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Effect of dietary linseed and rapeseed supplementation on fatty acids profiles in the ostriches. Part 2. Fat*

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The aim of the study was to determine the effect of linseed and rapeseed dietary supplementation on the fatty acids profiles of two ostrich fat depots: breast and subcutaneous (above the leg). The study was carried out on 40 ostriches raised in five groups – control (C) or with 4% (L4) or 8% (L8) linseed, or 5% (R5) or 10% (R10) rapeseed in the diet, from hatching to 12 months of age. Fat samples of breast (BF) and leg fat (LF) were taken for fatty acids analysis. Generally ostrich fat has high contents of PUFA (BF – 23.9, LF – 20.2 g/100 g FAME), especially linoleic acid (BF – 16.4, LF – 12.5 g/100 g FAME) and linolenic acid (BF – 5.7 and LF – 6.2 g/100 g FAME). Ostrich BF had a higher content of n-6 FA and total PUFA and lower n-3 FA than the LF. Both fat depots had desired PUFA/SFA ratios above 0.4, but not desirable n-6/n-3 ratios. BF had significantly higher (0.69) PUFA/SFA ratio than LF (0.55). Both L4 and L8 caused higher total PUFA content (27.8, 25.6 g/100 g

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FAME, respectively) and higher PUFA/SFA ratios (0.74, 0.75, respectively) and lower n-6/n-3 ratios (1.5, 1.8, respectively) compared to C. The rapeseed supplementation decreased the LA content in ostrich fats (R5- 14.1, R10-13.4g/100g FAME), causing a lower n-6/n-3 (4.1, 4.6, respectively) ratio compared to C (6.1). The supplementation of ostrich diets with linseed improved the nutritional value of ostrich fat by increasing the n-3 FA, total PUFA content and PUFA/SFA ratio. Although the leg fat had a lower PUFA content, both depots of ostrich fats can be recommended as valuable ingredients for value-added meat products fit for human consumption.

KEY WORDS: fat / fatty acids / oilseed / ostrich

The ostrich is gaining interest as a livestock animal worldwide because of its potential to produce dietetic red meat, eggs and fat as well as valuable skin and feathers [Horbańczuk and Sales 2001, Cooper *et al*. 2004, 2007, Horbanczuk *et al*. 2007, 2008, Poławska *et al*. 2011]. Although the world market for ostrich products includes mainly meat and skin [Horbanczuk *et al*. 1998, Sales and Horbańczuk 1998, Cooper and Horbanczuk 2004] the industry has started to use other ostrich products such as fat as a new source of animal fat [Horbańczuk *et al*. 2003, Basuny 2007, Basuny *et al*. 2011]. This includes the oil rendered from various fat depots that is used in cosmetics [Horbanczuk 2002]. Additionally, the food industry in Europe including Poland, uses ostrich fat as an ingredient in value-added products for human consumption, as well as a supplement to pet food, mainly for dogs and cats [Horbanczuk 2002]. Fat from the ostrich carcass is situated in specific depots: in the abdomen (internal fat) as well as on breast (also known as the breast fat pad) and on the back above the legs, this subcutaneous fat is also known as external or subcutaneous fat [Sales *et al*. 1999]. Since the scientific knowledge about the quality of ostrich fat as well as factors that influence its composition is very limited [Sales and Franken 1999, Cooper *et al*. 2000, Horbanczuk *et al*. 2004, Hoffman *et al*. 2005, 2012], the aim of this study was to assess the influence of dietary linseed and rapeseed supplementation on the fatty acids profiles of ostrich fat.

Material and methods

The experiment was carried out on 40 ostriches (*Struthio camelus* var. *domesticus*) in five groups (n=8 birds per group), from hatching to 12 months of age on a commercial farm in Stypułów in western Poland (the farm is under scientific supervision of the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences). Ethical approval for the trail was obtained from the Local Ethical Commission (No 27/2009). The breeding conditions and dietary treatment was in details described in Poławska *et al.* [2013].

Sample preparation

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 approximately 96.3±5.5 kg. Slaughter procedures are presented

in Poławska *et al*. [2012]. Samples (fat) were taken from the breast (BF) and from the subcutaneous depot above the leg/drum stick (LF) regions from the left side of the carcasses and transported to the laboratory in insulated containers at ca. 4°C until analyses of the fatty acids.

