# CHAPTER 5

## POTENTIAL OF FRESHWATER FISH PRODUCTION WITH HIGH CONTENT OF OMEGA-3 FATTY ACIDS

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# POTENTIAL OF FRESHWATER FISH PRODUCTION WITH HIGH CONTENT OF OMEGA-3 FATTY ACIDS

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## I. Objectives of the Methodology

Fish consumption in the Czech Republic has been at a low level for a long time. At the same time, the country occupies the top position in the incidence of cardiovascular disease. Therefore, it is necessary to promote the fish market, for example by increasing the nutritional value of fish flesh. Omega-3 polyunsaturated fatty acids (omega-3 PUFA) are well known for their beneficial effects in the prevention and treatment of cardiovascular diseases. The main sources of these compounds in human nutrition is fish. Using an appropriate strategy, based on feeding, it is possible to significantly increase the content of omega-3 PUFA in the fillet of freshwater fish species. Objective of the methodology is to provide the information to fish farmers. Farmers can use the information to improve the quality of fish flesh by increasing the omega-3 fatty acid content. The methodology is also intended to provide instructions about proper processing and culinary preparation of fish flesh that ensures the preservation and full utilisation of omega-3 PUFA in human nutrition.

## II. Description of the Methodology

## 1. INTRODUCTION

### 1.1. Influence of fatty acids on human health

Omega-3 fatty acids are chemical compounds belonging to the group of unsaturated fatty acids, whose common feature is the first double bond between the third and the fourth carbon (counted from the methyl end). Originally omega-3 fatty acids are synthesised by plants, both single cell algae and crops. Plant organisms have the ability to synthesise two essential fatty acids – linoleic acid (LA; 18:2 n-6), which is the precursor of omega-6 HUFA, and α-linolenic acid (ALA; 18:3 n-3), which is the precursor of omega-3 HUFA. Animal organisms must receive these two fatty acids via the food chain, because animals (including humans) are not able to synthesise LA and ALA. However, humans are partly able to synthesise longer fatty acid from these precursors by elongation and desaturation (Figure 1), but the efficiency is low.

| n-6 | | | | n-3 |
|---|---|---|---|---|
| linoleic (LA) | C18:2 | | C18:3 | α-linolenic (ALA) |
| | ↓ | Δ-6-desaturase | ↓ | |
| γ-linoleic (GLA) | C18:3 | | C18:4 | octadecatrienic (stearidonic) |
| | ↓ | elongase | ↓ | |
| dihomo-GLA | C20:3 | | C20:4 | eicosatetraenoic |
| | ↓ | Δ-5-desaturase | ↓ | |
| arachidonic (AA) | C20:4 | | C20:5 | eicosapentaenoic (EPA) |
| | ↓ | elongase | ↓ | |
| adernic | C22:4 | | C22:5 | docosapentaenoic (DPA) |
| | ↓ | elongase | ↓ | |
| tetracosatetraenoic | C24:4 | | C24:5 | tetracosapentaenoic |
| | ↓ | Δ-6-desaturase | ↓ | |
| tetracosapentaenoic | C24:5 | | C24:6 | tetracosahexaenoic |
| | ↓ | β-oxidation | ↓ | |
| docosapentaenoic | C22:5 | | C22:6 | docosahexaenoic (DHA) |

*Figure 1. Fatty acid metabolism.*

Fish, especially freshwater species, are able to create HUFAs more efficiently, because they have developed enzymes, desaturases and elongases, which synthesise 20, 22 and longer carbon chains with four and more double bonds from their 18-carbon precursors. The best known of these fatty acids are EPA (eicosapentaenoic) and DHA (docosahexaenoic). Together, the omega-6 and omega-3 fatty acids are important components of the cell membranes and, simultaneously, precursors of many compounds in the human body, e.g. eicosanoids (prostaglandins, tromboxanes, leukotriens). Beside the other functions, these substances have an inflammatory (omega-6) and anti-inflammatory (omega-3) effect in the body. Organisms, whether human or animal, need both these groups. The ratio between omega-3 and omega-6 is very important, because it affects the metabolisation in the organism. Therefore, intake of omega-3 and omega-6 in a ratio of about 1:1–1:4 is extremely essential, However, in reality in the Czech Republic this ratio is up to 1:40 in favour of omega-6. This leads to the manifestation of cardiovascular disease, which is the main cause of death in the country. Fish consumption in the Czech Republic is very low, only 5.5 kg per capita and year, with only about 1.5 kg of freshwater fish (MZe CR, 2010). Consumption of fish with an increased proportion of omega-3 fatty acids is a good method for prevention of cardiovascular disease, as well as resulting in faster recovery from treatment (Adámková et al., 2011). For people affected by cardiovascular disease it is often recommended to increase the consumption of fish. The European Food Safety Authority (EFSA) recommends including fish in the diet at least twice a week. The daily intake of specific fatty acids recommended is as follows: 250 mg EPA+DHA, 2 g ALA and 10 g LA (EFSA, 2009) for the normal population. This recommendation is even higher for people suffering from cardiovascular disorders. In addition, a balanced intake of the essential LA and ALA is important, because of their role in HUFA biosynthesis. Excess intake of LA in the diet causes a decrease in omega-6 HUFA content, because conversion of ALA to HUFA depends on the ratio between omega-3 and omega-6 fatty acids in the diet (Pickova, 2009). Adequate intake of omega-3 fatty acids results in:

- ✓ Increased production of 'good' HDL cholesterol at the expense of 'bad' LDL cholesterol
- ✓ Reduced triglyceride fraction in blood serum
- ✓ Reduced blood pressure
- ✓ Limitation of inflammatory diseases
- ✓ Reduced risk of myocardial infarction, atherosclerosis, multiple sclerosis, cancer, stroke, etc.
- ✓ Strengthening of the brain function and nervous system, especially in prenatal development

