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Influence of linseed oil on selected parameters of blood and production performance of turkey hens

Wpływ oleju lnianego na wybrane wskaźniki krwi oraz efekty produkcyjne indyczek

Summary. The aim of this study was to compare the effectiveness of introducing soybean or linseed oil to feed mixtures for turkey hens based on the evaluation of their production effects and changes in their selected blood parameters. The experiment was carried out on 240 turkey hens of Big 6 line, aged from 1 up to 16 weeks. The birds were assigned to two groups, 120 turkeys each. Turkey hens from group I received a soybean oil addition to their feed mixtures, while the source of fat to birds from group II was linseed oil. In the 15th week of turkey hens rearing, their blood was sampled and hematological parameters (the number of WBC and RBC, Ht value and Hb concentration) as well as the levels of selected anti- and pro-oxidative parameters were determined. After the rearing, the birds were slaughtered, and their carcasses were subjected to slaughter analysis. The results obtained indicate that the application of linseed oil as the source of fat improved the rearing effects of turkey hens only to a slight extent, and effected beneficial changes in the parameters of antioxidative defense (increase of the total anti-oxidative potential, FRAP). In the turkey hens receiving linseed oil, a significant decrease in the concentration of MDA and H_2O_2 was recorded additionally, which indicates the inhibiting influence of this additive on the intensiveness of lipid peroxidation process.

Key words: turkey hens**,** soybean oil, linseed oil, blood, performance

INTRODUCTION

Proper feeding is one of the most important factors determining the apt course of metabolic processes in birds, and thereby their health status and rearing effects. Fats constitute the basic nutrient in poultry diet and fulfill a number of significant functions in the body. As it results from studies of many authors, the type of applied fat might influ-

ence immune responses [Nuernberg *et al*. 2005, Rey *et al*. 2004, Hassan *et al*. 2011], processes of erythropoiesis as well as antioxidative defense mechanisms [Friendship and Henry 1996; Hassan *et al.* 2011]. In poultry feeding, soybean oil is the most frequently applied source of fat, as it is reach in valuable unsaturated fatty acids of the n-6 family (especially linoleic acid). Nowadays, however, great hopes are also fostered in the feasibility of linseed oil application, for it is characterized by a high content of long-chain fatty acids of the n-3 family (especially eicosapentaenoic acid – EPA and docosahexaenoic acid – DHA) and a very beneficial n-3 to n-6 acids ratio (2.5:1) [Weill *et al*. 2002]. These acids are implicated to exhibit the anti-inflammatory effect as they inhibit the synthesis of pro-inflammatory cytokines [Jeffery *et al*. 1996, Świątkiewicz and Koreleski 2007]. Linseed oil contains also lignans that belong to a group of the so-called phytoestrogenes. They are deemed to be responsible for the regulation of lipid metabolism and for capturing free radicals which initiate processes of lipids peroxidation and thus are found detrimental to the organism [Kouba and Mourot 2011; Wood *et al*. 2003]. The antioxidative activity of phytoestrogenes was revealed to be even significantly higher than that of tocopherols [*Cos et al.* 2003].

Taking into account the health-promoting, i.e. immunomodulatory, anti-inflammatory and antioxidative, effects of linseed oil components, it would be interesting to analyze whether its application as the source of fat in feed mixtures for turkey hens improves rearing parameters and stimulates immune response and mechanisms of antioxidative defense. The objective of this study was, therefore, to compare the effectiveness of introducing soybean or linseed oil to feed mixtures for turkey hens based on the evaluation of their performance and changes in their selected blood parameters.

MATERIAL AND METHODS

The experiment was carried out on 240 turkey hens of Big 6 line, aged from 1 up to 16 weeks. The birds were randomly assigned to 2 experimental groups, 120 turkeys each, allocated for 5 replications, 24 birds each. The birds were kept in cages 2.5 × 4m in size, under zoohygienic conditions recommended for turkeys fattening. The turkey hens from all groups were fed full-dose mixtures *ad libitum* and had free access to water. Feed mixtures were produced once a week on the farm and stored in a cool and dark place in order to protect them from oxidative processes. All the mixtures contained wheat, maize meal, soybean meal, soybean/linseed oil, and were composed maintaining the isoprotein and isoenergy balance. Feed mixtures formula was provided in the work by Czech *et al.* [2012]. Birds from both groups received standard feed mixtures, in which the content of nutrients was consistent with NCR [1994] and the source of fat was soybean oil (group I) or linseed oil (group II).

