Europ.Poult.Sci., **79.** 2015, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2015.73

Effect of garlic, black pepper and hot red pepper on productive performances and blood lipid profile of broiler chickens

Einfluss von Knoblauch, schwarzem Pfeffer und scharfem, rotem Pfeffer auf die Mastleistung und die Blutfettwerte von Broilern

Nikola Puvača[1,*], **Ljiljana Kostadinović**[2], **Dragana Ljubojević**[3], **Dragomir Lukač**[1], **Jovanka Lević**[2], **Sanja Popović**[2], **Nikolina Novakov**[1], **Bojana Vidović**[1] and **Olivera Đuragić**[2]

[1] University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

[2] University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia

[3] Scientific Institute of Veterinary Medicine, Novi Sad, Serbia

Correspondence: nikola.puvaca@stocarstvo.edu.rs

Manuscript received 19 August 2014, accepted 28 November 2014

Introduction

Apart from an important role of medicinal herbs, aromatic plants and spices in daily human nutrition for enhancement of taste, aroma and colour of food, these additives have also been efficiently used in animal nutrition for improvement of animal health and wellbeing. With the ban of antibiotics use in animal nutrition due to the emergence of microbe resistance, alternative growth promoters must be found (STEINER, 2009). Removal of antibiotics as growth promoters has led to animal performance problems, increase of feed conversion ratio, and a rise in the incidence of certain animal diseases (WIERUP, 2001). The alternatives to antibiotics as growth stimulators are numerous (SIMON, 2005; STEINER, 2009; KOSTADINOVIĆ and LEVIĆ, 2012). Plant-derived additives used in animal nutrition to improve performance have been called "phytogenic feed additives" (WINDICH et al., 2008). This form of feed additives has recently become of particular interest for use in poultry production and there have been an increasing number of scientific publications since the ban of in-feed antibiotics growth promoters in 2006. In commercial broiler production mainly powder forms or essential oils of oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*), garlic (*Allium sativum*), black pepper (*Piper nigrum*) and chilli (*Capsicum annum*) are used singly or in combination as feed additives (GRASHORN, 2010; PUVAČA et al., 2013). Garlic is one of the most traditionally used plants as a spice and herb. Garlic has been used for a variety of reasons, most of which have been approved scientifically: anti-atherosclerosic, antimicrobial, hypolipidemic, anti-thrombosis, anti-hypertension, anti-diabetes etc. (MANSOUB, 2011). There are a lot of active components in garlic like ajoene, s-allyl cysteine, diallyl sulphide and the most active one allicine (RAHMATNEJAD and ROSHANFEKR, 2009). Allicine possibly reduces LDL, triglyceride and cholesterol in serum (ALDER and HOLUB, 1997) and tissues (STANAĆEV et al., 2012; PUVAČA et al., 2014), and it has been used in treatments against cardiovascular diseases (TANAMAI et al., 2004). Garlic has been found to lower serum and liver cholesterol, inhibit bacterial growth, inhibit platelet growth and reduce oxidative stress (CAVALLITO et al., 1994). In broilers, it was reported that garlic, as a natural feed additive, has improved broiler growth and feed conversion ratio, and decreased mortality rate (STANAĆEV et al., 2011). In addition, this additive has a relatively low market price, and it is added in small amounts of 0.2 to 2%, thus not increasing the production costs, which is of particular importance to manufacturers (ZEKIĆ et al., 2014). Black pepper is well-known as a spice due to its pungent quality. Black pepper was found to improve feed digestibility (MOORTHY et al., 2009). It also proved to be rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase, and it has been shown that piperine can dramatically increase absorption of selenium, vitamin B complex, β carotene and curcumin as well as other nutrients (KHALAF et al., 2008; TAZI et al., 2014).

Piperine enhances the thermogenesis of lipids and accelerates energy metabolism in the body and also increases the serotonin and β-endorphin production in the brain (AL-KASSIE et al., 2011a). Pepper has been found to have antioxidant properties (MITTAL and GUPTA, 2000) and anticarcinogenic effect, especially when combined with chili (NALINI et al., 2006). Among its chemical and biological activities, piperine is characterised by antimicrobial (REDDY et al., 2004) and anti-inflammatory (PRADEEP and KUTTAN, 2004) properties. Piperine is an active alkaloid that modulates benzopyrene metabolism through cytochrome P450 enzyme (CYP), which is important for the metabolism and transport of xenobiotic and metabolites (REEN et al., 1996). Investigation of ABOU-ELKHAIR et al. (2014) showed that black pepper in broiler nutrition had influence on improved health status through increase of serum globulin concentration. Hot red pepper plays an important role in decreasing the deposition of cholesterol and fat in the body, contributes to decreased levels of triglycerides and supports the vascular system in the body. Efficient hot red pepper compounds consist of capsaicin, capsisin and capsantine, some of which allay rheumatic aches. HENCKEN (1991) explained that hot red pepper is rich in vitamin C, which has a considerable impact on improving production through contributing to the reduction of heat stress. Consumption of hot red pepper induces a considerable change in energy balance in birds when individuals are given free access to feed (YOSHIOKA et al., 2001). Recent studies on poultry performance have shown that blends of active compounds for hot red pepper have chemopreventive and chemotherapeutic effects. In research of AL-KASSIE et al. (2012) addition of hot red pepper significantly affected the heterophil/lymphocytes (H/L) ratio, which reflects the role of hot red pepper, especially its active compound capisicine, which is involved in stress hormones, and which supports the immune system of birds and enhances its resistance against disease through decreasing (H/L) ratio.

The aim of this study was to investigate the effect of spice herbs such as garlic (*Allium sativum* L.), black pepper (*Piper nigrum* L.) and hot red pepper (*Capsicum annuum* L.) in broiler chicken nutrition on productive performances and blood lipid profile.