## 1.2. Fatty acids in aquaculture

The main sources of HUFAs in the human diet are fish and fish oil. The world capture fishery has reached (or somewhere already exceeded) the limit called overfishing. This is a situation when ocean fish stocks are over-captured and cannot naturally restore their populations. Some species are actually threatened with extinction if their populations are not supplemented by artificial breeding. Nowadays, aquaculture is the fastest growing industry in the animal husbandry sector. Aquaculture production has grown by up about 8.5% annually for last 25 years and currently more than 50% of all fish for human consumption is produced in aquaculture. The requirements for fish meal and fish oil, as the main components of feedstuffs, are increasing accordingly. Simultaneously, the price of these raw materials is increasing and it is not possible to increase production. Therefore farmers are under increasing pressure from governments to replace fish meal and oil with alternative, sustainable sources, mainly products of terrestrial agriculture (Pickova and Mőrkőre, 2007). These resources are cheaper, their production is sustainable and they are much more environmentally friendly for ocean fish populations. Many studies on many marine and freshwater species have been performed and the results confirm that up to 70% of fish oil in the diet can be replaced by vegetable oil without any negative effect on growth and survival of farmed fish. With a suitably chosen technology, it is also possible to restore a high content of HUFAs in the fish flesh, as their content is reduced to some extent when fish oil in the diet is replaced by plant oils.

## 2. FACTORS INFLUENCING THE FATTY ACID COMPOSITION

Factors influencing the fatty acid composition of fish lipids are summarised by Kalač and Špička (2006) and for carp by Mraz and Pickova (2011, and can be divided into internal and external. These factors are interrelated and often correspond closely. The list is quite long, but is difficult to say which (except nutrition) is the most important. Each factor is always affected by the others.

### 2.1. Internal factors

#### 2.1.1. Fish species

There are huge differences in fillet fatty acid composition between fish species. One important fact must be considered – some species are very fatty (over 10% fat), e.g. eel (*Anguilla anguilla*), silver carp (*Hypophthalmichtys molitrix*) and bighead carp (*Aristichtys nobilis*); some have medium fatty levels (2–10%), e.g. common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*) and tench (*Tinca tinca*); and some have a low fat content (under 2%), e.g. pikeperch (*Sander lucioperca*) and perch (*Perca fluviatilis*). The amount of fat significantly affects fatty acid composition. In the body of fatty species, a substantial part of the fat is stored in the form of storage lipids (triacylglycerols), which usually contain a higher percentage of MUFA (e.g. oleic acid), while the percentage of PUFA is relatively lower. In case of low fat species, the body fat is preferably stored in the form of structural lipids (phospholipids). These usually contain fatty acids with longer carbon chains and they are more unsaturated (i.e. EPA and DHA) than fatty acids stored in the form of triacylglycerols, so there is a relatively higher content of PUFA in low fat species. Table 1 shows the average proportion of fatty acid groups in the fat of common freshwater fish species.

*Table 1. Examples of fatty acid composition (%) in fillet lipid of several freshwater fish species (reviewed by Steffens, 1997).*

| Fatty acids | Rainbow trout | Common carp | Silver carp | Bighead carp | Eurasian perch |
|---|---|---|---|---|---|
| SFA | 22.5 | 30.4 | 25.1 | 23.9 | 27.3 |
| MUFA | 36.3 | 40.7 | 40.7 | 42 | 13.4 |
| PUFA | 41.2 | 28.9 | 34.2 | 34.1 | 59.3 |
| EPA+DHA | 25.2 | 14.1 | 15.6 | 20.6 | 37.9 |
| Σ omega-3 | 33.2 | 18.5 | 23.2 | 26.2 | 45.4 |
| Σ omega-6 | 8 | 10.4 | 11 | 7.9 | 13.9 |
| omega-3/omega-6 | 4.2 | 1.8 | 2.1 | 3.3 | 3.3 |

### 2.1.2. Genetic origin

In recent years, many investigations have focused on clarification of the genetic influence on fatty acid biosynthesis. The aim is to determine whether there is variability within species. For livestock, it has been confirmed that fat content as well as proportion of omega-3 fatty acid are highly inheritable factors (Karamichou et al., 2006; Kerry and Ledward, 2009). Obviously, fish farmers are mainly interested in the economically most important fish species. Leaver et al. (2011) used the method of gene expression to confirm the differences between Atlantic salmon (*Salmo salar*) populations and found a high heritabilty of fat content and proportion of omega-3 fatty acids, similarly to livestock. As regards common carp, at least two subspecies are known within the genus *Cyprinus*, namely European carp (*Cyprinus carpio carpio*) and Asian carp (*Cyprinus carpio haematopterus*). Zajic et al. (2011) found differences in fatty acid composition between several strains (crossbreds) of Ropsha scaly carp, with a higher proportion of PUFA in lipids in pure-bred Ropsha carp (a mix of *C.c. carpio* and *C.c. haematopterus*) compared with other crossbreds of this strain under identical conditions. The confirmation of differences in the ability for HUFA biosynthesis could be used in the common carp selection programme for the production of offspring with a higher content of healthy PUFAs in the lipids.

### 2.1.3. Gender and stage of maturation

Some studies have demonstrated an influence of gender on the amount of fat in common carp. Females have been reported to reach a higher fat content compared with males at the same age (Kocour et al., 2007). This could be explained by the later sexual maturation of females. However, Buchtová (2007) did not demonstrate the effect of gender on fatty acid composition in carp. Hence, the differences between males and females have not yet been confirmed.

### 2.1.4. Type of tissue

The body fat is not stored in all parts of the fish equally. Basically, fish flesh can be divided into white and red muscle. Furthermore, there is adipose tissue located in the abdominal (belly) part of the fillet and visceral fat around the inner organs (Figure 2). Many lipids are also stored in the hepatopancreas and in the gonads, both in males and females. All these parts of the fish can show significant differences in fat content and thus also in fatty acid composition. The higher the fat content in a tissue, the higher the representation of SFA and MUFA. This is due to the fact that PUFA are usually the fraction of phospholipids that forms the membranes, while SFA and MUFA are stored as triacylglycerols, with energetic functions. Mraz et al. (2009) studied the distribution of body fat and fatty acid composition in common carp and found the lowest fat content in white muscle (0.95%), followed by red muscle (16.7%) and the belly part (30%).

