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The effect of raw and extruded linseed on the chemical composition, lipid profile and redox status of meat of turkey hens*

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The aim of the study was to estimate an effect of extruded and raw linseed supplementation to feed mixtures on the quality of turkey hen muscles. Phase 1 of the experiment included 480 1-week-old turkey hens divided into four groups of 120 each (6 replications with 20 birds each). In phase 1 of this experiment turkey hens in groups L2, L4 and L6 received the complete mixture with 2%, 4% and 6% linseed, respectively. On the basis of the results obtained in phase 1, we selected the most effective proportion of linseed, which was given to the birds in group L4. Phase 2 was conducted on 360 birds divided into three groups of 120 each (6 replications with 20 birds each). The birds in group L4-E received linseed in the same amount as group L4, but in this variant linseed was extruded. In terms of modification of fatty acids the most beneficial was the 4% raw linseed supplement in the mixtures for turkey hens (ω-3/ω-6 in breast muscle - 5.96; in thigh muscle-1.34). However, the use of this amount of raw linseed slightly increased oxidation processes in the muscles analysed (increased MDA and LOOH). Replacing 4% raw linseed with 4% extruded linseed reduced the intensification of oxidation processes (increase LOOH about 27±2%), but resulted in a decrease in the total quantity of PUFA in the muscles of the turkey hens. In view of the highly beneficial effect of the 4% raw linseed supplement on the proportion of PUFA, as well as the high production cost of extrudate, which reduces meat quality in terms of PUFA, it seems to be advisable to use 4% raw linseed in feed mixtures for turkey hens, regardless of the small degree of oxidation.

KEY WORDS: antioxidants / extrusion / fatty acids / linseeds / turkey hen

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Due to its significant proportion of polyunsaturated fatty acids (PUFA), such as linoleic, linolenic and arachidic acids, as well as low cholesterol content, the fat of poultry meat is considered to be of greater value than that of beef or pork [Yanovych *et al*. 2013]. Although poultry meat has been regarded as one of the main sources of PUFA in human diets, in particular ω-3, research is conducted to improve the ratio of SFA (saturated fatty acids) to PUFA and that of ω-3 to ω-6 in poultry meat [Sioen *et al*. 2006, Poławska *et al.* 2011].

The fatty acid profile of monogastric animal tissues is directly influenced by the lipid composition of feed mixtures. Not only the content of fatty acids is important, but also the ratio of saturated to unsaturated and of monounsaturated to polyunsaturated acids [Schwingshackl and Hoffmann 2012]. Studies showed that the content of ω-3 fatty acids in poultry meat, particularly α-linolenic acid (ALA – 18:3), may be easily improved by increasing the levels of ω-3 PUFA in poultry diets with oily fish by-products. Apart from the feed strategies used in the past to increase the ω-3 PUFA content of poultry meat, such as the use of oily fish by-products, researchers have recently focused on the addition of vegetal sources such as linseed [Yanovych *et al*. 2013, Poławska *et al.* 2013].

Linseed (*Linum usitatissimum*) has a normalizing gastro-intestinal function and positive effects on the health of birds. Therefore, linseed may represent a valid alternative to genetically modified soybean due to its high content of oil (35%) rich in PUFAs (particularly ALA) and its cultivation sustainability. ALA (α-linolenic acid) is essential in the diet, because the organism cannot synthesize it. ALA and other ω-3 fatty acids compete with ω-6 fatty acids for the same desaturase enzymes involved in their metabolism [Kouba and Mourot 2011]. Linseed, in addition to unsaturated fatty acids treated as vitamin F, also contains considerable quantities of group A, B_6, D and E vitamins. Moreover, it is a valuable source of lignans [Kasote 2013], which apart from the function of plant oestrogens also have strong antioxidant properties [Yamashita *et al*. 2003]. Research showed that owing to their presence, as well as the high activity of natural vitamin E contained in linseed, the intensity of lipid peroxidation processes is substantially decreased [Jóźwik *et al*. 2013]. This can have a highly beneficial effect on the ω-3 to ω-6 ratio, and also result in a reduction in lipid peroxidation products (malondialdehyde – MDA and lipid peroxides – LOOH) [Kasote 2013], thereby increasing the stability of meat after slaughter. However, whole linseed contains antinutrients (e.g. linamarin) that may be inactivated by heat treatment such as extrusion [Anuonye *et al*. 2012]. Delgado-Licon *et al*. [2009] found a correlation between the extrusion procedure and the content of bioactive compounds and antioxidant capacity in the end product. The extrusion process can lead to a reduction in the contents of bioactive compounds, including antioxidant activity.

