Fakulta rybářství
a ochrany vod
Faculty of Fisheries
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of Waters

Jihočeská univerzita
v Českých Budějovicích
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Impact of production systems on lipid quality of common carp (*Cyprinus carpio*)

Vliv chovu na kvalitu tuku kapra obecného (*Cyprinus carpio*)




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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. AQUACULTURE – OVERALL BACKGROUND

Aquaculture involves the farming of aquatic organisms such as fish, crustaceans and aquatic plants. Today, aquaculture is the fastest growing sector of animal production worldwide. During the last 25 years, aquaculture production grew by up to 8.5% annually (FAO, 2012) and currently covers roughly half the global demand for fish and fish products for human consumption. Annually, almost 100 million tons of fish are captured from the ocean, of which around 70 million tons are intended for direct human consumption. This amount includes hundreds of species. Some of these, especially predatory species, are actually threatened with extinction (Naylor et al., 1998; Naylor et al., 2000). Therefore, the constantly increasing demand for fish and fish products for human consumption must be covered by fish farming – by aquaculture.

Two key biological sources of feeding components for aquaculture are fish meal and fish oil. Both these raw materials have their origins in traditional ocean capture fisheries (De Silva et al., 2011). Back in the 1980s, most of the feed resources needed for the cultivation of carnivorous and omnivorous fish and crustaceans originated from pelagic forage fish (capelin, sand eel, anchovy, horse mackerel, pilchard and menhaden) (Olsen, 2011). Thanks to major investments in research, there has been a change in this over the last decade, with a tendency towards greater use of agricultural feed resources for both fish and crustacean production (Gatlin et al., 2007; Naylor et al., 2009). This change has been driven by the limited availability of marine feed resources and the lower production costs obtained with plant resources from agriculture. The strategy of increasing the fraction of plant products in formulated pellet feeds has been successful for aquaculture (Olsen, 2011; Tacon et al., 2011). Aquaculture production continues to grow, although the production of fish meal and fish oil is stagnating, as well as their use in aquaculture (Figure 1). The demand for fish oil in aquaculture will outstrip the supply within the next few years (Tacon and Metian, 2008). This demand for feed raw materials can be met by new additives such as plant proteins and oils.

Most fish species farmed in European aquaculture are predatory fish species, feeding naturally on smaller fish. Therefore the natural feed for these species contains fish raw products such as fish meal and fish oil. Fish meal represents the most suitable protein source, with good digestibility and containing all essential amino acids in appropriate proportions for fish nutrition (Cho and Kim, 2011).

Fish oil is obtained as a by-product in the production of fish meal. Generally, from 100 kg of fish (or fish processing by-products), 20–23 kg of fish meal and 5 kg of fish oil can be produced (Pike and Jackson, 2010). In recent years, about 25 million tons of fish per year have been diverted directly to the production of these commodities, which represents about 25% of captured fish worldwide (FAO, 2012). These facts have led to a global discussion about using part of this 25% for direct human consumption, especially in the developing countries (Tacon and Metian, 2008, 2009). This discussion is constantly growing and is supported by other considerations, such as the use of large quantities of fresh fish and fish products in the pet food industry, which is the second largest sector where the fish is not used primarily for human consumption (De Silva and Turchini, 2008). Annual world production of fish oil fluctuates widely, as does its price. Nowadays, fish oil production varies within the range 0.9–1 million tons per annum (of which about 80% is exclusively used in aquaculture), with a market price of around 1000–2000 USD per ton (FAO, 2012).

Fish oil is considered an excellent lipid source, especially in terms of polyunsaturated (PUFA) and highly unsaturated (HUFA) fatty acids (Rice, 2009). Fish oil composition

reflects the process whereby HUFAs produced mostly by microalgae are magnified in the food chain and consumed by humans as fish. These substances are highly valued with regard to human nutrition, as further described below. In addition, the content of fat-soluble vitamins, primarily A and D (Rice, 2009), and also antioxidants (e.g. vitamin E, carotenoids) in fish oil is of importance.

In comparison with marine species, freshwater fish generally contain less n-3 HUFA. This is due to the composition of the food chain. In marine environments, large amounts of n-3 HUFA are naturally present in algae, which are the sole primary producer in the food chain. Predatory species, standing at the top of the food chain, gain access to n-3 HUFA through pelagic fish. These small, oily fish are also the raw material for production of fish meal and fish oil. Therefore, the carnivorous fish produced in fed aquaculture contain high levels of n-3 HUFA in lipids. More than half the entire annual production of fish oil is utilised in feeds for salmonids; mostly Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). However, production of salmonid species is about 2.5 million tons per year, which represents only about 4% of total fish production in aquaculture (FAO, 2012). Therefore it is obvious that the ratio between consumption and production is highly unsatisfactory in salmonid culture.

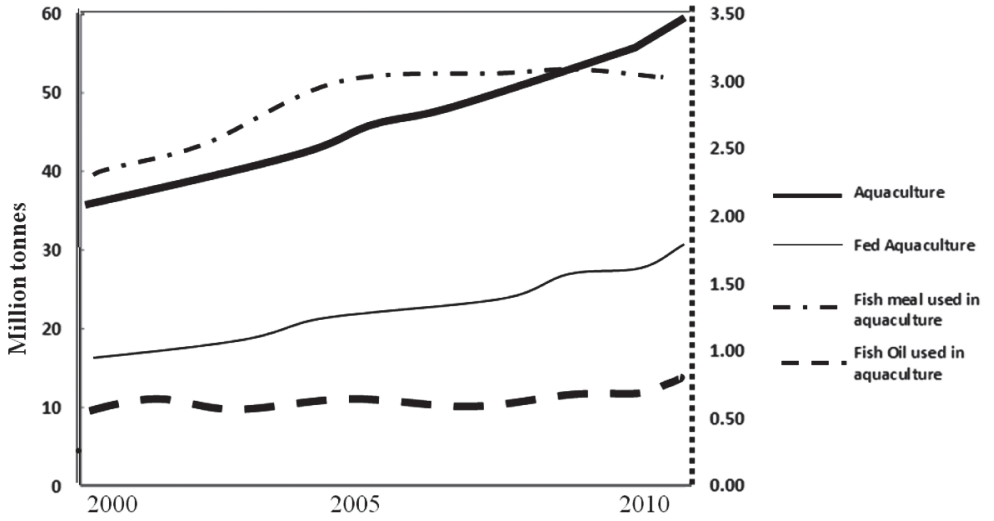

Figure 1. *Global growth of aquaculture production and stagnation of usage of fish meal and fish oil over the period 2000–2011 (data adapted from FAO, 2012).*

By developing new feeding strategies, > 50% of the fat in fish feed can be replaced by vegetable oils, such as rapeseed oil instead of fish oil (Bell et al., 2003; Izquierdo et al., 2005). The substitution takes place during the main period of growth. Feed companies ensure that the content of n-3 HUFA stays similar to that in wild fish (Pike and Jackson, 2010), mainly by using fish oil in the finishing period of 10–12 weeks (Robin et al., 2003).

As reviewed by Turchini et al. (2009), one aim of research in aquaculture should be to design eco-friendly, cost-effective aquafeeds that ensure the optimal utilisation of fisheries-derived raw products and guarantee maximum fish growth, health and maintenance of product quality. In addition, fish species such as common carp are not dependent on sources of fish meal and fish oil and have potential in improving fatty acid composition via their ability to biosynthesise n-3 HUFA from C18 sources.

1.2. CULTURE OF COMMON CARP (*CYPRINUS CARPIO*)

Common carp (*Cyprinus carpio*) is one of the most important fish species in aquaculture throughout the world. Annual world production is around 3000000 tons and European production 145000 tons (FAO, 2012). Nowadays, the traditional aquaculture system in the Czech Republic comprises 42000 ha of ponds. The proportion of Bohemian common carp in the total yield has remained stable over the years at between 86% and 90% of the total production of around 20000 tons per year, which is the largest production of carp in Europe.

The technology of carp culture in ponds is very well known and has been practised for many centuries. The quality of farmed fish in the Central European region has been studied by many authors (Buchtová et al., 2008, 2010; Mráz and Pickova, 2011; Mráz et al., 2012a; Steffens and Wirth, 2007). During the development of carp farming, the emphasis was on mastering different rearing stages, from broodstock through the management of fry production to market carp culture. In recent years, there has been increasing interest in fish flesh quality and its improvement.

Carp is an omnivorous species, feeding on plankton and benthos as well as detritus in natural conditions (Adamek et al., 2004; Mráz et al., 2012a). Culture of common carp in the Czech Republic is carried out in large earthen ponds, and there are two main production systems: extensive culture, where only natural feed (plankton and benthos) is utilised, and semi-intensive culture, where the fish stocks are supplemented, mainly with cereals. In the former case, the extensively produced carp are relatively rich in PUFA, especially n-3 fatty acids (ALA, EPA, DHA) (Mráz et al., 2012a; Paper II). These fatty acids are present in the natural feed available in the ponds (Mráz and Pickova, 2011; Mráz et al., 2012a). Carp also have relatively low requirements both for n-3 and n-6 fatty acids and for amount of dietary fat. The optimal dietary fat content for carp is 8–12%, of which only 1% should be linoleic and 0.5% alpha-linolenic acid (Kaushik, 1995). This suggests that the requirements can be fulfilled by plant 18-carbon fatty acids (Takeuchi, 1996). In addition, common carp, in contrast to marine fish, is able to convert ingested ALA into its longer derivatives, including EPA and DHA (Farkas, 1984; Takeuchi, 1996; Tocher, 2003). If carp are supplemented with cereals they grow faster, but at the same time their adipose tissue content can be more than double that of extensively farmed carp (Mráz and Pickova, 2011).

There are several possibilities for effectively achieving an increase in the n-3 HUFA level in the lipids of common carp in a sustainable and environmental friendly way:

1) The use of diets with a source of ALA, exploiting the ability of carp to biosynthesise n-3 HUFA *de novo* from its precursors. For rearing in ponds, these sources can be rapeseed mouldings, rapeseed oil or cake, linseed and hempseed.

2) Improved utilisation of natural feed in ponds, i.e. plankton and benthos, which are known to be excellent sources of n-3 HUFA (see above). Development of natural feed in ponds is usually supported by addition of missing nutrients in the form of manure, slurry, etc. This is a cheap and highly effective approach, but addition of fertiliser to ponds is restricted by law and by environmental standards.