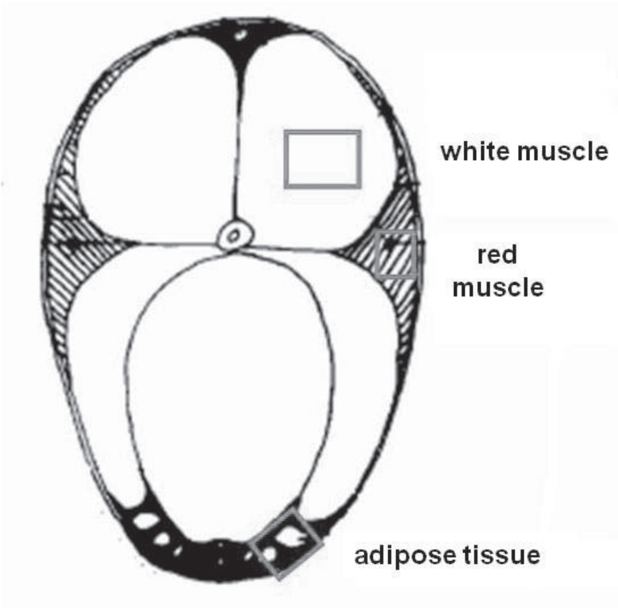


*Figure 2. Distribution of major types of muscle in edible parts of fish (adapted from Kiessling et al., 1991).*

#### 2.1.5. Fish age

As mentioned above, the fatty acid composition in fish tissue depends on the fat content. The higher the fat content, the higher the storage lipid content and the higher the proportion of MUFA. Younger individuals grow intensively and therefore under natural conditions they have lower levels of energy reserves than older individuals. In contrast, older fish grow more slowly and thus they have a higher amount of storage fat in adipose tissue as triacylglycerols. In semi-intensive and intensive culture, the age factor can be significantly influenced by feeding intensity of the fish stock.

#### 2.1.6. Health status

When considering the relationship between health status and fatty acid composition of fish, it is important to highlight the fact that fish in poor condition or in deteriorative health status feed poorly and behave similarly as during starvation (see section 2.2.4.).

### 2.2. External factors

#### 2.2.1. Salinity

It has been confirmed that the salinity of the water environment is one of the most important factors affecting the fatty acid composition in fish tissue. While the tissues of marine fish usually contain a higher proportion of omega-3 fatty acids, freshwater species tend to have a higher amount of omega-6. This is due to differences in the food chain between salt and fresh water environments. There is evidence that some species migrating from one environment to another are subjected to significant changes in the composition of fatty acids.

### 2.2.2. Nutrition

Influencing the fatty acid composition by nutrition is very complicated in ruminants, where the microorganisms in the stomachs play a negative role. It is easier in the case of monogastric species (e.g. pigs), where it is possible to increase the content of one fatty acid by supplying it in the diet. Freshwater fish species have the ability to biosynthesise HUFA (including EPA and DHA) *de novo* from the precursors LA and ALA. Marine species and some freshwater predatory fish (pikeperch, pike) have lost this ability and therefore HUFA are essential for them and they must receive them via the diet. This is due to their position in the food chain and to the composition of their natural feed. While there is huge availability of HUFA in sea algae and plankton, freshwater plankton and benthos are rather rich in ALA, although there are also some HUFA in freshwater environments. Table 1 shows several examples of the muscle fatty acid composition of some freshwater fish species. Obviously, the highest content of HUFA is found in predatory (carnivorous) fish (trout, perch), because these species are higher in the food chain compared with their prey. A relatively high content of HUFA is present in the flesh of herbivorous fish (silver carp) because, as mentioned above, there are also quite high levels of HUFA in freshwater plankton and benthos and, simultaneously, these species are able to biosynthesise HUFA themselves.

When composing the diets for the two most farmed species in the Czech Republic (common carp, rainbow trout), it is important to supply their essential fatty acid requirements. An optimal diet fat content for carp is 8–12%, of which 1% should be LA and 0.5% ALA respectively. For rainbow trout, a minimum dietary fat content of 18–22% is recommended, of which 0.8% should be LA and 1% acid.

Another important aspect is the storage of feed and feeding mixtures, as well as the raw materials for production. Feeds in general, and fats in particular, are very susceptible to oxidation. Impaired feed then causes health complications, reluctance to feed and growth disorders in farmed fish and subsequently a lower quality of the final products. To prevent oxidation of fat in the feed, it is important to use only fresh fats with low peroxide number. Antioxidants (e.g. α-tocopherol) are commonly used to protect feeds, while in addition proper storage (dark, dry place) as well as timely consumption are necessary.

Table 2 presents a list of vegetable oils potentially suitable as a lipid source in feedstuffs for fish. Oils above the red line have a high proportion of omega-3 PUFA and their use should be preferred. Oils below the red line contain mostly omega-6 PUFA and are not as appropriate. These oils are suitable rather in a mixture with other oils (e.g. rapeseed oil + palm oil).

*Table 2. Vegetable oils suitable for aquaculture feedstuffs (Pickova and Mőrkőre, 2007).*

| Vegetable oil | Content of omega-3, % | Ratio omega-6/omega-3 |
|---|---|---|
| Linseed oil | 60 | 0.2 |
| Hempseed oil | 22 | 2.5 |
| Rapeseed oil | 13 | 2 |
| Olive oil | 1 | 8 |
| Soy oil | 8 | 7 |
| Palm oil | 0.5 | 20 |
| Corn oil | 1 | 60 |
| Cottonseed oil | 0 | >100 |
| Sunflower oil | 0.5 | >100 |

### 2.2.3. Bioactive compounds

An alternative and promising option for the future seems to be addition of biologically active compounds to fish feed. These specifically active compounds affect the fish metabolism and cause higher production and storage of omega-3 fatty acids in the lipids.

