PL-ISSN 0015-5497 (print), ISSN 1734-9168 (online)
Folia Biologica (Kraków), vol. 62 (2014), No 2
doi:10.3409/fb62_2.127

The Effect of Different Fat Sources in the Diet on the Composition of Adipose Tissue in Arctic Foxes (*Alopex lagopus* L.)

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Accepted February 19, 2014

NOWICKI S., PRZYSIECKI P., FILISTOWICZ A., POTKAŃSKI A., FILISTOWICZ A., WÓJTOWSKI J. 2014. The effect of different fat sources in the diet on the composition of adipose tissue in arctic foxes (*Alopex lagopus* L.). Folia Biologica (Kraków) **62**: 127-133.

The study investigated the effect of vegetable and animal fat in the feed ration on the fatty acid profile of reserve fat in the arctic fox. Varying proportions of saturated and unsaturated fats in the feed ration comprised the experimental factor. In order to differentiate contents of saturated and unsaturated fatty acids in the experimental feed rations, various percentages of rapeseed oil and turkey fat were applied. The subcutaneous and circum-organal fat in this study differed considerably in terms of contents of individual fatty acids and between individual groups of acids. The circum-organal fat contained much higher amounts of unsaturated acids, but at the same time it was characterized by a lower amount of monounsaturated acids. Significant differences were also found in the contents of as many as 11 fatty acids. Recorded results indicate a potential modification of reserve fat in the arctic fox, a model representative of carnivorous animals (*Carnivora*). The possibility of the partial substitution of animal fat with vegetable oil (rape oil) in the nutritive diet of artic foxes was confirmed.

Key words: Arctic fox *(Alopex lagopus)*, reserve fat, fatty acid profile.

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Fat may account for as much as 30% of the body weight in arctic foxes, since at slaughter approx. 1-3 kg of the subcutaneous fat of the adipose tissue is obtained from one animal (BATURA *et al*. 1989). Rendered vulpine fat is light in colour, almost odourless and has a similar composition to avocado oil, used e.g. to produce cosmetics (SZCZEPAŃSKA *et al*. 1984). Considering its easy absorption, smoothing irritations, this fat is used in therapeutic ointments (among others zincic) and also for production of hand and foot creams. This fat has moistening, nutritive and regenerative properties and is used in cosmetics for dry, sensitive skin and also aging skin (KOSKO 1983).

Rheological, technological and performance properties of fat are primarily dependent on the composition of fatty acids and their degree of unsaturation (PIKUL & WÓJTOWSKI 2008; CAIS-SOKOLIŃSKA *et al*. 2011; SZUMACHER-STRABEL *et al*. 2011). Variation in the quantitative composition of fatty acids in reserve fat in *Carnivora* is a highly complex problem, dependent e.g. on rearing conditions and nutrition (MUSTONEN *et al*. 2007; NIEMINEN *et al*. 2007). The feed ration for foxes kept on fur farms contains abattoir waste of different farm animals as well as feeds of plant origin, e.g. ground grain. Additionally, in order to enhance the level of energy in the feed ration, a considerable addition of animal fats (e.g. suet, leaf fat, lard) and vegetable and animal oils is used. These factors influence the quantitative composition of fatty acids in vulpine fat to a significant degree (BATURA *et al*. 1989).

The aim of the study was to estimate the effect of vegetable oil addition (rapeseed oil) and animal fat (turkey) to nutritious doses used in nourishment of foxes on the profile of fat acids of the subcutaneous and circum-organal depot fat and also its possible utilization in the cosmetic industry.

Material and Methods

Experimental animals

Analyses were conducted on a fur farm of arctic foxes located in western Poland. The experiment comprised 60 arctic foxes of both sexes (30 females and 30 males), originating from 10 females. Cubs stayed with their mothers in free-standing cages from birth to weaning, i.e. until the age of 7 weeks. After weaning cubs were transferred to a pavilion, with 3 foxes per cage of 200 cm × 100 cm × 80 cm. Animals were divided into three groups: the control, experimental 1 and experimental 2, with 10 males and 10 females in each group, with animals of the same sex from each litter assigned to each group.

Experimental diets

The experimental factor comprised varying proportions of saturated and unsaturated fats in the feed ration. The feed was prepared from typical components available in the area in which the farm is located. The total share of added fat (rapeseed oil and turkey fat) in feed rations was identical, amounting to 6%. In order to differentiate contents of saturated and unsaturated fatty acids in the experimental feed rations, different proportions of rapeseed oil and turkey fat were applied (Table 1).