3) A relatively new technology is that known as 'biofloc' (BFT). By adding a carbon source (e.g. molasses), floc-forming bacterial production is enhanced (Avnimelech, 1999). These flocs become fish feed. This technology makes better use of nutrients, is suitable for cyprinids (Mahanand et al., 2013) in aquatic environments and the micro-organisms are potentially a good source of n-3 fatty acids.

The lipid composition of fillets from commonly farmed carp is generally highly variable. There are no standard values for such fish, because the lipid composition varies depending on many factors, e.g. fish size, breed, the amount of natural feed available in the pond and farming intensity. However, in general, carp fed diets supplemented with cereals have higher lipid contents, high levels of MUFA, lower levels of PUFA and HUFA, including EPA+DHA and a higher n-6/n-3 ratio compared with fish fed natural feed only (Csengeri, 1996). A 200 g serving of fillet from carp fed wheat supplementation, rapeseed/linseed pellet supplementation and in natural conditions contains 1.06 g n-3 PUFA and 326 mg EPA + DHA, 1.72 g PUFA and 453 mg EPA + DHA, and 0.75 g PUFA and 354 mg EPA + DHA, respectively. Thus there is great potential for improvement of feeding strategies in carp production to achieve higher fatty acid production and a better fatty acid profile (Mráz et al., 2012a + Paper II).

1.3. FATTY ACIDS – CHEMICAL AND BIOLOGICAL BACKGROUND

Lipids are divided into two main classes – polar lipids and neutral lipids. Neutral lipids consist mainly of triacylglycerols, and partly monoacylglycerols and diacylglycerols, whereas polar lipids include mainly phospholipids (Henderson and Tocher, 1987). These lipids consist mainly of fatty acids.

Polyunsaturated fatty acids are hydrocarbon chains with two or more double bonds situated along the length of the carbon chain. Depending on the location of the first double bond relative to the methyl end, they can be classified as either n-6 or n-3. Linoleic acid (LA; 18:2n-6), the parent fatty acid of the n-6 family, is an essential fatty acid and cannot be endogenously synthesised by mammals (Das, 2006). LA is found in vegetable oils, seeds and nuts. Alpha-linolenic acid, ALA (18:3n-3), the parent fatty acid of the n-3 family, is the essential n-3 fatty acid. ALA is found in leafy vegetables, nuts, flaxseed and some vegetable oils (especially rapeseed and linseed oils) (Anderson and Ma, 2009; Wall et al., 2010). Both LA and ALA can be further metabolised to HUFA through a series of desaturation and elongation steps. LA is metabolised to arachidonic acid (AA, 20:4n-6), while ALA can be metabolised to eicosapentaenoic (EPA, 20:5n-3) and ultimately docosahexaenoic (DHA, 22:6n-3) fatty acids, which in aquaculture are often termed highly unsaturated fatty acids (HUFA; ≥ 20 C atoms and ≥ 3 double bonds) (Tocher, 2010).

HUFA of n-3 and n-6 series have three main functions in organisms: to act as an energy source (generally in the form of triacylglycerols), to act as structural components of cell membranes (mostly phospholipids) and to act as a precursor of eicosanoids (Horrobin, 1995; Scollan et al., 2001; Das, 2006).

The nomenclature, metabolism and functioning of fatty acids are further described in Paper IV.

1.4. FATTY ACIDS AND HUMAN HEALTH

There is convincing evidence that consumption of fatty acids, especially n-3 HUFA, has beneficial effects on human health (Kris-Etherton et al., 2002; Mozaffarian and Rimm, 2006; Simopoulos, 1991, 2002, 2008). Fish also provide significant levels of a number of other potentially protective components. These include vitamin D, vitamin B12, selenium, iodine, choline and taurine, as well as a well-balanced amino acid composition (Lund, 2013). Current intakes of highly unsaturated n-3 fatty acids, EPA and DHA are low in most individuals living in Western countries (Calder and Yaqoob, 2009; Pickova, 2009), whereas consumption of saturated fats and n-6 fatty acids is nowadays the highest in human history. The exact values of EPA and DHA consumption by Czech population have not been conclusively determined. Hibbeln et al. (2006) presented the consumption of n-3 HUFA as a percentage of total daily intake of energy. The value for Czech population is 0.07%, which is comparable with Austria (0.071%), Germany (0.084%) or Poland (0.066%). In the countries with traditionally high fish consumption this intake is much higher (e.g. Iceland – 0.435%; Japan – 0.374%; Sweden – 0.139% etc.). According to the consumption of different fish species in the Czech Republic and their average representation of EPA and DHA we can calculate value around 150 mg per day, which is, given to the nutritional recommendations, very low number. The changes in fatty acid intake over thousands of years based on paleo-nutrition studies are depicted in Figure 2. As man turned from hunter-gatherer to farmer, intake of n-3 fatty acids began to fall, and this accelerated as farming became intensive. More recently, the increase in plant oil consumption has led to an increase in n-6 fatty acid consumption.

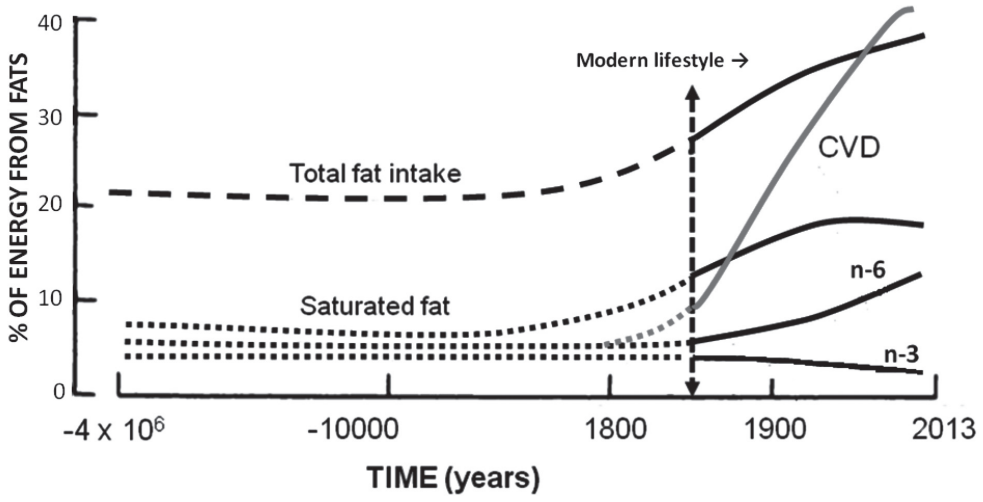

Figure 2. Changes in the composition and representation of fat in human nutrition during evolution in relation to the occurrence of cardiovascular diseases (adapted from Leaf and Weber, 1987). CVD: cardiovascular diseases.

Daily intake of saturated fatty acids (SFA) should contribute no more than 10% of dietary energy, because SFA consumption increases blood cholesterol levels (Williams, 2000). However, it is now clear that it is mainly lauric (12:0), myristic

(14:0) and palmitic (16:0) fatty acids, and their trans isomers in particular, which are responsible for increasing plasma total and LDL cholesterol concentrations, whilst the other major SFA, stearic acid (18:0), has been shown not to increase total cholesterol or LDL-cholesterol concentrations (Hunter et al., 2010). Excessive intake of SFA is considered adverse in terms of plasma LDL cholesterol concentration, but consumption of monounsaturated fatty acids (MUFA) is much more positive (Williams, 2000). A huge human intervention study performed by Livingstone et al. (2012) demonstrated that supplementation of dairy cow diets with a source of MUFA or PUFA may have beneficial effects on the CVD risk in consumers of dairy produce by partially replacing milk SFA, thus reducing entry of SFA into the food chain. In human nutrition there is generally sufficient or excess SFA, sufficient or excess MUFA and sufficient or excess n-6 PUFA. Nowadays, a fat component which is lacking is n-3 PUFA (and HUFA), the unique source of which is fish, both marine and freshwater. Evidence of anti-atherogenic, anti-thrombotic, anti-inflammatory and immune-suppressive actions of n-3 PUFA from fish and fish oils has been provided in a range of epidemiological and experimental studies in humans (Daviglus et al., 1997; Mozaffarian and Rimm, 2006).

Recommendations for optimal lipid intake to minimise metabolic syndrome in humans are strongly related to lifestyle and are constantly being re-evaluated according to new research findings. General guidelines recommend that the total fat intake should be around 30% of the daily energy intake (Linseisen et al., 2009), but should not exceed 35% (Dostalova et al., 2012). European Food Safety Authority (EFSA), American Heart Association (AHA). Furthermore, the World Health Organisation (WHO) recommends consumption of at least two portions (or 400 grams) of fatty fish per week to maintain good health. The recommendation for daily intake of individual fatty acids is: 2 g ALA, 10 g LA (n-3/n-6 ratio 1:5) and 200–500 (250) mg EPA+DHA for the normal population (Dostalova et al., 2012; EFSA, 2009; 2010; Kris-Etherton et al., 2002; WHO/FAO, 2003). In the case of pregnant women, infants and children, these doses should be even higher. For patients with cardiovascular problems or with hypertriglyceridaemia, daily intake of EPA+DHA should be increased up to 4 g EPA+DHA (Kris-Etherton et al., 2002).

It is a sad fact that despite strong evidence that consumption of fish flesh and fish products is beneficial for human health, fish consumption in the Czech Republic is very low compared with that in other European Union countries. The EU average is around 13 kg fish per capita and year, while in the Czech Republic the average is only around 5.5 kg (Ženíšková and Gall, 2012). Thus, it would be beneficial to generally increase fish consumption and also to increase the content of the beneficial n-3 fatty acids in locally produced fish and other products (Mráz and Picková, 2011).

1.5. PRINCIPLES OF N-3 FATTY ACID BIOSYNTHESIS IN FISH METABOLISM

All living organisms have the ability to produce MUFA of 16:1n-7 and 18:1n-9 by desaturation of their saturated forms (Glencross, 2009). All vertebrates, including fish, lack desaturases beyond Δ-9. Thus linolenic (18:2n-6) and alpha-linolenic (18:3n-3) (Figure 3) acids cannot be synthesised endogenously and are therefore termed essential fatty acids (EFA). However, plant cells have the ability to synthesise 18-carbon fatty acids.