In salmonids, one promising path seems to be the addition of sesamin, a natural lignan present in sesame oil. Trattner et al. (2008a) provide strong evidence that sesamin is a good modulator of fatty acid metabolism in hepatocytes of Atlantic salmon. In agreement with these results, Trattner et al. (2008b) showed in an *in vivo* study that dietary sesamin increased the desaturation and elongation of ALA towards DHA by up to 37% in rainbow trout. The β-oxidation of lipids was clearly increased, as well as expression of CPT1 (carnitine palmitoil transferase) and genes involved in β-oxidation. Furthermore, sesamin had an effect on the expression of Δ5 and Δ6 desaturases. Dietary sesamin was also tested in common carp culture by Mraz et al. (2010), but no such effect was observed.

Another potentially suitable compound could be lipoic acid. This substance operates as an antioxidant in fat. Trattner et al. (2007) demonstrated the positive effect of dietary lipoic acid on the EPA content in polar lipids of the freshwater species *Piaractus mesopotamicus*.

### 2.2.4. Starvation

During starvation, fish consume their energy reserves. This leads to changes in the amount and composition of fat. In carp culture in the Czech Republic, there is typically a period of starvation before carps are sold (purging). Purging is a very important part of the rearing process for common carp in Central Europe and is commonly conducted between October and December. Fish are kept in clear water without feeding in order to empty the gut, decrease the entrail proportion and eliminate possible tainted flavour. This leads to weight loss and storage fat mobilisation. When storage fat is metabolised, the saturated and monounsaturated fatty acids are digested first, so the relative proportion of PUFA increases (Tocher et al., 1989). This fact can be utilised in the purging technology and, to some extent, to influence the fatty acid composition of common carp intended for human consumption. In a study of fatty acid composition in carp flesh during starvation by Vacha et al. (2007), carp supplemented with cereals slightly increased the proportion of HUFA, whereas a decrease was observed in the group kept under natural conditions. Csengeri (1996) found a continual decrease in oleic acid during starvation of carp, while the proportion of HUFA slowly increased over time. An increase in the relative content of HUFA has also been observed in other important fish species, such as channel catfish (*Ictalurus punctatus*), Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). The effect of starvation is highly influenced by the type of feeding that precedes this process.

### 2.2.5. Season (ambient temperature)

In general, fish species living in cold water (trout) have a higher proportion of long-chain omega-3 fatty acids (more than 20 carbons in chain) than warm water species (carp). This is due to the principle of action of fatty acids in the cell membranes. At low temperatures, the membranes consist of longer and more unsaturated fatty acids, because these have a higher fluidity due to the substantially lower freezing point of highly unsaturated fatty acids compared with shorter-chain and saturated fatty acids.

In temperate climate conditions (as found in the Czech Republic), the temperature varies during the year in relation to natural feed supply. The fillet fat content changes from spring to winter, leading to changes in fatty acid composition. Common carp is fattest in late summer and early autumn. During the winter, the fat reserves gradually decline and at the beginning of spring the fillet fat content is at its lowest. Kmínková et al. (2001) monitored changes in the fatty acid composition of common carp during the year and found that the proportion of individual fatty acids varied depending on activity and feed availability. Some other studies (Guler et al., 2008) have reported that the SFA content remains unchanged during the year, whilst there is a variation in the percentage of MUFA, PUFA and HUFA. These fluctuations in fatty acid composition can be successfully influenced by proper fish nutrition and rearing.

### 2.2.6. Processing and cooking

One of the most important factors influencing the final quality of fish intended for human consumption is processing and cooking of fish meat. Compliance with all hygiene principles during processing, especially monitoring of the freshness and storage temperature, is necessary, because fat is vulnerable to oxidation. On the other hand, PUFA are relatively stable during cooking. It is recommended that fish be prepared at temperatures up to 100 °C.

The final quality of fish and/or fish products is influenced by the fat or oil used for frying. Changes in the fatty acid composition after processing were observed by Sampels et al. (2009), who found that the ratio between omega-3 and omega-6

PUFA was up to 400 times lower (to the detriment of omega-3) than that of the fresh fish when an omega-6 – rich oil was used.

From the above, it is clear that the most important factor affecting the fatty acid composition of fish is their nutrition. When applying the appropriate feed and in combination with other factors, it is possible to produce freshwater fish rich in omega-3 HUFA.

## 3. MODIFICATION OF FATTY ACID COMPOSITION IN FISH FLESH

### 3.1. Finishing feeding technology

In controlled conditions (in the Czech Republic particularly in salmonid culture), fish oil from marine pelagic species is usually used as the source of fat for feeds. In the past this raw material was easily available and relatively cheap, but with increasing production in aquaculture, fish oil is becoming less available and its price is rising. Therefore, new ways have to be found to keep the aquaculture in constant growth and, simultaneously, to avoid an increased use of fish oil.

One possible way to avoid the use of high proportions of fish oil is to adopt a finishing feeding strategy in which a diet with vegetable oil is fed during the fattening period as a partial replacement for fish oil. For the final period (weeks, months), a diet with 100% fish oil is used again to partially or completely restore the omega-3 HUFA proportion in the fish flesh (Mraz et al., 2011a).

*Example:*

In trout initially fed a diet with vegetable oil, the average weight, fat content and EPA+DHA content of fish at the end of the period was 100 g, 8% and 1%, respectively. The diet was then changed to a conventional diet containing fish oil until the trout reached a market size of 250 g and a fat content of 10%. In trout fed the conventional fish oil diet throughout the whole fattening period, the EPA+DHA content was 9%.

