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Influence of linseed oil on content of the fatty acids in feed mixtures and muscles of turkey hens and blood profile lipids

Wpływ oleju lnianego na zawartość kwasów tłuszczowych
w paszy i mięśniach indyczek oraz profil lipidowy krwi

Summary. The objective of the study was to evaluate the use of linseed oil to feed turkey hens and its impact on the change in the fatty acids profile of feed and, consequently, in the muscle (growth of fatty acids of n-3), as well as on the improvement of the indices of the blood lipid profile (decrease of total cholesterol and triglycerides and increase in HDL-cholesterol fraction). 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 2 groups, 120 turkeys each. Turkey hens from group I received a soybean oil addition to a feed mixture. In group II, the source of fat was linseed oil. In the 15th week of rearing the birds, their blood was sampled for the analysis of lipid markers, whereas in the 16th week the birds were slaughtered and samples of their breast and thigh muscles were collected for analyses of fat and cholesterol contents and fatty acids profile. The obtained results indicate that in the turkey hens receiving the linseed oil addition the content of fat and cholesterol decreased in both breast and high muscles, compared to the birds fed the feed mixtures containing soybean oil. An increase in the contribution of n-3 family fatty acids, especially of α-linolenic acid (the source of which was linseed oil), in the feed mixtures for turkey hens also caused a significant increase in the content of this acid in breast and thigh muscles. The addition of linseed oil was also observed to contribute to a reduced triacylglycerols content in the blood plasma of the birds.

Key words: turkey hens, linseed oil, fatty acids, muscles, blood

INTRODUCTION

Contemporary methods of livestock feeding enable modifying not only the fat-meat ratio but also contents and ratios of essential unsaturated fatty acids (EFAs). One of the

natural means to improve health-promoting properties of poultry meat is to feed birds with feed mixtures with a more desirable fatty acid profile, which is reflected in diversified fatty acid composition of fat in animal tissues (an increase in the content of n-3 fatty acids). Consumption of meat products with a higher content of n-3 fatty acids may reduce the level of triglycerides in the blood plasma, slightly increase low and high density lipoprotein cholesterol, and has a significant effect on serum cholesterol [Stillwell *et al*. 1993, Harris 1997].

The increase in the content of n-3 in muscle disease may be achieved by the addition of linseed oil [Wood *et al.* 2003]. It is characterized by a higher content of EFAs than of saturated fatty acids, which is of great significance from the point of view of both: health status of birds and dietary values of their meat. In addition, linseed oil is a source of compounds with antioxidative properties, e.g. phytoestrogens. The latter are responsible for the regulation of lipid metabolism and are capable of quenching free radicals that are detrimental to the body as they initiate lipids peroxidation processes [Kouba and Mourot, 2011, Wood *et al*. 2003]. A comparison of the effectiveness of linseed and soybean oils aims to determining which of them affects improvement of meat quality.

In view of the above mentioned valuable properties of linseed oil the objective of this study was to evaluate the use of linseed oil to feed turkey hens and the change impact fatty acids profile of feed and, consequently, in the muscle (growth of fatty acids of n-3), as well as to improve the indices of blood lipid profile (decrease of total cholesterol and triglycerides and increase in HDL-cholesterol fraction).

MATERIALS AND METHODS

Animals and diets

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 located for 5 replications, 24 birds each. The birds were kept in cages 2.5 × 4 m in size, under zoohygienic conditions recommended for turkeys fattening Regulation of the Minister of Agriculture and Rural Development dated 15 February 2010 [Rozporządzenie… 2010].

The turkey hens from all the groups were fed full-dose mixtures *ad libitum* and had free access to water. The feed mixtures were prepared once a week at the farm and stored in a cool and dry place to prevent oxidation processes. All the mixtures contained wheat, maize meal, soybean meal, soybean/linseed oil, and were composed maintaining the isoprotein and isoenergy balance (Table 1). Birds from both groups received standard feed mixtures, in which the content of nutrients was consistent with NRC [1994] and the source of fat was soybean oil (group I-SO) or linseed oil (group II-LO).

