KRMIVA

FUNCTIONAL POULTRY MEAT ENRICHED WITH BIOLOGICALLY ACTIVE SUBSTANCES FROM NEUTRAL EXTRACTIVES OBTAINED FROM SPRUCE NEEDLES

FUNKCIONALNO MESO PERADI OBOGAĆENO BIOLOŠKI AKTIVNIM TVARIMA IZ NEUTRALNIH EKSTRAKATA DOBIVENIH OD SMREKOVIH IGLICA

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SUMMARY

Neutral extractives of spruce needles were produced from forestry by-products: the green biomass of spruce needles. The extractes of spruce needles extract contains a significant amount of natural biologically active substances. In the course of our investigations, the evaluation of biologically active substances obtained from neutral extracts of spruce needles was carried out to assess their effect on quality of broiler chicken meat.

The feeding trial was conducted with broiler chickens of the cross ROOS – 308 by adding neutral extract of spruce needles in the form of loose powder to the composition of broiler chicken diet. It was found that the loose powder feed additives containing neutral extract of spruce needles (0.05% and 0.10% in pure substance) used to supplement the broiler feed improved the quality of poultry meat. The feed additives containing biologically active substances from spruce needles neutral extract involved in metabolic processes of the broiler chicken organism, increased the n – 3 polyunsaturated fatty acids (DHA) content in meat by 0.59 – 1.05% and the carotenoids' content in liver by 1.46 – 1.70 mg kg $^{-1}$, at the same time positively reducing the cholesterol level in meat by 17.18 – 26.16 mg 100 g^{-1} in comparison with the control group.

Key words**:** broiler chicken, neutral extractives of spruce needles, meat quality

INTRODUCTION

Forestry by-products: green biomass of spruce needles and non–lignified shoots, contain a significant amount of natural biologically active substances.

The major active ingredients of biologically active substances obtained from the green biomass of spruce needles are chlorophyll and its derivates, antioxidants, essential oils, fatty acids, vitamins, minerals, phytosterols, polyprenols etc. (Nekrasova et al., 2000; Ievins et al., 1986; Andersons et al., 1983.). The above biologically active substances have a broad therapeutic and disease-preventive influence both, on poultry and human organism (Bespalov and Nekrasova, 2004.).

Including the above biologically active substances, contained in spruce needles extracts in the poultry diet, they are transferred from feed to poultry products: meat and eggs.

Researchers (Grashorn, 2010; Leskanich and Noble, 1997.) have confirmed the transfer of biologically active substances from feed to products, resulting in products of innovative composition enriched with biologically active substances.

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The biomass of spruce needles contained a higher amount of various biologically active substances (carotenoids, fatty acids etc.). Extractives in the form of paste pose certain difficulties as to precise dosage to be added to poultry feed to achieve uniform binding with basic feed ingredients. Therefore it was necessary to produce a loose powder form of feed additive containing neutral extractivesw of spruce needles.

According to the patent (Patents A61K1/16 14568B, 2012) the paste containing neutral extractive of spruce needles was enriched with limestone and manufactured into a loose-form feed additive. The loose-powder readily binds with ingredients of the basic poultry feed and are easy to doze.

The purpose of our study was to find out whether the biologically active substances contained in the loose-form additive of neutral extractives of spruce needles had an impact on productivity of broilers and improve their meat quality.

MATERIALS AND METHODS

The feeding trial was conducted with broiler chickens of cross ROSS – 308 from 0 to 42 days of age (n = 300, Table 1).

The ingredient composition of the basic diet and nutritive value for all groups of broiler chickens was identical: wheat, soybean meal, corn, sunflower meal, rapeseed oil, flax oil, calcium carbonate, monocalcium phosphate, salt, and vitamin - mineral premix, DL - Methionine, L - Lysine HCl and L – Threonine and fatty acids.

In the basic diet, the content of crude protein, metabolizable energy and calcium at different ages of chickens was 19.0 – 24.0%, 11.90 – 12.80 $MJkg^{-1}$ and 0.85 – 1.00% Ca on the average. Additives of neutral extracts from biomass of spruce needles were not added to the control group diet.

The basic diet of the 2nd and 3rd group was supplemented with additives obtained from neutral extractives of spruce needles in the form of dry loose powder that was obtained by addition of calcium carbonate to the paste.

The additives of spruce needles neutral extracts in the form of loose powder were added to the diet of 2nd group in 0.70% dose (0.05% in pure substance) and to the diet of 3rd group in 1.40% dose (0.10% in pure substance).