Materials and methods

Animal trials

Biological tests were carried out under production conditions at the experimental farm "Pustara" in property of the Faculty of Agriculture, Department of Animal Science in Novi Sad. At the beginning of the experiment, a total of 1200 one-day old Hubbard broilers were distributed into eight dietary treatments with four replicates each. Every dietary tretment included 150 chickens, which were divided in four pens with 37–38 chicken per each pen. Chickens were reared on floor holding system with chopped straw as litter material in amount of 3 kg/m^2. Chickens were provided with a light regime of 23 h per day during the entire experimental period of 42 days with incandescent light source (5 w/m^2). Heating of chickens was provided locally with infrared light heaters of 250 W/37 chicks, and the whole house was supplied with 2 thermometers linked to the heater 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 were decresed for about 2°C, reaching 20–22°C at the end of 6th week of the experiment. For nutrition of chicks three mixtures were used, starter, grower and finisher throught pan feeders (1/37 broilers). For the first 14 days, during the preparatory period, chicks were fed with starter mixtures. 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 according to the experimental desing given in Table 1 and dietary chemical composition of used starter, grower and finisher mixtures which is given in Table 2. During the experiment chicks were fed and watered *ad libitum*. Chickens were watered throught a nipple water system with 1 nipple/10–15 broilers. Microclimate conditions 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 also monitored at pen level every seven days.

Table 1. Experimental design

Versuchsdesign

Experimental treatmens	Additive	Concetration of additives in chicken diets		
		In starter, g/100g 1 – 14 days	In grower, g/100g 15 – 35 days	In finisher, g/100g 36 – 42 days
T1	Control treatment	0.0	0.0	0.0
T2	Garlic powder	0.0	0.5	0.5
T3	Garlic powder	0.0	1.0	1.0
T4	Black pepper powder	0.0	0.5	0.5
T5	Black pepper powder	0.0	1.0	1.0
T6	Hot red pepper powder	0.0	0.5	0.5
T7	Hot red pepper powder	0.0	1.0	1.0
T8	Mixture of garlic, black pepper and hot red pepper (1:1:1)	0.0	0.5	0.5

Table 2. Chemical composition of dietary mixtures (g/100 g)

Chemische Zusammensetzung der Futterrationen (g/100 g)

Nutrients	Diet mixtures		
	Starter	Grower	Finisher
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
Metabolic Energy, MJ/kg	12.5	12.8	13.3

* Spice herbs is added *on top* on the basic diet

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 convesrion ratio (kg feed/kg body weight gain)] × 100

Blood lipids

At the end of the 6th week, twelve birds were randomly chosen from each treatment 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 analyses

Statistical analyses were conducted with statistical software program Statistica 12 for Windows, to determine if variables differed between treatments. Significant effects were further explored using analysis of variance (ANOVA) with repeated measurements, least square means (LSM) and standard errors of least square menas (SE_{LSM}), as well as Fisher's LSD post-hoc multiple range test with Bonferroni corrections to ascertain differences among treatment means. A significance level of $p < 0.05$ was used.

Results and Discussion

Based on the obtained results it can be concluded that the addition of garlic, black pepper and hot red pepper in the diet of broiler chickens led to significant ($p < 0.05$) differences in body weight (Table 3). Chickens have finished the preparatory period with uniform body weight with no significant differences ($p > 0.05$).

Table 3. Body weights of chickens at different ages (g)

Körpergewichte der Broiler bei unterschiedlichem Alter (g)

Experimental treatments		Age of chickens						
		1 day	7 days	14 days	21 days	28 days	35 days	42 days
T1	LSM	42.8[a]	163[a]	389[a]	786[bc]	1162[b]	1644[c]	2076[d]
	SE_{LSM}	0.47	1.52	3.64	8.38	11.8	12.2	24.2
T2	LSM	42.1[a]	160[a]	390[a]	819[a]	1202[a]	1743[b]	2371[b]
	SE_{LSM}	0.47	1.63	3.84	8.41	11.8	12.2	24.0
T3	LSM	42.2[a]	160[a]	386[a]	805[ab]	1205[a]	1737[b]	2336[bc]
	SE_{LSM}	0.47	1.64	3.79	8.5	11.8	11.9	23.4
T4	LSM	42.4[a]	159[a]	384[a]	754[d]	1117[c]	1578[d]	2077[d]
	SE_{LSM}	0.47	1.62	3.79	8.41	11.8	12.4	24.4
T5	LSM	42.4[a]	160[a]	387[a]	728[e]	1056[d]	1504[e]	2078[d]
	SE_{LSM}	0.47	1.62	3.86	8.35	11.8	12.2	24.0
T6	LSM	42.5[a]	163[a]	385[a]	771[cd]	1194[ab]	1815[a]	2461[a]
	SE_{LSM}	0.47	1.63	3.86	8.29	11.8	12.3	24.1
T7	LSM	42[a]	162[a]	385[a]	762[cd]	1184[ab]	1812[a]	2442[a]
	SE_{LSM}	0.47	1.6	3.87	8.38	11.8	12.2	24.3
T8	LSM	41.8[a]	163[a]	385[a]	779[c]	1179[ab]	1718[b]	2298[c]
	SE_{LSM}	0.47	1.6	3.81	8.38	11.7	12.0	23.8