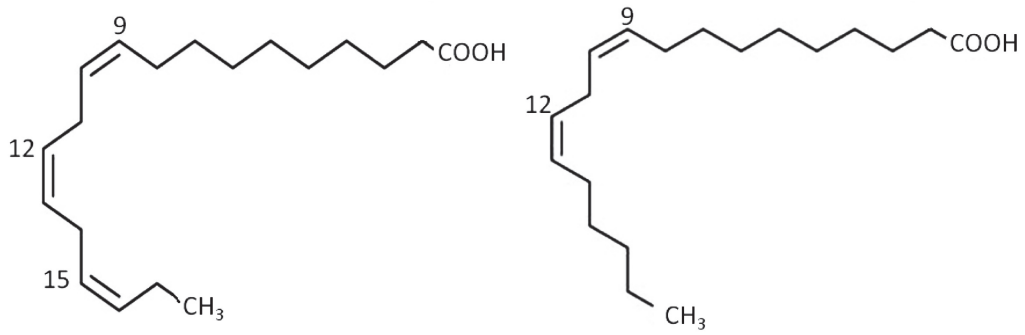

Figure 3. Chemical structure of two essential fatty acids: alpha linolenic acid (ALA; 9,12,15-octadecatrienoic) on the left and linoleic acid (LA; 9,12-octadecadienoic) on the right.

In contrast to marine fish, it is generally believed that freshwater fish, including common carp, are capable of converting both n-3 and n-6 C-18 PUFA to their higher unsaturated derivatives (HUFA) (Tocher, 2003; Tocher et al., 1989). Further metabolisation of alpha-linolenic acids is characterised by the action of the Δ-6 desaturase enzyme for the saturation of the fatty acid, followed by the action of the elongase enzyme for the addition of a carbon atom to the molecular chain, leading to action by Δ-5 desaturase to form EPA (20:5n-3). (Innis, 2003). The next step is further elongation of carbon chain through the DPA (22:5n-3) to 24:5n-3. Subsequently, by the effect of Δ-6 desaturase, 24:6n-3 is created. Further shortening the chain from 24:6n-3 synthesises DHA (22:6n-3) and this occurs in the peroxisomes by β-oxidation (Ferdinandusse et al., 2001).

Despite the many studies conducted on common carp (Tocher and Dick, 1999, 2000), the exact mechanisms controlling HUFA biosynthesis in this fish species are still unknown. In general, both desaturases and elongases are activated in fish fed a diet (vegetable oils) containing 18-carbon fatty acids (18:3n-3, 18:2n-6) in comparison with fish fed a diet (fish oil) containing HUFA (Tocher et al., 2001; Leaver et al., 2008).

1.6. BACKGROUND RESEARCH

The overall aim of this research project was to improve the quality of common carp in terms of content of healthy beneficial n-3 PUFA by using production practices ensuring sustainability and improved composition in terms of human health benefits. First, an overall analysis of carp lipid composition in different parts of fillet was carried out (Mráz and Pickova, 2009). Based on the results, it was concluded that carp has the potential to be an interesting functional food in terms of lipid composition. The effect of dietary sesamin was then tested in common carp culture to determine whether there is a similar influence as in salmonid fish, where sesamin enhanced the DHA content of tissue lipids (Trattner et al., 2008a,b). The study did not demonstrate such an effect in common carp (Mráz et al., 2010).

Furthermore, a special feeding mixture based on cereals with addition of rapeseed and flaxseed has been developed. Rape and flax seeds are well known as a good source of alpha-linolenic acid (18:3n-3) (Pickova and Mørkøre, 2007), which is the precursor

of n-3 HUFA. The composition of this pelleted diet is now protected by a utility model (Mráz et al., 2011a). Between 2008–2012, the pellets were tested in classical, semi-intensive (natural feed and supplementation) carp culture in experimental (Mráz et al., 2012a) and practical (Mráz et al., 2012b) conditions. A significantly increased content of n-3 fatty acids, both PUFA and HUFA, was observed in the fillet of such carp. The technology involved has been patented (Mráz et al., 2011b).

There are many studies describing the positive effects of marine fish consumption on human health (Horrocks and Yeo, 1999; Ruxton, 2011). However, little is known about the health benefits of carp flesh. Thus, the influence of a diet enriched with carp flesh with an elevated content of n-3 fatty acids (200 g twice weekly for 4 weeks) was evaluated in a group of subjects after cardiac revascularisation surgery for ischemic heart disease with a follow-up spa treatment. In the group with higher consumption of carp, significantly greater improvements in lipid parameters (total cholesterol, triacylglycerol, LDL and HDL cholesterol) in comparison with the standard spa diet were detected by Adamkova et al. (2011). These positive changes are consistent with the results published in association with marine fish consumption by Balk et al. (2006).

1.7. AIMS OF THIS THESIS

1) The first study sought to devise strategies for improvement of fatty acid composition in common carp. In this case, the possibility of rearing carp under controlled conditions in a recirculation aquaculture system (RAS) using the finishing feeding strategy (Robin et al., 2003; Jobling, 2004) was examined. The aim was to achieve a predicted increased level of n-3 HUFA compared with standard rearing procedures (Paper I).

2) The aim of the next study was to identify ways to close the production circle for carp with an increased content of n-3 PUFA. Carp for consumption in Central Europe are traditionally harvested during late autumn and kept in concrete-coated ponds with fresh water flow-through for some weeks before being sold, a process known as purging. During this time the fish are not fed, so that the digestive tract is emptied and unpleasant odours are eliminated. Therefore, this experiment explored the effect of purging on fillet fat content and FA composition in common carp from three different production systems: supplementation with cereal; supplementation with rapeseed/linseed pellets; and natural feed only (Paper II).

3) The aim of the third study was to investigate the changes caused by frying on fat uptake, fatty acid composition and oxidation in common carp fillets (Paper III).

The final study aimed to summarise existing knowledge about the metabolism of fatty acid and factors influencing fatty acid composition in the lipids of freshwater species to formulate a certified methodology, targeted at fish farmers and relevant professionals (Paper IV).

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CHAPTER 2

CULTURE OF COMMON CARP (*CYPRINUS CARPIO*) WITH DEFINED FLESH QUALITY FOR PREVENTION OF CARDIOVASCULAR DISEASES USING FINISHING FEEDING STRATEGY

Paper I:

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ORIGINAL ARTICLE

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Culture of common carp (*Cyprinus carpio*) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy

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Abstract

OBJECTIVES: Fish is the major source of n-3 polyunsaturated fatty acids (n-3 PUFA) which are well known to have positive effects in prevention of cardiovascular diseases. This study investigated the possibility to produce common carp with defined flesh quality using finishing feeding strategy and predict changes of fillet FA by a dilution model.

METHODS: During the 110-day experiment, fish were fed diets with two different vegetable oils (rapeseed/linseed blend, VO; olive oil, OO) only, or with a subsequent fish oil (FO) finishing treatment for 30 or 60 days. Fillet FA composition was measured and data were compared to the ones predicted by the dilution model.

RESULTS: The FO finishing treatment resulted in the higher percentage of SFA (from 19.1% to 23.6%; $p<0.001$), MUFA (from 46.8% to 51.9%; $p<0.001$), n-3 PUFA (from 3.6% to 7.4%; $p<0.001$) and lower n-6 PUFA (from 30.5% to 16.9%; $p<0.001$) and n-6/n-3 ratio (from 8.7 to 2.3; $p<0.001$) in groups previously fed the VO diet and in lower MUFA percentage (from 67% to 63%; $p<0.001$) and n-6/n-3 ratio (from 8.2 to 2.8; $p<0.001$) and higher n-3 PUFA percentage (from 1.5% to 4.5%; $p<0.001$) in group previously fed the OO diet. The dilution model gave a good prediction for fillet FA changes (slope of the regression line 0.97–1.00; R^2 value of 0.992–0.996).

CONCLUSION: The finishing feeding strategy is suggested for production of common carp with a required flesh FA composition for purposes of special nutritional needs, especially for primary and secondary prevention of cardiovascular disease.

Abbreviations:

DHA	- docosahexaenoic acid (22:6n-3)
EPA	- eicosapentaenoic acid (20:5n-3)
FA	- fatty acids
FAME	- fatty acid methyl esters
FO	- fish oil
HUFA	- highly unsaturated fatty acids (20 ≥ carbons, 3 ≥ double bonds)
MUFA	- monounsaturated fatty acids
OO	- olive oil
PUFA	- polyunsaturated fatty acids
SFA	- saturated fatty acids
VO	- vegetable oil blend (rapeseed/linseed blend)

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INTRODUCTION

The n-3 highly unsaturated fatty acids (n-3 HUFA; 20 ≥ carbons, 3 ≥ double bonds), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are beneficial for human health (Mozaffarian & Rimm 2006). These fatty acids (FA) play an important role in biological functions, including brain development, inflammatory response, homeostasis and prevention of cardiovascular disease (Calder 2006; Calder & Yaqoob 2010). Fish is the major dietary source of n-3 HUFA and worldwide promoted as healthy and beneficial for human health, especially in prevention of cardiovascular diseases. Several specific dietary recommendations have been developed related to fish consumption and intake of n-3 FA by nutrition and health authorities. For the general population, two servings of fish per week or 250 mg of EPA and DHA per day are recommended (EFSA 2009). Patients with documented cardiac heart disease and hypertriglyceridemia are advised to have daily intake of EPA+DHA even higher up to 1 and 2–4 g, respectively (Kris-Etherton 2002). However, it is difficult for the public to meet the nutritional recommendations for the FA by a diet since the FA composition of fish flesh is highly variable and influenced by many factors, mainly by feeding (Mráz & Pickova 2011). Therefore it would be valuable if fish producers could produce fish with high and defined content of n-3 HUFA.

Feed sources rich in n-3 HUFA, such as fish oil, are becoming scarce, while algae and various microorganisms that supply n-3 HUFA are expensive and not yet available on a commercial scale. Therefore it would be of great economic and sustainability value if a feeding strategy could be devised where these feedstuffs are not used for the entire feeding period but only for the final part, thus saving resources. In line with this there is a need to be able to predict changes of fish FA composition during the course of feeding.