The feed ration:

– The control ration contained 4.0% turkey fat and 2.0% rapeseed oil;

– Experimental ration 1 contained 5.5% turkey fat and 0.5% rapeseed oil;

– Experimental ration 2 contained 0.5% turkey fat and 5.5% rapeseed oil.

Contents of fatty acids in feed rations are presented in Table 2.

Table 1

Composition of feed ration (%)

Raw material	Group		
	C	E1	E2
Wheat Extrudat	13	13	13
Turkey heads and feet	13	13	13
Poultry chicken intestines	25	25	25
Cod fillet	5	5	5
Fish flour	5	5	5
Blood flour	2	2	2
Meat – bony flour/ meal	5	5	5
Wheat bran	1	1	1
Turkey fat	4.0	5.5	0.5
Rapeseed oil	2.0	0.5	5.5
Water	25	25	25
Dry mass	33.2	33.1	33.8
Level of metabolic energy (EM) MJ/ 1kg	7.08	7.06	7.10
Distribution of ME (%)			
from protein	35.79	35.85	35.65
from fat	46.81	46.72	47.02
from carbohydrates	17.40	17.43	17.33

C – Control group; E1; E2 – Experimental groups.

Table 2

Total levels of saturated and unsaturated fatty acids in feed rations (% FAME, $\bar{x} \pm SD$)

FA	C	E1	E2
SFA	30.81 ± 0.41	31.51 ± 0.42	28.55 ± 0.38
MUFA	44.17 ± 0.73	43.43 ± 0.65	45.64 ± 0.82
PUFA	24.95 ± 0.29	24.99 ± 0.27	25.73 ± 0.24
UFA	69.12 ± 0.93	68.42 ± 0.91	70.37 ± 0.98
UFA/SFA	2.24 ± 0.07	2.17 ± 0.07	2.46 ± 0.08

$\bar{x}$ – mean value; SD – standard deviation; C – Control group; E1; E2 – Experimental groups; FA – Fatty acids; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids – total sum of MUFA+PUFA.

Throughout the entire experimental period, starting from weaning to slaughter, foxes were fed ad libitum and the daily feed ration was regulated depending on the amount of consumed feed. The mass of the eaten fodder was monitored each day.

On the day of slaughter animals were on average 301 days of age (+/- 25 days). Animals were slaughtered in the normal running technological cycle. Slaughtering of foxes was conducted with the apparatus FOXFINAL F2011 produced according to UE orders of low voltage: 73/23ETTY, EMC – INSTRUCTION/ ORDER 89/336/ETY, EY1099/2009. The slaughtering of foxes was consistent with principles of putting animals to death contained in decrees of Council/ Board/ Committee (WE) number 1099/ 2099 from the 24th of September 2009. The electrical method is conducted by applying electrodes of the apparatus of FOXFINAL F2011 to the muffle and anus through at least a period of 3 seconds by using a current of at least of 0.3 ampere and at least 110 volts.

After slaughtering, the subcutaneous fat was removed from the meaty side of the leather (rough side of the leather) and also from dorsal, lateral and abdominal sides. After weighing, the subcutaneous fat was homogenized with the aim of unifying its composition. After mixing, 50 gram samples were collected for the analysis of the profile of fatty acids. Moreover from the mesentery of the small intestine (*lat*. *mesenterium*) and also the region of the kidneys, more or less 50 g samples of the circum-organal fat was collected for determination of the fatty acid profile. Weight of the animals, mass of the subcutaneous fat and also daily consumption of the fodder during the experiment are shown in the Table 3.

Table 3

Body weight of animals, mass of the subcutaneous fat and also daily consumption of the fodder over the duration of the experiment ($\bar{x} \pm SD$)

Item	Group		
	C	E1	E2
Body weight in the 7th week of life (kg)	1.11 ± 0.15	1.09 ± 0.16	1.08 ± 0.14
Weight on the day of slaughtering (kg)	10.64 ± 1.76	11.16 ± 2.78	10.35 ± 2.22
Mass of the subcutaneous fat (kg)	3.33 ± 0.93	3.55 ± 1.12	3.22 ± 1.39
Subcutaneous fat/ body weight (%)	30.91 ± 4.92	31.43 ± 4.88	30.10 ± 7.38
Average daily consumption of fodder 164 days (g kg^{-1})	545.33 ± 14.48	546.11 ± 14.25	543.25 ± 14.25

$\bar{x}$ – mean value; SD – standard deviation; C – Control group; E1; E2 – Experimental groups.