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

At the end of the third week, chickens in treatment T2 have achieved the highest body weight (819 g) with significant differences compared to treatments T1, T4 – T8. Almost the same tendency was observed at the end of the fourth week where the highest body masses were recorded in treatments with addition of 0.5 (T2) and 1.0 g/100 g (T3) of garlic powder (1202 and 1205 g) with significant differences compared to T1, T4 and T5, while the significant differences with treatments T6, T7 and T8 were absent. At the end of the second fattening period, addition of hot red pepper in treatments T6 and T7 exerted the stimulating effect and led to significant differences ($p < 0.05$) in body weight in relation to the control and other experimental treatments. After the completion of the experimental period, the highest achieved body weight of chicken was in treatment T6 (2461 g) which was followed by treatment T7 (2442 g), with significant differences ($p < 0.05$) compared to other treatments. Treatments with addition of garlic powder (T2, T3) achieved final body masses of 2371 and 2336 g which have been significantly ($p < 0.05$) higher than masses of chickens in treatments T1 (2076 g), T4 (2077 g) and T5 (2078 g). Addition of black pepper in treatments T4 and T5 led to a significantly ($p < 0.05$) lower body weight compared to other experimental treatments, but without significant differences ($p > 0.05$) compared to the control treatment T1. Investigation of FAYED et al. (2011) showed that the dietary addition of garlic powder (0.5 kg/t) to broiler chickens led to increased final body weights, which is also in agreement with the findings of ONIBI et al. (2009), who concluded that powdered garlic at 0.5% level may be incorporated as a growth promoter in the ration of Japanese quails. LEWIS et al. (2003) showed that addition of garlic, oregano, yarrow and other plant extracts to broiler chickens nutrition has some positive effects on performance but none of them were significant. Results obtained in the study of LEWIS et al. (2003) are not in agreement with the previous observations that indicate that pepper, oregano, cinnamon and their essential oils, oregano leaves and garlic and thyme powders did significantly affect body weight gain, feed intake or feed efficiency in broilers (DEMIR et al., 2003; HERNANDEZ et al., 2004; BAMPIDIS et al., 2005; AMOOZ MEHR and DASTAR, 2009). Our study has shown that the addition of garlic, black pepper and hot red pepper has positive effect on production results of chickens, which is also in agreement with previous findings of ASHAYERIZADEH et al. (2009) with the use of garlic, black cumin and wild mint; FADLALLA et al. (2010), STANAĆEV et al. (2011), ISSA and ABO OMAR (2012), MILOŠEVIĆ et al. (2013) and PUVAČA et al. (2014) with the use of garlic powder; AL-KASSIE et al. (2011a) with the use of black pepper and VALIOLLAHI et al. (2013) with the use of black pepper and ginger in broiler chicken nutrition. AHMAD (2005) reported higher weight gain in broilers fed rations supplemented with garlic, which probably may be due to the fact that allicin, an antibiotic substance found in garlic, inhibits growth of intestinal bacteria such as *S. aureus* and *E. coli* and inhibits aflatoxins producing fungi (MERAJ, 1998). ABOU-ELKHAIR et al. (2014) showed that the addition of black pepper and mixture of black pepper and turmeric powder to broiler chicken diet led to a higher final body weight of chickens during the fattening period of 35 days. Improvement of broilers body weight gain as a result of supplementation of black pepper powder was observed and reported by GHAZALAH et al. (2007) and MANSOUB (2011). HOSSEINI (2011) showed that black pepper increases digestion through arousing digestive liquids of stomach and eradicates infectious bacteria. Black pepper affects the absorption power, decreases material transit velocity and increases digestive enzymes. The most active component in black pepper, piperine, promotes pancreatic digestive enzymes such as lipase, amylase and proteases, which play important roles in the digestion process (PLATEL and SRINIVASAN, 2000). Research of AL-KASSIE et al. (2011b) revealed that the inclusion of hot red pepper at levels of 0.5%, 0.75% and 1% in the diets of broiler chicken of hybrid line Ross 308 improved body weight gain and feed conversion ratio. Investigation of THIAMHIRUNSOPIT et al. (2014) with the different forms of hot red peppers showed better growth performance results of chickens in experimental hot red pepper treatments in comparison to control treatments.

From the results given in Table 4 it can be seen that feed conversion ratio in preparation period of chicken was uniform and ranged between 1.3 and 1.4 kg of feed per kg of gain, without significant ($p > 0.05$) differences. In the grower phase the lowest achieved feed conversion ratio was in treatment T2 (1.7 kg/kg) and the highest in T4 and T5 (1.9 kg/kg) treatments. Feed conversion ratio in finisher phase was the highest in the control treatment T1 (3.0 kg/kg) with significant ($p < 0.05$) differences in comparison to the rest of the treatments. The lowest feed conversion ratio of 2.3 kg/kg was recorded in T2 and T5 treatments, followed by 2.4 kg/kg in T6, 2.5 kg/kg in T3 and T4, and 2.6 kg/kg in T7 and T8. Lower feed conversion ratio in experimental treatments shows that addition of garlic, black pepper and hot red pepper and their mixture have positive influence on feed utilization and efficiency. FAYED et al. (2011) showed that the addition of garlic powder to broiler chickens nutrition significantly improved feed conversion ratio which clarified that the broilers fed rations supplemented with garlic utilized their feed more efficiently than those fed rations without addition of garlic which also may be attributed to the antibacterial properties of garlic, which resulted in better absorption of the nutrients present in the gut and finely leading to improvement in feed conversion ratio. AL-KASSIE et al. (2011a) and ABOU-ELKHAIR et al. (2014) with the use of black pepper powder in chicken nutrition did not record positive an influence of the added spice on feed conversion ratio. AL-HARTHI (2002) found that broiler chicks fed with diets supplemented with hot red pepper showed improved feed conversion ratio and concluded that the effect may be due to its stimulative, carminative, digestive and antimicrobial properties, what is in agreement with our results. AL-KASSIE et al. (2011b) showed that the inclusion of hot red pepper in the diet improved body weight gain, feed consumption and feed conversion ratio of broiler chickens. THIAMHIRUNSOPIT et al. (2014) showed significantly higher average daily gain and lower feed conversion ratio of chickens on dietary treatment with hot red pepper than those chickens in the control treatment. In our study, for the entire experimental period, feed conversion ratio was the lowest in treatments T2 and T5 (1.8 kg/kg) and the highest in control treatment T1 (2.1 kg/kg), without significant ($p > 0.05$) differences.