In creation of a model for predicting the fatty acid content in fish flesh, these data were used as input in a mathematical equation designed for salmonids (Robin et al., 2003; Jobling, 2004):

$$\mathbf{P_T = P_K + [( P_0 - P_K ) / (Q_T / Q_0 )]}$$

where:

$P_T$ ..... Predicted percentage of a fatty acid at time T
$P_K$ ..... Percentage of fatty acid measured at time T in the fillet of control fish continuously fed the reference/finishing diet
$P_0$ ..... Percentage of a fatty acid in the fillet of tested fish at the beginning of the finishing feeding period
$Q_T$ ..... Quantity of total fatty acids in the tested fish at time T
$Q_0$ .... Quantity of total fatty acids in the tested fish at the beginning of the finishing feeding period

In this example, the values for the variables listed above for trout were entered:

$Q_0$ = **0.6** (6%) x 0.1 (0.1 kg)
$Q_T$ = **2.5** (10%) x 0.25 (0.25 kg)
$P_K$ = **9** (9% EPA+DHA in control)
$P_0$ = **1** (1% EPA+DHA at the beginning of the finishing feeding period)

Then:

$$P_T = 9 + [(1 - 9) / (2.5 / 0.6)]$$

$$\mathbf{P_T = 7.08\%\ EPA+DHA}$$

Thus at the end of fattening period using a finishing feeding strategy, trout in this example have a muscle EPA+DHA content of 7.08%. Generally, about 85% of total fats are fatty acids. The fat content in this example is 10%, and thus these trout will contain approximately 600 mg EPA+DHA per 100 g serving (see section 1.1.).

## 3.2. Fattening with a mixture containing HUFA precursors

The most reliable way to achieve a high content of HUFA in fish flesh is probably by using the feed containing either HUFA or its precursors. The simplest way is to use a feeding mixture with fish oil containing a large amount of n-3 HUFAs. However, use of fish oil is currently economically and environmentally unsustainable.

Unlike marine species, most freshwater fish have the ability to biosynthesise HUFA via their own metabolism by specific enzymes, desaturases and elongases. Therefore, it is possible to add HUFA precursors directly to the fish diet. The precursor of n-6 HUFA is LA (18:2n-6), which is metabolised towards mainly arachidonic acid (AA; 20:4n-6), whereas the precursor of n-3 HUFA is ALA (18:3n-3), which is metabolised towards eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acid. The best sources of these precursors in an appropriate balance are rapeseed, linseed and hempseed, and their oils (Table 2). Rapeseed and linseed contain precursors of n-3 and n-6 HUFA in suitable proportions and are relatively cheap components for aquaculture feeds.

In carp culture, it is for example possible to replace commonly used cereals (whole grains) with pellets containing rapeseed. An even better option is to use a mixture, which is based on cereals with addition of rapeseed mouldings and linseed. However it is very important, especially in the case of linseed, to choose the correct variety. The reason is that several varieties of flax cultivated today contain the opposite n-3/n-6 ratio (in Czech Republic e.g. varieties JANTAR and LOLA). These varieties have been bred for higher yields, but their use as a component of aquafeeds is inappropriate. A suitable content of 18:3n-3 for fish in linseed is 30% or more. In combination with natural feed (plankton and benthos) present in the pond environment, production of carp with a significantly elevated proportion of n-3 fatty acids in flesh can be achieved.

*Example:*

At the beginning of April, a pond with an area of 2 hectares and average natural productivity of 250 kg/ha was stocked with three-year-old carp, average weight 1 kg per individual. Stocking density was fixed at 500 kg/ha. The plan was to use 2000kg of feeding mixture with feed conversion ratio (FCR) 2 and to achieve 1 kg weight gain per carp, i.e. a total of 1000 kg per pond. Fish were transferred from the same overwintering pond and had to be in good condition, with a Fulton's coefficient (CF) of above 2.7. The pond was filled with water from a natural stream in order to replenish evaporated water, but high attention was paid to avoiding leakage of plankton from the pond. During the vegetation season, the fish were supplemented three times a week with rapeseed/linseed pellets. The composition of the diet is shown in Table 3.

*Table 3. Composition of the diet with HUFA precursors.*

| Component | Specific composition of the mixture (%) | Range (%) |
|---|---|---|
| Rapeseed mouldings | 15 | 12–20 |
| Extruded linseed | 15 | 10–20 |
| Linseed oil | 0 | 0–4 |
| Rapeseed oil | 0 | 0–4 |
| Wheat+flour+bran | 55 | 50–60 |
| Wheat | 6.5 | 6–15 |
| Soybean meal | 6.5 | 5–10 |
| Limestone | 1.5 | 1–2 |
| Premix for carp (Carp 0.3)* | 0.3 | 0.3 |
| Wafolin** | 0.2 | 0.2 |

* Carp 0.3 is a commercially available mixture of vitamins, minerals and nutrients, designed as a component of the diet of carp; **Wafolin is commercially available product based on lignosulphonate, intended to improve the physical stability of the pellets.

Feed ration varied from 1% to 3% of the actual stock weight and was adjusted depending on water temperature, oxygen saturation and the amount of available natural feed. The properties of the feeding mixture were based on an optimal combination of rapeseed mouldings and extruded linseed, which supply a relatively cheap ALA-rich source for the diet. The presence of ALA in the mixture both increases its content in fish flesh and is a precursor for n-3 HUFA biosynthesis of EPA and DHA. Another important factor is that the mixture has the optimal n-3/n-6 ratio of 1:1 to 1:2, which, together with a sufficient amount of essential fatty acids, is suitable for growth of the carp and synthesis of EPA and DHA. This fact is also important for reduced storage of the less beneficial SFA and MUFA in the fish muscle. The diet was given to fish in form of pellets to avoid losses and separation of the individual components.

The carp were harvested at the end of October and transported to purging ponds, where they were purged for several weeks and then processed into fillets. During filleting, the abdominal (belly) part of the fillet containing the majority of storage fat, mostly in the form of SFA and MUFA, was removed. The adjusted raw fillet had the characteristic quality and quantity of lipids in a 200 g portion shown in Table 4.

This whole procedure of a carp farming strategy using a feed mixture containing HUFA precursors is based on Utility model No. 21926 and Patent No. 302744 (The Authority of Industrial Property) (Mraz et al., 2011c).