Over the course of trial, the main indicators of poultry productivity and meat quality were recorded and evaluated. Meat samples of broiler chickens in age of 42 days were analysed for fatty acid, levels of cholesterol and carotenoids. Neutral extracts of spruce needles were analysed for total carotenoids, α – tocopherol, polyprenol, sterol and fatty acid contents. The chromatography method was used to identify fatty acids, carotenoids, α – tocopherol, polyprenol and sterol. The Blur colorimetric method was used for cholesterol analysis; pH level in intestinal content on duodenum, jejunum, ileum, caecum and rectum (15 broilers in each part of guts) of broiler chickens of 42 days was analysed according to LV ISO 2917:2004 with Jenway3510 pH meter.

Table 1 Experimental design

Tablica 1. Plan istraživanja

Group - Grupa	Basic diet* - Osnovni obrok	Ratio of NESN – Udio NSEN
1st – control - kontrola	Basic diet - Osnovni obrok	-
2nd – trial – 2. pokus	Basic diet - Osnovni obrok	0.70% (0.05% in pure substance – u čistoj tvari)
3rd – trial – 3. pokus	Basic diet - Osnovni obrok	1.40% (0.10% in pure substance - u čistoj tvari)

*The composition complies with standard requirements (ROOS Broiler Management, 2009).

** NESN - Neutral extractes of spruce needles – Neutralni ekstrakt smrekovih iglica

From the data obtained on the fatty acid composition of broiler meat, the index of atherogenic (IA) (1) and index of thrombogenic (IT) (2) was calculated by using the following formulae (Manuela et al., 2011):

$$\text{Index of atherogenic IA} = \frac{(4\text{xC14:0}) + \text{C16:0} + \text{C18:0}}{\Sigma\text{MUFA} + \Sigma\text{PUFA n} - 6 + \Sigma\text{PUFA n} - 3}$$

$$\text{Index of thrombogenic IT} = \frac{\text{C14:0} + \text{C16:0} + \text{C18:0}}{0.5\text{xMUFA} + 0.5\text{xPUFA n} - 6 + 3\text{xPUFA n} - 3 + \frac{\text{PUFA n} - 3}{\text{PUFA n} - 6}}$$

The statistical analysis was performed using SPSS 17. The results of the experiment were analyzed using ANOVA, t-test. Statistical significance was fixed at $p<0.05$. The data were presented as mean and standard errors.

RESULTS AND DISCUSSION

The neutral extractives of spruce needles contain (in pure substance) 2607.64 mgkg^{-1} of total carotenoids, 110.15 mg 100g^{-1} α – tocopherol, 3.0 – 6.0% polyprenolsi 6.0 – 8.0% sterol, as well as the following amounts of fatty acids: 1.02% myristic acid, 4.02% palmitic acid, 2.19% stearic acid, 3.02% linoleic acid, 1.19% eicosapentaenoic (EPA) and 2.83% docosahexaenoic (DHA) fatty acids of total lipids as well as other biologically active substances. Thus, neutral extractives of spruce needles contain a significant amount of anti-oxidants, polyprenol and sterol ensuring functional composition of the poultry meat.

The impact of the above biologically active substances on the productivity and meat quality of broilers was evaluated under trial conditions. The effect of biologically active substances on broiler chicken productivity contained in neutral extract obtained from spruce needles is shown in Table 2. On the average, the live weight of broiler chickens at the age of 42 days ranged from 3120.33 to 3298.61 g, the live weight gain per day was from 73.25 to 77.51 g and feed conversion kept within 1.67 and 1.70 kg kg^{-1} (Table 2).

Feeding diets with added neutral extracts from biomass of spruce needles to 3rd group in doses 1.40% (0.10% in pure substance) had favourable

Table 2 Productivity of broiler chicken

Tablica 2. Proizvodnost brojlera

Parameters - Pokazatelji	1st group – control 1. grupa - kontrola	2nd group – trial 2. grupa - pokus	3rd group – trial 3. grupa - pokus
Live weight at the age of 42 days, g Tjelesna masa u dobi od 42. dana, g	3120.33±87.17	3181.05±101.39	3298.61±105.70
% to control - % od kontrole	100.00	101.94	105.71
Live weight gain per day, g – Dnevni priras po danu, g	73.25	74.72	77.51
% to control - % od kontrole	100.00	102.00	105.81
Feed conversion, kg kg^{-1} – Konverzija hrane, kg kg^{-1}	1.70	1.67	1.67
% to control - % od kontrole	100.00	98.23	98.23

Table 3 pH level in intestinal content

Tablica 3. Razina pH u sadržaju crijeva

Parameters - Pokazatelj	1st group – control 1. grupa - kontrola	2nd group – trial 2. grupa - pokus	3rd group – trial 3. grupa - pokus
Duodenum	5.80	6.00	5.97
Jejunum	5.82	5.75	5.74
Ileum	5.82	5.65	5.44
Caecum	5.73	5.71	5.76
Rectum	5.94	5.90	5.85

impact on productivity of broiler chickens in comparison with the control group.

