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The effect of dietary oil seeds on the fatty acid profile and metabolism in ostrich liver*

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The study was carried out on 40 ostriches randomly allocated into five groups and each group was randomly assigned to a different dietary treatment. Experimental diets were made on the basis of a control diet, where a part of the gross energy was replaced with energy from linseed (4 or 8% - treatment L4 and L8, respectively) or rapeseed (5 or 10% - treatment R5 and R10, respectively). The ostriches were slaughtered at 12 months of age and within 30 minutes after slaughter liver samples (50 g) were taken.

The results indicate that the linseed supplementation, especially 4%, to the ostrich diet improves the nutritional value of ostrich liver by decreasing the n-6/n-3 ratio and by increasing the PUFA/SFA ratio. It makes ostrich liver more valuable for the production of meat products e.g. pates. The rapeseed supplementation to the ostrich diet has no influence on the fatty acid profile of ostrich liver compared to the control diet. Dietary supplementation by linseed changes the fatty acid metabolism in ostrich liver.

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Over the last several years there has been a growing interest in ostrich farming worldwide [Cooper and Horbanczuk 2004, Horbanczuk *et al*. 2004, 2007, 2008, Poławska *et al.* 2011, 2013a]. One of the reasons of this interest is that these birds provide valuable products especially meat, skin, feathers and eggs [Horbanczuk *et al*. 1998, Sales and Horbanczuk 1998, Sales *et al*. 1999, Cooper *et al*. 2007, 2008, Kawka *et al*. 2012]. Moreover, ostrich meat by-products like stomach and liver are also used for daily consumption and production of, among others, traditional meat products such as pates [Hoffman and Mellett 2003]. In their research, it was shown that pâtés from ostrich liver can be a valuable option for the industry, which is mainly putting fresh meat and meat products into the market. However, there is a lack of information about the nutritional value, and more specific on the fatty acid profile, of ostrich liver. Thus, the aim of this study was to determine the fatty acid profile of ostrich liver, and possible improvements in its fatty acid profile by different diets.

Material and methods

Animals, diets and sampling

The study was carried out on 40 ostriches (Struthio camelus var. domesticus) raised on a commercial farm in Stypułów in western Poland, which is under scientific supervision of the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences. Ethical clearance was obtained from the Local Ethical Commission (No 27/2009).

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) and had free access to water during the entire study period. From the age of 5 months (ca. 40 kg BW), birds were randomly allocated into five groups and each group was randomly assigned to a different dietary treatment. Experimental diets were made on the basis of a control diet (150 $g.kg^{-1}$ crude protein and 2550 $kcal.kg^{-1}$), where a part of the gross energy was replaced with energy from linseed (4 or 8% - treatment L4 and L8, respectively) or rapeseed (5 or 10% – treatment R5 and R10, respectively). All diets were iso-protein and iso-energetic. The composition of the experimental diets were presented earlier in Poławska *et al*. [2012]. The fatty acid profile of the different diets is presented in Table 1.

The ostriches were slaughtered in an European-Union approved commercial abattoir for cattle and pigs in Wolbrom (Poland) at 12 months of age when their live weight had reached on average 96.3±5.5 kg, as previously described in Poławska *et al*. [2012]. Within 30 minutes after slaughter liver samples (50 g) were taken and transported to the laboratory in insulated containers. The samples were homogenized and chemical composition was determined. For fatty acid analysis, 5g samples were taken and maintained at -20°C until analysis.

Table 1. Fatty acids profile of the diets (g/100g FAME)

Fatty acid	Diet/Group[1]				
	C	L4	L8	R5	R10
Σn-6	39.86	36.7	33.35	39.25	34.18
Σn-3	6.38	18.68	28.27	7.00	7.87
PUFA	46.62	56.15	62.00	46.86	42.52
MUFA	31.73	23.91	20.71	35.21	41.35
SFA	21.65	19.94	17.29	17.93	16.13
n-6/n-3	6.25	1.96	1.18	5.61	4.34
PUFA/SFA	2.15	2.82	3.59	2.61	2.64

[1]C – control group; L4 – diet with 4% of linseed supplementation; L8 – diet with 8% of linseed supplementation; R5 – diet with 5% of rapeseed supplementation; R10 – diet with 10% of rapeseed supplementation; Σn-6 - sum of n-6 polyunsaturated fatty acids; Σn-3 – sum of n-3 polyunsaturated fatty acids; PUFA – total sum of polyunsaturated fatty acids; MUFA – total sum of monounsaturated fatty acids;SFA – total sum of saturated fatty acids;n-6/n-3 – ratio of n-6 to n-3 fatty acids; PUFA/SFA – ratio of polyunsaturated to saturated fatty acids.

