

Characterization of Fatty Acid Composition in the European Beaver (*Castor fiber* L.)

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Abstract

Lipids obtained from the muscular and adipose tissues of the European beaver were isolated by the modified Folch method. Fatty acids were converted to methyl esters and separated by high-resolution gas chromatography (HR-GC). The content and composition of beaver fat depended on the sex of a given animal. The adipose subcutaneous tissue of the female contained the most fat (approximately 70.5%). The fat content of muscular tissue was very low in both male and female beavers. Adipose tissue lipids of the beaver contained fatty acids ranging in chain length from 12 to 22 carbon atoms. Polyunsaturated fatty acids have the highest proportion in of total FA content of adipose tissue in the beaver, which distinguishes these lipids from the lipids of adipose tissues in other mammals. The results of the present study also confirmed a unique FA composition in the tail fat of the beaver, including a very high content of alpha-linolenic acid (ALA, 18:3 n-3) (on average 20.0%) and the sum of n-3 fatty acids (on average 20.45%). In addition, a very low content of the sum of saturated fatty acids (on average 14.93%) was observed, and an extremely low content, as for animal fat, of palmitic acid 16:0 (on average 10.53%).

Keywords: European beaver (*Castor fiber*), fatty acids, PUFAs

Introduction

The beaver (*Castor fiber*), the largest amphibious rodent except for the capybara (*Hydrochoerus capybara*), is found in the temperate zone of Europe, Asia and North America. The genus beaver comprises two species differing in tail proportions, color, details of skull structure and caryotype [1]. The European beaver (*Castor fiber* Linnaeus 1758) lives in Europe and Asia, and the Canadian beaver (*Castor fiber canadensis* Kuhl 1820) – in North America and Europe, where the species was acclimatized. At present both species are numerous in the Warmia and Mazury Region (Northeastern Poland), and often co-exist in the

same area (e.g. Uniszewo near Olsztyn). Body length of a beaver may reach 1 m, and body weight of an adult varies from 9 to 30 kg. Sierżanin (1961) described exceptionally large beavers found in Belarus, whose body weight was as high as 54 kg. Beavers are highly adaptive and can survive in various conditions [2].

The average beaver carcass contains 62.8% meat, 14.5% fat and 22.4% bone [24]. Beavers have high amounts of depot adipose tissue. They maintain energy reserves in the form of adipose tissue beneath the skin (subcutaneous fat), which also contributes to insulation. Subcutaneous adipose tissue is deposited unevenly, forming the thickest layer around the abdominal region and tail (10-20 mm). Its weight accounts for 12.1% of the total body weight of a beaver after skinning and evisceration

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[16]. Tail fat, containing over 80% unsaturated fatty acids, seems especially interesting [3]. In the water, the beaver can use its tail as a rudder. Another function of the beaver tail is thermoregulation. Beavers of both sexes have a pair of large scent glands, located laterally with respect to the anal orifice, which produce musky castoreum oil (Castoreum oil is a semi-liquid musky-scented mass, dried to a red-brown powder, which was considered a panacea in Europe until the beginning of the 20th century). Once highly valued as a therapeutic agent, today castoreum oil is used as an ingredient of some perfumes.

The therapeutic properties of castoreum oil and beaver fat, especially tail fat, have been known for ages. The Old-Polish Encyclopedia [4] gives examples of the most common applications of some elements of beaver carcass: "Castoreum oil has no negative effects on a healthy human being. When administered to an ill person, it can stimulate the functions of the brain and spinal cord, and especially the lower abdomen, in the case of pain, irritability and hypersensitivity in this region. When used in high doses, it increases excitement, heat sensation and skin ventilation, and accelerates pulse rate. Castoreum oil can be successfully administered in such diseases and states as hysteria, hypochondria, nervous exhaustion, anxiety state, headache, fainting, throbbing of the hearth, thoracic spasms, dizziness, migraine, lower abdomen neuralgia and during parturition".