At the end of the experimental period, blood was sampled for analyses from the brachial vein of 10 birds from each group. Blood was sampled to heparinized test tubes 10 mL in volume under the supervision of a veterinarian. Monotests developed by Cormay company were used to determine spectrophotometrically samples of blood plasma for contents of selected biochemical markers: uric acid (UA), bilirubin (BIL), and creatinine (CREAT). Spectrophotometric assays were also applied to analyze blood plasma samples for the activity of antioxidative enzymes: superoxide dismutase (SOD) with the

adrenaline method modified regarding the wavelength of 320 nm, the method was modified to increase the selectivity of transient reaction products at this length of light [Bartosz 2004] and catalase (CAT) - according to Bartosz [2004]. As for the antioxidant status parameters, assays were also made for the ferric reducing ability of plasma (FRAP), vitamin C and glutathione (GSH + GSSG) according to Bartosz [2004]. In addition, the biological material was analyzed for levels of lipid peroxidation products: peroxides (H_2O_2) – according to Gay and Gębicki [2000], and malondialdehyde (MDA) as the end product of tissue lipids oxidation – according to Salih *et al*. [1987]. Hematological tests included the determination of white blood cells (WBC) and red blood cells (RBC) number with the manual chamber technique, after dilution in Natt - Herrick solution, hematocrit (Ht) level using the microhematocrit method, and hemoglobin (Hb) content – following the Drabkin's method [Pinkiewicz *et al*. 1971, Feldman *et al.* 2000].

On completion of the rearing period (16 weeks of age), ten birds from both experimental group were slaughtered following the LEC euthanasia protocol (all the birds from a group/ subgroup were weighed and then chosen for slaughter analysis on the grounds of the mean values of body weight measurement). During the partial dissection, there were sampled breast and leg muscles as well as edible giblets (liver, heart, stomach). The slaughter procedure was approved by the II Local Ethical Commission for Experiments with Animals in Lublin (approval no. 9/2009).

During the experiment, the turkey hens' body weight (taken on each last day of the week of their lives), survival rate and feed intake were recorded. On the basis of the productive performance, the value of the WEO index was calculated following the formula given below:

$$WEO = \frac{\text{mean body weight after rearing (kg)} \times \text{liveability (\%)} \times 100}{\text{day of rearing} \times \text{feed conversion (kg kg}^{-1})}$$

Digital data achieved were subjected to a statistical analysis, by determining mean values and standard errors of the means using Statistica ver.6.1 software, according to the model:

$$Y_i = \mu + a_i + e_i$$

where: μ = overall mean
a_i – influence of the oil additive, $i = 1$,
e_i – random error.

The significance of differences between means was determined with the one-way analysis of variance test ANOVA, at significance levels of 0.05 and 0.01.

RESULTS AND DISCUSSION

At the initial fattening period, rearing effects of the turkey hens receiving linseed oil addition in their feed mixture were not significantly different from those obtained for the control birds (Table 1). Starting from the 13th week of rearing, however, the body

weight of the turkey hens receiving linseed oil was significantly ($P \leq 0.05$) higher than in the group with soybean oil addition. Generally, throughout the whole experimental period, these birds were achieving slightly greater body weight gains than the birds receiving soybean oil in their feed. Feed conversion per one kilogram of body weight gain in the group with linseed oil addition was, however, slightly ($P > 0.05$) lower than in the control group (ca. 3%). These positive tendencies resulted in a higher (by 47 points) value of the European productivity index – WEO. More beneficial productivity effects may be due to the better availability of this fat to birds, which was indicated in a study by Blanch *et al.* [1996]. In turn, results of the experiment by Mossab *et al.* [2002] carried out on turkeys only until the 7th week of their life, indicated an insignificant influence of linseed oil application on birds' productivity. A research by Nam *et al*. [1997] showed that the application of linseed in broiler chickens feeding improved their production performance.

Results of slaughter analysis presented in Table 2 show that the type of fat applied had no significant effect on the analyzed slaughter traits of turkey hens. As it results from the study by Crespo *et al.* [2002] carried out on broiler chickens, the application of linseed oil caused an increased carcass fatness. A similar dependency ($P \leq 0.05$) was observed also in the reported research (Table 2).