Table 4. Feed conversion ratio (kg/kg)

Futterverwertung (kg/kg)

Experimental treatments		Periods of nutrition			
		Starter phase 1 – 14 days	Grower phase 15 – 35 days	Finisher phase 36 – 42 days	Entire period 1 – 42 days
T1	LSM	1.3[ab]	1.8[ab]	3.0[a]	2.1[a]
T2	LSM	1.4[ab]	1.7[b]	2.3[b]	1.8[a]
T3	LSM	1.4[ab]	1.8[b]	2.5[b]	1.9[a]
T4	LSM	1.4[ab]	1.9[a]	2.5[b]	1.9[a]
T5	LSM	1.3[b]	1.9[ab]	2.3[b]	1.8[a]
T6	LSM	1.4[a]	1.8[ab]	2.4[b]	1.9[a]
T7	LSM	1.4[ab]	1.8[b]	2.6[b]	1.9[a]
T8	LSM	1.4[ab]	1.8[b]	2.6[b]	1.9[a]

Treatments with different letter indexes in the same column are statistically significantly different ($p < 0.05$); SE_{LSM} values for the starter phase was equal to 0.01; grower phase 0.05; finisher phase 0.14 and for entire period 0.15.

Table 5 gives an overview of European broiler index (EBI) and chicken mortality rate. Addition of these feed additives led to a significant ($p < 0.05$) increase in values of EBI of the experimental treatments in comparison to the control treatment T1. The highest mortality rate (5.1%) and the lowest EBI (220.4%) were recorded in the control treatment. Mortality rate of 0.0% was recorded in treatment T8 and 280% EBI which was significantly ($p < 0.05$) higher than in treatments T1, T4 and T5. The highest recorded EBI values were 299% in treatment T6 and 295% in treatment T2 without significant ($p > 0.05$) differences between them, but with significant ($p < 0.05$) differences to other experimental treatments. Positive results concerning EBI points were recorded also in research of Arczewska-Wlosek and Swiatkiewicz (2012) with addition of blend extract containing garlic, sage, thyme and oregano to chicken diets. Positive broiler production was also recorded in investigation of Fadlalla et al. (2010) with garlic, Tazi et al. (2014) with black pepper powder in the amount of 1% dietary addition and Al-Kassie et al. (2011b) with the addition of hot red pepper to chicken dietary mixtures.

Table 5. European broiler index and chicken mortality (%)

Europäischer Produktionsfaktor und Mortalität (%)

Experimental treatments		EBI	Mortality
T1	LSM	220^{g}	5.1^{a}
T2	LSM	295^{ab}	3.2^{ab}
T3	LSM	284^{cd}	1.3^{bc}
T4	LSM	244^{f}	1.3^{bc}
T5	LSM	260^{e}	0.6^{bc}
T6	LSM	299^{a}	2.6^{ac}
T7	LSM	289^{bc}	2.6^{ac}
T8	LSM	280^{d}	0.0^{c}

Treatments with different letter indexes in the same column are statistically significantly different ($p < 0.05$); SE_{LSM} values for the EBI was equal to 2.77 and for the mortality rate 0.96.

Addition of garlic, black pepper and hot red pepper as feed additives to broiler chicken nutrition in this experiment led to a high improvement of blood lipid profile. From the results given in Table 6 it can be noticed that the highest amounts of triglycerides (65.9 mg/dl), total cholesterol (97.2 mg/dl) and LDL (36.7 mg/dl) were in treatment T1 with significant ($p < 0.05$) differences in comparison to the other treatments. Addition of black pepper in the amount of 1.0 g/100 g (T5), significantly ($p < 0.05$) decreased the concentration of triglycerides (14.4 mg/dl), while the addition of garlic powder in the same amount (T3) significantly ($p < 0.05$) decreased LDL concentrations in blood serum. This effect can be explained by the possible inhibition of the Acetyl CoA syntheses enzyme that is necessary for the biosynthesis of fatty acids. Afzal et al. (1985) reported that polyunsaturated fatty acids prevent atherosclerosis through the formation of cholesterol esters. They further reported the presence of higher polyunsaturated fatty acids like arachidonic and eicosapentaenoic in garlic which could well be responsible for preventing atherosclerosis. In research with the lipid profile of chicken meat Fayed et al. (2011) showed that the cholesterol concentration in thigh and breast muscles decreased significantly with garlic powder supplementation to broiler chicken diets. Eidi et al. (2006) reported that garlic extract significantly decreased the total cholesterol, triglycerides in diabetic rats. In our study the lowest concentration of total cholesterol was recorded in treatments T6, T4, T2 and T7 ($p < 0.05$). The highest concentration of HDL (44.8 and 39.6 mg/dl) was recorded in treatments T3 and T2 with addition of 1.0 and 0.5 g/100 g of garlic powder. The significant effect of garlic powder on the mean values of HDL compared to control and other treatments can be explained by the hypocholesterolaemic mechanism and the hypolipidemic action of garlic powder. The compound allicin combines with the -SH group that is important in activation of Acetyl CoA which is essential for the biosynthesis of cholesterol. Although results of this study indicate the significant ($p < 0.05$) increase of HDL by addition of garlic powder, reports on the effects of garlic on HDL in different species are inconsistent (Chi et al., 1982; Qureshi et al., 1983). Both levels of garlic, black pepper and hot red pepper in our study decreased LDL levels compared to the levels in chickens of the control treatment. This effect can be explained by the possible mechanism of antioxidant and antiperoxide lowering action on LDL or the decrease in hepatic production of very low density lipoprotein (VLDL) which serves as the precursor of LDL in the blood circulation (Kim et al., 2009). In investigation of Ghaedi et al. (2014) addition of black pepper decreased triglycerides and the total cholesterol while the concentration of HDL was increased. Al-Kassie et al. (2011a) showed that broilers fed with black pepper mixtures had significantly lowered cholesterol, heterophil and lymphocyte ratio (H/L), red blood cell count, packed cell volume and haemoglobin compared with the control group, from which can be seen that the H/L ratio could serve as a good indicator to examine the stress level of chickens. In the research of the same authors the reduction of the total blood cholesterol in broiler chickens fed with addition of black pepper in amount of 0.5 and 1 g/100 g was about 16 mg/100 ml. Also as well as addition of garlic and black pepper, addition of hot red pepper to the broiler diet in different amounts from 0.25 to 1% had influence on decreased concentration of blood cholesterol, and other blood biochemical parameters (Al-Kassie et al., 2012; Alaa, 2010). Furthermore, addition of spice herbs and medicinal plants can facilitate activity of enzymes which are involved in the conversion of cholesterol to bilious acids and subsequently will result in lower cholesterol concentration in the carcass.

