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THE EFFECT OF DIETARY FISH OIL ON THE LIPID AND FATTY ACID COMPOSITION AND OXIDATIVE STABILITY OF GOOSE LEG MUSCLES

Dmytro Yanovych[1], Anna Czech[2], Zwenyslava Zasadna[1]

[1]Instrumental Methods of Control Laboratory, State Scientific-Research Control Institute of Veterinary Preparations and Feed Additives, 79019 L'viv, Ukraine
[2]Department of Biochemistry and Toxicology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
Corresponding author: annaczech@poczta.fm

Abstract

The aim of this study was to evaluate the effect of fish oil added to feed mixtures for geese on their growth, lipid profile and antioxidant status of leg muscles. Ninety-six Arzamas geese were randomly divided into two groups and fed from 30 to 60 days of life standard mixtures containing soybean oil (control) or fish oil at 5%. The addition of fish oil to the feed mixtures for geese increased the content of EPA and DHA, and decreased the ratio of *n-6* to *n-3* fatty acids in fat of breast muscles compared to birds fed the control mixture. It also resulted in a considerable increase in the content of phospholipids and decreased the content of glycerol, free fatty acids as well as triacylglycerols and esterified cholesterol. The type of fat source had no effect on the content of lipid peroxidation products and the activity of antioxidant enzymes.

Key words: goose, fish oil, leg muscles, lipids, fatty acids, antioxidation

The use of essential fatty acids (linoleic and α-linolenic acids) in animal and human nutrition is, to a large extent, connected with their conversion to unsaturated fatty acids, which on the one hand contribute to improving the quality of cell membranes by incorporating into phospholipids, and, on the other hand, are applied in the synthesis of eicosanoids (prostaglandins, leukotrienes), i.e. biologically-active substances characterized by a wide spectrum of regulatory activities (Saldeen et al., 1998).

In the 1970s, it was confirmed that Eskimos and inhabitants of Greenland do not suffer from ischaemic heart diseases, which nowadays constitute ca. 50% of all diseases in highly-developed countries. This has been attributed to the anticholesterolemic action of *n-3* polyunsaturated fatty acids, in particular EPA and DHA. A rich

source of these acids is fish oil and fat of mammals found in the north, which are the main components of the Eskimo diet (Masters, 1996).

Fish oil, like vegetable oil, is a valuable source of energy. In the fat from whale liver, commonly known as whale oil, the *n-3* polyunsaturated fatty acids (PUFA) constitute ca. 30% of total fatty acids. Whilst vegetable oils (linseed, rapeseed, soybean) provide only ~10% of the *n-3* PUFA, which is insufficient to cover the metabolic demands of the body. Hence, a number of experiments have been conducted in recent years to increase the amount of *n-3* polyunsaturated fatty acids in food products of animal origin, including poultry products (eggs or meat), through the addition of fish oil to feedstuffs among others (Woods and Fearon, 2009). Studies have shown that after feeding laying hens with 3–5% of fish oil, the content of EPA and DHA in egg yolk fat increased ca. 2–6 times (Villaverde et al., 2006), which significantly improved their nutritional value. Similar results were obtained when analysing the content of *n-3* PUFA in thigh muscles of broiler chickens fed diets supplemented with fish oil (Hoffman et al., 2005).

The fact that the available literature provides sparse data concerning the effect of fish oil in goose feeding and the positive results obtained in experiments with hens (laying hens, broilers) might constitute the basis for conducting research and increasing the accumulation of *n-3* polyunsaturated fatty acids in their muscles. Hence, the aim of this study was to evaluate the effect of fish oil on growth of geese as well as on lipid and antioxidant profile of their leg muscles.

Material and methods

The experimental material consisted of 30-day-old Arzamas geese reared until 60 days of life. The experiment was conducted on 96 geese randomly divided into two groups, with 48 geese per group (4 replications with 12 birds), taking into account their body weight.