Under the influence of natural biologically active substances contained in feeding ration incorporating 1.40% (0.10% in pure substance) of neutral extractives of spruce needles in loose powder form the live weight of broilers and consequently the daily live weight gain increased by 5.71 to 5.81%, in comparison with the control group ($p<0.05$).

It can be supposed that the addition of neutral extractives of pine needles to the broiler feed influenced the pH level of intestinal tract, activity of the digestion enzymes and the digestibility and absorption process levels.

Feeding diets containing neutral extractives of spruce needles to broilers (2nd and 3rd trial groups) the pH level in duodenum had a slight tendency to increase (by 0.17 to 0.20 units). It means that under the influence of supplements investigated the contents of duodenum turned slightly more alkaline in comparison with the control group ($p>0.05$). In comparison with the control group the pH level of the jejunum and ileum contents for 2nd and 3rd group of broilers was reduced by 0.07 to 0.38 units.

The reduced pH level in intestine contents indicates a more intensive digestibility and conversion of feed contained there in comparison with the control group. Consequently the additives containing neutral extractives of spruce needles promoted the digestive processes in intestinal tract. A 1.40% (0.10% in pure substance) dose of additive containing neutral extractives of spruce needles in the form of loose powder provided more intensive pH level reduction in ileum contents than application of a 0.70% (0.05% in pure substance). A 1.40% dose of neutral extractives in the form of lose powder increased the digestibility of feed materials relatively more efficiently and thus increased the live weight of broilers (Gauthier, 2002).

From the consumer's point of view, the impact of biologically active substances obtained from neutral extract of spruce needles on fatty acids and cholesterol content in muscle tissue, i.e., broiler chicken meat was essentially important. Broiler meat contained on the average 26.21 to 28.32% saturated (SFA), 30.39 to 32.56% monounsaturated (MUFA) and 36.41 to 39.03% polyunsaturated fatty acids (PUFA) of total lipids (Table 4).

Biologically active substances from neutral extract of spruce needles enhanced reduction of the saturated fatty acid content unfavourable for consumers by 1.15 – 2.11%, at the same time promoting the increase of monounsaturated fatty acids favourable to human system by 0.99 – 2.17% and the amount of polyunsaturated fatty acids : 1.28 – 2.62% in comparison with the control group ($p<0.05$).

Saturated fatty acids present in meat are involved in atherogenic and thrombogenic processes in human organism (Higgs, 2000). Of saturated fatty acids, just myristic acid has a hyper cholesterolemic effect, four times higher than palmitic acid, but stearic acid is considered neutral.

Myristic acid shows a tendency to increase the haematic cholesterol concentration (myristic acid is more atherogenic).

The neutral additives of extractives spruce needles favourably reduced the myristic acid content in muscle tissue by 1.13% (2nd group) and by 1.15% (3rd group), in comparison with the control group ($p<0.05$).

Table 4 Amount of fatty acids in broiler chicken meat

Tablica 4. Sadržaj masnih kiselina u mesu brojlera

Fatty acids, % of total lipids – Masne kiseline, % od ukupnih masti	1st group – control 1. grupa - kontrola	2nd group – trial 2. grupa - pokus	3rd group – trial 3. grupa - pokus
Σ Saturated (SFA)	28.32	27.17	26.21
Including - uključujući:			
Myristic acid (C14:0)	2.33	1.20	1.18
Palmitic acid (C16:0)	18.49	18.50	18.02
Stearic acid (C18:0)	7.50	7.47	7.01
Σ Monounsaturated (MUFA)	30.39	31.38	32.56
Σ Polyunsaturated (PUFA)	36.41	37.69	39.03
Including - uključujući:			
Σ PUFA (n – 3)	5.63	6.95	8.31
Σ PUFA (n – 6)	30.78	30.74	30.72
Σ PUFA(n – 6):Σ PUFA(n–3)	5.46:1	4.42:1	3.69:1
Σ PUFA(n – 3):Σ PUFA(n–6)	0.18	0.23	0.27
Eicosapentaenoic acid EPA(C20:5n-3)	0.42	0.57	0.58
Docosahexaenoic acid DHA (C22:6n-3)	1.22	1.81	2.27
IA (Atherogenic Health lipid indices)	0.53	0.44	0.42
IT (Thrombogenic Health lipid indices)	0.59	0.52	0.46