In the Middle Ages beaver fat (internal fat accumulated in the abdominal cavity) and bear fat were applied to heal wounds inflicted with a sword or a spear, as well as to take arrows out of the bodies of warriors. At that time the beaver was considered a fish, and eaten usually while fasting. The most appreciated carcass element was the tail [5].

Beavers are herbivorous animals and eat the bark and cambium of trees and bushes, such as trembling poplar, poplar, willow, alder and birch, as well as the thick roots and stems of shore and aquatic plants. However, there are certain exceptions to the dietary habits of beavers. For instance, in 1997, near Suwałki (Poland), one or two beaver families peeled the bark off pine trees to a height of 1 m within an area of almost 1 ha in a 50-year-old forest. In Wigry National Park (Poland) pine-tree barking is today considered a common phenomenon. The reasons why beavers prefer this untypical diet, rich in resins, remains unknown. A mixed diet is indispensable for herbivorous animals such as beavers [6]. A varied diet enables the elimination of large amounts of secondary metabolites and detoxification of these compounds, and prevents sodium depletion. It is also possible that feeding on typical vegetable matter (pine-tree bark) is aimed at nutrient supplementation [6,7]. It should also be noted that seasonal and geographical variations in the diet are observed in all beavers [8, 9].

The objective of the present study was to determine the fatty acid (FA) composition in the adipose tissue obtained from various body parts of the European beaver, a male and a female, and to compare this composition with the FA composition in the depot adipose tissue of a terrestrial animal (pork fat) and the FA composition in the brown fat of the muskrat (*Ondorata zibethicus*) – another amphibious mammal that has much in common with the beaver.

Materials and Methods

The experimental materials comprised samples of muscular and depot adipose tissues of beavers – a female and a male: periintestinal tissue (fat around the intestines), perirenal tissue, subcutaneous tissue, leaf fat, thigh, tenderloin and tail fat. Upon consent of the Ministry of Environmental Protection, the animals were harvested in the Forest Division of Srokowo. Of 11 harvested animals, ten adults – three male and seven female - were selected for further investigations. They were in good physical condition, not exhausted or injured during harvest, and anesthetized within a short period afterwards.

Analytical Procedure

Total Lipids Extraction

Total lipids were isolated from the tissues by a modified method proposed by Folch et al. [10], using a chloroform: methanol extraction mixture (2:1 v/v) [11].

Quantitative Analysis of Lipid Classes

The isolated fat was divided into lipid classes, i.e. phospholipids (PLs), free fatty acids (FFAs), triacylglycerols (TAGs), cholesterol (CH) and cholesterol esters (CEs) by the SPE technique, on Bakerbond® amine columns (500 mg), according to the elution design presented in Table 2, as described by Kałużny et al. [12]. The proportions of particular lipid classes in the analyzed samples were determined on a weight basis. The composition of eluted fractions was confirmed by TLC.

Analysis of Fatty Acids

The isolated lipids, water- and solvent-free, were converted into methyl esters of fatty acids (FAMES), in accordance with the European Standard (EN: ISO, 5509 2000) [13]. For comparative purposes, the FA composition of pork adipose tissue from leaf fat was analyzed too. FAMES, grouped by hydrocarbon chain length and the degree of saturation, were separated by HR-GC using a 6890 Hewlett-Packard gas chromatograph with a split/splitless injector and a flame-ionization detector (FID), on an Rtx 2330 column (100m x 0.25 mm) (Restek, Bellefonte, PA, USA). A qualitative and quantitative analysis of fatty acids was performed using standard FAME solutions (Supelco Bellefonte, Pennsylvania, USA; Larodan Fine Chemicals, Malmö, Sweden).

Results and Discussion

Energy storage, thermal isolation and protection of inner organs were traditionally considered the principal functions of white adipose tissue (WAT) [20].