The samples of the feed mixtures for laboratory analyses were collected twice at each feeding period. Feed mixtures were assayed for contents of dry matter, crude protein and crude fat according to AOAC methods [2000]. At the end of week 15 of turkey hens life, blood samples were collected for analyses. Blood was sampled to heparinized test tubes 10 ml in volume under the supervision of a veterinarian. On completion of the rearing period (16 weeks of age), ten birds from each experimental group were slaughtered following the II Local Ethical Commission for Experiments with Animals in Lublin 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).

Table 1. Composition of full mixtures for turkey hens (%)
Tabela 1. Skład recepturowy mieszanek pełnoporcjowych dla indyczek (%)

Specification Wyszczególnienie	Starter	Grower I	Grower II	Grower III	Finisher I
Feeding period week of age Okres żywieniowy	1–2	3–5	6–9	10–12	13–16
Corn/Kukurydza	25.6	27.4	23.8	35.2	47.4
Wheat/Pszenica	20.0	25.0	30.0	25.0	25.0
Wheat bran Otręby pszenne	3.0	–	–	–	–
Soybean meal 46% Protein/Śruta sojowa poekstrakcyjna 46%	43.0	41.7	38.8	32.7	20.4
Fish meal/Mączka rybna	3.5	–	–	–	–
Limestone Kreda pastewna	1.2	1.7	1.7	1.4	1.5
Soybean oil or linseed oil Olej sojowy lub olej lniany	0.5	1.0	2.5	3.0	3.0
Cytromix Plus[1]	0.2	0.2	0.2	0.2	0.2
Farmix[2]	3.0	3.0	3.0	2.5	2.5

Cytromix Plus[1] – citric acid, fumaric acid, phosphoric acid (62%).
Farmix[2] – mineral and vitamin premix provided the following per kilogram of diet – 3 000 000 IU of vitamin A; 900 000 IU of vitamin D_3; 10 000 mg of vitamin E; 500 mg of vitamin K_3; 700 mg of vitamin B_1; 2000 mg of riboflavin; 1200 mg of vitamin B_6; 6 mg of vitamin B_{12}; 400 mg of folic acid; 72 mg of biotin; 15 000 mg of niacin; 120 000 mg of choline; 4 200 mg of calcium pantothenicum; 30 000 mg of Mn; 18 000 mg of Zn; 12 000 mg of Fe; 3 000 mg of Cu; 200 mg of I; 60 mg of Se; 40 mg of Co; 15 g of Ca.

Cytromix Plus[1] – kwas cytrynowy, kwas fumarowy, kwas fosforowy (62%).
Farmix[2] – elementy mineralne i witaminy w 1 kg diety – 3 000 000 IU witamina A; 900 000 IU witamina D_3; 10 000 mg witamina E; 500 mg witamina K_3; 700 mg witamina B_1; 2 000 mg ryboflawina; 1200 mg witamina B_6; 6 mg witamina B_{12}; 400 mg kwas foliowy; 72 mg biotyna;15 000 mg niacyna; 120000 mg cholina; 4 200 mg pantotenian wapnia; 30 000 mg Mn; 18 000 mg Zn; 12 000 mg Fe; 3 000 mg Cu; 200 mg I; 60 mg Se; 40 mg Co; 15 g Ca.

Chemical analyses

The content of total cholesterol (CHOL), HDL cholesterol fractions, triacylglycerols (TAG) in blood plasma was assayed with the colorimetric method using monotests by Cormay company. Marks were made on spectrophotometer UNICAM 939.

The low density lipoprotein (LDL-chol) was calculated using Friedewald *et al.* [1972] formula:

$$\text{LDL-chol (mmol l}^{-1}) = \text{total cholesterol} - \text{HDL-chol} - \text{triacylglycerols}/2.2$$

During the dissection turkeys 400-g samples of breast and thigh muscles were collected from the right half-carcasses 6 birds per group. Turkey muscle samples cooled at 2–4°C, during cooling species of 2 h.

