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Oxidative stability of ostrich meat related to duration of linseed and lucerne supplementation to the bird's diet

Artur Jóźwik[1], Ewa Poławska[1], Żaneta Zdanowska-Sąsiadek[1], Paulina Lipińska[1], Magdalena Kawka[1], Dominika Guzek[2], Nina Strzałkowska[1]

[1]Institute of Genetics and Animal Breeding, Polish Academy of Sciences, 05-552 Jastrzębiec, Poland
[2]Warsaw University of Life Sciences, 02-776 Warsaw, Poland
aa.jozwik@ighz.pl

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Abstract

The effect of dietary linseed and lucerne supplementation on the oxidative stability of ostrich meat expressed by changes in concentrations of malondialdehyde (MDA) and glutathione (GSH), and in activity of superoxide dismutase (SOD), was studied. The feeding regimens were as follows: C – control group, L – 4% supplement of linseed, L-L45, L-L55, L-L65, and L-L75 – 4% supplement of linseed and supplement of lucerne added to the birds' diet at 45, 55, 65, and 75 kg b.w. The highest level of GSH was recorded in L-L65 group, whereas the highest activity of SOD was observed in C, L-L65 and L-L75 groups. Among all groups, the long-term linseed and lucerne supplementation reduced the antioxidant potential of ostrich meat, especially in L-L45 and L-L55 groups, which was reflected in the highest level of MDA and the lowest activity of SOD. Thus, the optimal results after linseed and lucerne supplementation with regard to ostrich meat oxidative stability were reported in groups L-L65 and L-L75, approximately three to four months prior to slaughter.

Keywords: ostrich, linseed, lucerne, feed additives, oxidative stability, meat.

Introduction

Ostrich meat is relatively rich in polyunsaturated fatty acids (PUFA) as compared to more traditional meats (8,9, 13-15, 19,20, 22, 24,25), and it therefore has lower oxidative stability and is more susceptible to oxidative processes. The oxidative deterioration of meat lipids negatively affects the nutritional value of the meat through formation of hydroperoxides to short chain aldehydes or other oxygenated compounds (6). It should be noted that malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) are recognised as markers of peroxidation in animal tissues since they participate in degradation of hydroperoxide products. There is a shortage of information about the oxidative stability of ostrich meat after dietary supplementation with n-3 fatty acids including plant oils as well as lucerne. Therefore, the aim of the study was to evaluate the effect of the duration of dietary supplementation with linseed and lucerne on the oxidative stability, expressed by changes of MDA, GSH, and SOD in ostrich meat.

Material and Methods

Animals, diets, and sampling. The study was conducted on 48 ostriches raised in 6 groups, from hatching to 12 months of age, on a commercial farm. After hatching until 5 months of age, the birds were reared together and fed a commercial ostrich starter diet (215 g/kg^{-1} crude protein and 2850 $kcal/kg^{-1}$ gross energy). From the age of 5 months, the birds were randomly allocated into groups and each group was randomly assigned to a different dietary regimen. Experimental diets were prepared on the basis of a control diet (150 g/kg^{-1} crude protein and 2550 $kcal/kg^{-1}$ gross energy) to which linseed and lucerne were added. The feeding regimens were as follows: C – control diet, L – 4% supplement of linseed, L-L45, L-L55, L-L65, and L-L75 – 4% supplement of linseed and supplement of lucerne added to the birds' diet at 45, 55, 65, and 75 kg b.w. The diets were fed for approximately 6, 5, 4, and 3 months prior to slaughter.

The ostriches were slaughtered in the European-Union approved commercial abattoir at 12 months of

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age when their body weight had reached approximately 89 ± 9.2 kg. The ostriches were fasted for 24 h prior to electrical stunning, and their bleeding and evisceration were performed according to standard slaughtering procedures for ostriches (18). The carcasses were halved, and legs and drumsticks were removed and cooled at *ca*. 4ºC for 24 h before deboning. Meat samples were taken from the *M. gastrocnemius pars interna* (GN) of the left side of the carcasses and transported to the laboratory in insulated containers, where they were preserved at -20ºC until further analyses.