The aim of the study was to estimate an effect of extruded and raw linseed supplementation to feed mixtures on the quality of turkey hen muscles.

Material and methods

A two-phase experiment was conducted on the material consisting of 1-week-old BIG 6 turkey hens raised until their 16th week of life.

Phase 1

The purpose of phase 1 of the experiment was to select the most effective dosage of raw linseeds (2, 4, 6%) based on analyses of the chemical composition of the muscles.

Phase 1 was conducted on 480 1-week-old turkey hens divided into four groups of 120 each (6 replications with 20 birds each). The turkeys in all the groups received linseed oil in their complete feed mixtures, with synthetic vitamin E (dl-tocopherol) at 30 g t^{-1} as an additive protecting the fat against oxidation. The turkey hens in groups L2, L4 and L6 received the complete mixture with 2, 4 and 6% linseed, respectively.

Phase 2

The purpose of phase 2 of the experiment was to determine whether the use of extruded linseeds in the feed for turkey hens would have a more beneficial effect on meat quality in comparison with the mixtures containing raw linseeds.

On the basis of the results obtained in phase 1 we selected the most effective proportion of linseed, which was given to the birds in group L4. The birds in group L4-E received linseed in the same amount as group L4, but in this variant linseed was extruded in order to eliminate antinutrients (including linamarins).

Phase 2 was conducted on 360 birds divided into three groups of 120 each (6 replications with 20 birds each). As in phase 1, the birds in the control (group K2) received a combination of linseed oil with synthetic vitamin E (30 g t^{-1} dl-tocopherol).

During the experiment the birds in all the groups received *ad libitum* complete feed mixtures balanced according to the recommendations of *Nutrient Requirements for Poultry* [NRP 2005] for each period of rearing (Starter - weeks 1-3 of life; Grower I - weeks 4-6 of life; Grower II - weeks 7-11 of life; Grower III – weeks 12-16 of life) and had permanent access to drinking water. In order to meet energy requirements, the mixtures were supplemented with linseed oil.

All the mixtures in phases 1 and 2 were composed of maize, wheat, wheat bran and 46% post-extraction soy meal, and were isonitrogenous and isoenergetically balanced (the composition was presented in Czech *et al*. [2015].

The experiment was carried out with the approval of the Second Local Ethics Committee for Animal Experiments in Lublin (approval no. 9/2009).

During each stage of rearing (Starter – weeks 1-3 of life; Grower I – weeks 4-6 of life; Grower weeks II – 7-11 of life; Grower III – weeks 12-16 of life), feed samples were collected twice for analysis. The content of basic chemical components (crude protein, ether extract) and of minerals (zinc, copper, iron, manganese and selenium) were determined in the feed. Additionally, the fatty acid profiles of the feed were determined as well as the contents of vitamin E, vitamin C, total antioxidant status (FRAP) and the malondialdehyde (MDA). Raw and extruded linseed were analysed for concentrations of antinutrients (linamarin).

Table 1 presents the metabolic energy value and the selected values of the fatty acid profile in mixtures for turkey hens of phases 1 and 2 of the experiment.

The results of all the analyses are presented in Czech *et al*. [2015].

In both experimental phases, after rearing (week 16) the birds were slaughtered and carcass analysis was performed (6 birds each from each experimental group). The birds were humanely slaughtered at the processing plant by severing the carotid artery, jugular vein, trachea and the oesophagus. In order to carry out the stunned slaughter method, the individuals were put in an electrical water bath for 5 seconds at 250 mA.

During dissection 400-g muscle samples (breast and thigh) were collected from right half-carcasses. Muscle samples were freeze-dried, then ground and homogenised using a home-style coffee grinder. The content of basic chemical components was determined according to the AOAC procedure [2000] (976.06 for crude protein, 920.39 for the ether extract, 934.01 for dry matter and 942.05 for ash) and the ASA method was used to determine the content of minerals (zinc, copper, iron, manganese and selenium).

The fatty acid profile was determined in the muscle samples by gas chromatography. Chromatographic separation was performed in a Varian CP 3800 gas chromatograph. The chromatograph operating conditions for fatty acid separation were as follows: the CP WAX 52CB DF capillary column 0.25 mm of 60 m length, gas carrier - helium, flow rate 1.4 ml/min, column temperature 120°C gradually increasing by 2°C/min up to 210°C, determination time 127 min, feeder temperature 160°C, detector temperature 160°C, other gases - hydrogen and oxygen. Total cholesterol (CHOL) was determined in the breast and leg muscles according to Rhee *et al*. [1982], while vitamin C according to Omaye *et al*. [1979].