Such a finishing feeding strategy has been suggested and developed for carnivorous fish species, including medium fatty fish species such turbot (*Psetta maxima*) (Robin *et al.* 2003), fatty fish such as Atlantic salmon (*Salmo salar*) (Jobling 2003 and 2004b) and lean fish species such as Atlantic cod (*Gadus morhua*) (Jobling *et al.* 2008) and Murray cod (*Maccullochella peelii peelii*) (Turchini *et al.* 2006). The results so far have been promising and a finishing feeding strategy for commercial applications has been proposed. The overall conclusions from all these different studies are in agreement with a general dilution model suggested by Robin *et al.* (2003).

Common carp (*Cyprinus carpio*) is one of the most cultured fish species in the world (FAO 2008). Thus, from a worldwide nutrition perspective, a method to increase the amount of n-3 HUFA in carp fillet is valuable. Optimization of the FA composition of carp has been examined in previous studies (Domaizon *et al.* 2000; Chen *et al.* 2011; Mráz *et al.* 2012; Steffens 1997; Steffens & Wirth 2007). Mráz & Pickova (2011) concluded that adjusting the lipid composition in the feed is the most effective tool to achieve the desired n-3 HUFA content. However, previous studies have not investigated the response of muscle FA composition to dietary changes and the duration of finishing feeding period needed to achieve desired changes in n-3 HUFA content. Therefore the aim of this study was to examine the applicability of the finishing feeding strategy in common carp production with defined and tailored flesh quality for specific needs in human nutrition.

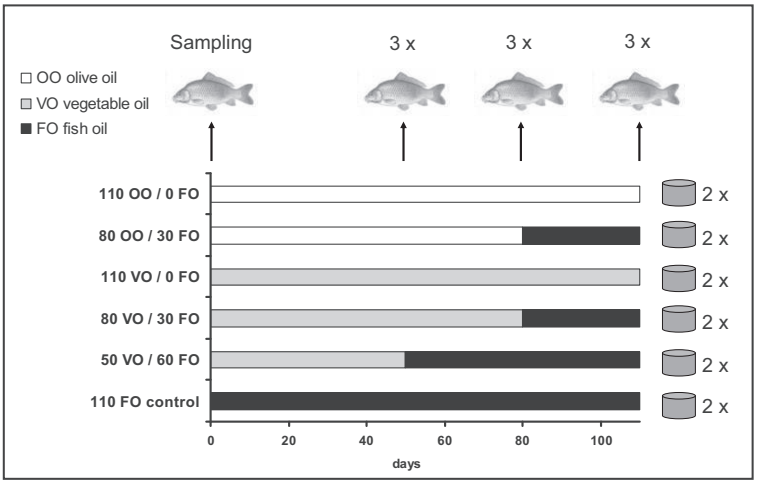

Fig. 1. Experimental design of the 110-day feeding trial. Fish (10 per tank, duplicate tanks per treatment) were fed one of three diets containing vegetable oil (VO), olive oil (OO) or fish oil (FO) alone for 110 days, or with a 30 or 60 day finishing period with FO. The different diets were: 110 OO/0 FO; 80 OO/30 FO; 80 OO/30 FO; 110 VO/0 FO; 110 VO/0 FO; 80 VO/30 FO; 50 VO/60 FO and 110 FO control. Fish were sampled at 0, 50, 80 and 110 days.

MATERIALS AND METHODS

Diets

The experimental diets used were based mainly on vegetable components (Table 1). No fish meal was used, in order to avoid high background levels of n-3 HUFA in the diet. The diets differed only in lipid source. The basal diet contained either a blend of vegetable oils (VO; rapeseed/linseed 3:2) or olive oil (OO) and the finishing feeding diet contained fish oil (FO). All diets were manufactured by extrusion. The proximate and FA composition of the experimental diets are listed in Table 1.

Tab. 1. Formulation (g 100g^{-1}), proximate composition (% on as is basis) and FA composition (% of identified FAs) of the experimental diets.

	VO Vegetable oil	OO Olive oil	FO Fish oil
Soybean meal	30	30	30
Wheat	18	18	18
Soycomil[a]	15.1	15.1	15.1
Maize	12	12	12
Fish oil	0	0	9
Linseed oil	3.6	0	0
Rapeseed oil	5.4	0	0
Olive oil	0	9	0
Corn gluten[b]	6	6	6
Wheat germ	5	5	5
Yeast vitex[c]	3	3	3
Aminovitan KPd[d]	0.6	0.6	0.6
Salt	0.5	0.5	0.5
Limestone	0.4	0.4	0.4
DL-methionine[d]	0.4	0.4	0.4
Dry matter	94.8	95.3	96.9
Protein	34.1	34.4	34.6
Fat	8.5	8.3	8.8
Fiber	3.5	3.1	3.7
Carbohydrates	44.1	44.8	44.2
Ash	4.6	4.7	5.6
SFA[e]	11.8	15.5	27.9
MUFA[f]	31.0	61.1	34.6
PUFA[g]	57.2	23.3	37.5
18:2n-6	53.0	21.7	16.2
20:2n-6	0	0	0.3
20:4n-6	0	0	0.3
18:3n-3	4.2	1.7	2.7
18:4n-3	0	0	2.2
20:5n-3	0	0	6.3
22:5n-3	0	0	0.6
22:6n-3	0	0	8.8
n-6/n-3	12.7	13.1	0.8

[a] ADM (Archer Daniels Midland Company), Olomouc, Czech Republic; [b] Bodit Tachov, s.r.o., Stribro, Czech Republic; [c] Biocel, a.s., Paskov, Czech Republic; [d] Zavod Biochemickych Sluzeb, s.r.o., Slusovice, Czech Republic; [e] SFA: saturated fatty acids; [f] MUFA: monounsaturated fatty acids; [g] PUFA: polyunsaturated fatty acids

Fish and experimental design

Two-year-old common carp (*Cyprinus carpio*) of the mirror scaly type with an average weight of 780g were used for the experiment. The fish were transferred from an earthen pond to the experimental facility at the Research Institute of Fish Culture and Hydrobiology in Vodnany, Czech Republic. Six fish were sampled to determine the initial lipid content and composition (data not shown). The fish were placed in tanks (1 m^3) and divided into 12 groups of 10 fish each. The tanks were supplied with oxygenated water from a recirculating system after mechanical and biological filtration (flow 0.1 l s^{-1}; dissolved oxygen 7–10 mg l^{-1}; temperature 20°C). The water level in the tanks was set to 0.4 m (tank volume 400 l). The fish were subjected to a light:dark (12h:12h) regime. During the 110-day experiment, the fish groups were fed only VO (110 VO/0 FO), only OO (110 OO/0 FO) or only FO (110 FO control), or VO or OO with a subsequent 30 or 60-day FO finishing treatment (80 OO/30 FO, 80 VO/30 FO, 50 VO/60 FO), with duplicate groups for each treatment. The experimental design is shown in Figure 1. The diets were supplied by automated continual feeders for 9 hours at a feeding ratio of 1.5% of current biomass. The fish stock biomass was determined every second week. Three fish were randomly sampled from each tank after 50, 80 and 110 days for lipid analysis. Samples of the fillets were immediately frozen in liquid nitrogen and stored at –80 °C until further analysis.

Lipid analysis

Lipid analyses were performed as described in detail by (Mraz & Pickova 2009). In brief, lipids from the fillet and feed samples were extracted with hexane and isopropanol according to Hara & Radin (1978). The FA were methylated (Appelqvist 1968) and the fatty acid methyl esters (FAME) were analyzed with a gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization detector and split injector and fitted with a 50 m long × 0.22 mm i.d. × 0.25 µm film thickness BPX 70 fused-silica capillary column (SGE, Austin, TX, USA) according to (Fredriksson Eriksson & Pickova 2007). The FA were identified by comparison with a standard FA mixture (GLC standard 461, Nu-Chek Prep, Elysian, MN, USA) and specific retention times. Peak area integration was performed using Star chromatography workstation software version 5.5 (Varian AB, Stockholm, Sweden). The FA were quantified using internal standard methyl 15-methylheptadecanoate (Larodan Fine Chemicals AB, Malmö, Sweden).

Dilution model

The data obtained from lipid analyses of fillet tissues from groups 80 OO/30 FO, 80 VO/30 FO and 50 VO/60 FO were compared against predicted data calculated according the dilution model designed by Robin *et al.* (2003) and shown in Equation 1, as verified by Jobling (2004a).

$P_T = P_R + [(P_0 - P_R) / (Q_T/Q_0)]$ (Equation 1),

where

P_T = Predicted percentage of a fatty acid at time T

P_R = Percentage of a 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 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.

The predicted percentage of specific FA, e.g. EPA at a specific time point (P_T) (in this case the end point after 110 days), was calculated by taking the percentage of the specific FA measured at time T (110 days) in fish continuously fed the finishing diet (P_R = value for 110 FO control at 110 days) and the corresponding percentage in the other experimental groups (80 OO/30 FO, 80 VO/30 FO or 50 VO/60 FO) at time point P_0 (50 or 80 days), directly before the finishing feeding period. Q_0 was taken as the average total FA content (lipid content × body mass) of the experimental fish before the finishing feeding period and Q_T as the final total FA content of fish from the corresponding group at the end of the experimental period. All values represent the mean of six replicates (three fish per duplicate tank).

Statistical analysis

Where applicable (n>2), all data are presented as mean values ± standard deviation (SD) and differences were regarded as significant at $p<0.05$. The SAS General Linear Model (GLM), Tukey´s test (SAS Institute Inc., Cary, NC, USA, version 9.2) was used to compare fillet FA composition among the dietary treatments.

RESULTS

Survival, growth and feed conversion data for the different groups are presented in Table 2. The fillet lipid content was not affected by any dietary treatment over the course of the feeding trial ($p>0.05$). The final fillet lipid content varied between 9–10% at the end of the trial.