Determination of malondialdehyde. The lipid peroxidation in muscle homogenates was determined by the measurement of the formation of MDA. In brief, 0.2 mL of the homogenate was added to 0.8 mL of a solution containing 15% (w/v) TCA, 0.375% (w/v) thiobarbituric acid, and 0.25 mol/L hydrochloric acid. The protein precipitate was removed by centrifugation (10 min by 10 000 rpm), and the supernatants were transferred to glass test tubes containing 0.02% (w/v) butylated hydroxytoluene (BHT) to prevent further peroxidation of lipids during subsequent steps. After that the samples were heated for 15 min at 100°C, cooled, and centrifuged to remove precipitant protein. The absorbance of each sample was read at 586 nm by spectrophotometer (Cary 50 Bio UV/ Vis). The MDA analysis was performed at least in triplicates and the results were presented in µmol/g of meat.

Determination of superoxide dismutase. Total SOD activity in tissue homogenates was assayed based on the inhibition of a superoxide induced NADH oxidation with a commercial kit (Dojindo Lab., Kumamoto, Japan). A decrease in the rate of NADH oxidation depended on the enzyme concentration, and saturation levels were attainable by recording the corresponding readings from spectrophotometer set at 520 nm wavelength. The enzyme activity analysis was performed at least in triplicates and the results were presented in U/g meat.

Determination of reduced glutathione. A commercial kit (ELISA kit for glutathione, Uscn Life Science Inc.) was used for determination of GSH. The analysis was based on the development of a yellow colour when 5,5′-dithiobis (2-nitrobenzoic acid) was added to sulphydryl compounds. The colour was fairly stable for about 10 min and the reaction was little affected by variation of temperature (22°C) by ratio 200 rpm. The reaction was read in Hybrid Multi-Mode Microplate Reader (Synergy 4) at 412 nm. The stage of GSH analysis was performed at least in triplicates and the results were presented in SH group concentration (µmol/L).

Statistical analysis. The statistical analysis was performed with the Statistica software (StatSoft ver. 9.0). The analysis of variance (ANOVA) was used to determine the influence of linseed and lucerne supplementation on oxidative parameters. The significance of differences between means was tested with the Tukey post-test.

Results

The influence of dietary supplement of linseed and lucerne on concentrations of MDA, GSH, and SOD in the gastrocnemius muscle is presented in Figs 1-3. When a diet supplemented with linseed and lucerne was fed to the ostriches for five and six months, the concentration of MDA achieved the highest level (13 and 12.1 µmol/g respectively), as compared to control group (6 µmol/g) (Fig. 1). No differences regarding the concentration of MDA between birds from L-L65 and L-L75 groups and control group were observed. Fig. 2 shows changes in GSH level in the muscle. An increase in GSH concentration in groups: L-L65 and L-L75 was found. The level of GSH was the highest in L-L65 group (183 µmol/g) and statistically significant ($P<0.001$) compared to the other groups. In L-L75 group, the GSH level was higher (144 µmol/g) as compared to other groups, although the differences were not significant.

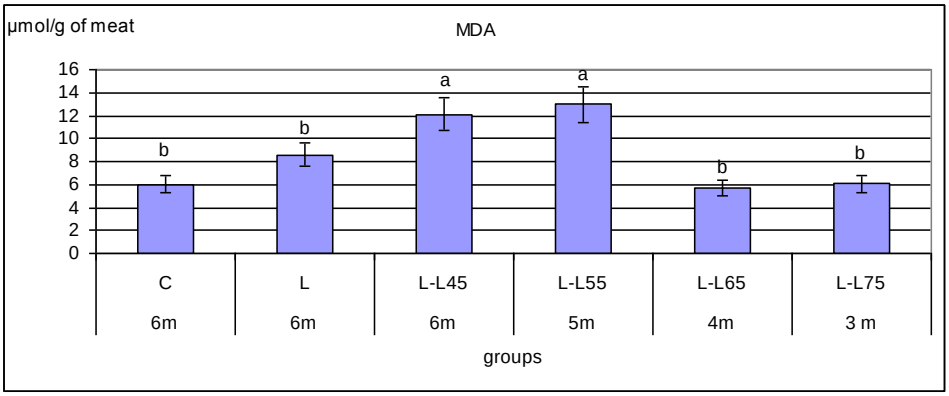


Fig. 1. The changes of the level of malondialdehyde (MDA) in ostrich meat in relation to the duration of dietary supplementation with linseed and lucerne (C – control group, L – 4% supplement of linseed, L-L45, L-L55, L-L65 and L-L75 – 4% supplement of linseed and supplement of lucerne applied in the doses of 45, 55, 65, and 75 kg b.w., 6m, 5m, 4m, 3m –months prior to slaughter)
a-b – the values marked with different letters vary significantly ($P < 0.05$)