Table 1. The productivity of turkey hens
Tabela 1. Efekty produkcyjne indyczek

Item – Wskaźnik	Feeding groups/Grupy żywieniowe						P value
	SO			LO			
Body weight/week of life – Masa ciała/tydzień życia							
1	1.49	±	0.38	1.58	±	0.16	NS
7	2.47	±	0.33	2.61	±	0.57	NS
8	3.23	±	0.24	3.27	±	0.24	NS
9	3.33	±	0.34	4.04	±	0.81	NS
10	4.42	±	0.37	4.93	±	0.83	NS
11	5.22	±	0.50	5.90	±	0.94	NS
12	6.38	±	0.59	6.98	±	0.77	NS
13	6.95 [b]	±	0.56	7.78 [a]	±	0.40	*
14	7.81 [b]	±	0.61	8.54 [a]	±	0.57	*
15	8.57 [b]	±	0.65	9.30 [a]	±	0.48	*
16	9.01 [b]	±	0.73	9.95 [a]	±	0.56	*
Body weight gains – Przyrosty masy ciała, kg							
1–7 wk	0.98			1.03			
7–12 wk	3.72			4.37			
12–16 wk	2.91			2.87			
Feed intake, kg/bird/week Spożycie paszy, kg/szt./tydzień	1.76			1.79			
Feed conversion ratio, kg/ kg 0–16 Zużycie paszy, kg/kg 0–16	2.47			2.39			
WEO pts	330[b]			367[a]			

*$P \leq 0.05$; NS: non significant, SO – soybean oil, LO – linseed oil.
*$P \leq 0{,}05$; NS: nieistotne, SO – olej sojowy, LO – olej lniany.

Table 2. Results of slaughter analysis of turkey hens after 16-week rearing period (% body weight prior to slaughter)
Tabela 2 Wyniki analizy rzeźnej indyczek po 16 tygodniach odchowu (% masy ciała przed ubojem)

Item/Wskaźnik	Feeding groups/Grupy żywieniowe						P value
	SO			LO			
Dressing percentage/ Wydajność rzeźna, %	83.2	±	1.29	83.7	±	1.35	NS
Breast muscle/Mięsień piersiowy, %	21.8	±	0.92	22.8	±	0.68	NS
Femoral muscle/Mięsień udowy, %	8.60	±	0.36	8.81	±	0.44	NS
Shank muscle/Mięsień podudzia, %	6.69	±	0.27	7.04	±	0.31	NS
Liver/Wątroba, %	1.38	±	0.60	1.45	±	0.82	NS
Stomach/Żołądek, %	1.26	±	0.08	1.25	±	0.08	NS
Heart/Serce, %	0.30	±	0.05	0.32	±	0.02	NS
Abdominal fat/Tłuszcz sadełkowy, %	0.47^{b}	±	0.05	0.58^{a}	±	0.06	*

*P ≤ 0.05; NS – non significant, SO – soybean oil, LO – linseed oil.
*P ≤ 0,05; NS – nieistotne, SO – olej sojowy, LO – olej lniany.

Table 3. Hematological parameters in blood of turkey hens
Tabela 3. Parametry hematologiczne krwi indyczek

Item/Wskaźnik	Feeding groups/Grupy żywieniowe						P value
	SO			LO			
White blood cells/Krwinki białe, $10^9\ l^{-1}$	24.34^{b}	±	2.01	26.85^{a}	±	2.06	*
Hemoglobin/Hemoglobina, g l^{-1}	8.98	±	0.32	8.91	±	0.23	NS
Red blood cells/Krwinki czerwone, $10^{12}\ l^{-1}$	2.24	±	0.13	2.38	±	0.12	NS
Hematocrit/Hematokryt, $l\ l^{-1}$	0.27	±	0.09	0.30	±	0.02	NS

*P ≤ 0.05; NS: non significant, SO – soybean oil, LO – linseed oil.
*P ≤ 0.05; NS: nieistotne, SO – olej sojowy, LO – olej lniany.

The analysis of results of hematological tests (Table 3) demonstrated that the application of linseed oil had no effect on hematocrit value and of hemoglobin and red blood cells contents. However, it resulted in a significant ($P \leq 0.05$) increase of the total number of leukocytes (WBC) in the blood of turkey hens from LO group. As the birds were in good condition, this was not so much a sign of a disease but a sign which might have been related to the immunomodulatory properties of the active components of linseed oil, namely, a high content of omega-3 fatty acids, which influence various immune mechanisms, inter alia, modulate the process of phagocytosis, the activity of NK cells and the production of cytokines [Calder *et al*. 2011, Kelley 1988].