The birds were reared under standard hygienic conditions according to the recommendations of Faruga and Jankowski (1996). Throughout the experiment, birds from all groups were fed *ad libitum* diets balanced according to the recommendations of the National Research Council (NRC, 1994) for the appropriate period of rearing: starter from 1 to 29 days and grower from 30 to 60 days of life (Table 1). Birds had free access to drinking water. The mixtures used in goose feeding were based on maize, wheat and barley.

Birds from group I (control) received standard mixtures, in which the source of fat was soybean oil (5%). Birds from group II received the same mixture, but with 5% fish oil replacing soybean oil. During the experiment, body weight (on each final day of week of life), survival rate and feed intake were recorded.

The geese were slaughtered by decapitation (12 geese from each group, three per replication) on day 60 of life. During dissection, samples of leg muscles were collected for analytical research. The thigh samples from each bird were mixed and packed in plastic bags (approximately 20 g per bag) and immediately stored at –20ºC for three months.

Table 1. Composition and nutrient content of experimental mixture for geese (g/kg)

Item	Days of fattening	
	1–29	30–60
Maize	320	135
Wheat	308	200
Barley		420
Wheat bran		80
Sunflower meal	140	50
Fodder yeast	100	
Limestone	25	35
Fish meals	10	5
NaCl	2	5
Soybean oil/fish oil		50
Premix[1)]	20	20
Calculated[2)] in 1 kg of mixture:		
Metabolizable energy (MJ)	2797	2629
Analysed nutrients in 1 kg of mixture (g):		
total protein	203	164
crude fat	26	58
crude fibre	50	65
calcium	10.6	10.1
total phosphorus	7.8	6.8
sodium	2.7	2.4
Lys	9.5	7.6
Met + Cys	6.7	5.6

[1)]1 kg contains: vitamin A (retinol acetate) – 400,000 IU, vitamin D_3 (cholecalciferol) – 100,000 IU, vitamin E (alpha-tocopherol, 50%) – 1,300 mg, vitamin K_3 – 200 mg, vitamin B_1 – 66 mg, vitamin B_2 – 230 mg, vitamin B_{12} – 800 mg, folic acid – 90 mg, niacin – 1,800 mg, calcium pantothenate – 400 mg, choline – 20,000 mg, iron – 2,300 mg, zinc – 2,500 mg, manganese – 3,000 mg, copper (copper sulfate × $5H_2O$, 24.5%) – 300 mg, iodine – 30 mg, cobalt – 40 mg, lysine – 33 g, methionine – 40 g, calcium – 290 g, digestible phosphorus – 110 g.

[2)]Metabolizable energy corrected for zero nitrogen balance – ME_N (kcal/kg) = 14.7 × CP + 32.9 × EE + 17.2 × Starch + 14.9 × Sugars.

Analysis of mixtures

Samples of the diets were collected for analysis twice during each feeding period. The mixtures were analysed for nutrient content: crude protein (CP, method 976.06), ether extract (EE, 973.18), crude fibre (CF, 962.10), and amino acids lysine, methionine and cysteine (994.12) according to AOAC (2000) protocols. The mineral solution for determination of the minerals was prepared by wet ashing and after the appropriated dilutions, the total phosphorus content was determined with the colorimetric procedure (method 965.17), whereas calcium content (968.08) using an atomic absorption spectrophotometer, and sodium content using a flame spectrophotometer (968.08). Lipids in the examined feed mixtures were extracted with a chloroform-methanol mixture according to Folch et al. (1957). Fatty acid composition of fat (% of total fatty acids) in experimental mixtures is given in Table 2.

