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# Concentration of trace elements *vs* redox status in blood, liver, and muscles of turkey hens fed diets with the addition of soybean or linseed oil

Anna Czech, Katarzyna Ognik, Iwona Sembratowicz, Agnieszka Chałabis-Mazurek[1]

Department of Biochemistry and Toxicology, Faculty of Animal and Breeding,
[1]Department of Toxicology and Environmental Protection, Faculty of Veterinary Medicine,
University of Life Science in Lublin, 20-950 Lublin, Poland
annaczech@poczta.fm

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**Abstract**

The undertaken study aimed at analysing the effect of linseed oil used in diets for turkey hens on contents of pro- and antioxidants in their tissues. Additionally, correlations were analysed between the contents of these compounds in blood, liver, and muscles of the birds. The experiment was conducted on 240 one-week-old turkey hens reared until 16 weeks of age, randomly allocated into two feeding groups. Hens from the first group received soybean oil in their complete feed mixture, whereas linseed oil was the source of fat for turkey hens from the second group. The oil content of the diet was on the level appropriate for the period of feeding: Starter - 0.5%; Grower I - 1.0%, Grower II - 2.5%; Finisher I - 3%. The redox parameters were assayed in breast and thigh muscles, blood, and liver. Introduction of PUFA n-3 high linseed oil to a feed mixture contributed to the enhancement of lipid peroxidation processes, which was indicated by a significant increase in concentrations of $H_2O_2$ and malondialdehyde in muscles, and by an increase of superoxide dismutase activity and concentrations of vitamin C and minerals ($Cu^{+2}$, $Zn^{+2}$, $Fe^{+2}$, $Se^{+2}$). This points out the need of strengthening the antioxidative defence by the use of suitable antioxidants. The use of linseed oil as a feed material for turkey hens additionally contributed to changes in parameters determining the ferric reducing ability of plasma (FRAP). The increased FRAP values resulted from a significant increase in vitamin E level.

**Key words:** turkey hens, linseed oil, antioxidant parameters, muscles, blood, liver.

## Introduction

Linseed and its products have long been used in animal feeding and human nutrition owing to the beneficial effects of their protein, dietary fibre, lignans, omega-3 fatty acids, and other biologically-active substances that are integral tissue constituents of live organisms (25). Investigations of poultry and livestock demonstrated that the addition of *e.g.* linseed oil or linseeds to feed mixtures enabled modifying the quality of meat products and stimulating antioxidative processes (10, 14, 16). The quality of meat products may be modified most of all through increasing the content of PUFA that originate from *e.g.* linseed products, including especially peroxidation-susceptible PUFA n-3. This, however, poses a potential risk of oxidative stress intensification, which should be understood as disturbance of the balance between generation of reactive oxygen species (ROS) and mechanisms of their inactivation (23). High quantities of ROS are synthesised during peroxidation, and they are known to induce multiple changes in the activity of antioxidative systems aiming at risk elimination. A measure of the organism's redox status is the balance between oxidative factors – namely products of lipids peroxidation (peroxide radicals, malondialdehyde) and endogenous and exogenous antioxidant substances (antioxidative enzymes, low-molecular antioxidants or cations of divalent metals) (1). Effects of peroxidative processes may be observed not only in the qualitative composition of fatty acids in animals' tissues but also in the quantities and ratios of pro- and antioxidative markers. This may be of a great significance in quality assessment of food products, which may in turn determine their dietetic values. Therefore, in view of high popularity and high intake of poultry meat,

a continuous control over these products in terms of contents of substances with antioxidative properties seems to be highly advisable. The present study aimed at analysing the effect of linseed oil addition to feed mixtures for turkey hens on the contents of pro- and antioxidants in their muscles. An additional analysis was carried out to determine correlations between contents of these components in blood, liver, and muscles.