As it results from Table 4, the significant differences were recorded in changes of the values of pro-oxidative and anti-oxidative parameters in turkey hens' blood depending on the applied source of fat. The positive effect of linseed oil application is recorded decrease ($p \leq 0.05$) in the content of lipid peroxidation products, i.e. hydrogen peroxide –

Table 4. Pro-oxidative and antioxidative parameters in blood of turkey hens
Tabela 4. Parametry prooksydacyjne i antyoksydacyjne krwi indyczek

Item/Wskaźnik	Feeding groups/Grupy żywieniowe						P value
	SO			LO			
Uric acid/Kwas moczowy, μmol l^{-1}	120.4	±	14.92	139.8	±	19.7	NS
Creatinine/Kreatynina, μmol l^{-1}	19.86	±	2.28	16.27	±	3.28	NS
Bilirubin/Bilirubina, μmol l^{-1}	8.93	±	0.71	8.32	±	9.45	NS
Urea/Mocznik, mmol l^{-1}	0.98	±	0.27	0.86	±	0.12	NS
Superoxide dismutase Dysmutaza ponadlenkowa, U ml^{-1}	28.52	±	0.76	29.4	±	0.73	NS
Catalase/Katalaza, U ml^{-1}	4.99	±	1.61	5.21	±	1.46	NS
Vitamin C/Witamina C, mg l^{-1}	0.36	±	0.09	0.38	±	0.09	NS
Glutatione/Glutation, μmol l^{-1}	52.22	±	8.82	59.8	±	8.46	NS
FRAP, μmol l^{-1}	0.181^{b}	±	0.04	0.191^{a}	±	0.03	*
Peroxides/Nadtlenek wodoru, μmol l^{-1}	2.53^{a}	±	0.51	1.86^{b}	±	0.54	*
Malondialdehyde/Aldehyd dimalonowy, μmol l^{-1}	0.824^{a}	±	0.12	0.650^{b}	±	0,13	*

*P ≤ 0.05; NS – non significant, SO – soybean oil, LO – linseed oil.
*P ≤ 0.05; NS – nieistotne, SO – olej sojowy, LO – olej lniany.
FRAP – the ferric reducing ability / całkowity potencjał antyoksydacyjny.

H_2O_2 (by 26.5%) and malondialdehyde – MDA (by 21.1%). These observations point to the antioxidative properties of linseed oil, that result probably from the presence of lignans. These properties were confirmed in the experiments by Bhatia *et al.* [2006] and Hosseinian *et al.* [2006]. Worthy of notice are also certain beneficial tendencies which were recorded in the group with linseed oil addition, although, they were not confirmed in statistical calculations. These include a slight increase in the level of uric acid (by 16.1%) and glutathione (by 14.6%) in turkey hens' blood. Both substances belong to the group of endogenous low-molecular antioxidants (uric acid is the main component of the total antioxidative potential of blood plasma (FRAP), which in the LO group was significantly ($p \leq 0.05$) higher in comparison to SO group. In turn, besides fulfilling the antioxidative function, glutathione also plays a significant role in detoxification [Bartosz 2004]. Therefore, an increased concentration of glutathione and uric acid in blood plasma should be deemed very desirable.

CONCLUSIONS

Due to a slight improvement in the rearing effects and a positive effect of linseed oil on changes in blood parameters, especially these of antioxidative defense, which was represented by a significant increase in the value of the total antioxidative potential and a decrease in the amount of lipid peroxidation products (hydrogen peroxide and malondialdehyde), the application of linseed oil as the source of fat in the feeding of turkey hens can be considered advisable.

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Streszczenie. Celem badań było porównanie efektywności wprowadzenia do paszy dla indyczek oleju sojowego lub lnianego na podstawie oceny efektów produkcyjnych oraz wybranych wskaźników krwi. Doświadczenie przeprowadzono na 240 indyczkach typu Big 6 (od 1 do 16 tygodnia życia). Ptaki podzielono na dwie grupy po 120 sztuk w każdej. Indyczki należące do grupy I otrzymywały olej sojowy do mieszanki paszowej, zaś źródłem tłuszczu dla ptaków z grupy II był olej lniany. W 15 tygodniu odchowu indyczek pobrano krew, w której oznaczono wskaźniki hematologiczne (liczba WBC i RBC, wartość Ht oraz stężenie Hb), a także poziom wybranych parametrów anty- i prooksydacyjnych. Po zakończonym odchowie ptaki ubito, a tuszki poddano analizie rzeźnej. Uzyskane wyniki wskazują, że zastosowanie oleju lnianego jako źródła tłuszczu nieznacznie poprawiło efekty odchowu indyczek oraz wpłynęło korzystnie na kształtowanie się parametrów obrony antyoksydacyjnej (wzrost całkowitego potencjału antyoksydacyjnego FRAP). U indyczek otrzymujących olej lniany zanotowano także istotne obniżenie koncentracji MDA oraz H_2O_2, co wskazuje na hamujący wpływ tego dodatku na intensywność procesu peroksydacji lipidów.

Słowa kluczowe: indyczki, olej sojowy, olej lniany, krew, efekty odchowu