In addition, in the tissue homogenates (breast and leg muscles) enzymatic activity of superoxide dismutase (SOD) and catalase (CAT) was determined. The SOD activity is established by measuring the rate of auto-oxidation of adrenaline at 30°C on the basis of the increase of absorbance at 320 nm (that corresponds to monitoring of the increase in concentrations of various adrenaline oxidation products - according to Misra, in: Greenwald [1985]). Besides, the activity of catalase (CAT) was established, following the Clairborne method [1985]. The analysis consisted in the measurement of the substrate decomposition rate (hydrogen peroxide) catalyzed by this enzyme.

The concentration of lipid peroxides (LOOH) was determined according to Gay and Gębicki [2002] and that of malondialdehyde (MDA) according to Salih *et al*. [1987]. The method is based on a thiobarbituric acid (TBA) reaction with lipid peroxidation end products in the acid environment and at an increased temperature to generate a coloured adduct. To eliminate quantities of the complex series of adducts from TBA, the assay is run in the presence of inhibitors, e.g. BHT.

Recorded traits were analysed using one-way ANOVA, separately for different experimental phases taking into account feed additives (four levels in the 1st and three levels in the 2nd phase). Significance of differences between treatment means was determined by Duncan's test.

Table 1. Metabolic energy (MJ/kg^{-1}) and selected fatty acids (%) in feed mixtures of turkey hens in phases 1 and 2 experiment [Czech *et al.* 2015]

Item	Feeding period (in weeks of age)															
	Starter (1-3)				Grower I (4-6)				Grower II (7-11)				Grower III (12-16)			
	Feeding group															
	K1	**L2**	**L4**	**L6**	**K1**	**L2**	**L4**	**L6**	**K1**	**L2**	**L4**	**L6**	**K1**	**L2**	**L4**	**L6**
	Phase 1 experiment															
ME	11.9	11.6	11.9	12.3	12.5	12.5	12.4	12.3	12.6	12.5	12.5	12.3	12.9	12.5	12.7	12.7
18:2 ω-6	31.51	30.09	25.99	22.37	31.07	29.99	26.02	22.31	30.81	30.20	25.72	22.03	31.16	29.51	25.82	22.39
18:3 ω-3	8.01	12.23	18.00	20.26	8.02	12.60	18.02	20.53	7.69	12.08	17.89	20.34	7.95	12.87	17.68	20.13
SFA	21.43	16.40	17.30	20.06	22.20	16.95	17.31	19.98	22.18	18.47	17.35	20.32	23.17	18.11	17.49	20.05
MUFA	35.80	37.61	35.71	34.31	35.60	36.62	35.58	34.12	36.03	35.49	35.96	34.28	35.82	35.71	36.14	34.32
PUFA	41.96	44.24	46.09	44.71	41.34	44.72	46.16	44.95	40.98	44.27	45.77	44.48	40.19	44.47	45.54	44.60
ω-6/ω-3	3.93	2.46	1.44	1.10	3.87	2.38	1.44	1.09	4.01	2.50	1.44	1.08	3.92	2.29	1.46	1.11
	Phase 2 experiment															
	K2	**L4**	**L4-E**		**K2**	**L4**	**L4-E**		**K2**	**L4**	**L4-E**		**K2**	**L4**	**L4-E**	
ME	11.62	11.86	11.99		12.04	12.30	12.79		12.31	12.47	12.41		12.80	12.67	12.86	
18:2 ω-6	32.30	25.39	34.50		33.6	25.66	31.40		27.35	25.38	35.72		32.04	26.16	34.20	
18:3 ω-3	9.56	25.06	15.55		9.55	26.30	16.30		9.53	20.00	13.99		9.44	24.15	11.13	
SFA	19.51	14.20	20.66		19.49	14.05	23.04		16.53	16.91	25.73		18.5	16.51	21.14	
MUFA	34.70	31.62	25.66		32.77	30.45	25.35		45.32	36.93	23.57		35.24	30.53	30.34	
PUFA	44.53	53.35	52.72		46.27	54.65	50.69		37.10	45.40	49.89		45.04	52.21	47.64	
ω-6/ω-3	3.38	1.01	2.22		3.52	0.98	1.93		2.87	1.27	2.55		3.39	1.08	3.07	

ME – metabolic energy; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Results and discussion