Analysis

Fatty acids were extracted from homogenised samples (1 g) of ostrich livers with the chloroform-methanol procedure of Folch *et al*. [1957]. Fatty acid methyl esters (FAME) were analyzed using a GC-7890 Agilent gas chromatograph equipped with a 60 m Hewlett-Packard-88 capillary column (Agilent J&W GC Columns, USA) with 0.25 mm inner diameter and 0.20 µm film thickness. A 1 µl sample was injected at a split ratio of 1:40. As a carrier gas helium was used at a flow rate of 50mL.min^{-1}. The injector and detector were maintained at 260°C. Column oven temperature was programmed to increase from 140°C (held for 5 min) at a rate of 4°C.min^{-1} to 190°C and then to 215°C at a rate 0.8°C.min^{-1} [Poławska *et al.*, 2012c]. Individual fatty acids were identified by comparison of retention times to those of a standard FAME mixture (Supelco 37 Component FAME Mix, 47885-U – 10 mg.ml^{-1} in methylene chloride, analytical standard, Sigma-Aldrich Co.) and expressed as a g/100g FAME.

Calculations and statistical analysis

The sums of saturated fatty acids – SFA (≤12:0 + 14:0 + 15:0 + 16:0 + 18:0 + 20:0), monounsaturated fatty acids - MUFA (16:1n-7 + 18:1n-7 + 18:1n-9) and polyunsaturated fatty acids – PUFA (18:2n-6 + 18:3n-3 + 20:4n-6 + 20:5n-3 + 22:6n-3) were calculated. The sum of *n-6* fatty acids was calculated as the sum of 18:2n-6 + 20:4n-6 and the sum of *n*-3 fatty acids as the sum of 18:3n-3 + 20:5n-3 + 22:6n-3. The ratios of PUFA/SFA and *n-6/n-3* were also calculated.

The following indices for enzyme activities involved in fatty acid metabolism were also calculated, based on the ratio of product and precursor fatty acids.

Δ9-desaturase: Δ9d = C 18:1 n-9/C 18:0
Δ4-desaturase: Δ4d = C 22:6 n-3/C 22:5 n-3
elongase 14 : E14 = C 14:0/C 12:0
elongase 16 : E16 = C 16:0/C 14:0
elongase 18 : E18 = C 18:0/C 16:0

Also the index of competitiveness (CI) – competition for lipogenic enzymes between n-6 and n-3 fatty acids was determined using the following formula:

CI = (18:2 n-6/20:4 n-6) / (18:3 n-3/20:5 n-3).

CI is a new index proposed by the authors. CI takes into account the metabolism of FA on n-6 and n-3 FA, according to substrate amount, which are important for human health. In authors opinion this index can wider present the influence of dietary FA on FA content of tissues.

An analysis of variance using STATISTICA (ver. 9, StatSoft Inc., USA) was conducted with diet as a factor. Tukey-tests were calculated at a 5% significance level to compare means for significant effects.

Results and discussion

The fatty acid profile of ostrich livers is presented in Table 2. The oil seed supplementation influenced the SFA profile and proportion. The linseed supplementation in the ostrich diet decreased total SFA proportion in liver (38.5 and 41.7 g/100g FAME L4 and L8 group, respectively) as compared to control group (45.9 g/100g FAME). There were no significant differences in SFA proportion between R5 and R10 group (42.7 and 42.4 g/100g FAME) as compared to C group, as well as compared to L8 group. The differences in total SFA proportion were strictly connected with differences in palmitic acid (16:0) proportion. Similar results obtained Nam *et al*. [1997] in breast muscles of chicken fed linseed supplemented diet.

The 4% linseed supplementation to ostrich diet significantly decreased the palmitoleic acid (16:1 n-7) proportion in ostrich liver (3.99 g/100 g FAME) as compared to control group (6.26% $\sum_{FA}$) (Tab. 3). Moreover liver of L4 and L8 group had lower proportion of oleic acid (18:1 n-9) (25.7 and 25.8%g/100g FAME respectively) as compared to C group (29.3g/100g FAME). The differences in proportion of those fatty acids influenced the difference in total MUFA proportion between L groups (L4 and L8) and C group (31.6; 32.8 and 37.3 g/100g FAME respectively).