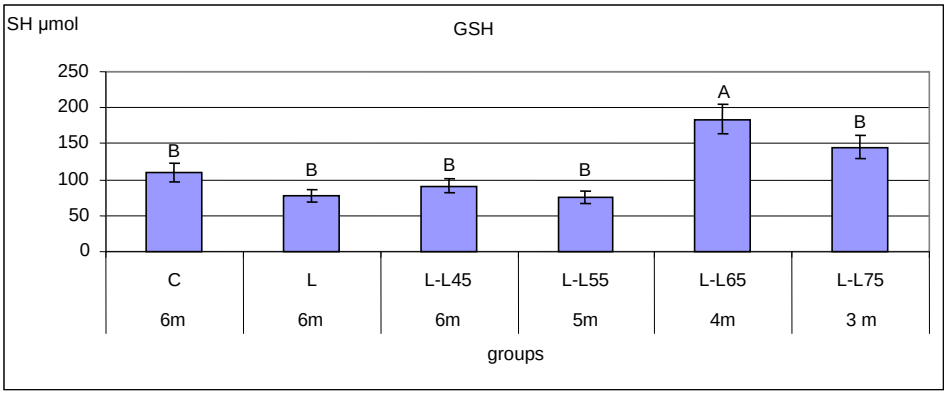


Fig. 2. The changes of the level of reduced glutathione (GSH) in ostrich meat in relation to the duration of dietary supplementation with linseed and lucerne (C – control group, L – 4% supplement of linseed, L-L45, L-L55, L-L65, and L-L75 – 4% supplement of linseed and supplement of lucerne applied in the doses of 45, 55, 65, and 75 kg b.w., 6m, 5m, 4m, 3m –months prior to slaughter)
A-B – the values marked with different letters vary significantly ($P < 0.01$)

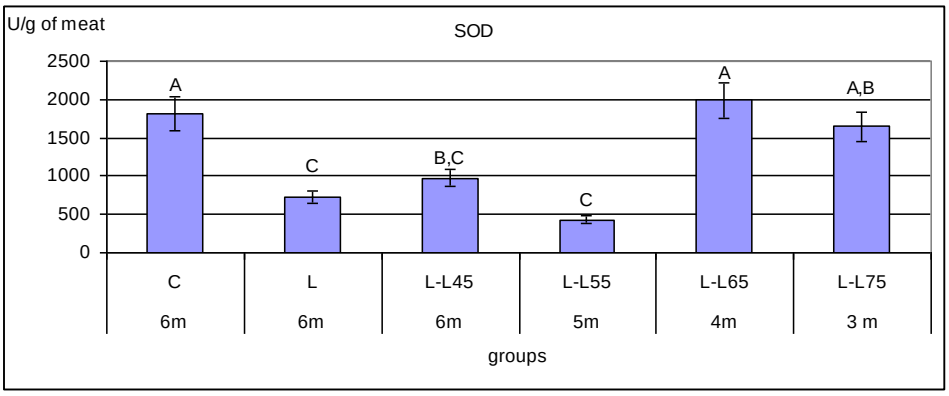


Fig. 3. The changes of the activity of superoxide dismutase (SOD) in ostrich meat in relation to the duration of dietary supplementation with linseed and lucerne (C – control group, L – 4% supplement of linseed, L-L45, L-L55, L-L65, and L-L75 – 4% supplement of linseed and supplement of lucerne applied in the doses of 45, 55, 65, and 75 kg b.w., 6m, 5m, 4m, 3m –months prior to slaughter)
A-B-C – the values marked with different letters vary significantly ($P < 0.01$)

The long-term supplementation with linseed and linseed with lucerne decreased the activity of SOD (Fig. 3). This change was statistically significant ($P < 0.01$) in the L, L-L 45, and L-L55 groups.