According to Yasin *et al*. [2012], the fatty acid profile may be modified through diet. In the present study the use of linseed, either raw (Phase 1 – Tab. 2) or extruded (Phase 2 – Tab. 3), caused no substantial differences between the groups in the contents of basic chemical components in breast and thigh muscles of turkey hens. An exception was the higher ($P \leq 0.05$) fat content in the breast muscle in group L4 (4% linseed) when compared to group L6 (6% linseed), and in the thigh muscle in group L2 (Phase 1). A slight increase in cholesterol content was noted in the case of the 6% linseed supplement (group L6). The results of previous research on cholesterol content in tissues of farm animals receiving linseed are unclear. A study by Xiccato and Trocino [2003] on rabbits showed that the application of essential unsaturated fatty acids in the right proportions between types of acids can reduce the total cholesterol level in muscles and storage fat. This is very important for human nutrition, as consumption of meat with a reduced cholesterol content has been shown to significantly lower the concentration of total cholesterol and its atherogenic fraction in the lipid profile of plasma [Willett 2012].

As it was mentioned above, the fatty acid composition of feed directly affects the fatty acid profile in monogastric animal tissues. The results of our study show that adding linseed to the feed for turkey hens significantly differentiated the fatty acid composition of both the breast and thigh muscles (Phase 1, Tab. 2). Owing to the high content of polyunsaturated fatty acids (PUFA) in the feed mixtures containing linseed [Czech *et al*. 2015 – Tab. 1], the total proportion of these acids in bird muscles increased. At the same time, the proportion of saturated fatty acids (SFA) decreased, particularly palmitic acid (L2, L4) and stearic acid (L4, L6) in the breast muscles (Table 2). Saturated fatty acids (especially lauric (12:0), myristic (14:0) and palmitic (16:0) acids) are among the most important hypercholesterolemic agents [Cichosz and Czeczot 2011], thus reducing their contents in meat is considered highly beneficial. Owing to supplementation with linseed, changes in the quantitative proportions of unsaturated fatty acids (MUFA:PUFA) were noted as well (Phase 1). A decrease ($P \leq 0.05$) was noted in the content of monounsaturated fatty acids – MUFA (ω-9 (16:1) and ω-9 (18:1) – in the breast muscle in all groups of turkey hens receiving linseed with respect to group K1, and an increase in the content of polyunsaturated fatty acids – PUFA (ω-6 (18:2), ω-3 (18:3) and ω-6 (20:4) (Tab. 2). Similar results, i.e. an increase in polyunsaturated fatty acids in pork as a result of linseed supplementation, were obtained in a study by Grela and Kowalczuk [2009]. Beneficial changes for consumers observed in meat of turkey hens receiving linseed in their feed also include a pronounced increase in the concentrations of α-linolenic acid (ALA) and linoleic acid (LA), in the lipids of thigh muscles analysed (Tab. 2). Gonzales-Esquerra and Lesson [2000], who administered a 10% linseed supplement to chickens, observed a nearly five-fold increase in ALA content in the breast and thigh muscles, and the proportion of long-chain ω-3 PUFA significantly increased as well. In a study using linseed oil as a fat source for chickens, a considerable increase in ω-3 acids – ALA, DHA and EPA – was also observed [Zelenka

Table 2. Contents of basic chemical components (%), cholesterol (mg $100g^{-1}$) and fatty acid profile (%) in muscles of turkey hens in phase 1 experiment