Chemical analysis

Contents of fatty acids in feed rations, components (rapeseed oil and turkey fat) as well as samples of subcutaneous and circum-organal fats were analyzed using the same method. Samples of examined materials were extracted with diethyl ether, next fatty acids were esterified in a 2 ml 0.5 M NaOH solution with methanol (at 20g NaOH per 1 l methanol), and run in derivatisation processes using bortrifluorid in fatty acid methyl esters (FAME). After the addition of 7 ml of 0.34 M NaCl solution and 1 ml hexane and thorough mixing the obtained organic phase containing long-chain fatty acids was used in chromatographic analyses. Chromatographic analyses were performed with the use of a gas chromatograph (Varian CP-3380) equipped with a flame ionization detector and a CP – Select CB for FAME (CP 7420 Varian) column of 100 m, ID 0.25 mm, stationary phase bed of 0.25 μm. Ultra pure Helium 5.0 was used as a carrier gas at a constant flow rate of 3 ml/min.

Samples of 2 μl were transferred to the column with the carrier gas stream divided at 1:99. Oven temperature was programmed as follows: 140°C for 9 min., followed by an increase by 4°C / min. for 25 min. until 240°C. Fatty acids were identified based on their retention times, and results were expressed as the percentage of the sum of identified fatty acids (% wt).

Fatty acids were identified using a standard (37 FAME Mix, Supelco, Bellefonte, USA, catalogue no. 47885-U), while quantitative analysis was based on peak area using Varian Star Chromatography, Workstation (ver. 5.31) software.

Statistical analysis

Statistical analysis was conducted using a two-way analysis of variance (group, sex) based on the fixed model (SAS 2006). The Duncan test was applied to determine the significance of differences between groups.

Results

Profiles of fatty acids of the subcutaneous and circum-organal depot fat of the examined animals are shown in Table 4. The highest proportion of saturated fatty acids was found in the feed containing 5.5% turkey fat. The level of metabolic energy and the percentage of energy from basic nutrients (protein, fat, carbohydrates) in the control and experimental rations fell within the limits given in feeding standards for foxes (BARABASZ *et al*. 1994). In the examined subcutaneous fat a total of 47 fatty acids were detected out of the 54 analyzed. The dominant group comprised monounsaturated acids, which were found at over 50%. Oleic acid predominated in this group at 41.10-42.43%. Palmitoleic acid was detected at 5.58-5.68%. The group of monounsaturated acids was represented mainly by linoleic acid, found at 18.78-19.07%, with a total content recorded at 22.09-22.33%. Saturated acids accounted jointly for 25.23-26.44%, including palmitic acid at 15.58-16.52%, stearic acid at 7.13-7.49% and myristic acid at 1.52-1.59%, respectively. Subcutaneous fat and superficial fat in this study differed considerably in the contents of individual fatty acids and within individual groups of acids. Superficial fat contained much higher amounts of unsaturated acids, while at the same time it was characterized by a lower content of monounsaturated acids. Significant differences were also found in contents of as many as 11 fatty acids ($P<0.05$). The composition of the feed ration in this experiment differed considerably in terms of contents of individual fatty acids in the analyzed subcutaneous fat and superficial fat. Significant differences were found in the total saturated fatty acids (1.22% and 1.20%) and monounsaturated acids (1.43% and 1.40%) between both types of fat in the experimental groups ($P<0.05$). A similarly significant difference was observed in the contents of individual fatty acids between the same groups, i.e. palmitic acid (0.94% and 1.24%) and oleic acid (0.95% and 1.35%). Specified in examined animals, the profile of fatty acids of the subcutaneous fat, particularly in respect of contents of SFA, MUFA and PUFA meet the requirements of fats used for cosmetic purposes, in this also for oil produced from fruits and leaves of avocado (KOSKO 1983).