Table 6. Biochemical blood parameters and lipid profile (mg/dl)

Biochemische Blutparameter und Lipidkennwerte (mg/dl)

Experimental treatments		Triglycerides	Total cholesterol	HDL	LDL	non HDL	HDL/LDL
T1	LSM	65.9^{a}	97.2^{a}	19.2^{e}	36.7^{a}	78.0^{a}	0.5^{cdefg}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T2	LSM	19.3^{cd}	54.1^{bd}	39.6^{b}	5.8^{e}	14.5^{de}	7.7^{b}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T3	LSM	22.4^{b}	55.7^{b}	44.8^{a}	0.9^{f}	10.9^{f}	48.9^{a}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T4	LSM	16.5^{cef}	54.1^{bd}	29.7^{d}	16.6^{b}	24.4^{b}	1.8^{bg}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T5	LSM	14.4^{f}	55.5^{b}	35.6^{c}	13.4^{c}	19.9^{c}	2.6^{bf}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T6	LSM	16.7^{e}	52.4^{cd}	35.5^{c}	9.4^{d}	16.9^{ce}	3.8^{bd}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T7	LSM	17.7^{de}	54.3^{bcd}	35.7^{c}	10.3^{d}	18.6^{c}	3.6^{be}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T8	LSM	20.7^{bc}	55.8^{b}	38.5^{bc}	8.3^{de}	17.3^{cd}	4.7^{bc}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33

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

Conclusions

Based on the obtained results, it can be concluded with certainty that the addition of garlic, black pepper and hot red pepper in broiler chicken nutrition has positive effect on production performances. Addition of garlic and hot red pepper in the amount of 0.5 g/100 g has led to the highest final body weights, lower feed conversion ratio and higher feed utilization, with the highest percentage of European broiler index. Also it can be concluded that significant lowering of plasma cholesterol, triglycerides, LDL and increase of HDL by these spice herbs supplementation in broiler diet indicate that garlic, black pepper and hot red pepper are effective in regulation of lipid metabolism in a favourable manner for prevention of atherosclerosis or coronary heart diseases in humans who use this kind of chicken products in their daily nutrition. Therefore the general conclusion would be that the addition of these spice herbs has positive influence on chicken production and blood lipid profile, but further investigation of their mode of action is still necessary.

Summary

This experiment was conducted to investigate the effect of various medicinal herbs such as garlic (*Allium sativum* L.), black pepper (*Piper nigrum* L.) and hot red pepper (*Capsicum annuum* L.) in broiler chicken nutrition on productive performances and blood lipid profile. In total 1200 Hubbard broilers have been assigned to eight treatments with four replicates, each. In the control treatment (T1) the chickens were fed with a commercial compound feed based on corn flour and soybean meal. Experimental treatments were formed by supplementing the commercial feed with medicinal herbs as follows: garlic 0.5 (T2) and 1.0 g/100 g (T3), black pepper 0.5 (T4) and 1.0 g/100 g (T5), hot red pepper 0.5 (T6) and 1.0 g/100 g (T7) and mixture of garlic, black pepper and hot red pepper (1:1:1) in total of 0.5 g/100 g (T8). During the first two weeks chickens received a starter mixture without addition of medicinal herbs, thereafter, chickens were fed with grower and finisher mixtures according to the plan given.

At the end of the experiment (day 42 of life) chickens in experimental treatments T6 and T7 achieved significantly ($p < 0.05$) higher final body masses (2461 and 2442 g) compared to the chickens in the control and other treatment groups. Feed conversion ratio for the entire fattening period ranged from 1.8 kg/kg (T2, T5) to 2.1 kg/kg (T1) with no significant differences ($p > 0.05$). European broiler index (EBI) was the lowest in treatment T1 (220) and highest in treatment T6 (299) with significant differences ($p < 0.05$). The highest amounts of triglycerides, total cholesterol, low density lipoprotein (LDL) and non high density lipoprotein (non HDL) was recorded in chicken blood in treatment T1 with significant ($p < 0.05$) differences compared to the treatments with addition of medicinal herbs. The significantly lowest level of high density lipoproteins (HDL) was determined in control treatment T1 ($p < 0.05$). It can be concluded that feeding diets with supplements of spice herbs results in better production level and much better lipid profile status compared to the control treatment.