Item	Breast muscle				Thigh muscle			
	K1	L2	L4	L6	K1	L2	L4	L6
Dry matter	26.20	26.24	26.53	26.41	27.94	29.1	27.12	27.29
Crude protein	23.51	23.57	23.69	24.10	19.74	19.33	19.97	19.91
Fat	1.52^{ab}	1.50^{ab}	1.69^{a}	1.15^{b}	7.08^{b}	8.70^{a}	6.06^{b}	6.28^{b}
Crude ash	1.15	1.13	1.13	1.14	1.10	1.06	1.06	1.06
Cholesterol	81.58	82.71	84.97	86.10	97.40	110.9	114.3	113.8
Fatty acid profile								
14:0	0.93	0.96	0.96	1.08	0.98	0.79	0.82	0.90
16:0	20.25^{a}	16.03^{b}	16.24^{b}	18.02^{ab}	25.55	22.41	23.33	21.63
16:1 ω-9	4.05^{a}	2.01^{c}	3.38^{b}	2.17^{c}	3.44	3.06	3.12	4.34
18:0	10.12^{a}	11.53^{a}	8.88^{b}	8.05^{b}	10.43	9.14	9.72	7.52
18:1 ω-9	33.59^{a}	29.31b	29.18^{b}	27.32^{b}	35.24	33.17	29.96	31.03
18:1 ω-7	1.80	1.75	1.74	1.67	1.29^{b}	1.64^{a}	1.28^{b}	1.60^{a}
18:2 ω-6	25.46^{b}	30.53^{a}	30.99^{a}	33.22^{a}	16.56^{b}	22.87^{a}	20.31^{a}	23.91^{a}
18:3 ω-3	0.88^{c}	2.90^{a}	3.20^{a}	2.50^{b}	1.87^{c}	2.60^{c}	7.32^{a}	4.82^{b}
20:1 ω 9	0	0	0	0	0.45	0.62	0.37	0.44
20:1 ω-7	0.59^{ab}	0.40^{b}	0.36^{b}	0.79^{a}	0.92	0.85	0.68	0.76
20:4 ω-6	0.17^{c}	0.67^{b}	0.87^{ab}	1.19^{a}	0.32	0.27	0.26	0.24
20:5	0	0	0	0	0.35	0.36	0.35	0.12
other[1]	2.16	3.91	4.2	3.99	2.60	2.22	2.48	2.69
total	100	100	100	100	100	100	100	100
SFA	31.30	28.52	26.08	27.15	36.96	32.34	33.87	30.05
UFA	66.54	67.57	69.72	68.86	60.44	65.44	63.65	67.26
MUFA	40.03^{a}	33.47^{b}	35.66^{b}	31.95^{b}	41.34	39.34	35.41	38.17
PUFA	26.51^{b}	34.10^{a}	34.06^{a}	36.91^{a}	19.10^{b}	26.1^{a}	28.24^{a}	29.09^{a}
ω-3/ω-6	0.034	0.093	0.104	0.073	0.111	0.112	0.356	0.200
ω-6/ω-3	29.13	10.76	9.64	13.76	9.03	8.90	2.81	5.01

[abc]Withi rows means bearing different superscript differ significantly at P≤0.05.
SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.
[1]SUM = 6:0+10:0+11:0+13:0+14:1+15:0+15:1+17:1+CLA+20:0+20:3+21:0+22:1.

et al. 2008]. In the present study, increasing the proportion of ω-3 fatty acids (mainly ALA) in muscles of turkey hens resulted in a tendency towards a decrease in the ω-6 to ω-3 ratio (Tab. 2, Phase 1). The smallest and most beneficial ω-6 to ω-3 ratio was recorded in the muscles of birds receiving 4% linseed (Tab. 2). The diet of Western societies has been shown to be deficient in ω-3 acids, with a wide range of ω-6 to ω-3 ratios, from 15:1 to even 25:1, while the optimal ratio is 1:1 [Schwingshackl and Hoffmann 2012]. Excessive consumption of ω-6 acids reduces metabolism of ω-3 acids and disturbs the physiological balance of compounds synthesised from these acids, including leukotrienes and prostaglandins [Lands 2014]. A decrease in the ω-6 to ω-3 ratio can reduce the risk of numerous diseases, including circulatory diseases and cancers [Kouba *et al*. 2011].

The results of the present study show that the use of 4% raw or extruded linseed in the seed mixtures caused no significant changes in the contents of basic chemical

Table 3. Contents of basic chemical components (%), cholesterol (mg $100g^{-1}$) and fatty acid profile (%) in muscles of turkey hens in Phase 2 experiment