Lipids of the examined turkey hens muscles and mixtures were extracted with a chloroform-methanol mixture (02:01), according to Folch *et al*. [1957] method. Muscle samples were freeze-dried and then ground and homogenized using a home-style coffee grinder.

Muscle and mixtures samples were also determined for fatty acid profile with the gas chromatography method. Chromatographic separation was carried out on a Varian CP3800 gas chromatograph. Fatty acids were determined by GLC using a 100-m capillary column (SP 2560, Supelco, Bellefonte, PA) with a split ratio of 100:1 and He as a carrier gas for a column flow rate of 1.0 ml/metal. Injector and detector temperatures were 250°C and column temperature was ramped from 140° to 240°C at 3.5°C/metal.

In homogenates from breast muscles and leg muscles, total cholesterol (CHOL) was assayed with the method by Rhee *et al.* [1982].

Statistical analyses

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, with Duncan's multiple confidence interval, at significance levels of 0.05 and 0.01.

RESULTS AND DISCUSSION

In analyzing the fatty acid profile in feed mixtures administered to the birds (Table 2), it was noticed that in all feed mixtures (irrespective of the fattening period) with linseed oil used as a source of fat, the content of α-linolenic acid (ALA, C18:3n-3) was significantly higher when compared to the feed mixtures with soybean oil. Worthy of attention is also the n-6 to n-3 fatty acids ratio which in the feed mixtures with soybean oil ranged from 3.68 to 4.98, whereas in the feed mixtures with linseed oil was lower and ranged from 1.29 to 2.88. The differences in the fatty acid profile of experimental diets resulted from the composition of vegetable oils, including a relatively high content of palmitic and oleic acids, an even higher content of linoleic acid and a low content of linolenic acid in soybean oil [Kavouridou *et al*. 2008]. Linseed oil, compared with soybean oil, contains substantially less linoleic acid and significantly more linolenic acid [Crespo and Esteve-Garcia 2002]. The addition of linseed oil influenced also the fatty acid profile of breast and thigh muscles of the turkey hens.

Poultry meat is a rich source of polyunsaturated fatty acids (PUFAs), owing to which its nutritive value is higher when compared to pork or beef [Wood *et al*. 2003]. A significantly higher (almost 4-fold) content of PUFAs in muscles of poultry compared to beef cattle was demonstrated in a study by Rule *et. al*. [2002]. In addition, lipids contained in

Table 2. Mixture chemical composition and fatty acids profile of mixtures fat for turkey hens
Tabela 2. Skład chemiczny mieszanek i profil kwasów tłuszczowych w tłuszczu paszy dla indyczek