Replacing OO or VO with FO as the lipid source in the diet of common carp resulted in fillets with clearly different FA profiles (Figure 2). In the groups previously fed the VO diet the percentage of SFA (from 19.1% to 23.6%; $p<0.001$), MUFA (from 46.8% to 51.9%; $p<0.001$), n-3 PUFA (from 3.6% to 7.4%; $p<0.001$), EPA (from 0.36% to 1.53%; $p<0.001$) and DHA (from 0.71% to 3.16%;$p<0.001$) were positively correlated to the length of the FO finishing feeding period while the percentage of n-6 PUFA (from 30.5% to 16.9%; $p<0.001$) and the n-6/n-3 ratio (from 8.7 to 2.3; $p<0.001$) were negatively correlated. In the group previously fed the OO diet, the finishing treatment resulted in a lower percentage of MUFA (from 67% to 63%; $p<0.001$), a lower n-6/n-3 ratio (from 8.2 to 2.8; $p<0.001$) and a higher percentage of n-3 PUFA (from 1.5% to 4.5%; $p<0.001$), EPA (from 0.24% to 0.84%; $p<0.001$) and DHA (from 0.49% to 1.54%; $p<0.001$). The percentages of EPA and DHA both increased linearly with cumulative FO consumption (R^2 value >0.99) (Figure 3).

Although the fillet FA composition (Figure 2) changed significantly in response to the dietary FA composition (Table 1), the FA composition in the fillet did not reach that in the feed. The most obvious differences were seen in percentage of MUFA and PUFA (Figure 2 and Table 1). The percentage of MUFA in the VO and FO diet was 31% and 35%, respectively. The percentage of MUFA was considerably higher in the fillet, varying between 47% and 54% ($p<0.001$). The PUFA content in the VO and FO diet was 57% and 38%, respectively, but the corresponding values in the fillet were significantly lower and varied from 20% to 34% ($p<0.001$).

At the end of the experiment, the observed FA composition in the fillet samples from fish receiving the finishing feed (80 OO/30 FO, 80 VO/30 FO and 50 VO/60 FO) was compared against the values predicted using the dilution model designed by Robin *et al.* (2003) (Figure 4). The data showed that the dilution model gave a good prediction for the 10 most important FA or FA groups in the fillet of common carp, with a slope of the regression line close to 1 (0.97, 0.99 and 1.00, respectively) and with an R^2 value of 0.996, 0.993 and 0.992, respectively. Similar regression statistics were obtained for all FA identified (data not shown).

DISCUSSION

This study investigated possibility to produce common carp with defined flesh quality (high content of n-3 PUFA, EPA, DHA) for prevention of cardiovascular

Tab. 2. Fish performance (data presented are mean of duplicate values).

	Survival (%)	Final body weight (g)	Feed conversion (kg feed kg yield^{-1})
110 VO/0 FO	100	1610	1.76
80 VO/30 FO	95	1466	1.96
50 VO/60 FO	100	1407	1.73
110 OO/0 FO	100	1648	1.57
80 OO/30 FO	100	1491	1.74
110 FO control	95	1624	1.69

Abbreviations: VO, vegetable oil mixture; FO, fish oil; OO, olive oil

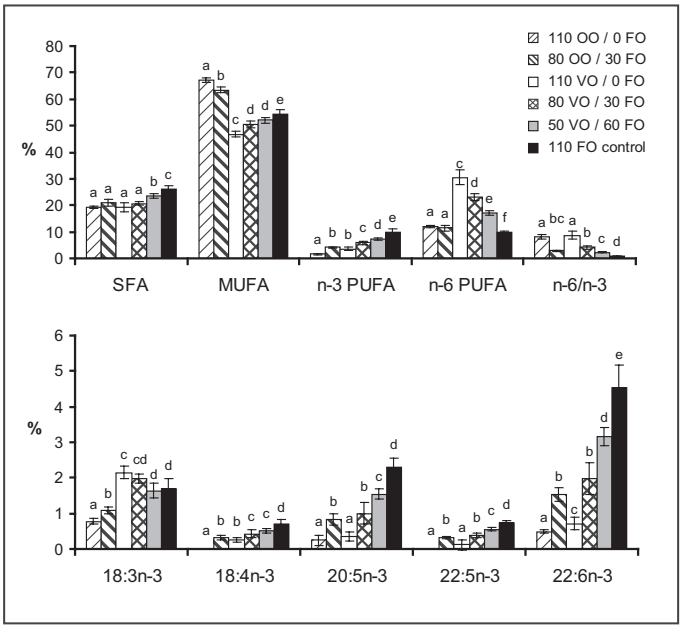

Fig. 2. Fatty acid (FA) composition (% of identified) in the fillet of the experimental fish at the end of the experiment (n=6; mean ± SD). Different letters indicate significant difference among the treatments. Abbreviations: SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.

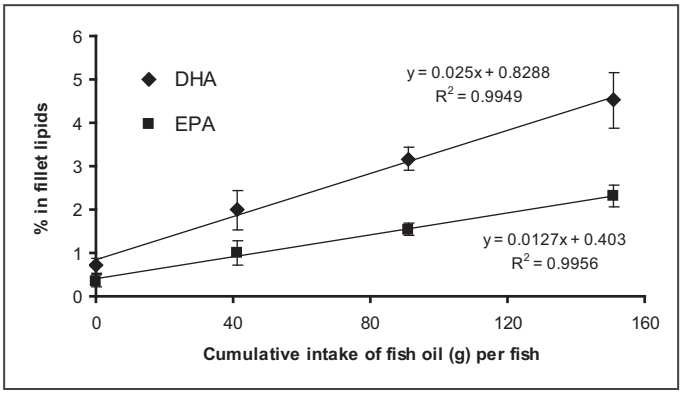

Fig. 3. Percentage of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (% of total identified FA) in fish fillet lipids in relation to the cumulative intake of fish oil (g) per fish (n=6; mean ± SD).

diseases using finishing feeding strategy and predict changes of fillet FA by a dilution model.

In the present study, the fillet FA composition highly reflected the FA composition of the diet and was significantly correlated to the length of the feeding period. This agrees with previous findings that it is possible to boost the content of beneficial EPA and DHA in fish fillet by n-3 HUFA supplementation prior to harvest (Bell *et al.* 2004; Torstensen *et al.* 2005, re Atlantic salmon; Benedito-Palos *et al.* 2009, re gilthead sea bream; Steffens 1997; Steffens & Wirth 2007, re common carp; Turchini *et al.* 2006, re murray cod).

However, when the dietary composition was used as the reference value for prediction of the FA composition in the groups continuously fed the same diet (110 FO control, 110 VO/0 FO and 110 OO/0 FO; Figure 5) the observed percentage of MUFA were significantly higher than the predicted values ($p<0.001$) while percentage of PUFA were significantly lower ($p<0.001$). This could indicate that the carp synthesized a significant amount of MUFA de novo from excess energy, or that MUFA are the preferred FA group for storage in common carp. This is supported by the fact that regardless of the low amount of MUFA in the natural and supplemented diet

of carp, MUFA is the major FA group stored in carp fillet and is probably produced from energy obtained from cereals (Buchtová *et al.* 2010, Mráz *et al.* 2012).

Using the dilution model proposed by Robin *et al.* (2003) showed to give an excellent prediction of the FA composition in fillet of carp of marketable size. This confirms previous findings for carnivorous fatty fish species such as Atlantic salmon (Jobling 2003), where the lipids are predominantly represented by storage fat (triacylglycerols). The dilution model is clear in its straightforwardness and can therefore be applicable for fish farmers, enabling production of high quality fish as well as minimizing the use of expensive feed. A small disadvantage is that the model does not account for the FA composition of the feed used, but for the FA composition in the fillet of fish continuously fed the finishing diet, which is hence needed as a reference value. However if the reference value has been established for a species and diet once it might be used continually.

The European Food Safety Authority recommends a daily intake of 250 mg EPA+DHA per person (EFSA 2009) and two servings of oily fish per week. A 200 g serving of carp from the 110 FO control and 110 VO/0 FO group contained 1190 mg and 180 mg EPA+DHA, respectively. According to the predictions by the dilution model and experimental values obtained here, we concluded that the finishing feeding treatment needs to be applied for 70 days to achieve the recommended daily value of 250 mg EPA+DHA in two 200 g servings a week (250 mg × 7 days = 1750 mg/2 servings = 875 mg/serving). Reducing FO feeding to this shorter period would significantly reduce fish production costs and lead to more sustainable use of limited FO resources.

Currently common carp is mostly produced in ponds on the basis of natural feed (plankton and benthos) with cereal supplementation. Since there are huge differences in natural productivity among ponds there is also a huge variability of FA composition in carp flesh (Mráz & Pickova 2012). As a consequence there is no standard of quality which makes it difficult to advertise carp as a healthy product. The finishing feeding strategy could therefore be used in production of carp with defined flesh quality to fulfill dietary needs for humans, especially in connection to cardiovascular recovery. Fish farmers could easier control the final carp flesh quality and produce fish with standardized and tailored quality. In line with this they could declare the content of n-3 PUFA and EPA+DHA on the product label which could increase the market value of carp and support consumption of this locally produced fish. From the consumers point of view this would be desirable as they thereby could easier meet the nutritional recommendations. It would also be easier to set up dietary interventions using carp in prevention and treatment of cardiovascular diseases.

In conclusion, the finishing feeding strategy is suggested for the production of common carp with tailored flesh FA composition, for contributing to healthy fat profile of e.g. EPA and DHA content, for cardiovascular disease prevention of the Central Europe population.

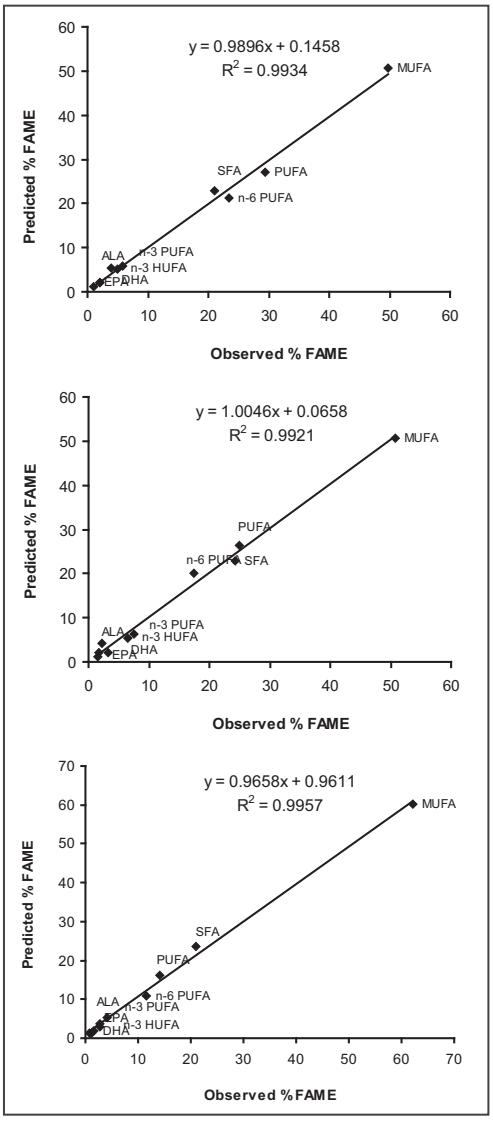

Fig. 4. Prediction plot of fillet fatty acid composition (%) for carp groups A) 80 VO/30 FO; B) 50 VO/60 FO; and C) 80 OO/30 FO. The measured values represent the mean for 6 fish. The thick line shows the regression line. FAME = fatty acid methyl esters, for other abbreviations see Figure 2.