Table 2. Fatty acid composition (% of total fatty acids) of experimental mixtures

Fatty acid	Days of fattening		
	1–29	30–60	
		soybean oil	fish oil
C12:0	0.01	0.02	0.01
C14:0	0.75	0.58	0.82
C15:0	0.11	0.03	0.02
C16:0	21.75	25.01	17.42
C16:1	3.51	6.21	7.52
C17:0	0.97	0.02	0.03
C17:1	0.71	0.11	0.15
C18:0	6.55	5.32	6.41
C18:1	28.15	39.15	33.96
C18:2 *n-6*	18.06	17.18	7.59
C18:3 *n-3*	5.79	1.09	1.05
C20:1	4.96	0.49	9.88
C20:2	0.02	0.14	0.45
C20:3	0.44	0.09	0.68
C20:4 *n-6*	5.15	0.03	3.16
C20:5 *n-3*	1.21	2.07	4.02
C22:1	0.01	0.04	0.03
C22:2 *n-6*	1.22	0.03	0.74
C22:6 *n-3*	0.27	2.39	0.27
SFA	30.14	30.98	24.71
MUFA	37.34	46	51.54
PUFA	32.16	23.02	23.75
n-6	24.43	17.24	11.49
n-3	7.27	5.55	11.13
n-6/n-3	3.36	3.11	1.03

Determinations of lipids and fatty acid profile in leg muscles

Lipids of the examined goose tissues were extracted with a 2:1 chloroform-methanol mixture according to Folch et al. (1957). The lipid fractions (phospholipids, mono- and diacylglycerols, free cholesterol, free fatty acids, triacylglycerols, esterified cholesterol) were separated using thin-layer chromatography with Silica Gel in the eluent system: hexane-diethyl ether-glacial acetic acid (70:30:1), according to Kates (1975).

The composition of fatty acids in fat of goose tissues was determined by gas-liquid chromatography. Methyl esters of fatty acids were obtained by direct pre-extraction with methanol in closed glass ampoules in a thermostat at a temperature of 65°C for 24 hours in the medium of 3% of hydrochloric acid in absolute methanol. The separation of methyl esters of fatty acids was conducted using a "Chrom – 5" chromatograph (Czech Republic) with a flame ionization detector, on a column (2.5 m in length, 4 mm in diameter, Chromosorb sorbent 60–80 mesh, stationary phase – polyethylene glycol succinate, evaporator temperature 200°C, column tem-

perature 185°C, hydrogen flow rate – 30 ml/min, air flow rate – 400 ml/min). The fatty acid peaks obtained on the chromatogram were identified with the use of standard fatty acid esters and calculated based on logarithmic law of homologous series.

Determinations of lipid peroxidation products in leg muscles

Lipid hydroperoxide content was determined by the thiocyanate method according to Tagashira and Ohtake (1998). The reagent was prepared by mixing equivalent amounts of a methanolic solution of KSCN (3%) and a ferrous-ammonium sulphate solution (45 mM in 0.2 mM HCl). Solutions absorbance measured at 500 nm was recorded. The lipid peroxide value was determined using a calibration curve prepared with standard cumene hydroperoxide.

Malondialdehyde

The MDA concentration was checked by measuring the UV absorbance at 245 nm and using the extinction coefficient of 13 700 (Esterbauer et al., 1984). The working solution was diluted to the appropriate conditions and reacted with the TBN phosphoric acid solution to form the standard curve.

Determination of antioxidative enzyme activities in leg muscles

Superoxide dismutase

The enzymatic activity of SOD was determined according to the method of Mirsa and Fridrovich (1972) based on the inhibition of adrenaline self-oxidation into an adrenochrome by the SOD enzyme. The absorbance was measured spectrophotometrically at a wavelength of 320 nm; the enzymatic activity was plotted on the basis of absorbance increase in the experimental and control samples.

Glutathione peroxidase

The incubation mixture contained 1970 µl of the assay mixture and 30 µl of the filtered sample. The activity of glutathione peroxidase (GSH) was measured at 22°C, recording the oxidation of NADPH by the decrease in absorbance of the incubation mixture at 340 nm during 3 min (Günzler and Flohé, 1985). The GSH activity was expressed as nmol of oxidized NADPH min^{-1} mg^{-1} protein.

The concentration of proteins in homogenate fractions was determined with the method of Lowry et al. (1951).