Wskaźnik Item	SO					LO				
	1–2	3–5	6-9	10–12	13–16	1–2	3–5	6–9	10–12	13–16
Dry matter g kg^{-1}/Sucha masa, g kg^{-1}	889.2	889.5	888.4	891.2	899.6	888.7	889.6	886.5	890.9	889.0
Crude fat, g kg^{-1} f.m. Tłuszcz surowy, g kg^{-1} m.n.	55.19	52.47	50.61	51.82	32.44	56.47	55.24	53.68	54.79	40.11
Obliczenia w 1 kg mieszanki/Calculated in 1 kg of mixture:										
EM/ME; kcal kg^{-1}	2719	2799	2859	2989	3111	2709	2780	2900	2981	3001
Fatty acids/Kwasy tłuszczowe, %										
14;0	1.15	2.15	0.1	0.15	1.22	2.17	1.55	0.4	0.41	0.74
16;0	12.22	17.24	4.64	6.13	10.38	15.44	14.1	8.71	8.9	9.97
16;1n-9	1.49	2.75	0.02	0.03	0.02	2.42	1.8	0.03	0.04	0.04
18;0	3.13	3.07	3.77	4.27	7.4	2.9	3.38	3.81	3.97	4.69
18;1	24.08	19.19	31.43	30.02	44.04	20.89	29.66	27.53	27.33	30.8
18;2 n-6	39.63	37.74	49.23	45.27	28.13	33.89	31.29	34.36	33.31	28.95
18;3 n-3	11.33	9.25	9.88	12.24	7.19	13.85	11.76	23.06	23.77	22.52
20;0	0.28	0.24	0.32	0.47	0.32	0.23	0.32	0.26	0.29	0.24
20;1	1.87	1.65	0.4	0.66	0.73	1.81	1.7	0.81	0.81	0.86
20;4 n-6	0.11	0.14	0	0	0.01	0.14	0.09	0.01	0.01	0.01
20;5	1.81	2.45	0.01	0	0.03	2.38	1.65	0.02	0.02	0.03
22;6	2.61	3.97	0	0	0	3.67	2.48	0.08	0.08	0.08
Other/Inne	0.29	0.16	0.2	0.76	0.53	0.21	0.22	0.92	1.06	1.07
∑	100	100	100	100	100	100	100	100	100	100
SFA	16.78	22.7	8.83	11.02	19.32	20.74	19.35	13.18	13.57	15.64
UFA	82.93	77.14	90.97	88.22	80.15	79.05	80.43	85.9	85.37	83.29
MUFA	27.44	23.59	31.85	30.71	44.79	25.12	33.16	28.37	28.18	31.7
PUFA	55.49	53.55	59.12	57.51	35.36	53.93	47.27	57.53	57.19	51.59
n-3	11.33	9.25	9.88	12.24	7.19	13.85	11.76	23.06	23.77	22.52
n-6	39.74	37.88	49.23	45.27	28.14	34.03	31.38	34.37	33.32	28.96
n-6/n-3	3.51	4.10	4.98	3.70	3.91	2.46	2.67	1.49	1.40	1.29

ME/EM – metabolizable energy/energia metaboliczna.
f.m./m.n.– fresh mass/naturalna masa.
SFA – saturated fatty acids/nasycone kwasy tłuszczowe.
UFA – unsaturated fatty acids/nienasycone kwasy tłuszczowe.
MUFA – monounsaturated fatty acids/jednonienasycone kwasy tłuszczowe.
PUFA – polyunsaturated fatty acids/wielonienasycone kwasy tłuszczowe.

Table 3. Contents of nutrients in muscles and fatty acids profile in muscles fat of turkey hens
Tabela 3. Zawartość składników pokarmowych w mięśniach i profil kwasów tłuszczowych w tłuszczu mięśni indyczek

Components Składniki	Breast muscle Mięsień piersiowy		P value	Mięsień udowy Thigh muscle		P value
	SO	LO		SO	LO	
Dry matter, g kg^{-1} Sucha masa, g kg^{-1}	262.2	264.1	NS	266.7	270.9	NS
Crude fat, g kg^{-1} f.m. Tłuszcz surowy g kg^{-1} m.n.	17.8	12.1	*	79.4	66.8	*
Cholesterol, mg kg^{-1} f.m. Cholesterol, mg kg^{-1} m.n.	645.0	605.0	*	1210	1090	*
Fatty AIDS Kwasy tłuszczowe(%)						
12;0	0.15	0.16	NS	0.14	0.14	NS
14;0	1.23	1.13	NS	1.24	1.18	NS
14;1	1.52	1.62	NS	0.46	0.44	NS
16;0	27.08	26.51	NS	27.81	27.86	NS
16;3 n-3	0.28	0.37	NS	0.1	0.15	NS
18;0	9.62	9.02	NS	7.59	7.47	NS
18;1	28.55	29.81	*	32.61	32.79	NS
18;2 n-6	25.89	23.76	*	26.54	24.79	*
18;3 n-3	1.91	4.07	**	2.23	3.88	*
20;0	0.14	0.14	NS	0.14	0.13	NS
20;1	0.28	0.3	NS	0.32	0.44	NS
20;2	0.34	0.31	NS	0.19	0.17	NS
20;3 n-9	0.3	0.28	NS	0.12	0.11	NS
20;4 n-6	2.28	2.2	NS	0.11	0.12	NS
20;5	0.19	0.2	NS	0.14	0.13	NS
22;5	0.17	0.1	NS	0.05	0	NS
Other/Inne	0.07	0.06	NS	0.23	0.24	NS
∑	100	100		100	100	
SFA	38.22	36.96		36.92	36.78	
UFA	61.71	63.02		62.87	63.02	
MUFA	30.35	31.73		33.39	33.67	
PUFA	31.36	31.29		29.48	29.35	
n-3	2.19	4.44		2.33	4.03	
n-6	28.17	25.96		26.65	24.91	
n-6/n-3	12.86	5.85		11.44	6.18	