Item	Breast muscle			Thigh muscle		
	K2	L4	L4-E	K2	L4	L4-E
Dry matter	26.71	26.39	26.12	27.42	27.07	28.39
Crude protein	24.77	24.51	24.30	19.92	20.50	20.16
Fat	0.77	0.61	0.62	6.44	6.02	6.83
Crude ash	1.18	1.19	1.18	1.04	1.03	1.02
Cholesterol	86.10	84.97	82.71	103.1	113.2	117.7
Fatty acid profile						
14:0	0.62	0.64	0.62	0.75^{b}	0.62^{b}	1.06a
16:0	22.25^{a}	19.09^{b}	19.77^{b}	25.00^{a}	20.08^{b}	21.08^{b}
16:1 ω-7	0.36^{a}	0.29^{b}	0.35^{a}	0	0	0
16:1 ω-9	3.10^{a}	2.15^{b}	2.79^{a}	3.50	3.24	3.25
17:0	0	0	0	0.25^{b}	0.23^{b}	0.34^{a}
18:0	11.77^{a}	7.83^{b}	6.81^{b}	9.74^{b}	7.55^{c}	13.42^{a}
18:1 ω-9	29.85^{b}	25.96^{c}	33.18^{a}	38.25^{a}	30.95^{c}	36.12^{b}
18:1 ω-7	1.77	1.44	1.56	2.15^{a}	1.51^{b}	1.98^{ab}
18:2 ω-6	25.55^{c}	34.64^{a}	28.99^{b}	15.59^{c}	24.48^{a}	19.31^{b}
18:3 ω-3	1.03^{c}	4.17^{a}	1.78^{b}	2.60^{b}	9.41^{a}	0.56^{c}
20:1 ω-9	0.42^{b}	1.18^{a}	0.49^{b}	0	0	0
20:2	0.53^{a}	0.28^{b}	0.00^{b}	0	0	0
20:1 ω-7	0	0	0	0.85^{b}	0.59^{c}	1.72^{a}
20:4 ω-6	1.09^{b}	1.22^{b}	1.52^{a}	0	0	0
22:5	0.32^{b}	0.08^{c}	0.45^{a}	0	0	0
22:6	0.43	0.43	0.54	0	0	0
other[1]	0.91^{b}	0.60^{b}	1.15^{a}	1.32	1.34	1.16
total	100	100	100	100	100	100
SFA	34.64^{a}	27.56^{b}	27.20^{b}	35.74^{a}	28.48^{b}	35.90^{a}
UFA	64.45^{b}	71.84^{a}	71.65^{a}	62.94^{b}	70.18^{a}	62.94^{b}
MUFA	35.50^{b}	31.02^{c}	38.37^{a}	44.75^{a}	36.29^{b}	43.07^{a}
PUFA	28.95^{c}	40.82^{a}	33.28^{b}	18.19^{b}	33.89^{a}	19.87^{b}
ω-3/ω-6	0.039	0.116	0.058	0.167	0.384	0.029
ω-6/ω-3	25.86	8.60	17.14	6.00	2.60	34.48

[abc]Withi rows means bearing different superscript differ significantly at P≤0.05.
SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.
[1]SUM = 6:0+10:0+11:0+13:0+14:1+15:0+15:1+17:1+CLA+20:0+20:3+21:0+22:1

components or in cholesterol concentration in muscles of turkey hens in comparison to the K2 group (Phase 2, Tab. 3).

By comparing the fatty acid profile in breast muscles turkey hens from the group receiving the feed with extruded linseed (L4-E) vs. the control (K2) it was much improved, as indicated by a significantly higher content of PUFAs, including 18: 2 (ω -6); 18: 3 (ω-3) 20: 4 (ω-6) and 22: 5. Also, birds receiving the feed with raw linseed (L4) were characterised by a favourable fatty acid profile compared to the control (K2) and the attribute concerned both breast and thigh muscle. In addition, the obtained differences were more favorable than in the case of extruded linseed (Tab. 3).

In breast muscles of birds fed the mixture with the added linseed extrudate a significant increase in the proportion of UFA was recorded (except for ω-7 (18: 1) at the expense of PUFA (both ω-3 and ω-6) (Tab. 3). Since the decrease in the proportion of α-linolenic acid (the main representative of ω-3 acids) was greater than that of linoleic acid (ω-6), the ratio of ω-6 to ω-3 acids increased considerably, which is an undesirable phenomenon, as it should not exceed 4:1 [Schwingshackl and Hoffmann 2012]. The increase observed in the proportion of arachidic acid (20:4 ω-6) (Tab. 3), one of the most important precursors of pro-inflammatory eicosanoids, thromboxanes and prostaglandins, was unfavourable as well. Similar changes in the fatty acid profile were also recorded in the thigh muscle (except for ω-6 acid (20:4), which was not present). Moreover, the proportion of saturated fatty acids increased at the expense of unsaturated fatty acids, which is nutritionally undesirable (Tab. 3, Phase 2). The modifications observed in the fatty acid composition of the muscles of turkey hens were the result of changes in the fatty acid profile of the feed mixtures, caused by the addition of linseed extrudate in place of unprocessed seeds.

The modification of the fatty acid profile of the feed caused by the addition of linseed, i.e. the increased proportion of UFA, particularly ω-3 PUFA, which are particularly susceptible to peroxidation, created a potential risk of increased oxidative stress [*Yanovych et al*. 2013]. The redox status of the organism is measured as the balance between oxidative agents, i.e. lipid peroxidation products (peroxide radicals and malondialdehyde), and endogenous and exogenous antioxidant substances [Jóźwik *et al*. 2012]. The study shows that the introduction of linseed containing large amounts of PUFA (particularly susceptible to oxidation) into the feed for turkey hens, resulted in the potential risk of inducing oxidative stress, understood as an imbalance between generation of ROS and their inactivation. Susceptibility of fatty acids to oxidation increases geometrically, in proportion to the number of unsaturated bonds in individual acids. Moreover, a study by Cichosz and Czeczot [2011] shows that the oxidative stability of fatty acids also depends on the position of unsaturated bonds; ω-3 acids undergo autooxidation much more easily than ω-6 acids.