Fat isolated from the tissues and internal organs of the beaver differed with regard to the content of lipids repre-

Table 1. Content of particular lipid classes in fat isolated from the tissues of the European beaver (m±SD, mg/100mg fat from tissues).

| Tissue | FFA | CH | TAG | PL | CE |
|--------------------|-------------------|-----------|------------|-------------|------------|
| | m ± Δm [mg ± Δmg] | | | | |
| Tail | 5.6 ± 0.8 | 3.6 ± 0.4 | 84.1 ± 9.2 | 3.1 ± 1.1 | 3.4 ± 1.9 |
| Subcutaneous fat | 6.2 ± 1.6 | 2.5 ± 1.2 | 71.9 ± 4.9 | 15.2 ± 3.2 | 3.9 ± 1.3 |
| Liver | 8.8 ± 1.1 | 8.3 ± 2.1 | 16.9 ± 6.0 | 56.8 ± 13.5 | 8.9 ± 1.7 |
| Periintestinal fat | 5.3 ± 0.9 | 6.1 ± 1.8 | 42.1 ± 2.2 | 8.1 ± 2.1 | 38.2 ± 8.6 |
| Tenderloin | 8.5 ± 1.9 | 7.2 ± 3.5 | 38.9 ± 5.3 | 36.7 ± 5.2 | 8.4 ± 1.4 |
| Leaf fat | 3.6 ± 2.5 | 5.8 ± 1.2 | 82.1 ± 7.5 | 0.9 ± 0.2 | 7.3 ± 0.6 |

senting particular classes (Table 1). The main lipid fraction of depot adipose tissues of the beaver (subcutaneous, leaf fat, periintestinal, tail fat) are TAGs (39.3-77%), since fat used as an additional source of energy is deposited as TAGs. The percentage of phospholipids was the highest in liver fat and tenderloin fat, while periintestinal fat contained the largest amounts of cholesterol esters, compared to the other tissues (Table 1).

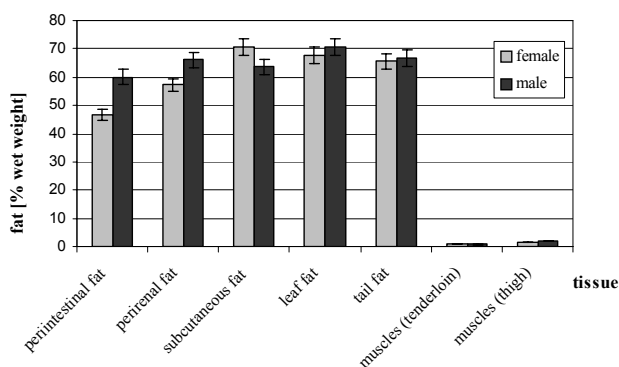


Fig. 1. Fat content of particular depot adipose tissues and muscular tissue of the European beaver. Data in the Figure include standard deviations.

The examined tissues contained various amounts of fat, depending on their type and the sex of the beavers (Fig. 1). The average amount of fat isolated from the depot adipose tissues of the female (N=7), i.e. periintestinal, subcutaneous, perirenal, leaf fat and tail tissues, was 61.53% in relation to raw tissue. The average amount of fat isolated from the depot tissues of the male was generally higher than in the female – 65.50% (Wald-Wolfowitz test, p<0.011). The highest amount of fat was isolated from subcutaneous tissue on the female – 70.54% (fresh weight) and from leaf fat of the male beaver – 70.84% wet weight.

The tail in the female and male contained averages of 65.64 and 66.71% fat, respectively. Tenderloin and thigh muscles contained much less fat than in internal adipose tissues, i.e. 1.12 and 1.54% respectively in the female, and 0.74 and 1.83% in the male. The differences between the amount of fat in a female’s and male’s muscles were not statistically significant (Wald-Wolfowitz test, p=0.184).

A gas chromatography analysis of fatty acids in adipose tissue lipids of the beaver revealed the presence of fatty acids ranging in chain length from 12 to 22 carbon atoms (Fig. 2).