Statistical methods

Statistical analysis was performed using Statistica v. 6.1 package. Significance of the difference between mean values was estimated by means of one-way ANOVA, assuming the significance level at 0.05 and 0.01.

Results

Table 3 presents production performance of geese receiving supplemental soybean oil (control) or fish oil. The initial body weight of the birds from both groups

was at a similar level. It should be noted, however, that the geese fed mixtures with fish oil were slightly lighter, by ca. 3%. This is crucial, as after 30 days of receiving fish oil, the geese from this group were characterized by higher body weight (by ca. 8.5%) and by higher daily weight gains than the birds from the control group. The differences obtained were, however, statistically insignificant (P>0.05).

Table 3. Production performance parameters

Item	Feeding groups		
	soybean oil	fish oil	significance
Body weight (g):			
at the beginning of the experiment	1847±12.6	1824±8.8	NS
at the end of the experiment	3324±6.5	3611±75.0	NS
Average daily gains (g/bird):	49.2±8.99	59.6±7.25	NS

NS: not significant.

Table 4 presents data concerning the composition of lipids and their fractions. The addition of fish oil did not result in any changes in the total content of lipids or free cholesterol in bird muscles (P>0.05). The content of phospholipids was significantly higher in birds supplemented with fish oil, and the differences reached ca. 22% (P≤0.01). An opposite relationship was observed for concentrations of mono- and diacylglycerols (P≤0.05), free fatty acids (P≤0.05) as well as triacylglycerols (P≤0.01) and esterified cholesterol (P≤0.05).

Table 4. Total lipids and lipid fractions in goose muscles

Item	Feeding groups		
	soybean oil	fish oil	significance
Total lipids (g %)	2.14±0.09	2.40±0.08	NS
Lipid fractions (%):			
phospholipids	20.89±0.67	25.53±0.84	**
mono and diacylglycerols	18.14±0.49	16.5±0.42	*
free cholesterol	19.09±0.37	20.73±0.58	NS
free fatty acids	10.63±0.25	8.93±0.38	*
triacylglycerols	8.12±0.26	7.36±0.14	**
esterified cholesterol	23.14±0.58	20.95±0.30	*

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

The fish oil addition led to a change in fatty acid (FA) composition in goose muscles (Table 5). Taking into account the content of saturated fatty acids (SFA), it was noted that in the muscles of geese receiving dietary fish oil, the total content of SFA increased by as much as 29% and the total content of all the determined saturated fatty acids was significantly higher (P≤0.01) in relation to the control group. A similar relationship as for SFA was observed for MUFA, yet the difference was smaller at 13%. In turn, the content of PUFA in leg muscles of the geese receiving dietary fish oil was almost 30% lower compared to the control group. Nevertheless,

attention should be paid to the content of *n-3* fatty acids, which was as much as 74% higher in geese from group II compared to the control group. The ratio of *n-6* to *n-3* acids is very significant from the dietary point of view. In the mixture fed to geese in group II, the ratio of these acids (1.03) was three times lower than in mixtures for the control group (Table 2). The ratio of *n-3* to *n-6* in leg muscles amounted to 3.89 in control geese and was considerably lower (1.07, $P \leq 0.05$) for geese supplemented with fish oil. It should also be noted that the content of EPA and DHA was significantly higher ($P \leq 0.01$) for birds receiving dietary fish oil (2.5-fold and 13.3-fold, respectively).