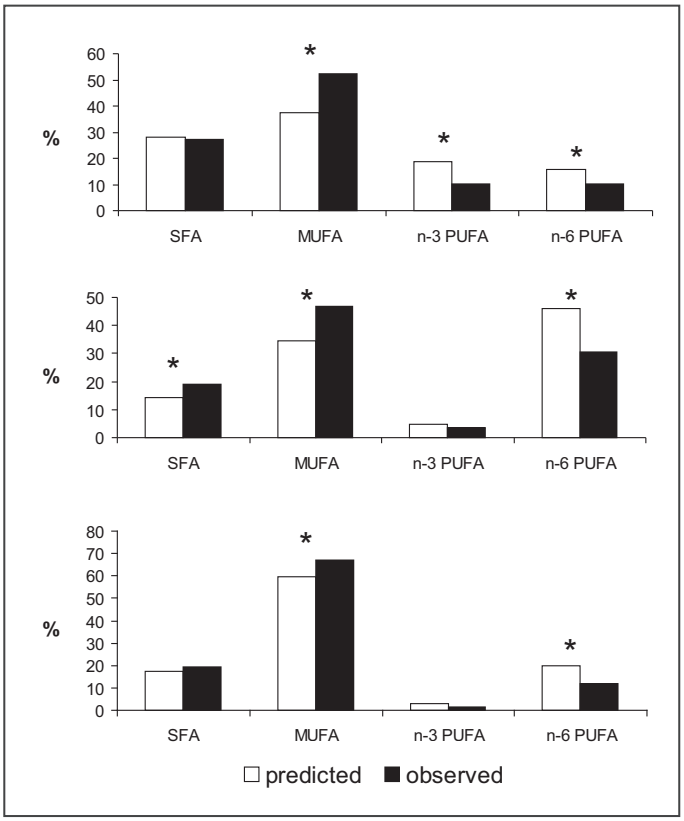

Fig. 5. Observed fatty acid composition (%) in fillet of fish from the carp groups A) 110 FO control; B) 110 VO/0 FO; and C 110 OO/0 FO, compared with the composition predicted by the dilution model when the fatty acid composition of the FO, VO and OO diet, respectively, was used as the reference value. * indicates significant difference ($p<0.05$) between predicted and observed data. For abbreviations see Figure 2.

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CHAPTER 3

FILLET QUALITY CHANGES AS A RESULT OF PURGING OF COMMON CARP (*CYPRINUS CARPIO* L.) WITH SPECIAL REGARD TO WEIGHT LOSS AND LIPID PROFILE

Paper II:

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Fillet quality changes as a result of purging of common carp (*Cyprinus carpio* L.) with special regard to weight loss and lipid profile

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ABSTRACT

Purging is a very important part of the rearing process for common carp (*Cyprinus carpio* L.) 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 stored fat mobilisation. This study investigated the effect of a purging period of up to 70 days on lipid content and quality of common carp flesh. Four-year-old, market-size carp (weight 1700–2600 g) from three different production systems (C: cereal supplemented; P: linseed/rapeseed pellet supplemented; N: natural feed) were sampled every 14 days for weight, fillet yield and lipid analysis. Fillet yield was highest after 14 days and decreased thereafter. Throughout the experiment, fillet fat content decreased continuously in groups C and P, but remained stable in group N. Initially, carp from groups C and P mainly metabolised mono-unsaturated fatty acids (MUFAs), but with prolonged starvation fish from all groups started to metabolise more polyunsaturated fatty acids (PUFAs). After 70 days of purging, all groups showed almost identical saturated FA (SFA), MUFA and PUFA values. Our conclusion is that carp are able to metabolise selected FA for their energy needs when they are in good condition and have surplus fat stores. However, when body fat content is low, they may metabolise all FA types equally to sustain metabolic functions.

1. Introduction

Common carp (*Cyprinus carpio*) is one of the most commonly reared fish globally, with a production volume of around 3 000 000 tons annually (FAO, 2010). Carp for consumption in Central Europe are traditionally harvested during late autumn and kept in concrete coated ponds with fresh water flow for some weeks before being sold, a process known as purging. During this time the fish are not fed, so that the digestive tract is emptied and unpleasant odours are eliminated (Einen et al., 1998). Purging is necessary to achieve good product sensory quality. Common carp in natural conditions decrease feeding and activity with decreasing water temperature in winter to save energy. The optimal temperature range for carp is 20–28 °C, and in general carp stop feeding in the range 12–4 °C and stop movement at water temperatures below 6–4 °C in natural conditions (Bauer and Schlott, 2004).

In preparation for the winter starvation period, carp naturally store fat as reserve energy in muscle and abdominal wall tissues, mainly in the form of energy-rich triacylglycerols (TAGs). During the winter starvation period these TAG reserves are metabolised gradually for maintenance of the organism (Csengeri, 1996).

Lipids have an important function as an energy source in the body (McCue, 2010). Different fatty acids (FAs) also have metabolically important functions in the body, and are involved in the determination of the physical and chemical properties and capacities of biological membranes (Wiseman, 1996). They also serve as precursors in the synthesis of several different chemical messengers and eicosanoid hormones, as well as other regulating factors (Horrobin, 1995; Kinsella, 1988). In general, TAG serves mainly as an energy source, whereas phospholipids (PLs) are mainly constituents of biological membranes (Sargent et al., 1999).

Under semi-intensive rearing conditions, carp have access to natural feed (plankton and benthos) in the pond and are also fed a supplement, often cereals. While cereals are rich in carbohydrates with moderate levels of fat and n−6 polyunsaturated fatty acids (PUFAs), plankton and benthos contain high amounts of n−3 PUFA (Bell et al., 1994; Domaizon et al., 2000). The carbohydrate-rich diet leads to a high muscle fat content (Mraz and Pickova, 2009), more than 10% in intensively reared carp (Keshavanath et al., 2002), by de novo synthesis of FA (Henderson, 1996). In addition, cereals contain n−6 PUFA, which are generally not present in the natural diet of fish in such amounts and therefore affect the lipid composition of fish tissues. Mraz and Pickova

Abbreviations: BF_3, boron trifluoride methanol complex; CF, condition factor; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FFA, free fatty acid; FAME, fatty acid methyl ester; MUFA, monounsaturated fatty acid; PL, phospholipid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TAG, triacylglycerol; TLC, thin layer chromatography.

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(2009) suggested that cereals cause an increase in n−6 PUFA in carp, while the proportions of n−3 PUFA decrease. Mraz et al. (2012) studied the effect of three different production systems (supplementation with cereals or pellets containing rapeseed cake, and a natural diet only) on lipid composition in common carp and found a significantly higher content of n−3 PUFA in fish fed rapeseed pellets compared with fish fed cereals. This and other studies (Pickova and Mørkøre, 2007; Runge et al., 1987; Schwarz, 1996) indicate the possibility of influencing fish lipid composition towards a higher content of n−3 PUFA, which is favourable from a human nutrition perspective (Calder and Yaqoob, 2009; Leaf and Weber, 1987; Simopoulos, 2002, 2008).

However, few previous studies have examined changes in lipid content and FA composition during the purging period of common carp. Csengeri (1996) and Vacha et al. (2007) observed some changes in FA composition during a long-lasting purging period in carp supplemented with different cereals. There was a slight increase in n−3 PUFA in the groups fed cereal, while n−3 PUFA decreased in the control group kept in natural conditions without supplemental feeding before purging. In a study on common carp, Csengeri (1996) concluded that monounsaturated fatty acids (MUFAs), especially oleic acid (18:1 n−9), are mainly utilised for energy production during prolonged starvation, while PUFAs are partly preserved. Similar results have been published for other fish species, for example channel catfish (*Ictalurus punctatus*; Luo et al., 2009), Atlantic salmon (*Salmo salar*; Einen et al., 1998), Murray cod (*Maccullochella peelii peelii*; Palmeri et al., 2008a, 2009a) and hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*; De Silva et al., 1997). Different reduced feed ratio levels in rainbow trout (*Oncorhynchus mykiss*) were studied by Kiessling et al. (1989), who found that higher diet restriction resulted in higher n−3 PUFA percentage in fish flesh. Decreased muscle fat content has been reported in brown trout (*Salmo trutta*) starved for 2 months (Regost et al., 2001).

Previous studies suggest that the lipid content and composition of the edible parts of different fish species are affected during starvation (Palmeri et al., 2008b, 2009b; Thanuthong et al., 2012). In addition, previous nutrition most likely plays an important role in the changes (Tucker, 2000). The aim of the present study was to explore the effect of purging on fillet fat content and FA composition in common carp from three different production systems — supplementation with cereal; supplementation with rapeseed/linseed pellets; and natural feed only.

2. Materials and methods

2.1. Experimental design

Duplicate groups of 4-year-old, market-size common carp were reared in three different production systems for one season (April–September) in the experimental unit of the Faculty of Fisheries and Protection of Waters in Vodňany, Czech Republic. The production systems involved three different types of feed: natural feed only (N); supplementation with cereal (C) and supplementation with rapeseed/linseed pellets (P). Each treatment was carried out in two ponds to balance the effect of the pond environment.