Fatty acid analyses

Fatty acids were extracted from homogenised samples (1 g) of fat with the chloroform-methanol procedure of Folch *et al*. [1957]. After esterification, the fatty acid methyl esters (FAME) were analyzed using a GC-7890 Agilent gas chromatograph as earlier presented in Poławska *et al.* [2013]. 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 in methylene chloride, analytical standard, Sigma-Aldrich Co.) and expressed in g/100 g FAME.

Calculations and statistical analysis

The sum of saturated fatty acids – SFA (C12:0+ C14:0+ C16:0+ C17:0+ C18:0+ C20:0+ C22:0+ C24:0), monounsaturated fatty acids – MUFA (C14:1+ C15:1+ C16:1+ C17:1+ C18:1n-9+ C18:1n-7) and polyunsaturated fatty acids – PUFA (C16:3+ C18:2n-6+ C18:3n-3+ C20:2+ C20:3n-6+ C20:4n-6+ C20:5n-3+ C22:6n-3) was calculated. The total omega-6 fatty acids was calculated as the sum of C18:2+ C20:3+ C20:4 fatty acids and the total omega-3 fatty acids as the sum of C18:3+ C20:5+ C22:6 fatty acids. The ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) and ratio of omega-6 to omega-3 fatty acids (n-6/n-3) was also calculated.

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

Results and discussion

The fatty acids profiles of ostrich fat from the breast depot and the subcutaneous depot above the leg are presented in Tables 1 and 2. Generally, there were no significant differences ($P>0.05$) in SFA content between the two fat depots within a specific diet, including the dominant fatty acid C16:0 (Tab. 1). However, the breast fat (BF) depot had a lower content of C18:0 (3.3 g/100 g FAME) than the leg fat (LF) depot (5.9 g/100 g FAME). For the sum of the SFA, the LF depot had a tendency ($P=0.059$) to contain more SFA than the BF depot. This phenomenon could probably be explained by differences in the functions and lipogenic activity of these fat depots. Mourot *et al*. [1995] confirmed in pigs that some tissues/depots (backfat, ham fat) have higher potential to synthesis de novo fatty acids (mainly SFA) whilst others tend to deposit them (mainly neck fat and dewlap). Similarly, Wiseman and Agunbiade [1998] obtained higher contents of SFA in the inner layer of backfat compared to the outer layer in pigs. On the basis of literature, they speculated that SFA are selectively

Table 1. Selected saturated and monounsaturated fatty acids content (g/100g FAME) in ostrich leg and breast fat

Item	Type of fat (F)[1]	Group(G)[2]						Statistic	
		C	L4	L8	R5	R10	mean	SEM	F x G
14:0	LF	0.87	0.73	0.68	0.78	0.73	0.75	0.06	ns
	BF	0.83	0.68	0.75	0.77	0.69	0.75		
	mean	0.84	0.71	0.72	0.77	0.71			
16:0	LF	26.09	26.13	26.84	26.03	26.77	26.40	0.74	ns
	BF	29.11	26.01	27.52	25.83	24.93	26.80		
	mean	28.10^{A}	26.07^{AB}	27.22^{AB}	25.91^{B}	25.72^{B}			
18:0	LF	5.67	6.24	6.01	5.59	5.70	5.85^{A}	0.18	*
	BF	3.61	6.12	3.14	1.52	2.92	3.33^{B}		
	mean	4.30^{AB}	6.18^{A}	4.42^{AB}	3.04^{B}	4.11^{AB}			
ΣSFA[3]	LF	36.74	34.99	35.98	41.64	40.88	37.77	0.97	ns
	BF	36.16	40.90	33.12	32.24	32.64	34.72		
	mean	36.35	37.75	34.39	35.76	36.17			
16:1	LF	11.70	6.07	6.76	6.23	6.36	7.17	0.53	ns
	BF	7.89	6.14	6.95	6.66	5.75	6.76		
	mean	9.16^{A}	6.10^{B}	6.87^{B}	6.50^{B}	6.02^{B}			
18:1n-9	LF	32.47	30.05	31.22	38.62	39.96	34.06	0.58	ns
	BF	33.42	22.95	32.37	36.70	38.80	33.24		
	mean	33.10^{B}	26.73^{C}	31.86^{BC}	37.42^{A}	39.30^{A}			
ΣMUFA[3]	LF	44.78	37.79	38.47	45.05	46.89	41.99	0.93	ns
	BF	43.30	30.57	41.28	45.45	46.34	41.89		
	mean	43.80^{ab}	34.42^{c}	40.04^{bc}	45.30^{a}	46.58^{a}			