The linseed supplementation (L4 and L8 group) increased the linolenic (18:3 n-3) proportion (10.8 and 9.4 g/100g FAME , respectively) as compared to control group (2.3 g/100g FAME) and influenced the proportion of total n-3 fatty acids in linseed supplemented group: an almost four fold higher proportion in L4 (11.7 g/100g FAME) and three fold higher proportion in L8 (10 g/100g FAME) as compared to control group (2.9% g/100g FAME) was observed. Total PUFA proportion was also significantly higher in linseed supplemented groups (above 25 g/100g FAME) than in

Table 2. Influence of the dietary group on the fatty acid profile (g/100g FAME) in ostrich liver

FA	Dietary group[1]									
	C		L4		L8		R5		R10	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
≤12:0	0.47	0.65	0.23	0.08	0.23	0.10	0.23	0.10	0.26	0.10
14:0	0.68	0.19	0.49	0.15	0.56	0.21	0.64	0.25	0.58	0.25
15:0	0.36	0.31	0.31	0.09	0.39	0.09	0.28	0.06	0.32	0.09
16:0	34.0^{a}	4.26	25.8^{c}	1.44	28.9^{bc}	3.97	31.6^{ab}	4.48	29.8^{abc}	5.36
18:0	9.27	1.57	9.94	1.35	10.5	2.62	8.35	1.44	10.4	1.35
20:0	0.20	0.15	0.24	0.09	0.15	0.09	0.21	0.15	0.25	0.12
16:1 n-7	6.26^{a}	1.41	3.99^{b}	1.74	5.15^{ab}	1.71	6.07^{a}	1.57	4.86^{ab}	1.26
18:1 n-9	29.3^{a}	1.47	25.7^{b}	2.15	25.8^{b}	2.74	30.7^{a}	1.59	29.5^{a}	3.96
18:1 n-7	1.81	0.25	1.84	0.13	1.80	0.17	1.97	0.24	2.01	0.40
18:2 n-6	11.10	3.69	15.2	0.71	12.6	3.74	12.7	2.78	13.9	4.42
18:3 n-3	2.26^{b}	0.75	10.8^{a}	3.2	9.37^{a}	2.68	2.87^{b}	0.83	2.76^{b}	0.86
20:4 n-6	2.83	1.28	3.16	0.69	2.92	1.12	2.47	1.01	3.91	1.54
20:5 n-3	0.41	0.36	0.40	0.15	0.34	0.19	0.30	0.09	0.35	0.18
22:6 n-3	0.25	0.06	0.42	0.18	0.28	0.19	0.25	0.11	0.29	0.09
Σn-6	13.9	4.79	18.3	0.96	15.5	4.20	15.2	3.22	17.8	5.57
Σn-3	2.90^{b}	0.83	11.7^{a}	3.18	9.99^{a}	2.90	3.42^{b}	0.89	3.40^{b}	0.83
SFA	45.9^{a}	3.35	38.5^{c}	1.58	41.7^{bc}	3.23	42.7^{ab}	3.36	42.4^{ab}	5.44
MUFA	37.3^{a}	2.42	31.6^{b}	3.36	32.8^{b}	3.97	38.8^{a}	2.29	36.4^{a}	3.78
PUFA	16.8^{c}	5.39	30.0^{a}	3.96	25.5^{ab}	5.90	18.6^{c}	3.89	21.2^{bc}	6.10
Σ n-6/n-3	4.87^{a}	1.17	1.75^{b}	0.77	1.65^{b}	0.61	4.52^{a}	0.78	5.35^{a}	1.48
PUFA/SFA	0.38^{c}	0.15	0.78^{a}	0.12	0.62^{b}	0.17	0.44^{c}	0.12	0.52^{bc}	0.19

[1]C – control group; L4 – diet with 4% of linseed supplementation; L8 – diet with 8% of linseed supplementation; R5 – diet with 5% of rapeseed supplementation; R10 – diet with 10% of rapeseed supplementation; SFA – total sum of saturated fatty acids; MUFA – total sum of monounsaturated fatty acids; PUFA – total sum of polyunsaturated fatty acids; ; n-6/n-3 - ratio of n-6 to n-3 fatty acids; PUFA/SFA – ratio of polyunsaturated to saturated fatty acids.
[abc]Within rows means bearing different superscripts differ significantly at P<0.05.

Table 3. Indices for enzyme acitivities involved in the lipid metabolism

Item	Group[1]									
	C		L4		L8		R5		R10	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
E 14	3.12	2.07	2.48	1.32	2.83	1.70	3.06	1.68	2.73	1.99
E 16	52.59	10.92	56.24	13.60	55.46	13.17	55.74	21.47	55.02	9.40
E 18	0.28^{b}	0.08	0.39^{a}	0.07	0.37^{a}	0.13	0.27^{b}	0.08	0.36^{ab}	0.08
Δ4d	0.99	0.77	1.14	0.44	0.89	0.41	0.83	0.28	0.90	0.25
Δ9d	3.25^{a}	0.62	2.66^{b}	0.61	2.64^{b}	0.80	3.78^{a}	0.70	2.90^{b}	0.65
CI	0.85^{a}	0.86	0.23^{b}	0.22	0.16^{b}	0.09	0.62^{ab}	0.30	0.56^{ab}	0.51