Discussion

Arctic fox (*Alopex lagopus* L.) in natural conditions accumulates a considerable quantity of subcutaneous and circum-organal fat in the autumn and early winter (AUDET *et al*. 2002). A similar phenomenon also occurs in arctic foxes kept in conditions of farm breeding (ROUVINEN & KIISKINEN 1989). The main purpose of the breeding of carnivorous fur-bearing animals is obtaining leather. However, additionally at slaughtering of farmed arctic foxes, about 1-3 kg of subcutaneous fat from specimens may be obtained (BATURA *et al.* 1989; KOSKO 1983; PRZYSIECKI 2000). This fat contributes up to 30% of the body weight of arctic foxes. In the nourishment of fur-bearing animals, the fat plays an important role, because it is not only the carrier of metabolic energy, but also positively affects the absorption of other substances, for instance vitamins soluble in fats (A, D, E, K), having a protective function in relation to protein

Table 4

The profile of fatty acids (% FAME, $\bar{x} \pm SD$)

FA	Subcutaneous fat			Circum-organal fat		
	C	E1	E2	C	E1	E2
C12:0	0.33 ± 0.03	0.29 ± 0.03	0.21 ± 0.02	0.38 ± 0.04	0.37 ± 0.04	0.29 ± 0.03
C14:0	1.59 ± 0.06	1.57 ± 0.05	1.53 ± 0.05	1.51 ± 0.04	1.49 ± 0.05	1.49 ± 0.05
C15:0	0.11 ± 0.03	0.10 ± 0.03	0.10 ± 0.02	0.11 ± 0.04	0.11 ± 0.03	0.10 ± 0.03
C15:1	0.02 ± 0.006	0.02 ± 0.008	0.02 ± 0.006	0.02 ± 0.005	0.02 ± 0.007	0.01 ± 0.003
C16:0	15.85 ± 0.65	16.52^{a} ± 0.56	15.58^{b} ± 0.63	17.30 ± 0.67	17.92^{a} ± 0.71	16.68^{b} ± 0.69
C16:1	5.58 ± 0.12	5.58 ± 0.09	5.68 ± 0.17	4.58 ± 0.21	4.66 ± 0.19	4.93 ± 0.23
C17:1	0.20 ± 0.005	0.19 ± 0.003	0.17± 0.005	0.22 ± 0.007	0.21 ± 0.005	0.17 ± 0.003
C18:0	7.14 ± 0.14	7.49 ± 0.18	7.31 ± 0.15	8.13 ± 0.16	8.09 ± 0.14	8.16 ± 0.19
C18:1n-9	42.04 ± 0.72	41.09^{a} ± 0.65	42.43^{b} ± 0.82	40.51 ± 0.69	40.29^{a} ± 0.55	41.64^{b} ± 0.76
C18:1n-11	2.78 ± 0.12	2.72^{a} ± 0.08	2.92^{b} ± 0.09	2.41 ± 0.07	2.37 ± 0.09	2.53 ± 0.07
C18:2n-9-12	18.76 ± 0.48	19.07 ± 0,57	18.78 ± 0.61	19.16 ± 0.59	19.12 ± 0.63	18.98 ± 0.61
C18:3n-9-12	1.91 ± 0.11	1.77 ± 0.09	1.75 ± 0.08	1.74 ± 0.10	1.60 ± 0.11	1.63 ± 0.08
SFA	25.45 ± 0.69	26.44^{a} ± 0.78	25.24^{b} ± 0.71	27.19 ± 0.82	24.22^{a} ± 0.65	27.86^{b} ± 0.79
MUFA	52.30 ± 0.81	51.11^{a} ± 0.74	52.55^{b} ± 0.86	49.64 ± 0.71	49.22^{a} ± 0.84	50.62^{b} ± 0.89
PUFA	22.14 ± 0.52	22.33 ± 0.63	22.10 ± 0.57	22.40 ± 0.65	22.27 ± 0.59	22.09 ± 0.51
UFA/SFA	2.93 ± 0.09	2.78 ± 0.07	2.96 ± 0.09	2.65 ± 0.07	2.95 ± 0.09	2.61 ± 0.10

$\bar{x}$ – mean value; SD – standard deviation; FA – Fatty acids; C – Control group; E1; E2 – Experimental groups; a, b – means in the row with different letters differ significantly ($P<0.05$).

and affects the rate of growth of body weight (LOREK & GUGOŁEK 1998). LOREK and GUGOŁEK (1993) and also LYNGS (1990) proved that an increase of energeticity of the diet by the addition of fat causes minor fodder utilization and thus protein on the unit of the increase of body weight, therefore also possessing an economic aspect. The fat addition to the nutritive dose in the nourishment of fur-bearing animals can reach up to 10 % (LOREK & GUGOŁEK 1995; LOREK & GUGOŁEK 1998). In carnivores and other simple-stomached animals, almost all fatty acids deposited in membrane phospholipids and storage triacylglycerols are unaltered, so the composition of fatty acids of such components of animal tissue reflects the diet over previous weeks or months (PRESTRUD & NILSSEN 1992). According to LOREK and GUGOŁEK (1993), the fundamental factor determining the possibility of increasing the fat contribution in the nutritive dose of arctic foxes is the physiological state of animals and the associated demand for this nutritive component. The second important factor is the ratio between quantity and quality of the fat and contents of the protein in the dose. It was ascertained that the contents of the fat in the dose should increase also the quantity and quality of the protein. In the applied nutritive doses, energy from the fat did not exceed the upper limits of the norm and the protein was characterized by high biological value. JAŃCZAK (2002) and JAŃCZAK *et al*. (2002a, 2002b) used an addition in the form of vegetable (rapeseed oil) and animal fats (tallow) in their fodder for artic foxes. The addition of animal fat (5% of tallow) improves the quality of hair coat and its licencial evaluation. The kind of fat did not influence traits of the animal exterior or the quality of leather.