Table 5. Fatty acid profile (% of total fatty acids) of goose leg muscles

Fatty acid	Feeding groups		
	soybean oil	fish oil	significance
C12:0	0.12±0.01	0.19±0.01	*
C14:0	1.24±0.05	1.67±0.04	**
C15:0	0.70±0.05	1.02±0.08	**
C16:0	14.85±0.84	17.23±0.22	**
C16:1	3.09±0.16	6.11±0.22	**
C17:0	0.87±0.02	1.89±0.37	**
C17:1	1.00±0.05	1.18±0.06	NS
C18:0	10.79±0.68	14.81±0.81	**
C18:1	23.94±1.13	24.82±0.26	NS
C18:2 *n-6*	28.27±0.92	12.10±2.05	**
C18:3 *n-3*	5.59±0.19	1.09±0.23	**
C20:1	0.19±0.02	0.08±0.02	**
C20:2	0.17±0.01	0.16±0.02	NS
C20:3	0.55±0.06	0.10±0.01	**
C20:4 *n-6*	2.43±0.16	0.43±0.06	**
C20:5 *n-3*	0.77±0.12	1.85±0.08	**
C22:1	0.50±0.05	0.23±0.09	NS
C22:2 *n-6*	1.52±0.09	0.73±0.03	**
C22:3	0.75±0.03	0.36±0.05	**
C22:4	0.19±0.04	1.83±0.11	**
C22:5 *n-3*	1.28±0.03	5.15±0.48	**
C22:6 *n-3*	0.45±0.02	6.00±0.78	**
C24:0	0.74±0.03	0.97±0.04	**
SFA	29.31±4.02	37.78±5.31	*
MUFA	28.72±9.45	32.42±8.97	NS
PUFA	41.97±7.01	29.8±3.05	**
n-6	32.22±12.81	13.26±5.09	**
n-3	8.09±2.01	14.09±2.88	*
n-6/n-3	3.89±0.82	1.07±0.59	*

*$P \leq 0.05$; **$P \leq 0.01$; NS: not significant.

In the present experiment, no statistically significant differences were noted, however, for the content of both lipid peroxidation products (lipid hydroperoxides,

malonaldehyde) and enzyme activities (superoxide dismutase, glutathione peroxidase) (Table 6).

Table 6. Lipid peroxidation and antioxidant enzyme activities in goose leg muscles

Item	Feeding groups		
	soybean oil	fish oil	significance
Lipid hydroperoxides (mM H_2O_2 mg protein)	1.62±0.10	1.66±0.10	NS
Malonaldehyde (nmol/g)	6.85±0.18	6.94±0.19	NS
Superoxide dismutase (U/g)	5.45±0.12	5.59±0.16	NS
Glutathione peroxidase (nmol GSH/mg protein)	3.95±0.18	4.49±0.17	NS

NS: not significant.

Discussion

Oil from sea fish and fats from edible sea products constitute a very rich source of *n-3* PUFA isomers. Undoubtedly, in recent years the focus of interest for zootechnicians, food technologists, biologists and physicians has been to find a method that would enable modifying fatty acid profile of fat of slaughter animals in order to improve their nutritive value. One such method involves the addition of fish oil to animal diets. On the one hand, this fat is a perfect source of energy by contributing to the improvement of production performance (He et al., 2007) and on the other hand, it is a factor that might increase the content of EPA (C20:5, *n-3*) and DHA (C22:6, *n-3*) acids in meat and other products of animal origin (Villaverde et al., 2006). The beneficial influence of fish oil addition on the rearing performance of broiler chickens was observed by He et al. (2007). The study by Fritsche et al. (1991) also indicates an increase in body weight gains in chickens supplemented with various sources of fat. However such significant relationships were not observed in present experiment with geese.

Studies conducted with various animal species confirm a significant effect of fish oil on fatty acid profile in their tissues. For example, EPA and DHA levels in hen eggs were found to increase significantly after adding fish oil to hen diets. In the present research, we also noted a significant increase in the content of *n-3* fatty acids, including both EPA and DHA, as well as phospholipids in leg muscles of geese receiving dietary fish oil. This corresponds with the fact that DHA constitutes a basic ingredient of neuron cell membrane phospholipids, which increases their survival rate and resistance to the effects of detrimental factors. Similar results were obtained in an experiment with broilers (Schreiner et al., 2005).

A decrease in both free and esterified cholesterol was observed in the leg muscles of geese fed dietary fish oil, compared to the control group. Similar results were obtained in a study which examined the effect of cinnamon oil in broiler chicken nutrition (Ciftci et al., 2010).