Key words

Broiler, nutrition, garlic, black pepper, hot red pepper, performance, bloog lipid profile, cholesterol

Zusammenfassung

Einfluss von Knoblauch, schwarzem Pfeffer und scharfem, rotem Pfeffer auf die Mastleistung und die Blutfettwerte von Broilern

Das Ziel der Studie war die Untersuchung des Einflusses des Zusatzes von verschiedenen Heilkräutern, wie Knoblauch (*Allium sativum* L.), Schwarzem Pfeffer (*Piper nigrum* L.) und scharfem Rotem Pfeffer (*Capsicum annuum* L.), zum Futter auf die Leistung und auf die Blutkennwerte von Broilern. Hierzu wurden 1200 Eintagsküken der Herkunft Hubbard auf 8 Behandlungen mit jeweils 4 Wiederholungen verteilt. Die Tiere der Kontrollgruppe (T1) wurden mit einem kommerziellen Alleinfutter auf Mais-Soja-Basis gefüttert. Bei den Behandlungsgruppen wurden der Basisfuttermischung die Heilkräuter in folgenden Dosierungen zugegeben: Knoblauch 0,5 (T2) und 1,0 g/100 g (T3); Schwarzer Pfeffer 0,5 (T4) und 1,0 g/100 g (T5); scharfer Roter Pfeffer 0,5 (T6) und 1,0 g/100 g (T7); Knoblauch, Schwarzer Pfeffer, scharfer Roter Pfeffer 0,5 g/100 g im Verhältnis 1:1:1 (T8). In den ersten zwei Lebenswochen wurden die Tiere mit kommerziellen Starter-Rationen ohne einen Zusatz an Heilkräutern gefüttert. Von der dritten bis zur sechsten Lebenswoche wurden Grower- und Finisher-Rationen mit den oben angeführten Zusätzen eingesetzt.

Am Versuchsende (42. Lebenstag) hatten die Broiler der Behandlungsgruppen T6 und T7 eine signifikant höhere Lebendmasse (2461 bzw. 2442 g) erreicht als die Tiere der übrigen Behandlungsgruppen ($P < 0,05$). Die Futterverwertung variierte zwar über die gesamte Mastdauer zwischen 1,8 kg/kg (T2, T5) und 2,1 kg/kg (T1), die Unterschiede waren aber nicht signifikant ($P > 0,05$). Der niedrigste Europäische Produktions-Index wurde in Behandlung T1 (220) und der höchste in Behandlung T6 (299) erreicht. Die Unterschiede waren signifikant ($P < 0,05$). In der Kontrollgruppe (T1) wurden im Vergleich zu den Behandlungsgruppen mit der Zulage an Heilkräutern die signifikant höchsten Triglyzerid-, Gesamt-Choelsterol-, Low Density Lipoprotein- (LDL) und Nicht-High Density Lipoprotein- (non HDL) Spiegel im Blut gemessen. Der geringste High Density Lipoprotein-Spiegel (HDL) lag ebenfalls in der Kontrollgruppe vor ($P < 0,05$). Die Untersuchung hat gezeigt, dass durch einen Zusatz an Heilkräutern zum Futter im Vergleich zur Kontrollgruppe bessere Produktionsergebnisse und ein deutlich günstigerer Blut-Lipid-Status erreicht werden kann.

Stichworte

Broiler, Fütterung, Knoblauch, schwarzer Pfeffer, roter Pfeffer, Leistung, Blutfettwerte, Cholesterol

Acknowledgments

This paper is a part of the project III 46012 which is financed by Ministry for Science and Technological development of the Republic of Serbia. Also the realization of one part of this experiment was supported by the Perutnina Ptuj – Topiko a.d., Petefi brigade 2, 24300 Bačka Topola, Serbia.

References

ABOU-ELKHAIR, R., H.A. AHMED, S. SELIM, 2014: Effects of black pepper (*Piper nigrum*), turmeric powder (*Curcuma longa*) and coriander seeds (*Coriandrum sativum*) and their combinations as feed additives on growth performance, carcass traits, some blood parameters and humoral immune response of broiler chickens. Asian Australian Journal of Animal Science **27**, 847-854.

AFZAL, M., R.A.H. HASSAN, A.A. EL-KAZINI, R.M.A. FATTAH, 1985: *Allium sativum* in the control of atherosclerosis. Agricultural and Biological Chemistry **49**, 1187-1188.

AHMAD, S., 2005: Comparative efficiency of garlic, turmeric and kalongi as growth promoter in broiler. M.Sc Thesis, Department of Poultry Sciences, University of Agriculture, Faisalabad, Pakistan.

ALDER, A.J., B.J. HOLUB, 1997: Effect of garlic and fish-oil supplementation on serum lipid and lipoprotein concentrations in hypercholesterolemic men. American Journal of Clinical Nutrition **65**, 445-450.

AL-HARTHI, M.A., 2002: Efficacy of vegetable diets with antibiotics and different type of spices or their mixtures on performance economic efficiency and carcass traits of broilers. Journal of Agriculture Science **27**, 3531-3545.

AL-KASSIE, G.A.M., M.A.M. AL-NASRAWI, S.J. AJEENA, 2011a: Use of black pepper (*Piper nigrum*) as feed additive in broilers diet. Research Opinions in Animal and Veterinary Sciences **1**, 169-173.

AL-KASSIE, G.A.M., M.A.M. AL-NASRAWI, S.J. AJEENA, 2011b: The effects of using hot red pepper as a diet supplement on some performance traits in broiler. Pakistan Journal of Nutrition **10(9)**, 842-845.

AL-KASSIE, G.A.M., G.Y. BUTRIS, S.J. AJEENA, 2012: The potency of feed supplemented mixture of hot red pepper and black pepper on the performance and some hematological blood traits in broiler diet. International Journal of Advanced Biological Research **2**, 53-57.

ALAA, A.A., 2010: The effect of the Capsicum annuum in the diet of broilers on the isolation and shedding rate of Salmonella paratyphoid. Kufa Journal for Veterinary Medical Sciences **1(1)**, 28-38.

AMOOZ MEHR, A., B. DASTAR, 2009: Effects of alcoholic extract of two herbs (garlic and thymus) on the performance and blood lipids of broiler chickens. Journal of Agricultural Sciences and Natural Resources **16**, 61-72.