[1]C- control group; L4 – diet with 4% of linseed supplementation; L8 – diet with 8% of linseed supplementation; R5 – diet with 5% of rapeseed supplementation; R10 – diet with 10% of rapeseed supplementation; E 14 – elongase 14; E 16 – elongase 16; E 18 – elongase 18; Δ4d – delta-4 desaturase ; 9d – delta-9 desaturase; CI– index of competitiveness.
[abc]Within rows means bearing different superscripts differ significantly at P<0.05.

control group (below 17 g/100g FAME). The effect of linseeds and sunflower seeds, as a source of MUFA fatty acids, on liver fatty acids profile in pigs, was determined by Guillevic *et al*. [2009b]. These authors confirmed that linseed supplementation increased the proportion of the total n-3 fatty acids, and more specific of 18:3n-3, when compared to control group, while no differences were observed between the sunflower supplemented groups and the control group in terms of n-3 PUFA. Similar relations observed Nam *et al*. [1997] and Lopez-Ferrer *et al*. [2001] in studies on chickens. The influence of linseed supplementation in ostriches was studied by Poławska *et al.* [2013b]. Authors confirmed that linseed supplementation increase omega-3 and linolenic acid content in different ostrich muscles when compared to muscles of standard fed ostriches.

The n-6 to n-3 ratio (Σ n-6/n-3) in rapeseeds and control groups was similar (above 4.5). The linseed supplementation decreased n-6 to n-3 ratio to values below 2. According to World Health Organisation [2003] the recommended ratio for consumers level is below 4, thus, ostrich liver from linseed groups can be recommended as raw material with a good nutritional value. However, also livers from the control and rapeseed groups have a n-6/n-3 ratio near the recommended one by WHO. Also the PUFA/SFA ratio of ostrich livers is near (C and R5 groups) or above (R10, L4 and L8 groups) the recommended ratio by WHO (>0.4). Similar results in muscles and livers of chicken and pigs obtained Nam *et al*. [1997], Lopez-Ferrer *et al*. [2001] and Guillevic *et al.* [2009b]. Also in ostrich muscles those relations were confirmed in ostriches fed 4% linseed supplemented diet [Poławska *et al.* 2013b].

The liver as one of the main fat depot sites has a high lipogenic metabolism activity as compared to the other depots in ostrich. The calculated lipogenic enzymes activities are presented in Table 3. Significant differences between dietary groups were only observed in the index for elongase 18 activity. In C and R5 groups in liver the index for activity was lower (0.28) than in L4 and L8 (on average 0.38). The direct influence on the index for elongase 18 activity can have SFA content of diets applied to ostriches, low SFA in the diet influence the index towards a higher activity of elongase 18, which is responsible for elongation of C-chains.

Although, the ostriches from L4 and L8 groups received a diet with the lowest content of MUFA, the index of the activity of for the enzyme Δ9 desaturase, responsible for desaturation of stearic acid to oleic acid, was significantly lower (2.6) than for the other groups (<2.9). Similar results were obtained by Skiba et al. [2011] in liver of pigs fed linseed and rapeseeds diets. Also, Guillevic *et al*. [2009a] confirmed those results on pigs fed linseed and sunflower diets. Those results can explain the hypothesis that diets rich in PUFA increase the PUFA proportions in tissues, by lowering MUFA proportion in the tissues, without affecting the proportions of SFA .

In this study also the index of competitiveness was determine. The CI values were significantly lowered in linseed groups (<0.23) as compared to the other groups (>0.56). It is probably related to a higher level of n-3 FA in the linseed diets. In liver of ostrich fed diets rich in n-3FA, the level of conversions of linolenic acid to

eicosapentaenoic acid (EPA) is lower, cause EPA is directly deposited from the diet, thus the organism regulate the omega-6 level (arachidonic acid) on its own metabolism regulatory system.

The results of calculated lipogenic enzymes activity suggests that even though diet applied to animal have great influence on fatty acid profile of depot sides, the organism have natural regulation systems of FA metabolism and the level of FA profile modifications by feeding system is limited.

The linseed supplementation, especially 4%, to the ostrich diet improves the nutritional value of ostrich liver by decreasing the n-6/n-3 ratio and by increasing the PUFA/SFA ratio. It makes ostrich liver more valuable for the production of meat products e.g. pates. The rapeseed supplementation to the ostrich diet has no influence on the fatty acid profile of ostrich liver compared to the control diet.

Dietary supplementation by linseed changes the fatty acid metabolism in ostrich liver.

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