***Table 4.*** *Content and composition of carp lipid in a 200 g serving when a diet with HUFA precursors is supplied.*

| Indicator monitored | Mean | Minimum | Maximum |
|---|---|---|---|
| Lipid content | 15 g | 10 g | 20 g |
| Saturated fatty acids (SFA) | 3 g | 2 g | 4 g |
| Monounsaturated fatty acids (MUFA) | 6 g | 4 g | 8 g |
| Polyunsaturated fatty acids (PUFA) | 3 g | 2.5 g | 3.5 g |
| Omega-3 PUFA + HUFA | 1 g | 0.8 g | 1.2 g |
| Omega-3 : omega-6 | 1:1.75 | 1:1.5 | 1:2 |
| Omega-3 HUFA | 600 mg | 400 mg | 800 mg |
| EPA + DHA | 300 mg | 200 mg | 400 mg |

Figure 3 shows several examples of aquaculture feeds that are potentially applicable for the rearing of freshwater fish species with an elevated content of omega-3 HUFA. Complete mixtures (Figure 3a) with high content of fish oil and fish meal are widely used in salmonid culture around the world, but also in perch or pikeperch farming facilities. These diets provide a high content of HUFA, so they are the best option in this respect. However, the situation concerning the state of marine pelagic species used for production of fish meal and fish oil urgently needs some alternatives. Complete extruded diets with the addition of the cultivated green algae *Chlorella* spp. (Figure 3b) could be one of these alternatives (see section 3.4.). In Czech pond aquaculture, there is major use of cereals (whole grains) (Figure 3c). However, cereals are not very useful in terms of n-3 fatty acid content. An interesting option for fish farming companies could be a mixture with rapeseed mouldings and extruded linseed (Figure 3d), with the composition and application as described in section 3.2. A similar feed to this is the extruded mixture for cyprinids, with a portion of linseed and rapeseed oil (Figure 3e). In this diet commodities such as fish meal and fish oil are fully replaced by alternative components. It is suitable especially in rearing of carp fry in controlled conditions and ensures a high intake of dietary ALA.

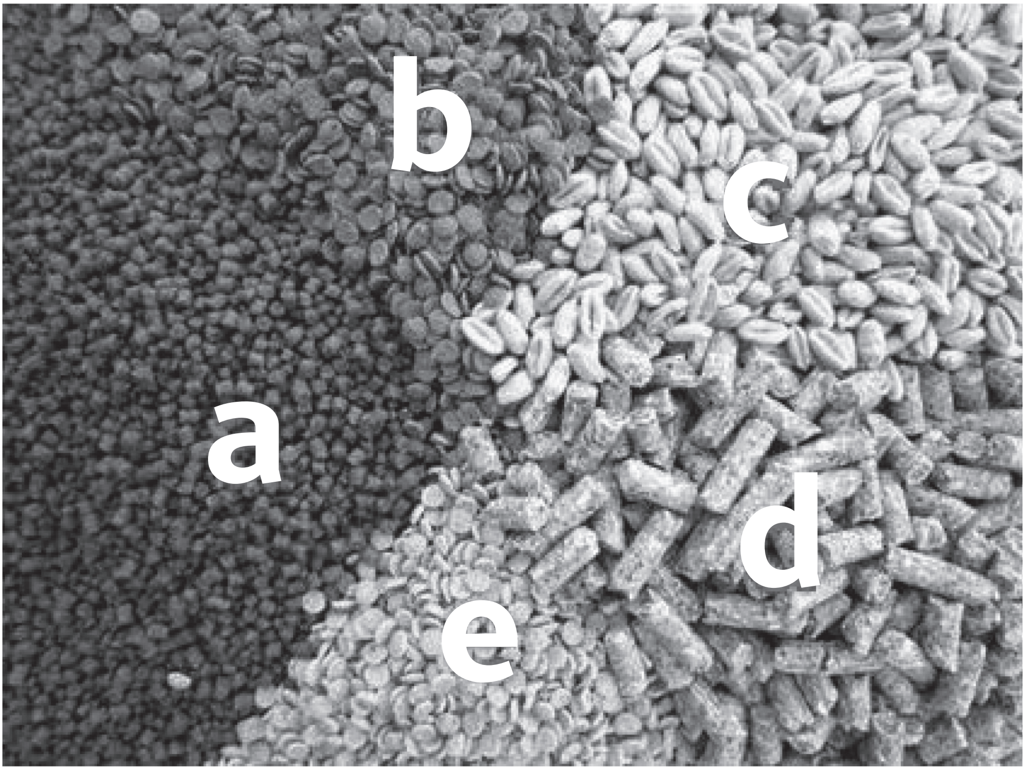


*Figure 3. Examples of aquaculture feeds applicable in the rearing of freshwater fish species. a) Complete, commercially available pellets with fish oil; b) complete extruded mixture with green algae addition (10%); c) wheat; d) supplementary rapeseed/linseed pellets; e) complete extruded diet with rapeseed and linseed oil.*

## 3.3. Utilisation of natural feed

Natural feed (plankton and benthos) is by far the cheapest way to achieve a high representation of n-3 HUFA, including EPA and DHA in carp flesh. In particular, plankton is very rich in n-3 HUFA. Plankton and benthos (Figures 4, 5 and 6) form the basis of pond production. Where cereals are used as a supplement for carp stock, the lipids in the carp will be rich in the MUFA oleic acid (18:1n-9) (up to 50% of the total fatty acid content in the fish lipids). Oleic acid is synthesised from the starch in the dietary cereals. In addition to starch, cereals also contain a high amount of n-6 fatty acids, LA (18:2n-6) in particular, which is unfavourable from a human nutrition point of view. On the other hand, an increased intake of natural feed leads to a lower content of oleic acid in the carp lipids and a higher synthesis of n-3 fatty acids. The fatty acid composition in plankton and benthos strongly depends on the season. The highest proportion of n-3 HUFA in natural feed is found in autumn (Mráz et al., 2011b), when the most abundant plankton organisms were copepods. The fatty acid composition of the natural feed compared with cereals (wheat) is shown in Figure 4.

Pond fish farming based on natural feed utilisation results in fish with the highest flesh quality, but at the expense of lower economic benefits for the fish farmer due to the lower production rate per area unit.