ARCZEWSKA-WLOSEK, A., S. SWIATKIEWICZ, 2012: The effect of a dietary herbal extract blend on the performance of broilers challenged with *Eimeria oocysts*. Journal of Animal and Feed Sciences **21**, 133-142.

ASHAYERIZADEH, O., B. DASTAR, M. SHAMS SHARGH, A. ASHAYERIZADEH, E. RAHMATNEJAD, S.M.R. HOSSAINI, 2009: Use of garlic (*Allium sativum*), black cumin seeds (*Nigella sativa* L.) and wild mint (*Mentha longifolia*) in broiler chickens diets. Journal of Animal and Veterinary Advances **8**, 1860-1863.

BAMPIDIS, V.A., V. CHRISTODOULOU, P. FLOROU-PANERI, E. CHRISTAKI, P.S. CHATZOPOULOU, T. TSILIGIANNI, A.B. SPAIS, 2005: Effect of dietary dried oregano leaves on growth performance, carcass characteristics and serum cholesterol of female early maturing turkeys. British Poultry Science **46**, 595-601.

CAVALLITO, C.J., J.S. BUCK, C.M. SUTER, 1994: Allicin, the antibacterial principle of Allium sativum. Determination of the chemical composition. Journal of the American Chemical Society **60**, 1952-1958.

CHI, M.S., H. KOH, T.J. STEWARD, 1982: Effects of garlic on lipid metabolism in rats fed cholesterol or lard. Journal of Nutrition **112**, 241-248.

DEMIR, E., S. SARICA, M.A. OZCAN, M. SUICMEZ, 2003: The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. British Poultry Science **44**, S44-S45.

EIDI, A., M. EIDI, E. ESMAEILI, 2006: Antidiabetic effect of garlic (*Allium sativum* L.) in normal or streptozotocin induced diabetic rats. Phytomedicine **9**, 624-629.

FADLALLA, I.M.T., B.H. MOHAMMED, A.O. BAKHIET, 2010: Effect of feeding garlic on the performance and immunity of broilers. Asian Journal of Poultry Science **4**, 182-189.

FAYED, R.H., H. ABEER, A. RAZEK, M. JEHAN, 2011: Effect of dietary garlic supplementation on performance, carcass traits, and meat quality in broiler chickens. Parameters **100**, 1000-1004.

GHAEDI, H., J. NASR, F. KHEIRI, Y. RAHIMIAN, Y. MIRI, 2014: The effect of virginiamycin and black pepper (*Piper nigrum* L.) extract on performance of broiler chicks. Research Opinion in Animal and Veterinary Sciences **4**, 91-95.

GHAZALAH, A.A., A.S.A. EL-HAKIM, A.M. REFAIE, 2007: Response of broiler chicks to some dietary growth promoters throughout different growth period. Egyptian Poultry Science Journal **27**, 53-57.

GRASHORN, M.A., 2010: Use of phytobiotics in broiler nutrition – an alternative to in feed antibiotics? Journal of Animal and Feed Sciences **19**, 338-347.

HENCKEN, H., 1991: Cooling the burn from hot peppers. Journal of the American Medical Association **266**, 2766-2770.

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

HOSSEINI, M.N., 2011: Comparison of using different level of black pepper with probiotic on performance and serum composition on broilers chickens. Journal of Basic and Applied Science Research **11**, 2425-2428.

ISSA, K.J., J.M. ABO OMAR, 2012: Effect of garlic powder on performance and lipid profile of broilers. Open Journal of Animal Sciences **2**, 62-68.

KHALAF, A.N., A.K. SHAKYA, A. AL-OTHMAN, Z. EL-AGBAR, H. FARAH, 2008: Antioxident activity of some common plants. Turkish Journal of Biology **32**, 51-55.

KIM, Y.J., S.K. JIN, H.S. YANG, 2009: Effect of dietary garlic bulb and husk on the physicochemical proper-ties of chicken meat. Poultry Science **88**, 398-405.

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. Journal of Animal Feed Science **19**, 244-256.

KOSTADINOVIĆ, Lj., J. LEVIĆ, 2012: Use of phytogenic products for pig and broiler diseases. Proceedings of International Feed Technology Symposium, Novi Sad, Serbia **15**, 64-74.

LEWIS, M.R., S.P. ROSE, A.M. MACKENZIE, L.A. TUCKER, 2003: Effects of dietary inclusion of plant extraction the growth performance of male broiler chickens. British Poultry Science **44**, S43-S44.

MANSOUB, N.H., 2011: Comparative effects of using garlic as probiotic on performance and serum composition of broiler chickens. Annals of Biological Research **2**, 486-490.

MERAJ, I.C.A., 1998: Effect of garlic and neem leaves supplementation on the performance of broiler chickens. M.Sc Thesis, Department of Poultry Science, University of Agriculture, Faisalabad, Pakistan.

MILOŠEVIĆ, N., V. STANAĆEV, L. PERIĆ, M. ĐUKIĆ STOJČIĆ, M. VELJIĆ, 2013: Effects of different levels of garlic powder in the diet on production parameters and slaughter traits of broiler chickens. European Poultry Science **77**, 254-259.

MITTAL, R., R.L. GUPTA, 2000: In vitro antioxidant activity of piperine. Methods and Findings in Experimental and Clinical Pharmacology **122**, 271-274.

MOORTHY, M., S. RAVIKUMAR, K. VISWANATHAN, S.C. EDWIN, 2009: Ginger, pepper and curry leaf powder as feed additives in broiler diet. International Journal of Poultry Science **8**, 779-782.

NALINI, N., Y. MANJU, V. MENON, 2006: Effect of spices on lipid metabolism in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. Journal of Medicinal Food **9**, 237-45.