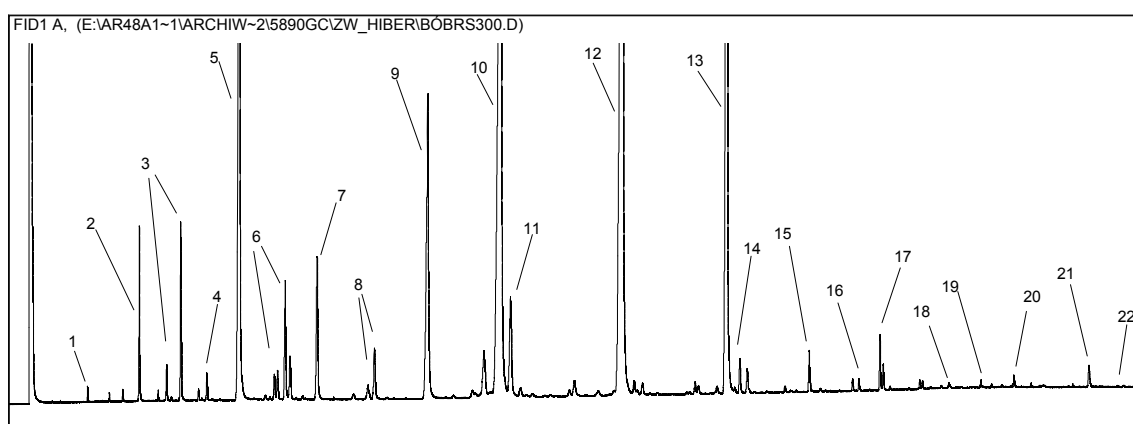


Fig. 2. Example of a gas chromatogram of the fatty acid composition of the depot adipose tissue of the European beaver. Separation conditions: carrier gas - H₂, flow rate - 1 ml/min., injector temperature - 250°C, detector temperature - 250°C. Analysis parameters: initial temperature - 155°C, initial time - 55 min., temperature increase – 1.5°C/min., final temperature - 210°C maintained for 80 minutes. Fatty acids: 1. 12:0, 2. 14:0, 3. isomers 14:1, 4. 15:0, 5. 16:0, 6. isomers 16:1, 7. 17:0, 8. isomers 17:1, 9. 18:0, 10. 18:1 9c, 11. 18:1 11c, 12. LA (18:2 n-6), 13. ALA (18:3 n-3), 14. 20:1, 15. 20:2 n-9, 16. DGLA (20:3 n-6), 17. AA (20:4n-6), 18. EPA (20:5 n-3), 19. 22:4 n-6, 20. 22:5 n-6, 21. DPA (22:5 n-3), 22. DHA (22:6 n-3).

Table 3. Concentrations of selected fatty acids in the depot subcutaneous tissue of the beaver, pork fat and the depot fat of muskrat and badger (wt % of total FA).

| Fatty acids | Beaver fat ¹ | Pork fat ¹ | Canadian beaver ² | Muskkrat brown fat ² | Eurasian badger ³ |
|---------------------|-------------------------|-----------------------|------------------------------|---------------------------------|------------------------------|
| 12:0 lauric | 0.1 | 0.1 | 0.2 | 0.1 | 0.3 |
| 14:0 myristic | 1.0 | 2.1 | 1.0 | 1.2 | 3.4 |
| 16:0 palmitic | 16.3 | 23.9 | 15.2 | 20.5 | 20.5 |
| 18:0 stearic | 5.3 | 12.4 | 7.3 | 7.3 | 8.8 |
| 16:1,9c palmitoleic | 2.6 | 1.1 | 2.0 | 1.3 | 7.0 |
| 18:1, 9c oleic | 25.4 | 42.6 | 13.5 | 27.0 | 30.4 |
| 18:2, n-6 | 21.4 | 4.45 | 34.1 | 21.0 | 10.6 |
| 20:3, n-6 | 0.1 | tr. | 0.3 | 0.2 | 0.6 |
| 20:4, n-6 | 0.4 | 0.4 | 1.3 | 0.6 | 2.1 |
| 18:3, n-3 | 17.2 | 0.2 | 10.1 | 11.5 | 2.0 |
| 22:5, n-3 | 0.2 | tr | tr | 0.1 | 1.6 |

¹ this study; ² [14], ³ [18].