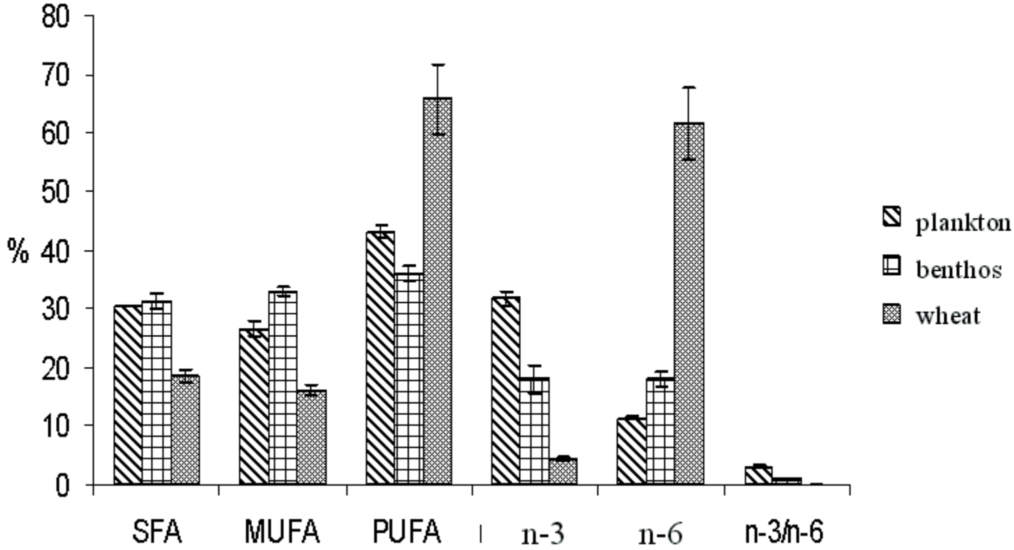


*Figure 4. Comparison of fatty acid composition in plankton, benthos and wheat.*

*Figure 5. A representative of pond benthos: Chironomus plumosus larvae (photo: M. Bláha).*

*Figure 6. Representatives of pond plankton: Cyclop spp. (left) and Daphnia spp. (right) (photo: M. Bláha).*

## 3.4. Utilisation of alternative HUFA sources

This publication is focused on freshwater fish species, but it is necessary to mention the possibilities of fish meal and fish oil replacements, recently used in marine aquaculture. These novel sources of protein and fat are also promising for freshwater aquaculture in the future. They are intended for the rearing of various invertebrate species and their subsequent use for the production of aquaculture feed. One such source is krill, which is the general name for a group of small crustaceans living in the ocean, feeding on plankton and extremely rich in n-3 HUFA. Krill capture for industrial and nutritional use does occur, but the possibility of rearing krill is currently being tested to secure sustainable production. One primary objective is to reduce the eutrophication of the sea, because krill consume huge amounts of nutrients. A secondary objective, the use of krill as a substitute for fish meal and fish oil, seems to be potentially feasible.

Another option is the controlled rearing of certain species of phytoplankton and zooplankton. Phytoplankton organisms (algae, e.g. *Chlorella* spp. see Figure 3b) can synthesise n-3 HUFA. At present this method is relatively expensive, but it is an interesting possibility for the near future, showing the way to obtain components for aquaculture feeds rich in n-3 HUFA and to ensure sustainable development of aquaculture.

## 3.5. Processing and culinary preparation of fish

Section 2.2.6. described how the culinary preparation of fish influences the fatty acid composition for human nutrition. During processing, it is beneficial to cut a strip off the abdominal part (Figure 6), which contains a high proportion of storage fat (up to 30% in carp) and which consists mainly of SFA and MUFA and very little n-3 HUFA (Mraz et al., 2009). Removing this section provides a significantly higher percentage intake of healthy beneficial n-3 fatty acids per serving.

When frying fish, it is recommended to use oil with a suitable n-3/n-6 ratio. We recommend commercially available rapeseed oil. An acceptable alternative is olive oil, which contains a high proportion of healthy neutral MUFA. Conversely, the use of sunflower oil is not recommended, because it contains a high proportion of n-6 fatty acids.

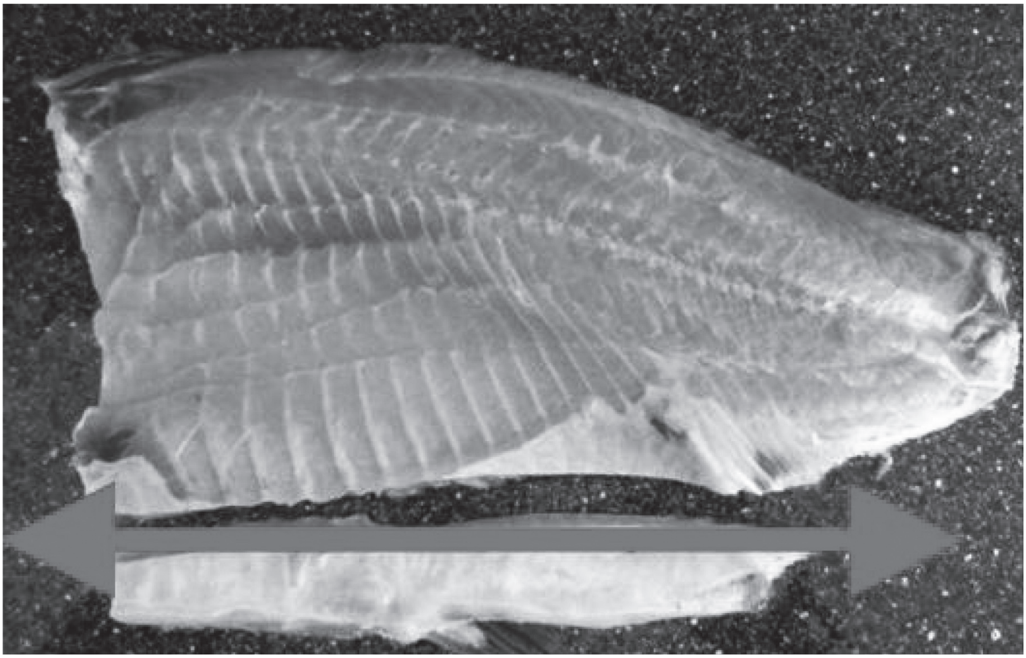

***Figure 7.*** *Carp fillet with removed belly (adipose) part.*

When buying semi-finished fish products, it is important to pay attention to the composition of such products. Sunflower or soybean oil (or mixtures thereof) are very often used for preparation of these products and their intake is not desirable in terms of health benefits for the consumer (see Table 2 for composition of some conventional oils). Use of unsuitable oil reduces the health benefits of fish consumption (Sampels et al., 2009) (see section 2.2.6.).