Differences in qualitative parameters of the hair coat and also the quality of leather between groups differing by additions of fats were not significant but animals receiving an addition of animal fat (tallow) were characterized by higher parameters of these traits. Similarly in the paper of NOWICKI *et al*. 2013, the addition of animal fat to the nutritive dose of arctic foxes and a significant effect on the improvement of the structure of the coat and also the class of obtained leather. PRESTRUD and NILSSEN (1992) haven studied the seasonal variation in the body composition of arctic foxes (*Alopex lagopus*) to determine the adaptive significance of the fat deposition in this species. Fat was deposited both subcutaneously and viscerally

in September-October and reached a maximum of about 20% of the skinned carcass mass in November. The amount of fat deposited did not decline between November and March of any year. The fat deposits were depleted from March through May, reaching about 6% of the carcass mass by summer. Fifteen percent of trapped foxes did not have any subcutaneous or visceral fat deposited in winter. The amount of the deposited fat varied among years ($P<0.05$) but did not change with age and was independent of sex. Females that reproduced the previous spring were less ($P<0.05$) fat in winter than other foxes. The fat deposition in arctic foxes probably is an adaptive response to a combination of the food shortage in severe winters or in brief periods during normal winters, increasing energy requirements during the reproductive season and thermoregulation during low temperatures (PRESTRUD & NILSSEN 1992). Investigations have indicated a relationship/ dependence between the environmental temperature and the degree of lipid saturation in animals (SHULTZ & FERGUSON 1974). Arctic mammals utilize lipids as fuel during cold exposure and in many cases employ the adipose tissue as insulation (NIEMINEN *et al*. 2007). It would seem plausible that adipose tissue from different sites in the animal body which utilizes lipids for insulation as well as metabolism might show different proportions of saturated vs. unsaturated fatty acids and therefore different amounts of specific fatty acids (SHULTZ & FERGUSON 1974). In the study of SHULTZ and FERGUSON (1974) in arctic foxes, monounsaturated and polyunsaturated fatty acids predominated in the subcutaneous adipose tissue, whereas saturated fatty acids predominated in the omental and inguinal tissues. The dietary fat source has a strong influence on the fatty acid composition of body fat depots and milk in mono-gastric animals (ENSER 1984). In general, body fat of fur bearing animals is highly unsaturated, which enhances peroxidation of raw skins, thus making their storage more difficult (ROUVINEN & KIISKINEN 1989). Moreover, the location of fat depot influences fatty acid composition. In the mink (*Mustela vison*) and the blue and arctic fox (*Alopex lagopus*), the fat in the internal organs is more saturated than the subcutaneous fat (ROUVINEN & KIISKINEN 1989; SHULTZ & FERGUSON 1974). In the experiment of ROUVINEN and KIISKINEN (1989) the dietary fat sources were beef tallow, mink fat, capelin oil and rapeseed oil. The fat level supplied by the diets was 20% in the dry matter of feed. In this study the composition of all body depots reflected the fatty acid composition of dietary fat (ROUVINEN & KIISKINEN 1989). Similarly as in this study, in subcutaneous fat the highest share was found for monounsaturated acids (43%, ROUVINEN & KIISKINEN 1989).

The present study confirms earlier results. Analyses confirmed the thesis that the diet of foxes is a major factor affecting the composition of fatty acids in the reserve fat.

Summing up the obtained results it may be stated that:

– a varied share of vegetable fat and animal fat in the feed ration changed the fatty acid profile of adipose tissue;

– significant differences were found in the composition of fatty acids in the subcutaneous fat and circum-organal fat;

– the results indicate the potential for modification of reserve fat in carnivorous animals (*Carnivora*), also in terms of their utilization in processing (the pharmaceutical and cosmetics industries).

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