Polyunsaturated Fatty Acids (PUFAs)

Polyunsaturated fatty acids constituted the highest proportion in the total FA content of adipose tissue in beavers (Table 2), which distinguishes these lipids from the lipids of adipose tissues in other mammals (Table 2). The group of PUFAs was dominated by linoleic acid (LA) (C18:2 n-6) and alpha-linolenic acid (ALA, C18:3 n-3). Their concentrations depended on the type of adipose tissue from which fat was isolated, and on the sex of the animals. LA content ranged from 18.71% of total FA in the thigh fat of the male, to 23.10% of total FA in the tail fat of the female. In all tissues of the female examined in this study, the LA level was higher than in the respective tissues of the male (except tenderloin). The differences in LA concentration varied from 0.61% of total FA in periintestinal tissue to almost 4% of total FA in thigh tissue. In three types of tissues (adipose tissue and tail) of the male, ALA level was statistically significantly higher than in the respective tissues of the female. The ALA content of muscle tissues (tenderloin and thigh) was approximately 2% higher in the female. Such high concentrations of LA and ALA in depot fat are a distinguishing feature of adipose tissue in the beaver. In the depot fat of the muskrat – that dwells in similar habitats and shares some traits with the beaver, LA concentration was also high (approximately 21%), whereas ALA content was 11.50% [14]. As regards fat content, the muscular tissues of the beaver resembles those of nutrias raised for fur and meat [19, 22]. To compare, pork fat contains on average 4.5% LA and only about 0.2% ALA (Table 3). The total percentage of the other identified PUFAs, i.e. eicosadienic acid (C20:2n-9), dihomo- γ -linolenic acid (DGLA, C20:3n-6), arachidonic acid (AA, C20:4n-6), docosatetraenoic acid (C22:4n-6), docosapentaenoic acid (C22:5n-6), eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in adipose tissue and tail was relatively small and did not

exceed 1.6% of total FA. In pork fat their percentage was about 1%. The fat extracted from muscular tissue (tenderloin and thigh) contain about 3.5% AA in the female and average 1.4% in the male. The n-3 to n-6 PUFA ratio (n-3/n-6 ratio) was almost 1:1 in the majority of the analyzed fats.

The only exception was muscular tissue fats of the beaver, where the n-3/n-6 ratio was almost 1:3. Such a high concentration of polyunsaturated acids was not reported in farm-raised beavers whose adipose tissues contained 9.80% linolic acid and 2.32-4.77% linolenic acid [3]. A high level of unsaturated fatty acids is normal in the lipids of all herbivorous and ruminant animals; however, the level of PUFAs in beaver fat is unusually high [14, 23, 24].

Monounsaturated Fatty Acids (MUFAs).

Monoenoic fatty acids accounted for 20.144 to 39.29% of total FA. Also in this case their percentage in total FA was related to type of tissue and sex of the animal. In the analyzed tissues, the concentration of monoenoic FA in the fat isolated from the female was higher than in the respective tissues of the male. These differences were significant and varied between 1.41 and 7.15% of total FA. Only in thigh fat were the concentrations of monoenoic FA in the tissues of the male higher than in the tissues of the female, at 29.26 and 23.23%, respectively.

The group of monoenoic FA was dominated by: oleomyristic (C14:1), oleopalmitic (C16:1 9c), oleic (C 18:1, 9c) and *cis*-octadec-11-enoic (C18:1, 11c) acids. Oleic acid dominated among them (C18:1 9c). It made up from 9.81% of total FA in the tenderloin fat of the male to 27.01% of total FA in the leaf fat of the female. The high concentration of oleic acid is typical of animal fat. In the brown fat of the muskrat oleic acid accounts for about 27% of total FA. The pork sample examined in the study contained 42.6% of this acid.