The results of the present study do in fact indicate intensification of the lipid peroxidation process due to the use of the linseed supplement, particularly at 4% and 6%, which is evidenced by the significant increase in the concentration of lipid peroxides and malondialdehyde in both muscles analysed (Phase 1, Tab. 4). According to Ognik *et al.* [2016], an increase in the level of ROS (including hydrogen peroxide and lipid peroxides) induces compensatory changes expressed as an enhanced synthesis and increased activity of antioxidant enzymes – SOD, GPx (glutathione peroxidase) and CAT. Owing to these changes, the degree of intensification of peroxidation processes under physiological conditions is small and does not pose a health threat.

However, a study by Ergun *et al*. [2005] shows that the body's reaction to oxidative stress is not necessarily manifested as an increase in the activity of SOD or CAT. The addition of linseed to the feed of turkey hens did not substantially affect catalase activity (a significant increase was recorded only in the thigh muscles of birds receiving

Table 4. Levels of anti- and pro-oxidant indices in muscles of turkey hens in phase 1 experiment

Item	Breast muscle				Thigh muscle			
	K1	L2	L4	L6	K1	L2	L4	L6
SOD (U mg^{-1})	10.47^{b}	11.14^{ab}	11.98^{ab}	13.15^{a}	8.88^{b}	8.05^{b}	10.93^{a}	11.00^{a}
CAT (U mg^{-1})	3.95	2.75	2.76	3.30	2.37^{b}	2.27^{b}	2.81^{b}	3.54^{a}
MDA (mg kg^{-1})	0.382^{b}	0.519^{a}	0.580^{a}	0.500^{a}	1.06^{b}	1.60^{a}	1.60^{a}	1.56^{a}
Vitamin C (mg g^{-1})	0.381^{b}	0.542^{a}	0.604^{a}	0.561^{a}	0.111	0.122	0.114	0.109
LOOH (µg kg^{-1})	0.303^{b}	0.216^{b}	0.420^{a}	0.390^{a}	0.309^{b}	0.330^{b}	0.397^{a}	0.374^{a}
Vitamin E (µg g^{-1})	0.400^{ab}	0.388^{a}	0.414^{b}	0.409^{b}	0.762^{a}	0.860^{a}	0.978^{b}	0.962^{b}
Copper (ppm)	0.572^{c}	0.918^{a}	0.768^{b}	0.791^{b}	0.943^{b}	1.03^{ab}	1.01^{ab}	1.09^{a}
Zinc (ppm)	4.01^{c}	4.01c	5.47^{a}	4.34^{b}	7.62^{b}	8.17^{b}	10.27^{a}	7.95^{b}
Ferrum (ppm)	3.80^{c}	6.08^{b}	8.75^{a}	6.77^{b}	13.68^{b}	17.65^{a}	19.19^{a}	18.11^{a}
Manganese (ppm)	0.112^{b}	0.105^{b}	0.122^{a}	0.109^{b}	0.130	0.158	0.159	0.140
Selenium (ppm)	0.870^{b}	1.15^{a}	1.17^{a}	0.802^{b}	0.732^{b}	0.644^{a}	0.725^{ba}	0.899^{a}

^{abc}Within rows means bearing different superscript differ significantly at P≤0.05.
SOD – superoxide dismutase; CAT – catalase; MDA – malondialdehyde; LOOH – lipid peroxides.

6% linseed). However, the use of a 4 or 6% linseed supplement caused a pronounced increase in SOD activity in both of the muscles analysed (Phase 1, Tab. 4). Dismutation of superoxide radical by SOD generates hydrogen peroxide, which is then reduced by glutathione peroxidase or catalase. Also noteworthy is the considerable increase in vitamin C content in the breast muscle tissue (Phase1, Tab. 4). Vitamin C is one of the most important aqueous-phase antioxidants and an increase in its concentration should also be considered in this context. Enhanced synthesis of vitamin C by the birds may have been a reaction to its increased demand in antioxidant reactions [Hacisevki 2009]. The increase noted in the present study in the concentrations of the micronutrients Cu^{+2}, Zn^{+2}, Fe^{+2}, Se^{+2} and Mn^{+2} in muscles of turkey hens (Phase 1, Tab. 4) should probably also be regarded as a means of adaptation to increased ROS concentration. These elements function as cofactors of antioxidant enzymes; copper, manganese and zinc are coenzymes of SOD, selenium is a coenzyme of GPx, and iron is essential for catalase activity. It should be emphasized that the antioxidant status may also be influenced by exogenous antioxidants supplied in feed. As it was stated above, linseed is one of the richest sources of lignanes [Yamashita *et al*. 2003]. It is possible that owing to their presence, as well as the high activity of natural vitamin E contained in linseed, lipid peroxidation was not overly intensive. This is indicated by the increased antioxidant potential of the blood plasma. Severe and long-lasting oxidative stress should be expected to result in a decrease in FRAP, due to the depletion of antioxidants and their degradation by ROS [Czech *et al*. 2014].