## Material and Methods

The experiment was carried out on 240 one-week-old BIG 6 turkey hens reared until 16 weeks of life in pens, 20 birds each. The hens were randomly allocated to two equal feeding groups: SO and LO. Turkeys from the SO group received an addition of soybean oil to a complete feed mixture, whereas linseed oil was the source of fat to the birds from the LO group. Throughout the experiment, the birds from both groups were administered feed mixtures *ad libitum,* that were balanced according to NRC recommendations (19) for respective feeding periods (Starter - 0-3 weeks of life; Grower 1 - 4-6 weeks of life; Grower 2 - 7-11 weeks of life; Finisher 12-16 weeks of life) (Table 1) and had free access to drinking water.

At the end of the 15$^{th}$ week of rearing, blood was sampled from the wing vein of 12 birds from each group (two from each cage), which at the beginning of the experiment were selected randomly and ear tagged. Blood was sampled after eight-hour fasting with free access to drinking water.

Activity of antioxidative enzymes in blood plasma was determined spectrophotometrically. Superoxide dismutase (SOD) was assayed with the adrenalin method (12) with modification in wavelength, *i.e.* 320 nm. The method was modified to achieve higher selectivity of transitory reaction products at this light bean length (1). The activity of catalase (CAT) was determined according to Clairborne (4), whereas the concentration of total glutathione (GSH + GSSG) – according to Gunzler and Flohe (13). In terms of antioxidative system markers, assays were carried out for ferric reducing ability of plasma (FRAP) according to Benzie and Strain (2), and vitamin C according to Omaye *et al.* (20). Plasma levels of lipid peroxidation products were also determined, namely: peroxides ($H_2O_2$) according to Gay and Gębicki (11), and malondialdehyde (MDA) product of tissue lipids oxidation, according to Ledwożyw *et al.* (18).

Once the rearing was completed (16 weeks of birds life), all birds were slaughtered. Samples of their breast thigh muscles and livers were collected (12 pieces from each experimental group). Their homogenates were assayed for the activity of SOD and CAT, and for concentrations of $H_2O_2$ and MDA.

Concentrations of minerals (zinc, copper, iron, manganese, selenium) were determined in samples of the liver, muscles and blood with the AAS method. Vitamin E was analysed in these tissues using a modified method of Wąsowska *et al.* (27).

Numerical data was processed with the analysis of variance (ANOVA) method, and the results were presented as mean values for groups and standard deviations (24).

## Results

The addition of linseed oil caused a significant increase of $H_2O_2$ and MDA concentrations in breast and thigh muscles of turkey hens (Tables 2, 3). Such changes were not observed in blood, nor in the liver. In the blood and liver concentrations of $H_2O_2$ and MDA were significantly lower in the group receiving the addition of linseed oil than in the group administered soybean oil (Tables 4, 5).

In the breast muscle and blood plasma of the birds from the LO group, analyses revealed a significant

**Table 1.** Composition of full mixtures for turkey hens (%)

| | Starter | Grower I | Grower II | Finisher I |
|---|---|---|---|---|
| Feeding period, week of age | 1-3 | 4-6 | 7-11 | 12-16 |
| Maize meal | 25.6 | 27.4 | 23.8 | 47.4 |
| Wheat | 20.0 | 25.0 | 30.0 | 25.0 |
| Wheat bran | 3.0 | - | - | - |
| Soybean meal 46% protein | 43.0 | 41.7 | 38.8 | 20.4 |
| Fish meal | 3.5 | - | - | - |
| Fodder chalk | 1.2 | 1.7 | 1.7 | 1.5 |
| Soybean oil or linseed oil | 0.5 | 1.0 | 2.5 | 3.0 |
| Cytromix Plus[1] | 0.2 | 0.2 | 0.2 | 0.2 |
| Farmix[2] | 3.0 | 3.0 | 3.0 | 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 ; 3000 mg of Cu ; 200 mg of I; 60 mg of Se; 40 mg of Co; 15 g of Ca