A feature distinguishing the tail fat of the European beaver from other animal fats is a very high concentration of oleopalmitic acid (C16:1, 9c), at 5.30% of total FA in the male and 7.20% of total FA in the female. It was found that beaver tissues contained from 1 to above 3% *trans*-octadecenoic FA.

Saturated Fatty Acids (SFAs)

Saturated fatty acids have different proportions in total FA composition, depending on the type of tissue and sex. The highest level of saturated FA, 42.34%, was recorded in the lipids isolated from the tenderloin fat of the male. The concentration of saturated fatty acids was the lowest (13.46) in the tail fat of the female. All tissues of the male contained more saturated fatty acids than the respective tissues of the female.

Palmitic acid (C16:0) dominated in the group of saturated FA. In the female its mean concentration ranged from 9.75% in tail adipose tissue to 22.60% in tenderloin fat. This level is relatively low, as compared with the palmitic acid content of adipose tissue in other mammals. In pigs, palmitic acid accounts for almost 24% of total FA in adipose tissue lipids, 20.5% in badger and 20.1% in muskrat (Table 3).

Another acid present in large amounts was stearic acid (C18:0). Depending on the type of tissue from which it was isolated, this acid made up 1.61 to 9.97% in the female, and 2.69 to 13.93% in the male. The depot subcutaneous tissue of the beaver contains about 6% stearic acid. A similar level of this acid is observed in this type of tissue in the muskrat and Canadian beaver (7.3% of total FA). Pork fat, which is the main source of saturated fatty acids in human diet, contained over 12% stearic acid (Table 3). It was found that beaver tissues contained above 2% odd-chain saturated FA (C15:0 and C17:0).

Conclusions

An analysis of lipids obtained from the tissues of the European beaver showed that FA composition was related both to the type of tissue from which it was isolated and to sex. Adipose tissue lipids in the beaver contained fatty acids typical of animal fats, such as palmitic acid and oleic acid. However, their mean content was generally lower than in the typical depot fat of terrestrial animals. PUFAs dominated in the FA composition of depot and internal adipose tissue lipids in the beaver, which is a distinguishing feature of this species. Their total content ranged from 34.68 to 43.53% of total FA; LA (approximately 21%) and ALA (approximately 17%) dominated among PUFAs. Only in lipids from muscular tissues dominated saturated FA. In the beaver, fat isolated from the adipose tissues of the female contained more monoenoic FAs and less saturated FAs than fat isolated from those tissues of the male. Another characteristic of the female's fat was a higher percentage of LA 18:2 n-6. The distinguishing features of the unique FA composition of the beaver's tail fat include, among others:

- a very high concentration of alpha-linolenic acid (ALA, 18:3 n-3) (20.00% on average) and the sum of n-3 fatty acids (20.45% on average);
- a low content of the sum of saturated fatty acids (14.93% on average), and an extremely low content, as for animal fat, of palmitic acid 16:0 (10.53% on average);
- a high level of oleopalmitic acid (6.37% on average), which enables differentiation between this type of fat and the fats of other animals, or the fat isolated from other tissues of the European beaver.

Study results presented by authors concerning of FA composition in selected tissues of the European beaver from Poland are similar to FA composition in tissues of the Canadian beaver presented by Käkälä et al. [14].

The results of the present study indicate that the optimum n-3 to n-6 PUFA ratio (n-3/n-6 ratio) makes beaver fat suitable for human nutrition. This fat can also be used in cosmetology, in place of very expensive lipids obtained from the alpine marmot, and in medicine. In addition, beavers are marked by a high carcass dressing percentage (54.5%) and a very high lean meat content of the carcass (73%) [15-17]. A distinguishing feature of beaver's meat is also a large amount of polyunsaturated fatty acids (PUFAs) in muscular fat, as well as a high percentage of mineral components [14, 16]. A similarly high level of PUFAs in the muscular fat of the European brown hare was reported by Valencak et al. [20]. Due to the considerable increase in the number of beavers in Poland and Europe, farm breeding of these animals has already been started. Thus, wider use of beaver carcasses can be expected in the near future.

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