## 4. CHEMICAL ANALYSIS OF FATTY ACID COMPOSITION

Laboratory analysis of fatty acid composition consists of several steps. The first step is sample collection. In the case of analysis of nutritional composition, it is better to sample the whole fillet (edible parts), mince it and then take a random sample. The amount required depends on the analytical method; in principle 1 g is enough. For fat extraction, the sample is homogenised and mixed with an organic solvent or a mixture thereof (e.g. HIP – hexane + isopropanol) (Hara and Radin, 1978). Salt addition results in modification of the polarity of the sample and the water and lipid phase separate. After centrifugation, to improve separation of the phases, the lipid phase is removed to an empty pre-weighed tube and the solvent is evaporated under nitrogen (Figure 8). The lipid content is then detected gravimetrically. The lipids can be either separated into lipid classes (triacylglycerols, phospholipids, free fatty acids, cholesterol, etc.) in order to determine the fatty acid composition in these classes (thin layer chromatography (TLC) method), or the fatty acid composition of the total lipids can be determined. Before the latter, it is necessary to prepare fatty acid methyl esters (FAME). These are prepared through esterification (Appelqvist, 1968), which is the reaction of an acid with an alcohol to form an ester and water. This reaction is very well known from the production of biodiesel from rapeseed oil. The FAME are then used for the last step, gas chromatography (GC) analysis (Figure 9). Individual fatty acids are detected by comparison of their retention time (peak) against a standard.

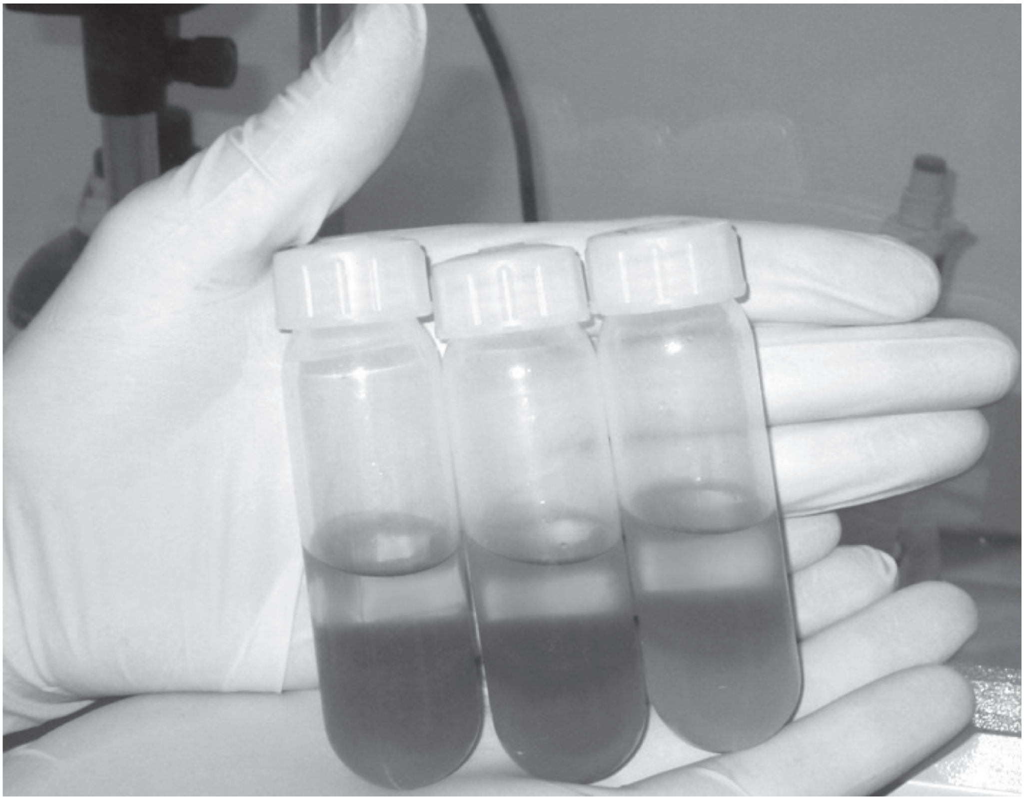

***Figure 8.*** *Lipids extracted from samples in Teflon tubes.*

***Figure 9.*** *Gas chromatograph Varian CP 3800 used for analysing the fatty acid composition.*

## III. COMPARISON OF "PROCEDURE NOVELTY"

This methodology does not replace an existing one. The topic of healthy nutrition for the population is currently very important and the use of fish as the nutritionally most valuable food is growing in importance. Fish consumption in the Czech Republic is at a very low level, while mortality connected with cardiovascular disease (atherosclerosis, myocardial infarction, etc.) is extremely high. Consumption of food with a high proportion of n-3 fatty acids can help prevent these diseases. The methodology presented here includes an overview of the influences on the fatty acid composition in fish flesh, brings a comprehensive overview of the functions and effects of fatty acids in human nutrition, and shows possibilities to influence fatty acid composition in freshwater fish. The methodology is the result of research in the field of fish quality in the world, as well as within the team of authors. Until now, a prescribed methodology for fish farmers has been lacking in the Czech Republic.

## IV. DESCRIPTION OF METHODOLOGY APPLICATION

This methodology could be a tool for large-scale and also small-scale fish farmers and feed processors. The list of the internal and external factors influencing the fat content and composition of fish should serve as a set of guidelines to assist farmers and feed processors in producing high quality fish for human consumption.

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## VI. LIST OF PUBLICATIONS PRECEDING THIS METHODOLOGY


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