Table 5 Amount of cholesterol in broiler chicken liver, blood and meat

Tablica 5. Sadržaj kolesterola u jetri, krvi i mesu brojlera

Parameters - Pokazatelj	1st group – control 1. grupa - kontrola	2nd group – trial 2. grupa - pokus	3rd group – trial 3. grupa - pokus
Cholesterol in liver, mg100 g^{-1} - Kolesterol u jetri, mg100 g^{-1}	353.13	333.99	295.32
± to control - ± prema kontroli	-	-19.14	-57.81
Cholesterol in blood, mg dl^{-1} - Kolesterol u krvi, mg dl^{-1}	118.55	100.30	91.80
± to control - ± prema kontroli	-	-18.25	-26.75
Cholesterol in meat, mg100 g^{-1} – Kolesterol u mesu, mg100 g^{-1}	73.43	56.25	47.27
± to control - ± prema kontroli	-	-17.18	-26.16

Table 6 Amount of total carotenoids in broiler chicken liver, blood and meat

Tablica 6. Sadržaj ukupnih karotenoida u jetri, krvi i mesu brojlera

Parameters – Pokazatelj	1st group – control 1. grupa - kontrola	2nd group – trial 2. grupa - pokus	3rd group – trial 3. grupa - pokus
Carotenoids in liver, mg kg^{-1} – Karotenoidi u jetri, mg kg^{-1}	0.71	2.17	2.41
± to control - ± prema kontroli	-	+1.46	+1.70
Carotenoids in blood, mg kg^{-1} -Karotenoidi u krvi, mg kg^{-1}	0.105	0.138	0.149
± to control - ± prema kontroli	-	+0.033	+0.044
Carotenoids in meat, mg kg^{-1} Karotenoidi u mesu, mg kg^{-1}	0.03	0.09	0.12
± to control - ± prema kontroli	-	+0.06	+0.09

The palmitic acid and stearic acid levels in muscle tissue were not influenced by addition of the additives investigated to broiler feed. The level of n-3 polyunsaturated fatty acids had expressedly increased: by 1.32 – 2.68% in comparison with the control group ($p<0.05$). Alongside with that, the increase of the EPA and DHA of the n-3 fatty acids content in broiler meat by use of the above dose neutral extractive additives is of special importance. The level of EPA and DHA fatty acids in broiler meat established was 0.57 - 0.58% and 1.81 - 2.27% respectively. The previously mentioned n-3 fatty acids are of high importance for human health because they decrease cardiovascular disease risk factors (WHO, 2003). The relations of SFA, MUFA and PUFA amounts in food products including the meat of broilers under trial to a certain extent determine their impact on consumer health. Saturated fatty acids promote atherogenic and thrombogenic processes in the human system, while MUFA and PUFA preclude them. Atherogenic (IA) and thrombogenic (IT) indices are calculated to that end. IA and IT indices in broiler meat stayed within the limits of 0.42 - 0.53 and 0.46 - 0.59 respectively. The lower the value of the indices calculated, the higher the quality of fatty acids in food products which in their turn are more favourable to human health (Chiofalo et. al., 2011). The addition of spruce needles' neutral extracts to broiler feed promoted reduction of IA and IT indices by 0.07 - 0.13 respectively, consequently, meat was more wholesome for human health.

A 1.40% (0.10% in pure substance) dose of neutral extractives investigated ensured a composition of fatty acids in meat more favourable to human system in comparison with 0.70% (0.05% in pure substance) dose. The IA and IT indices calculated in meat obtained from the 3rd trial group were lower than those in meat obtained from the 2nd trial group.

The composition and the amount of fatty acids were interconnected with the cholesterol level in meat (Freese, 2000; Van Dyck, Adams, 2003.).

The cholesterol level in meat of broilers was 47.27 – 73.43 mg$100g^{-1}$, in blood: 91.80 - 118.55 mgdl^{-1} on the average and in liver 295.32 -353.13 mg100 g^{-1} (Table 5). Due to addition to broiler feed of loose powder of spruce needles neutral extracts, the cholesterol level in blood was reduced by 18.25 – 26.75 mg dl^{-1}, in meat by 17.18 – 26.16 mg$100g^{-1}$ and in liver by 19.14 –57.81 mg100 g^{-1} respectively in comparison with the control group. The data obtained indicate that the biologically active substances contained in neutral extractive of spruce needles, including also polyunsaturated fatty acids, phytosterols and carotenoids are involved in metabolic processes of the animal system and favourably reduce the cholesterol level in blood, meat and liver.