*P ≤ 0.05; **P ≤ 0.01; NS: non significant.
*P ≤ 0.05; **P ≤ 0.01; NS: nieistotne.
f.m./m.n.– fresh mass/naturalna masa.
SFA – saturated fatty acids/nasycone kwasy tłuszczowe.
UFA – unsaturated fatty acids/nienasycone kwasy tłuszczowe.
MUFA – monounsaturated fatty acids/jednonienasycone kwasy tłuszczowe.
PUFA – polyunsaturated fatty acids/wielonienasycone kwasy tłuszczowe.

Table 4. Lipid parameters in blood plasma of turkey hens
Tabela 4. Wskaźniki lipidowe w osoczu krwi indyczek

Item/Wskaźnik	Feeding groups/Grupy żywieniowe		P value
	SO	LO	
CHOL; mmol l^{-1}	2.26 ± 0.27	2.19 ± 0.26	NS
HDL; mmol l^{-1}	1.27 ± 0.17	1.33 ± 0.20	NS
LDL; mmol l^{-1}	0.72 ± 0.19	0.68 ± 0.23	NS
TAG; mmol l^{-1}	0.60 ± 0.11	0.40 ± 0.11	*
% HDL	57.46 ± 6.18	61.12 ± 9.14	NS
CHOL/HDL	1.80 ± 0.21	1.70 ± 0.24	NS

*P ≤ 0.05; **P ≤ 0.01; NS – non significant.
*P ≤ 0,05; **P ≤ 0,0,1; NS – nieistotne.
CHOL – total cholesterol/cholesterol całkowity.
HDL – cholesterol fraction of high-density lipoprotein/frakcja cholesterolu o wysokiej gęstości lipoprotein.
LDL – cholesterol fraction of low-density lipoprotein/frakcja cholesterolu o niskiej gęstości lipoprotein.
TAG – triacylglycerols/triacyloglicerole.

poultry meat are more easily assimilated than lipids originating from other animal species [Kouba and Mourot 2011]. In view of that, great emphasis is being put on the modification of fatty acid profile with simultaneous reduction of fat content in carcass caused by different feeding of birds [Kouba *et al*. 2003, Wood *et al*. 2003]. Many studies show that the addition of various sources of fat to feed mixtures for poultry may significantly modify the fatty acid profile in their tissues and regulate fat content [Crespo and Esteve-García 2002]. This was confirmed in the present study, where a significantly lower fat content was noted in muscles (both breast and thigh ones) of turkey hens receiving linseed oil addition in their feed mixture (Table 3). According to many researchers [Crespo and Esteve-García 2002, Newman *et al*. 2002], reduced fat deposition in muscles of poultry is affected by the presence of PUFAs, including most of all fatty acids of the n-3 family, a rich source of which is – undoubtedly – linseed oil.