[1]LF– leg fat; BF– breast fat.
[2]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.
[3]ΣSFA – sum of saturated fatty acids (C12:0+ C14:0+ C15:0+ C16:0+ C17:0+ C18:0+ C20:0+ C21:0+ C22:0+ C24:0); ΣMUFA – sum of monounsaturated fatty acids (C14:1+ C15:1+ C16:1+ C17:1+ C18:1n-9+ C18:1n-7+ C20:1).
[A,B]Means within a row bearing with different superscripts differ significantly at: small letters – $P<0.05$; capitals $P<0.01$.
*Significant interaction at $P<0.05$; ns – not significant.

deposited in the inner layer while UFA are deposited in the outer layer and that the softer fat found in the outer layer is formed mainly by increased desaturation of SFA by specific enzymes. They also mentioned the possibility that the temperature gradient in the body can also influence where SFA are deposited. Ostrich breast fat, a main barrier from the environment and protection from mechanical injury, is a soft fat and its lower content of SFA may be explained by thus function, especially due to the ostriches specific body structure.

There were no significant differences in MUFA content and its main compound C18:1n-9 between fat depots (Tab. 1). Ostrich fat is generally characterised by a high content of PUFA (BF – 23.9 and LF – 20.2 g/100 g FAME), especially linoleic acid (C18:2n-6, LA) (BF – 16.4 and LF – 12.5 g/100 g FAME) and linolenic acid (C18:3n-

Table 2. Selected polyunsaturated fatty acids content (g/100g FAME) and the fatty acids ratios in ostrich leg and breast fat

Fatty acid	Type of fat (F)[1]	Group(G)[2]						Statistic	
		C	R5	R10	L4	L8	mean	SEM	F x G
18:2n-6	LF	14.64	7.93	8.14	15.04	15.34	12.51^{B}	0.34	*
	BF	16.65	17.80	17.32	15.44	14.54	16.37^{A}		
	mean	15.98^{a}	14.10^{bc}	13.39^{c}	15.23^{a}	14.90^{ab}			
18:3n-3	LF	2.42	3.65	2.46	10.78	8.73	6.21^{a}	0.35	ns
	BF	2.57	3.33	3.75	10.60	9.18	5.67^{b}		
	mean	2.52^{C}	3.45^{C}	3.20^{C}	10.70^{A}	8.98^{B}			
20:4n-6	LF	1.03	1.21	1.30	0.94	1.04	1.09	0.06	ns
	BF	1.02	0.92	1.28	2.16	1.54	1.06		
	mean	1.12^{b}	1.03^{b}	1.53^{a}	1.51^{a}	1.32^{a}			
20:5n-3	LF	0.19	0.15	0.15	0.18	0.18	0.17	0.02	ns
	BF	0.19	0.18	0.16	0.17	0.19	0.18		
	mean	0.19	0.17	0.16	0.18	0.19			
22:6n-3	LF	0.18	0.38	0.17	0.27	0.26	0.26^{a}	0.04	ns
	BF	0.11	0.08	0.06	0.15	0.15	0.11^{b}		
	mean	0.13	0.19	0.11	0.22	0.20			
Σn-6[3]	LF	15.67	9.14	9.44	15.98	16.38	13.60^{B}	0.36	ns
	BF	17.67	18.72	18.60	17.60	16.08	17.73^{A}		
	mean	17.00^{a}	15.13^{bc}	14.02^{c}	16.74^{ab}	16.21^{ab}			
Σn-3[3]	LF	2.80	4.18	2.78	11.23	9.17	6.64^{a}	0.37	ns
	BF	2.87	3.59	3.98	10.93	9.52	5.96^{b}		
	mean	2.85^{C}	3.81^{C}	3.47^{C}	11.09^{A}	9.36^{B}			
ΣPUFA[3]	LF	18.47	13.32	12.23	27.21	25.55	20.23^{B}	0.55	ns
	BF	20.54	22.31	22.58	28.53	25.59	23.91^{A}		
	mean	19.85^{C}	18.94^{C}	17.40^{C}	27.83^{A}	25.57^{B}			
Σn-6/Σn-3[3]	LF	5.87	2.19	4.74	1.43	1.79	2.93^{B}	0.63	ns
	BF	6.22	5.24	4.35	1.62	1.71	3.83^{A}		
	mean	6.10^{A}	4.10^{B}	4.55^{B}	1.52^{C}	1.75^{C}			
ΣPUFA/ΣSFA[3]	LF	0.51	0.32	0.30	0.78	0.72	0.55^{B}	0.03	ns
	BF	0.57	0.69	0.69	0.70	0.77	0.69^{A}		
	mean	0.55^{B}	0.55^{B}	0.50^{B}	0.74^{A}	0.75^{A}			