The biologically active substances investigated prevented the cholesterol synthesis in liver and its absorption in intestinal tract. (King, 2003; Grashorn, 2010). This resulted in reduction of the

cholesterol level in liver, blood and meat. Use of the higher dose of neutral extractives in broiler feed caused the reduction of cholesterol in liver by 57.81 mg100g^{-1}, in blood: by 26.75 mg dl^{-1} and in meat: by 26.16 mg100g^{-1} in comparison with the control group.

Total amount of carotenoids was in liver 0.71 – 2.41 mg kg^{-1}, in blood – 0.105 – 0.149 mg kg^{-1} and in meat of broilers– 0.03 – 0.12 mg kg^{-1} (Table 6). Neutral extractives of spruce needles contained an important amount of total carotenoids (2607.64 mg kg^{-1}).

The carotenoids contained in additives of neutral extractives transferred to organism of broilers, involved in metabolism and accumulated in liver and meat. This resulted in increase of the carotenoid content in the trial groups of broilers by 1.46 – 1.70 mg kg^{-1}, in liver – 0.033 – 0.044 mg kg^{-1}, in blood and 0.06 – 0.09 mg kg^{-1} in meat in comparison with the control group.

When was applied a higher dose i.e. 1.40% of neutral extractives in the form of loose powder, the meat and internal organs of broilers accumulated a higher amount of caroteneoids. In comparison with the control group, the carotenoids content in liver was higher by 1.70 mg kg^{-1}, in blood by 0.044 mg kg^{-1} and in meat 0.09 mg kg^{-1} ($p < 0.05$).

The additives of neutral extractives obtained from spruce needles can be used for enrichment of broiler meat and liver with natural carotenoids.

CONCLUSION

The loose powder feed additives containing neutral extracts of spruce needles in dose 0.70% and 1.40% (0.05% and 0.10% in pure substance) were used to supplement the broiler feed and meat with natural biologically active substances. Natural biologically active substances from neutral extracts of spruce needles are carried over from feed to organism of broilers increasing their productivity and meat quality.

0.70% and 1.40% (0.05% and 0.10% in pure substance) doses of loose powder feed additives containing neutral extracts of spruce needles to broiler feed caused the broiler live weight increase by 5.71%, enriched meat with n – 3 polyunsaturated fatty acids DHA contained in neutral extractives of spruce needles by 0.59 – 1.05% of total lipids, with carotenoids by 1.46 – 1.70 mg kg^{-1} in liver and by 0.06 – 0.09 mg kg^{-1}in meat while positively reducing cholesterol level in meat by 17.18 – 26.16 mg100g^{-1} in comparison with the control group.

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SAŽETAK

Neutralni ekstrakti smrekovih iglica proizvedeni su iz smrekovih popratnih proizvoda, zelene biomase smrekovih iglica. Ekstrakti smrekovih iglica sadrže značajnu količinu prirodnih, biološki aktivnih tvari. U tijeku naših istraživanja procijenjene su biološki aktivne tvari dobivene iz neutralnih ekstrakata smrekovih iglica radi ocjene njihovog djelovanja na kakvoću mesa pilića brojlera. Proveden je pokus hranidbe brojlera križanaca Ross-308 dodavanjem neutralnih ekstrakata smrekovih iglica u obliku rasutog praška u smjesu za njihove obroke. Pokazalo se da je rasuti prašak kao dodatak hrani brojlera koji je sadržavao neutralne ekstrakte smrekovih iglica (0,05% i 0,10% čiste tvari) poboljšao kakvoću mesa pilića. Dodaci hrani koji sadrže biološki aktivne tvari iz neutralnog ekstrakta smrekovih iglica uključeni u metaboličke procese brojlera povećali su sadržaj n-3 polinezasićenih masnih kiselina (DHA) u mesu za 0,59-1,05% ($P<0,05$) i sadržaj karotenoida u jetri za 0,46-1,70 $mgkg.^{-1}$($P<0,05$) te u isto vrijeme pozitivno smanjili razinu kolesterola u mesu za 17,18-26,16 $mg.100g^{-1}$($P<0,05$) u usporedbi s kontrolnom skupinom.

Ključne riječi: pilići brojleri, neutralni ekstrakti smrekovih iglica, kakvoća mesa