ALTOP, A. OZMEN, 2008: Performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita* L.) or thyme (*Thymus vulgaris* L.) leaves as growth promoter source. Czech J. Anim. Sci. **53**, 169-175.

PUVAČA, N., D. LJUBOJEVIĆ, LJ. KOSTADINOVIĆ, J. LEVIĆ, N. NIKOLOVA, B. MIŠČEVIĆ, T. KÖNYVES, D. LUKAČ, S. POPOVIĆ, 2015a: Spices and herbs in broilers nutrition: hot red pepper (*Capsicum annuum* L.) and its mode of action. World's Poult. Sci. J. **71**, 683-688.

PUVAČA, N., LJ. KOSTADINOVIĆ, D. LJUBOJEVIĆ, D. LUKAČ, J. LEVIĆ, S. POPOVIĆ, N. NOVAKOV, B. VIDOVIĆ, O. ĐURAGIĆ, 2015b: Effect of garlic, black pepper and hot red pepper on productive performances and blood lipid profile of broiler chickens. Arch. Geflügelk. **79**, 1- 13.

SALEH, N., T. ALLAM, A.A. EL-LATIF, E. GHAZY, 2014: The effects of dietary supplementation of different levels of thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. Global Vet. **12**, 736-744.

STATISTICA (Data Analysis Software System) v. 12.0., 2013: Stat-Soft, Inc., USA (www. statsoft.com).

TOGHYANI, M., M. TOHIDI, A.A. GHEISARI, S.A. TABEIDIAN, 2010: Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afr. J. Biotechnol. **9**, 6819-6825.

ZHANG, K.Y., F. YAN, C.A. KEEN, P.W. WALDROUP, 2005: Evaluation of microencapsulated essential oils and organic acids in diets for broiler chickens. Int. J. Poult. Sci. **4**, 612-619.

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