**Table 2.** Levels of redox markers in breast muscle of turkey hens

| Parameter tested | Dietary treatments | | Significance |
|---|---|---|---|
| | SO | LO | |
| SOD; U $mg^{-1}$ | 7.83 ± 0.710 | 10.60 ± 0.849 | ** |
| CAT; U $mg^{-1}$ | 2.94 ± 0.317 | 3.61 ± 0.137 | ** |
| Vitamin C; mg $g^{-1}$ | 0.428 ± 0.088 | 0.577 ± 0.084 | ** |
| Vitamin E; µg $g^{-1}$ | 0.472 ± 0.091 | 0.411 ± 0.113 | ns |
| MDA; mg $kg^{-1}$ | 0.398 ± 0.046 | 0.503 ± 0.039 | ** |
| $H_2O_2$; µg $kg^{-1}$ | 0.293 ± 0.039 | 0.354 ± 0.143 | * |
| Copper; ppm | 0.676 ± 0.145 | 0.649 ± 0.108 | ns |
| Iron; ppm | 4.29 ± 0.273 | 4.89 ± 0.661 | ** |
| Zinc; ppm | 8.06 ± 2.59 | 12.67 ± 2.04 | ** |
| Manganese; ppm | 0.112 ± 0.014 | 0.118 ± 0.010 | ns |
| Selenium; ppm | 0.704 ± 0.115 | 0.621 ± 0.119 | * |

*P ≤ 0.05; **P ≤ 0.01; NS: not significant

**Table 3.** Levels of redox markers in thigh muscle of turkey hens

| Parameter tested | Dietary treatments | | Significance |
|---|---|---|---|
| | SO | LO | |
| SOD; U $mg^{-1}$ | 7.56 ± 0.492 | 7.83 ± 0.931 | ns |
| CAT; U $mg^{-1}$ | 3.00 ± 0.196 | 2.72 ± 0.236 | * |
| Vitamin C; mg $g^{-1}$ | 0.114 ± 0.007 | 0.120 ± 0.015 | ns |
| Vitamin E; µg $g^{-1}$ | 0.931 ± 0.047 | 0.976 ± 0.070 | * |
| MDA; mg $kg^{-1}$ | 1.43 ± 0.083 | 1.61 ± 0.038 | * |
| $H_2O_2$; µg $kg^{-1}$ | 0.277 ± 0.061 | 0.368 ± 0.055 | ** |
| Copper; ppm | 0.774 ± 0.101 | 1.05 ± 0.081 | ** |
| Iron; ppm | 7.23 ± 0.669 | 7.65 ± 0.669 | * |
| Zinc; ppm | 16.60 ± 1.82 | 18.02 ± 1.56 | * |
| Manganese; ppm | 0.148 ± 0.019 | 0.153 ± 0.018 | ns |
| Selenium; ppm | 0.925 ± 0.135 | 1.03 ± 0.100 | ** |

*P ≤ 0.05; **P ≤ 0.01; NS: not significant

**Table 4.** Levels of redox markers in blood plasma of turkey hens

| Parameter tested | Dietary treatments | | Significance |
|---|---|---|---|
| | SO | LO | |
| SOD; U $mL^{-1}$ | 23.55 ± 2.13 | 29.94 ± 3.63 | * |
| CAT; U $mL^{-1}$ | 4.98 ± 1.92 | 5.22 ± 2.53 | * |
| Vitamin C; mg $L^{-1}$ | 0.196 ± 0.053 | 0.163 ± 0.049 | ns |
| Vitamin E; µg $mL^{-1}$ | 0.359 ± 0.056 | 0.388 ± 0.063 | ** |
| FRAP; µmol $L^{-1}$ | 53.25 ± 13.91 | 59.54 ± 14.68 | ** |
| MDA; µmol $L^{-1}$ | 0.823 ± 0.349 | 0.625 ± 0.288 | ** |
| $H_2O_2$; µmol $L^{-1}$ | 2.52 ± 1.07 | 1.58 ± 0.691 | ** |
| Copper; µmol $L^{-1}$ | 23.49 ± 4.00 | 26.77 ± 5.45 | ** |
| Iron; µmol $L^{-1}$ | 44.12 ± 8.38 | 49.88 ± 6.80 | ** |
| Zinc; µmol $L^{-1}$ | 27.17 ± 5.28 | 24.64 ± 4.18 | ** |
| Manganese; µmol $L^{-1}$ | 0.499 ± 0.044 | 0.500 ± 0.040 | ns |
| Selenium; µmol $L^{-1}$ | 88.21 ± 9.81 | 91.34 ± 9.38 | ns |