ONIBI, G.E., E. OLUWATOYIN, A. ADEBISI, N. FAJEMISIN, V. AYODE, J.I. ADETUN, 2009: Response of broiler chickens in terms of performance and meat quality to garlic (*Allium sativum*) supplementation. African Journal of Agricultural Research **4 (5)**, 511-517.

PLATEL, K., K. SRINIVASAN, 2000: Influence of dietary spices or their active principles on pancreatic digestive enzymes in albino rats. Nahrung **44**, 42-46.

PRADEEP, C.R., G. KUTTAN, 2004: Piperine is a potent inhibitor of nuclear factor-kB (NF-kB), c-Fos, CREB, ATF-2 and pro-inflammatory cytokine gene expression in B16F-10 melanoma cells. International Immunopharmacology **4**, 1795-1803.

PUVAČA, N., V. STANAĆEV, M. BEUKOVIĆ, D. LJUBOJEVIĆ, Lj. KOSTADINOVIĆ, N. DŽINIĆ, 2014: Effect of phytogenic feed additive (*Allium sativum* L.) on broiler chicken nutrition on breast meat quality and tissues cholesterol content. Proceedings of International Scientific Conference, Vukovar, Croatia **7**, 75-80.

PUVAČA, N., V. STANAĆEV, D. GLAMOČIĆ, J. LEVIĆ, L. PERIĆ, V. STANAĆEV, D. MILIĆ, 2013: Beneficial effects of phytoadditives in broiler nutrition. World's Poultry Science Journal **69**, 27-34.

QURESHI, A.A., N. ABUIRMEILEH, Z.Z. DIN, C.E. ELSON, W.C. BURGER, 1983: Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fractions of garlic. Lipids **18**, 343-348.

RAHMATNEJAD, E., H. ROSHANFEKR, 2009: Evaluation the effect of several non-antibiotic additives on growth performance of broiler chickens. Journal of Animal and Veterinary Advances **8**, 1757-1760.

REDDY, S.V., P.V. SRINIVAS, B. PRAVEEN, K.H. KISHORE, B.C. RAJU, U.S. MURTHY, J.M. RAO, 2004: Antibacterial constituents from the berries of Piper nigrum. Phytomedicine **11**, 697-700.

REEN, R.K., S.F. ROESCH, F. KIEFER, F.J. WIEBEL, J. SINGH, 1996: Piperine impairs cytochrome P4501A1 activity by direct interaction with the enzyme and not by down regulation of CYP1A1 gene expression in the rat hepatoma 5L cell line. Biochemical and Biophysical Research Communications **218**, 562-569.

SIMON, O., 2005: Micro-organisms as feed additives-probiotics. Advances in Pork Production **39**, 161-167.

STANAĆEV, V., D. GLAMOČIĆ, N. MILOŠEVIĆ, L. PERIĆ, N. PUVAČA, V. STANAĆEV, D. MILIĆ, N. PLAVŠA, 2012: Influence of garlic (*Allium sativum* L.) and copper as phytoadditives in the feed on the content of cholesterol in the tissues of the chickens. Journal of Medicinal Plants Research **6**, 2816-2819.

STANAĆEV, V., D. GLAMOČIĆ, N. MILOŠEVIĆ, N. PUVAČA, V. STANAĆEV, N. PLAVŠA, 2011: Effect of garlic (*Allium sativum* L.) in fattening chicks nutrition. African Journal of Agricultural Research **6**, 943-948.

STEINER, T., 2009: Phytogenics in animal nutrition Natural concepts to optimise gut health and performance. Nottingham University Press, 1-169.

TANAMAI, J., S. VEERAMANOMAI, N. INDRAKOSAS, 2004: The efficacy of cholesterol-lowering action and side effects of garlic enteric coated tablets in man. Journal of the Medical Association of Thailand **87**, 1156-1161.

TAZI, S.M.E., M.A. MUKHTAR, K. MOHAMED, M.H. TABIDI, 2014: Effect of using black pepper as natural feed additive on performance and carcass quality of broiler chicks. International Journal of Pharmaceutical Research and Analysis **4**, 108-113.

THIAMHIRUNSOPIT, K., C. PHISALAPHONG, S. BOONKIRD, S. KIJPARKORN, 2014: Effect of chili meal (*Capsicum frutescens* LINN.) on growth performance, stress index, lipid peroxidation and ileal nutrient digestibility in broilers reared under high stocking density condition. Animal Feed Science and Technology **192**, 90-100.

VALIOLLAHI, M.R., Y. RAHIMIAN, Y. MIRI, A. RAFIEE, 2013: Effect use ginger (*Zingiber officinale*), black pepper (*Piper nigrum* L.) powders on performance, some blood parameters and antibody titer against new castle vaccine on broiler chicks. Scholarly Journal of Agricultural Science **3**, 535-540.

WIERUP, M., 2001: The Swedish experience of the 1986 ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microbial Drug Resistance **7**, 183-190.

WINDICH, W., K. SCHEDLE, C. PLITZNER, A. KROISMAYR, 2008: Use of phytogenic products as feed additives for swine and poultry. Journal of animal Science **86**, 140-148.

YOSHIOKA, M., E. DOUCET, V. DRAPEAU, I. DIONNE, A. TREMBLAY, 2001: Combined effects of red pepper and caffeine consumption on energy balance in subjects given free access to foods. British Journal of Nutrition **85**, 203-211.

ZEKIĆ, V., N. PUVAČA, D. MILIĆ, M. BEUKOVIĆ, D. GLAMOČIĆ, N. VUKELIĆ, D. LUKAČ, S. ZEKIĆ, 2014: Effect of garlic powder in broiler chicken nutrition: Emphasis on production economic efficiency coasts and chicken meat quality. Custos e @gronegócio **10**, 86-98.

Correspondence: Nikola Puvača, Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia; E-mail: nikola.puvaca@stocarstvo.edu.rs