Replacement of 4% raw linseed with 4% extruded linseed in the feed mixture caused a reduction in oxidative processes in the muscles of the turkey hens, manifested as a decreased content of lipid peroxides and malondialdehyde (Phase 2, Tab. 5). This may also be indicated by the considerably reduced content of vitamin C. Ascorbate is a primary antioxidant in that it directly neutralizes radical species. Ascorbate is not very

Table 5. Levels of anti- and pro-oxidant indices in muscles of turkey hens in phase 2 experiment

Item	Breast muscle			Thigh muscle		
	K2	L4	L4-E	K2	L4	L4-E
SOD (U mg^{-1})	10.86	11.87	10.67	11.43^{b}	16.51^{a}	12.71^{b}
CAT (U mg^{-1})	4.68	5.16	4.26	8.15^{b}	11.44^{a}	9.24^{ab}
MDA (mg kg^{-1})	0.512	0.621	0.402	0.701^{ab}	0.802^{a}	0.625^{b}
Vitamin C (mg g^{-1})	0.113^{b}	0.212^{a}	0.141^{b}	0.201	0.182	0.151
LOOH (µg kg^{-1})	0.452^{a}	0.491^{a}	0.371^{b}	0.381^{a}	0.384^{a}	0.262^{b}
Vitamin E (µg g^{-1})	0.471	0.460	0.432	0.957^{b}	0.958^{b}	0.870^{a}
Copper (ppm)	0.522	0.533	0.431	1.57	1.24	1.22
Zinc (ppm)	4.02	4.61	4.28	8.37	10.05	10.22
Ferrum (ppm)	6.79^{b}	11.60^{a}	10.50^{a}	18.55^{b}	21.04^{ab}	22.35^{a}
Manganese (ppm)	0.151	0.142	0.133	0.211	0.232	0.211
Selenium (ppm)	0.682	0.681	0.621	1.05	1.02	1.101

^{abc}Within rows means bearing different superscript differ significantly at $P \leq 0.05$.
SOD – superoxide dismutase; CAT – catalase; MDA – malondialdehyde; LOOH – lipid peroxides.

reactive with the prevalent cellular oxidants such as hydrogen peroxide and it probably reacts mostly with hydrogen peroxide breakdown products [Hacisevki 2009].

However, we need to stress the increase in zinc concentration recorded in the blood of these birds, and to a lesser extent the concentration of iron as well. A study by Anuonye *et al*. [2012] showed that extrusion may have a beneficial effect on the bioavailability of certain micronutrients, particularly zinc and iron. This is probably due to degradation during this process of antinutrient phytates present in the seeds, in which these elements are present in insoluble complexes that are not easily assimilated. However, Nielsen *et al*. [2013] showed that absorption of Zn^{2+}, Mg^{2+} and P from the extruded product was significantly decreased, whereas absorption of Ca^{2+} and Fe^{2+} was at the same level for the two products, indicating that phytate needs to be dephosphorylated to a greater extent for Fe^{2+} and Ca^{2+} release to occur when compared to Zn^{2+}, Mg^{2+} and P.

The introduction of 2, 4 and 6% raw linseed to feed mixtures for turkey hens does not affect the chemical composition or cholesterol content in their breast and thigh muscles. Of all the levels of raw linseed applied in the feed mixtures, the most beneficial in terms of the modification of fatty acids was the 4% supplement. However, the use of this amount of raw linseed slightly increased oxidation processes in the muscles analysed.

Replacing 4% raw linseed in the mixtures for turkey hens with 4% extruded linseed reduced the intensification of oxidation processes, but resulted in a decrease in the total quantity of polyunsaturated fatty acids in the muscles of turkey hens. Replacing 4% raw linseed with 4% extruded linseed did not affect either the chemical composition or cholesterol content in the muscles of the turkey hens.

In view of the highly beneficial effect of the 4% raw linseed supplement on the proportion of PUFA, as well as the high cost of producing extrudate, which reduces meat quality in terms of PUFA, the use of 4% raw linseed in feed mixtures for turkey hens, while inducing a small degree of oxidation, appears to be more justified than the use of extruded linseed.

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