In birds of the group LO analyses demonstrated a significantly higher ($p \leq 0.05$) content of oleic acid (18:1) in breast muscles and of linolenic acid (18:3, n-3) in thigh and breast muscles, as well as a lower content of linoleic acid (18:2, n-6), compared to the birds receiving soybean oil addition with feed mixtures (Table 3). The modification of fatty acid profile in muscles of turkey hens was also reflected in the n-6/n-3 fatty acids ratio which in the muscles of birds from the LO group was almost twofold lower than in the SO group. In other experiments involving chickens [Febel *et al.* 2008] and turkeys [Delezie *et al.* 2010], differences in the n-6/n-3 PUFA ratio between birds fed diets supplemented with soybean oil and linseed oil were at a level of 8.1:1.9 and 9.6:1.7, respectively. This ratio is, however, too high in all groups because it is claimed that the n-6/n-3 ratio beneficial for health status should reach 5:1. It is suggested that these values be even lower and approximate 1: 1, or eventually 2:1. A decrease in the n-6/n-3 ratio may contribute to a reduced risk of many diseases, including among others cancers [Kouba and Mourot 2011].

Apart from the modification of fatty acid profile in muscles, the linseed oil and specifically its α-linolenic acid (ALA, C18:3n-3), may affect changes in lipid indices of blood. As demonstrated in earlier studies, the n-3 fatty acids reduce, among other things, the level of triglycerides (TG) in blood plasma, the level of LDL cholesterol fraction (the so-called "bad cholesterol", and influence an increase in HDL cholesterol fraction (the so-called "good cholesterol") [Kouba and Mourot 2011; Cunnane *et al.* 1993]. This was confirmed in the present study with turkeys. In the LO group, the level of triacylglycerols was significantly lower than in the birds from the SO group (Table 4). It is very beneficial, as the high level of TAG is acknowledged as an independent risk factor of many diseases, likewise the high level of total cholesterol and its LDL fraction [Kouba and Mourot 2011]. In contrast, no significant differences were reported in changes of the other markers of lipids metabolism (CHOL, LDL and HDL cholesterol fractions).

CONCLUSION

Results achieved in the study demonstrate that in the turkey hens receiving linseed oil addition to feed mixtures, contents of fat and cholesterol decreased in both breast and thigh muscles, compared to the birds fed feed mixtures with the addition of soybean oil. The increase in the content of n-3 fatty acids, and specifically of α-linolenic acid (the source of which was the linseed oil) in the feed mixtures for turkey hens caused also a significant increase in the content of this acid in breast and leg muscles of the birds. The addition of linseed oil contributed also to a reduced level of triacylglycerols in blood plasma of the birds.

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Streszczenie. Celem pracy była ocena zastosowania oleju lnianego w paszy dla indyczek w aspekcie jego wpływu na zmianę profilu kwasów tłuszczowych w paszy, a w konsekwencji w mięśniach (wzrost zawartości kwasów z rodziny n-3), a także na poprawę wskaźników gospodarki lipidowej krwi (obniżenie zawartości cholesterolu ogólnego, triacylogliceroli i wzrost zawartości frakcji HDL-cholesterolu). 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 dodany do mieszanki paszowej. Źródłem tłuszczu dla grupy II był olej lniany. W 15 tygodniu odchowu od ptaków pobrano krew do analizy na zawartość wskaźników lipidowych, natomiast w 16 tygodniu ptaki ubito i pobrano mięśnie piersiowe i ud do analizy zawartości tłuszczu, cholesterolu i profilu kwasów tłuszczowych. Uzyskane wyniki wskazują, że u indyczek otrzymujących w paszy dodatek oleju lnianego zmniejszyła się zawartość tłuszczu i cholesterolu zarówno w mięśniach piersiowych, jak i ud, w porównaniu z ptakami, które były żywione mieszankami z olejem sojowym. Wzrost zawartości kwasów z rodziny n-3, a konkretnie α-linolenowego (którego źródłem był olej lniany) w mieszankach dla indyczek spowodował również istotny wzrost zawartości tego kwasu w mięśniach piersiowych i ud. Dodatek oleju lnianego przyczynił się także do zmniejszenia zawartości triacylogliceroli w osoczu krwi ptaków.

Słowa kluczowe: indyczki, olej lniany, kwasy tłuszczowe, mięśnie, krew