The activity of SOD was higher in C, L-L65, and L-L75 groups (1818, 1987, and 1645 µmol/g respectively) than in groups with long-term supplementation with linseed and linseed with lucerne (725, 975, and 425, in L, L-L45 and L-L55 respectively).

Discussion

The type of diet has a strong impact on the oxidative stability of meat. A large proportion of high-PUFA in meat leads to its oxidative deterioration. The high intake of PUFA from plant oils used in poultry diet is a very important factor which increases the demand for antioxidants (19, 20, 23). In our study, long term linseed supplementation in ostrich diet decreased the antioxidant potential of meat expressed by, *i.a.*, higher level of MDA and lower activity of SOD. However, a shorter period of feeding regimen (for three and four months before slaughter, L-L75 and L-L65 groups) did not influence the concentration of MDA, which remained on a similar level as in control group. It should be emphasised that the MDA assay is a practical method for the determination of food lipid peroxidation (1, 4). According to Delles *et al.* (10), lipid peroxidation process probably starts immediately after slaughtering and under certain post-slaughtering circumstances. The biochemical changes during the conversion of muscle to meat such as post mortem aging cause the destruction of the balance between prooxidant and antioxidant factors. The rate and extent of lipid peroxidation in muscle tissues appears to be dependent on the degree of muscle tissue damage under pre-slaughtering circumstances, for example stress and physical injury, and post-slaughtering events, for instance pH, carcass temperature, shortening, and tenderising techniques such as electrical stimulation (2, 21). Anjum *et al.* (3) showed that broiler chicken diet supplemented with extruded flaxseed leads to an increase in MDA from 0.46 to 1.05 nmol/mg of meat. These authors reported the highest amount of MDA in the group fed the diet with 15% of flaxseed and this group also displayed the lowest lipid stability and

maximum free radical scavenging activity as compared to other ones.

In our study, the highest level of MDA was obtained in groups fed linseed and lucerne for the longest period (5 to 6 months). It seems that supplementation of ostrich diet with linseed and lucerne for less than 5 months prior to slaughter is the most beneficial from both economical and biological point of view. The reason might be that high flaxseed content in animal diet results in the raising of the free radical scavenging activity (7). Bianchi *et al.* (5) suggested that supplementation with lucerne and whole linseed had a positive effect on lipids composition and susceptibility to lipid oxidation of rabbit meat and meat products.

In birds receiving the long-term linseed and lucerne supplementation, especially in L-L55 group, a decreased antioxidant capacity of meat was observed, which was reflected in the highest concentration of MDA and the lowest activity of SOD. Renerre *et al.* (23) reported that in turkeys fed linseed oil, the level of antioxidant enzymes (SOD, GR) in the thigh and breast muscles increased, with the higher level found in the thigh muscle. It should also be emphasised that muscles can be categorised into different metabolic types: oxidative (red) or glycolytic (white), based on their chemical composition and enzyme activities (17). The oxidative muscles have more mitochondria and a higher content of myoglobin than the glycolytic ones. They mainly use fatty acids as substrates and have low activities of ATPase and phosphorylase, while the glycolytic muscles use mainly glycogen as an energy source and have higher activities of the estimated parameters (11,12). It is generally considered that oxidative muscles show higher activities of antioxidants (GSH) and enzymes such as SOD and other glutathione enzymes (GPx, GR etc.) than glycolytic muscles (26, 27).

In our study, the inclusion of linseed and lucerne into ostrich diet at a later stage of feeding allowed for the beginning of the adaptation processes in birds, which is associated with the elimination of the highest level of radical oxygen species (ROS). These interdependences revealed intensive mobilisation of the ostrich organism leading to the neutralisation of ROS. This mechanism also protected unsaturated fatty acids in cell membranes. Similar trends for SOD and GSH may have a synergistic effect because SOD reduces free radical damages to hydrogen peroxide.

In conclusion, it should be concluded that the optimal duration of ostrich diet supplementation with linseed and lucerne, with regard to meat oxidative stability expressed by changes of MDA, GSH, and SOD, was approximately three- to four months prior to bird's slaughter (groups L-L65 and L-L75). Further research is needed, with an aim to improve the oxidative stability of ostrich meat enriched with n-3 fatty acids and selected antioxidants, like vitamin E or selenium.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this paper.

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Animal Rights Statement: Ethical approval was obtained from the Local Ethics Commission.

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