*P ≤ 0.05; **P ≤ 0.01; NS: not significant

**Table 5.** Levels of redox markers in the liver of turkey hens

| Parameter tested | Dietary treatments | | Significance |
|---|---|---|---|
| | SO | LO | |
| SOD; U $mg^{-1}$ | 7.07 ± 1.23 | 8.16 ± 0.801 | ** |
| CAT; U $mg^{-1}$ | 4.48 ± 0.677 | 3.02 ± 0.518 | ** |
| Vitamin C; mg $g^{-1}$ | 0.066 ± 0.015 | 0.084 ± 0.011 | ** |
| Vitamin E; µg $g^{-1}$ | 2.64 ± 0.352 | 2.61 ± 0.235 | ns |
| MDA; mg $kg^{-1}$ | 0.689 ± 0.200 | 0.580 ± 0.128 | * |
| $H_2O_2$; µg $kg^{-1}$ | 0.314 ± 0.035 | 0.368 ± 0.076 | * |
| Copper; ppm | 1.84 ± 0.177 | 1.96 ± 0.329 | ns |
| Iron; ppm | 49.73 ± 3.69 | 54.48 ± 5.49 | ** |
| Zinc; ppm | 61.31 ± 7.94 | 71.62 ± 6.11 | ** |
| Manganese; ppm | 1.10 ± 0.137 | 1.12 ± 0.112 | ns |
| Selenium; ppm | 1.26 ± 0.129 | 1.62 ± 0.159 | * |

*P ≤ 0.05; **P ≤ 0.01; NS: not significant

increase in the activity of SOD and CAT (Tables 2, 4). In contrast, in the thigh muscle and liver of birds from this group, the activity of CAT was significantly lower than in the birds from the SO group (Tables 3, 5).

The content of vitamin C in the breast muscle and liver of turkeys from the LO group increased significantly. Such a dependency was not determined for vitamin E (Tables 2, 5).

In the birds receiving the feed mixture with linseed oil addition, the concentration of $Cu^{+2}$, $Zn^{+2}$, $Fe^{+2}$, and $Se^{+2}$ in thigh muscles; the concentration of $Zn^{+2}$ and $Fe^{+2}$ in the breast muscle and liver; and the concentration of $Cu^{+2}$ and $Fe^{+2}$ in blood plasma were significantly higher compared to turkey hens from the SO group. The use of linseed oil as a feed additive for turkey hens additionally contributed to an increased concentration of parameters determining the FRAP (Table 4).

## Discussion

The replacement of soybean oil commonly applied in turkey hens feed with linseed oil seems highly advisable, especially considering the fact that the composition of dietary fatty acids is reflected in the fatty acid composition of cellular membrane of lymphocytes and other cells of the immune system, but principally of muscles (17). However, the use of linseed oil – being a valuable source of polyunsaturated fatty acids – poses a potential risk of oxidative stress induction (7, 29). The oxidative processes are directly involved in modification of both fatty acid profile in tissues and of other components linked directly with redox processes ongoing in the body. Linseeds contain many components possessing antioxidative properties including, *e.g.* lignans: secoisolariciresinol and its diglucoside, as well as vitamins E and C, minerals, phytosterines, flavonoids, or phytooestrogens. Some reports also demonstrated a high content of glutathione in linseeds (3), which additionally affects their antioxidative capability. The content of these antioxidants in linseed oil is, however, significantly lower than in fresh seeds (21, 28). This suggests the necessity of applying additional dietary substances capable of suppressing the oxidative processes.