After harvesting, 80 individuals were randomly chosen from each group, labelled by groups with visible implant elastomer (VIE, Northwest Marine Technology, Ltd., USA) and placed in a storage pond with continuous inflow of fresh river water. The pond was 8 m × 4 m × 1.3 m deep, with stony walls and gravel on the bottom. Water temperature measured with a temperature datalogger Minikin I (EMS, Brno, Czech Republic) decreased continually during the experimental period (18.5 °C at the beginning; 2.5 °C at the end; Fig. 1). Dissolved oxygen (O_2) concentration and pH were recorded regularly twice a week (O_2 varied between 6 and 8.5 mg L^{-1}; pH 7.1–7.6). Condition factor (CF) was calculated on each sampling day as the ratio of individual weight (W, grams) to body length (BL, cm = distance between edge of the head and base of tail fin) as:

$$CF = \left(W * BL^{-3}\right) * 100.$$

On days 0, 14, 28, 42, 56 and 70, a subsample of 10 fish from each group were weighed and 6 fish from each treatment (3 each from the two ponds of the same treatment) were killed for sampling. The fish were stunned by a blow to the head and then the gills were cut. A 4 cm wide strip of the fillet (containing white muscle, red muscle and adipose tissue with skin) was taken from each fish at the same position, in the fillet behind the dorsal fin. These fillet samples were packed in aluminium foil and immediately frozen in liquid nitrogen. All samples were stored at −80 °C until further analysis.

2.2. Lipid analysis

Strips of fillets with skin were minced in a table cutter to ensure that all edible parts were represented in the sample analysed. All chemicals and solvents were purchased from Merck (Darmstadt, Germany). Lipid extraction was performed according to Hara and Radin (1978) with minor modifications. Briefly, 1 g of sample was weighed and homogenised in HIP (hexane–isopropanol 3:2, v/v). The homogenate was transferred to a centrifuge tube and 6.5 mL 6.67% Na_2SO_4 was added to separate lipid and non-lipid phases. After centrifugation, the total lipid phase (upper phase) was transferred into pre-weighed tubes and evaporated under nitrogen (for about 1 h). Total lipid content was determined gravimetrically.

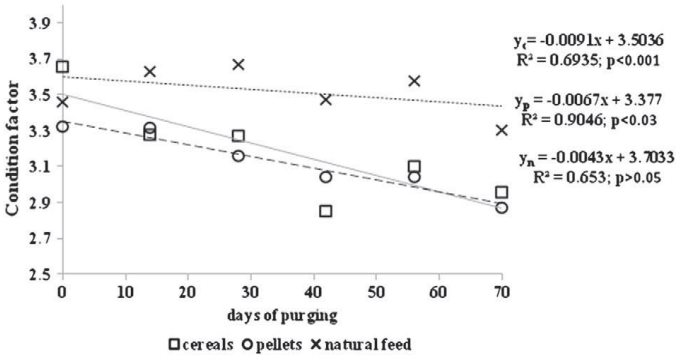

Fig. 1. Condition factor (CF) in carp in the group supplemented with cereals; the group supplemented with rapeseed/linseed pellets; and the group fed natural feed only during the purging period; P value indicates regression dependence within tested groups during purging period (mean values; n = 10).

Table 1
Average weight [g] of common carp in groups C, P and N during the purging period (mean ± standard deviation; n = 10); actual water temperature [°C] at the sampling times.

Group	Days of purging						
	1	14	28	42	56	70	*P value*
C	2690 ± 178[a]	2410 ± 146[a]	2400 ± 135[a]	2100 ± 116[a]	2270 ± 178[a]	2160 ± 122[a]	<0.001
P	2400 ± 176[b]	2380 ± 145[a]	2330 ± 121[a]	2170 ± 112[a]	2190 ± 156[a]	2050 ± 124[a]	<0.001
N	1750 ± 163[c]	1720 ± 160[b]	1760 ± 151[b]	1650 ± 101[b]	1700 ± 276[b]	1590 ± 226[b]	<0.05
t	18.5	13.5	6.9	7.9	1.2	2.5	

Different letters within the same day indicate significant difference ($P < 0.05$) among the groups; *P value* indicates regression dependence within tested groups; Abbreviations: t: temperature; C: cereal fed; P: pellet fed; N: natural fed.

2.3. Lipid class composition and separation

Lipid class composition was analysed according to Olsen and Henderson (1989) with minor modifications as described by Mraz and Pickova (2009). A Camag ATS 4 automatic TLC sampler was used to apply the samples dissolved in hexane (conc. 1 μg/μL) in 4-mm lines on pre-coated silica gel 60 TLC plates (20 × 10 cm; 0.20 mm layer; Merck, Darmstadt, Germany). The lipids were separated using a Camag ADC 2 developing chamber as described above. Derivatisation was performed by dipping the plate in solution phosphoric acid/ethanol followed by heating in an oven at 150 °C for 10 min. The proportions of the different classes of lipid (PLs—phospholipids, sterols, FFAs—free fatty acids, DAGs—diacylglycerols and TAGs—triacylglycerols) were densitometrically measured using a Camag TLC scanner 3. Lipid classes were identified by comparison against an external standard (TLC 18-4A, Nu-Check Prep, Elysian, USA).

2.4. Fatty acid analysis

Total lipids were separated into TAG and PL fractions using thin layer chromatography (TLC) on precoated TLC silica plates (20 × 10 cm Merck, Darmstadt, Germany). The lipids were separated using a Camag ADC 2 developing chamber with hexane-diethyl ether-acetic acid (85:15:1, v/v) as the mobile phase. Total PL and TAG were scraped from the plate and extracted with methanol and chloroform according to Pickova et al. (1997). Fatty acid methyl esters (FAMEs) were prepared with BF_3 following the method of Appelqvist (1968).

FAMEs were analysed using a gas chromatograph (Varian CP 3800; Stockholm, Sweden) equipped with an ionisation detector (FID), split injector and silica capillary column BPX 70 (SGE, Austin, TX) (Fredriksson Eriksson and Pickova, 2007). Helium was used as carrier gas at a flow rate of 0.8 mL min^{-1} and nitrogen was used as make-up gas. Retention time of the different FA was identified by comparison with a standard mixture (GLC-68A, Nu-check Prep, Inc., Elysian, MO). For quantification of the FA, an internal standard (15-methylheptadecanoate; Larodan Fine Chemicals AB, Malmö, Sweden) was used. FAs were expressed as percent of total identified FA.

2.5. Statistical analysis

All statistical analyses were performed using the Statistica CZ 10.0 software package. A proper regression analysis was performed to find the differences within experimental group during purging time. One-way analysis of variance (ANOVA) and Tukey's HSD test were used for the determination of differences among treatments, as well as for regression and correlation analysis. Differences were assumed to be statistically significant at $P < 0.05$.

3. Results

3.1. Water temperature, weight, fillet yield and condition factor

The mortality rate during the purging period in the experiment was in total 6.3% (14/240 fish). Water temperature decreased continuously from 18.5 °C to 2.5 °C at the end of experiment.

The average body weight at the start of experiment was 1750 ± 470 g in group N, 2690 ± 410 g in group C and 2404 ± 391 g in group P. With prolonged starvation time, total body weight decreased significantly in all groups. After 70 days of purging, the lowest weight decline was observed in group N (−9.2%; −161 g), followed by group P (−14.6%; −352 g) and the largest decline was measured in group C (−19.6%; −529 g) (Table 1).

Together with decreasing average weight, condition factor (CF) decreased during the purging period (Fig. 1). These decline is significant for groups C and P. Fillet yield (with skin) increased temporarily between days 1 and 14 in groups C and P, and then gradually decreased throughout the purging period (group C: 48.5%, 51% and 45.2%; group P: 46.1%, 48.7%, and 44.1% on days 1, 14 and 70, respectively). There were almost no changes in fillet yield in group N during the whole purging period (44.1% on day 1, 44.8% on day 70).

3.2. Fat content, fatty acid composition and lipid classes

There was a significant ($P < 0.01$) reduction in fat content in groups C and P during the experiment, while minor changes (non-significant) were observed in group N (Table 2). The largest decrease was measured in group C (−62%), followed by group P (−48%), and the smallest in group N (−9%).

The FA composition (mg/100 g^{-1} fillet) at the start of the experiment and after 14, 28, 42, 56 and 70 days of purging is presented in Table 3. Changes in percentage content of the main FA groups (MUFA, PUFA, n−3 PUFA) throughout the whole experiment are shown in Fig. 2a, b and c, respectively. The proportion of saturated FA (SFA) varied between 24.2 ± 0.92% and 28.6 ± 0.81%. The MUFA content decreased significantly ($P < 0.01$) in group C (Fig. 2a), from initially 54.2 ± 2.19% to 46.9 ± 4.49% at day 70. A decrease in MUFA was also observed in

Table 2
Average fat content [%] of common carp in groups C, P and N during the purging period (mean ± standard deviation; n = 6).

Group	Days of purging						
	1	14	28	42	56	70	*P value*
C	8.68 ± 2.8[a]	8.33 ± 3.4[a]	5.66 ± 1.1[a]	6.65 ± 3.8[a]	7.10 ± 2.4[a]	3.30 ± 1.4	<0.01
P	7.32 ± 4.5[ab]	6.58 ± 3.6[ab]	3.16 ± 1.3[b]	3.19 ± 1.7[b]	3.23 ± 2.3[b]	3.46 ± 2.1	<0.01
N	3.51 ± 0.8[b]	3.59 ± 2.1[b]	3.42 ± 0.9[b]	2.34 ± 1.1[b]	3.54 ± 1.2[b]	3.16 ± 0.9	>0.05

Different letters within the same day indicate significant difference ($P < 0.05$) among the groups, *P value* indicates regression dependence during purging days. Abbreviations: C: cereal fed, P: pellet fed, N: natural fed.

Table 3
Fatty acid composition of total lipid from the edible part of groups C, P and N carp fillet (mg/100 g^{-1} fillet).
At days 1, 14, 28, 42, and 56 and after 70 days of purging (mean ± standard deviation; n = 6).