The addition of fish oil also contributes to a change in the content of MUFA and individual monounsaturated fatty acids. According to Ayerza et al. (2002), a

decrease in the content of oleic (18:1) and palmitoleic (16:1) acids might be due to the presence of PUFA which suppress the activity of Δ9-desaturase, i.e. an enzyme which stimulates the synthesis of MUFA. Such an interaction between MUFA and PUFA was recorded in broiler experiments by Ayerza et al. (2002) and Saleh et al. (2009). Meanwhile, studies by O'Keefe et al. (1995) showed an increase of MUFA content in both breast and leg muscles of broilers receiving 4, 8 and 12% supplemental fish meal. Likewise, Hoffman et al. (2005) observed an increase in the content of MUFA in ostriches receiving dietary fish oil.

A study with broilers (Saleh et al., 2009) demonstrates a significant increase in the content of omega-3 fatty acids ($P<0.01$) in birds supplemented with 6% of dietary fish oil. The increase in *n-3* fatty acids content corresponded with a reduced *n-6:n-3* ratio of polyunsaturated fatty acids in this group compared to the control group ($P<0.01$). Similar relationships were observed in the leg muscles of geese in the present experiment, which was directly related to a decrease in *n-6* fatty acids and an increase in *n-3* fatty acids. A competitive interdependence between *n-6* and *n-3* fatty acids and keeping the balance between products of their conversion in an organism result from the mutual relation between linoleic and linolenic acids in dietary fats. Improper ratios between the content of 20- and 22-carbon fatty acids and *n-3* and *n-6* PUFAs in phospholipids of biological membranes might be one of the causes of cardiovascular diseases and ischaemic heart diseases (Frenoux et al., 2001).

In order to assure the appropriate absorption of polyunsaturated fatty acids, they should be consumed together with vitamin E, which is a natural antioxidant (Simopoulos et al., 1999). Antioxidants are also significant in the improvement of fat quality as they reduce peroxidation processes. Fish oil is also a rich source of natural antioxidants (Sikora et al., 2008). In the leg muscles of geese supplemented with fish oil, both the content of lipid peroxidation products and the activity of antioxidant enzymes were at a similar level compared to the control group, which indicates that the oil was of good quality.

In conclusion, it can be stated that the addition of fish oil to feed mixtures for geese contributed to an increase in the content of EPA and DHA, and to a decreased ratio of *n-6* to *n-3* fatty acids in leg muscle lipids. It also resulted in a significant increase in the content of phospholipids and a decrease in the content of glycerols, free fatty acids as well as triacylglycerols and esterified cholesterol. This type of fat source had no effect on the content of lipid peroxidation products and the activity of antioxidant enzymes.

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DMYTRO YANOVYCH, ANNA CZECH, ZWENYSLAVA ZASADNA

Wpływ oleju rybnego na profil lipidowy i skład kwasów tłuszczowych oraz stabilność oksydacyjną mięśni nóg gęsi

STRESZCZENIE

Celem badań była ocena wpływu oleju rybnego dodawanego do pasz dla gęsi na ich wzrost, profil lipidowy oraz status antyoksydacyjny w mięśniach nóg gęsi. 96 gęsi Arzamas podzielono losowo na dwie grupy. Ptaki były żywione od 30. do 60. dnia życia standardowymi mieszankami zawierającymi olej sojowy (grupa kontrolna) lub olej rybny na poziomie 5%.

Olej rybny dodawany do pasz dla gęsi przyczynił się do zwiększenia udziału EPA i DHA i zmniejszenia zdecydowanej przewagi kwasów z rodziny *n-6* do kwasów z rodziny *n-3* w tłuszczu. Spowodował również znaczny wzrost zawartości fosfolipidów, a spadek zawartości wolnego i zestryfikowanego cholesterolu. Rodzaj użytego tłuszczu nie wpłynął na zawartość produktów peroksydacji lipidów oraz aktywność enzymów antyoksydacyjnych w mięśniach nóg gęsi.