In order to increase the oxidative stability of polyunsaturated fatty acids, their intake should be completed with additives indicating antioxidative properties (6, 22). By reducing peroxidation processes, antioxidants may also significantly improve the quality of fat, as demonstrated in a study by Yanovych *et al.* (30). Results obtained in our experiment show explicitly that lipid peroxidation intensified upon the influence of linseed oil additive, which was indicated by significantly increased concentrations of $H_2O_2$ and MDA in thigh and breast muscles of turkey hens. Such a dependency was not determined in the liver, nor in blood.

The increased synthesis of lipid peroxidation products induces compensation changes expressed by enhanced production and activity of antioxidative enzymes – SOD, PGx, and CAT, which was confirmed in our study. In birds receiving the addition of linseed oil to a feed mixture a significant increase in the activity of both SOD and CAT was observed in the breast muscle and blood plasma. Due to these changes, the intensity of peroxidation processes under physiological conditions is low and does not pose a high health risk (5). Investigations of many authors have demonstrated, however, that body response to oxidative stress does not always have the influence on SOD, PGx, or CAT activity increase (9). In the reported experiment, the activity of CAT in the liver and thigh muscle of the birds from the LO group was significantly lower compared to the birds receiving soybean oil in their feed mixture.

It also was interesting to notice a significant increase in vitamin C concentration in tissues of the breast muscle and liver. The enhanced synthesis of vitamin C by the birds could result from its increased demand in antioxidative reactions (8).

The increased concentration of $Cu^{+2}$, $Zn^{+2}$, $Fe^{+2}$, and $Se^{+2}$ in thigh muscle, and of $Zn^{+2}$ and $Fe^{+2}$ in breast muscle and liver, determined in our study could have had an additional influence on the level of oxidative stress. The concentration of these trace elements in muscles was partially reflected by their blood level, but still the addition of linseed oil elicited a significant increase only in contents of $Cu^{+2}$ and $Fe^{+2}$, and additionally reduced $Zn^{+2}$ content. These elements are mobilised to inhibit ROS synthesis by being co-factors of antioxidative enzymes (copper, manganese, and zinc are co-enzymes of SOD, selenium is co-enzyme of PGx, and iron is co-enzyme of catalase). On the other hand, relatively high concentrations of investigated elements may initiate free radical reactions (1). Maintaining the appropriate level of the analysed trace elements in blood and tissues of turkey hens is a prerequisite to ensure the optimal activity of antioxidative enzymes. Nevertheless, as indicated by literature data, their contents may enormously vary in the process of enhanced oxidation. As reported by Truchliński *et al.* (26), in the process of enhanced peroxidation during turkey hens' exposure to stress, the contents of trace elements ($Cu^{+2}$, $Fe^{+2}$) were decreasing, which was correlated with increasing activities of antioxidative enzymes. In turn Kędzierska *et al.* (15) demonstrated an increase in the concentration of selected minerals (selenium and zinc in particular) in erythrocytes of dialysed patients (dialysis induces strong oxidative stress in blood cells). Most likely, the disparity of elements contents may result from different stages of oxidation enhancement observed in different studies. At the initial stage of oxidation both the activity of enzymes and the concentration of these elements may be lower, but when the body activates its enzymatic system of antioxidative defence, as a result

of exposure to oxidative stress, the level of trace elements and antioxidative enzymes may gradually decrease because reserves of trace elements are being depleted along with an increasing intensity of enzymatic antioxidative reactions.

The use of linseed oil as a feed additive for turkey hens additionally contributed to changes of parameters determining the FRAP. The increased FRAP values resulted from a significant increase in vitamin E level.

Summing up, it may be concluded that the origin of dietary fat affects lipid metabolism and changes in redox status of blood and muscles of poultry. The introduction of linseed oil with high content of PUFAs n-3 to a feed mixture of turkey hens contributed to enhancement of lipid peroxidation processes, which was indicated by a significant increase in the concentrations of $H_2O_2$ and MDA in muscles, an increase in SOD activity, and by concentrations of vitamin C and minerals ($Cu^{+2}$, $Zn^{+2}$, $Fe^{+2}$, $Se^{+2}$). The results obtained point to the need of strengthening the antioxidative defence by the use of appropriate antioxidants.

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