[1,2]See Table 1 for trait name abbreviations.
[3] Σn-6 – sum of n-6 polyunsaturated fatty acids; Σn-3 – sum of n-3 polyunsaturated fatty acids; ΣPUFA – sum of polyunsaturated fatty acids (C16:3+ C18:2n-6+ C18:3n-3+ C20:2+ C20:3n-6+ C20:4n-6+ C20:5n-3+ C22:2+ +C22:6n-3); Σn-6/Σn-3 – ratio of n-6 to n-3 fatty acids; PUFA/SFA – ratio of polyunsaturated to saturated fatty acids.
[A,B]Means within a row bearing with different superscripts differ significantly at: small letters – $P<0.05$; capitals $P<0.01$.
*Significant interaction at $P<0.05$; ns – not significant.

3, ALA) (BF – 5.7 and LF – 6.2 g/100g FAME). These results are in agreement with studies of Basuny *et al*. [2011], where ostrich fats were compared to fats of other farm animals. Basuny *et al*. [2011] obtained similar contents of SFA (36.5 g/100 g FAME), slightly higher contents of MUFA (46.8 g/100 g FAME) and lower contents of PUFA (18.2 g/100 g FAME). Though, the main compounds of ostrich fat in Basuny's *et al*. [2011] studies were similar (C18:0, C18:1n-9 and C18:3n-3) or on the same (C16:0 and C18:2n-6) levels as in this study.

Ostrich BF had higher contents of n-6 FA and total PUFA and lower of n-3 FA than LF (Tab. 4). BF also had a higher n-6/n-3 ratio (3.8) than LF (2.9), although both fat depots had ratios below the maximum recommended by World Health Organisation [2003] level (4.0). Both fat depots had desired PUFA/SFA ratios above 0.4, although the ratio from the BF was significantly higher (0.69) than that of the LF (0.55).

The fatty acid profiles of ostrich fat were significantly influenced by supplementation of the bird diet with oil seeds. The linseed supplementation (4 and 8%) caused lower contents of C16:1, C18:1n-9 and total MUFA (6.1 and 6.9 g/100 g FAME, 26.7 and 31.9 g/100 g FAME, 34.4 and 40 g/100 g FAME, respectively) compared to the control group (9.2, 33.1 and 43.8 g/100 g FAME, respectively) – Table 1. Moreover, linseed supplementation of 4% and 8% to the diet increased the content of ALA (10.7 and 9.0g/100g FAME, respectively) and total n-3 FA (11.1 and 9.4 g/100 g FAME, respectively) compared to C (ALA – 2.5 and n-3 – 2.9 g/100 g FAME, respectively) – Table 2. Both linseed treatments caused higher total PUFA contents (4% linseed – 27.8 g/100 g FAME and 8% linseed – 25.6 g/100 g FAME) and PUFA/SFA ratio (0.74 and 0.75, respectively) and lower n-6/n-3 ratio (1.5 and 1.8, respectively) in the ostrich fat depots compared to C. Although there are no studies in the readily sourced scientific literature reporting on the influence of oil seeds supplementation in the ostrich diet on the FA profile of fat depots or muscles, results of this study are in agreement with results obtained by Lopez-Ferrer *et al*. [2001] and Zelenka *et al*. [2008] on chickens fed on linseeds diets, as well as with the results of Beckerbauer *et al.* [2001] on emu fed diet rich in saturated and unsaturated fatty acids.

Rapeseed supplementation caused lower C16:1 (5% rapeseeds – 6.5 g/100 g FAME and 10% rapeseeds – 6.0 g/100 g FAME) (Tab. 1) content in fat depots compared to C group, although the total MUFA content did not differ among the treatments. Though not significant, there was a tendency to a higher content of C18:1n-9 and total MUFA in the rapeseed treatments, what could be caused by the higher content of MUFA in the rapeseed diets [Poławska *et al.* 2013]. The rapeseed supplementation decreased the LA content in the ostrich fat depots (14.1 and 13.4 g/100 g FAME, respectively in R5 and R10), what resulted in a lower ratio of n-6/n-3 (4.1 and 4.6, respectively) compared to C (6.1) – Table 2.

In conclusion, the supplementation of ostrich diets by linseed improved the nutritional value of ostrich fat depot by increasing the n-3 FA, total PUFA content and PUFA/SFA ratio. Although the leg fat had a lower PUFA content, both ostrich fat depots can be recommended as a valuable ingredient in value-added meat products.

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