Group	Sampling day	Fatty acid											
		14:0	15:0	16:0	16:1 (n−7)	18:0	18:1 (n−9)	18:1 (n−7)	18:2 (n−6)	18:3 (n−3)	18:4 (n−3)	20:3 (n−6)	20:4 (n−6)
C	day 1	109 ± 37^{a}	25 ± 9	1411 ± 507^{a}	21 ± 12	454 ± 140^{a}	3503 ± 1057^{a}	250 ± 95	583 ± 206	327 ± 157	51 ± 22^{a}	23 ± 8	37 ± 24
	day 14	87 ± 29^{a}	18 ± 5	1365 ± 491^{a}	531 ± 331^{a}	420 ± 169	3072 ± 1341^{a}	235 ± 97	505 ± 200	235 ± 93^{ab}	57 ± 28^{a}	20 ± 7	67 ± 13
	day 28	64 ± 37^{a}	13 ± 7	1044 ± 363^{a}	347 ± 180^{a}	212 ± 107^{a}	2576 ± 777^{a}	133 ± 67^{a}	330 ± 165	177 ± 90	82 ± 38^{a}	13 ± 7	31 ± 12
	day 42	91 ± 52^{a}	18 ± 9^{a}	1212 ± 635^{a}	572 ± 353^{a}	334 ± 175^{a}	2481 ± 1370^{a}	207 ± 114^{a}	429 ± 243	205 ± 102^{a}	104 ± 52	18 ± 8	11 ± 5^{a}
	day 56	75 ± 47^{a}	17 ± 10^{a}	928 ± 545^{a}	393 ± 228^{a}	291 ± 109^{a}	2122 ± 1280^{a}	173 ± 82^{a}	447 ± 212	220 ± 103	113 ± 66	16 ± 6	11 ± 7
	day 70	38 ± 30	9 ± 6	439 ± 298	157 ± 62	125 ± 92	849 ± 622	74 ± 49	216 ± 121	116 ± 64	29 ± 13	10 ± 5	47 ± 22
	P value	<0.001	<0.001	<0.001	<0.04	<0.001	<0.001	<0.001	>0.05	<0.05	<0.002	>0.05	>0.05
P	day 1	78 ± 38^{ab}	25 ± 13	1187 ± 612^{ab}	33 ± 20	255 ± 119^{ab}	2430 ± 1514^{b}	229 ± 124	870 ± 348	517 ± 211	30 ± 22^{ab}	27 ± 15	49 ± 22
	day 14	62 ± 31^{ab}	17 ± 7	956 ± 403^{ab}	346 ± 104^{ab}	280 ± 116	1956 ± 904^{ab}	186 ± 95	726 ± 266	386 ± 119^{a}	45 ± 20^{ab}	26 ± 11	84 ± 31
	day 28	34 ± 12^{b}	9 ± 5	513 ± 211^{b}	179 ± 79^{b}	131 ± 41^{b}	923 ± 357^{b}	84 ± 33^{b}	325 ± 131	194 ± 99	51 ± 21^{b}	13 ± 5	21 ± 8
	day 42	30 ± 9^{b}	9 ± 3^{b}	477 ± 233^{b}	150 ± 69^{b}	140 ± 91^{b}	973 ± 417^{b}	88 ± 47^{ab}	318 ± 174	167 ± 62^{ab}	62 ± 42	17 ± 9	8 ± 3^{ab}
	day 56	32 ± 15^{b}	9 ± 2^{b}	485 ± 230^{b}	170 ± 58^{b}	136 ± 77^{b}	950 ± 506^{b}	87 ± 32^{b}	353 ± 150	188 ± 111	71 ± 30	14 ± 7	11 ± 6
	day 70	33 ± 16	8 ± 3	508 ± 173	168 ± 109	158 ± 91	949 ± 375	86 ± 39	347 ± 141	211 ± 88	47 ± 18	17 ± 5	74 ± 30
	P value	<0.03	<0.02	<0.04	>0.05	>0.05	<0.04	<0.03	>0.05	>0.05	>0.05	>0.05	>0.05
N	day 1	51 ± 12^{b}	15 ± 5	633 ± 147^{b}	25 ± 13	147 ± 60^{b}	1166 ± 127^{b}	131 ± 22	376 ± 91	165 ± 73	14 ± 2^{b}	16 ± 2	10 ± 4
	day 14	33 ± 14^{b}	9 ± 5	505 ± 281^{b}	168 ± 65^{b}	183 ± 98	1058 ± 457^{b}	106 ± 53	372 ± 159	125 ± 59^{b}	21 ± 9^{b}	18 ± 4	86 ± 16
	day 28	43 ± 15^{b}	13 ± 5	525 ± 117^{b}	235 ± 57^{b}	134 ± 26^{b}	984 ± 232^{b}	110 ± 34^{ab}	367 ± 238	194 ± 82	52 ± 16^{b}	13 ± 7	9 ± 4
	day 42	23 ± 7^{b}	6 ± 3^{b}	356 ± 137^{b}	111 ± 32^{b}	121 ± 61^{b}	696 ± 337^{b}	65 ± 26^{b}	218 ± 108	66 ± 22^{b}	54 ± 26	13 ± 6	5 ± 2^{b}
	day 56	35 ± 11^{b}	8 ± 2^{ab}	456 ± 221^{b}	199 ± 84^{ab}	140 ± 55^{b}	951 ± 396^{b}	91 ± 49^{ab}	239 ± 125	112 ± 68	53 ± 21	10 ± 5	6 ± 2
	day 70	29 ± 14	7 ± 3	387 ± 116	105 ± 58	122 ± 55	844 ± 316	82 ± 25	245 ± 114	110 ± 43	19 ± 4	10 ± 4	43 ± 21
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

At day 1 and after 14 and 70 days of purging (mean ± standard deviation; n = 6).

Group	Sampling day	Fatty acid								
		20:5 (n−3)	22:5 (n−3)	22:6 (n−3)	SFA	MUFA	PUFA	n−3 PUFA	n−6 PUFA	n−3/n−6
C	day 1	148 ± 69	46 ± 24	73 ± 32	2034 ± 703^{a}	3976 ± 1203^{a}	1364 ± 518	680 ± 309	685 ± 220	0.99
	day 14	100 ± 31	36 ± 13	63 ± 7	1923 ± 700^{a}	4023 ± 1813^{a}	1135 ± 402	503 ± 174	632 ± 232	0.80
	day 28	90 ± 56	31 ± 11	57 ± 22	1786 ± 532^{a}	3085 ± 1033^{a}	937 ± 420	437 ± 224	400 ± 207	1.09
	day 42	117 ± 54	39 ± 16	68 ± 16	1716 ± 898^{a}	3293 ± 1849^{a}	1027 ± 491	532 ± 223	495 ± 272	1.07
	day 56	102 ± 50	34 ± 13	57 ± 21	1371 ± 517^{a}	2719 ± 1225^{a}	1032 ± 536	524 ± 330	507 ± 329	1.03
	day 70	61 ± 31	24 ± 11	50 ± 23	624 ± 331	1135 ± 616	577 ± 298	286 ± 146	291 ± 155	0.98
	P value	<0.02	>0.05	>0.05	<0.001	<0.001	<0.05	<0.05	>0.05	>0.05
P	day 1	130 ± 46	49 ± 21	109 ± 43	1588 ± 713^{ab}	2782 ± 991^{b}	1853 ± 642	862 ± 314	991 ± 444	0.87
	day 14	101 ± 28	43 ± 15	128 ± 55	1344 ± 526^{ab}	2642 ± 1200^{b}	1611 ± 533	725 ± 284	886 ± 324	0.82
	day 28	54 ± 33	24 ± 11	62 ± 31	715 ± 277^{b}	1207 ± 474^{b}	771 ± 335	484 ± 189	487 ± 155	0.99
	day 42	71 ± 28	33 ± 19	92 ± 47	689 ± 341^{b}	1233 ± 740^{b}	796 ± 400	428 ± 201	368 ± 202	1.16
	day 56	67 ± 29	27 ± 11	72 ± 45	697 ± 269^{b}	1227 ± 733^{b}	828 ± 402	425 ± 223	403 ± 175	1.05
	day 70	73 ± 23	33 ± 9	102 ± 41	722 ± 203	1294 ± 553	937 ± 228	473 ± 209	464 ± 177	1.02
	P value	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05
N	day 1	106 ± 35	44 ± 15	107 ± 40	765 ± 92^{b}	1346 ± 127^{b}	870 ± 145	441 ± 127	429 ± 86	1.03
	day 14	73 ± 37	38 ± 15	104 ± 42	747 ± 317^{b}	1427 ± 322^{b}	882 ± 279	371 ± 96	511 ± 188	0.73
	day 28	75 ± 24	30 ± 6	61 ± 17	756 ± 173^{b}	1360 ± 316^{b}	797 ± 369	381 ± 119	416 ± 158	0.92
	day 42	72 ± 46	34 ± 13	91 ± 39	530 ± 217^{b}	1090 ± 398^{b}	573 ± 214	317 ± 111	256 ± 130	1.24
	day 56	67 ± 24	27 ± 10	55 ± 21	668 ± 295^{b}	1256 ± 560^{b}	589 ± 246	313 ± 98	276 ± 102	1.13
	day 70	56 ± 31	21 ± 13	64 ± 31	558 ± 214	1088 ± 420	594 ± 134	277 ± 129	317 ± 182	0.87
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Mean values with different superscripts within different purging days differ significantly (P < 0.05) among the groups, *P value* indicates regression dependence within fatty acid during purging; Abbreviations: C: cereal fed; P: pellet fed, N: natural fed; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

group P (Fig. 2b) but was not statistically significant. Almost no changes were observed in group N (Fig. 2c), where the proportion of MUFA varied between 45.2 ± 3.41% and 48.2 ± 2.44% during the whole purging period. At the same time, the content of MUFA (mg/100 g^{-1} fillet; Table 3) was significantly different between the experimental groups at day 1 and after 14, 28, 42 and 56 days. There were no significant differences among the groups after the 70-day purging period. The proportion of PUFA increased linearly in groups C (significantly) and P (non-significantly) and, in contrast, there was a trend of slightly decreasing PUFA in group N (29.1 ± 3.97% at the beginning; 26.9 ± 4.23% at the end) (Fig. 3). There were no significant differences in total amount of PUFA (mg/100 g^{-1} fillet) among the groups during purging period. The similar trend as in total PUFA was observed for the proportion of n−3 PUFA, which increased significantly in groups C and P, while it was unchanged in group N (Fig. 2).

The composition of the lipid classes confirmed the negative correlation between increasing fat content and percentage of PL and, conversely, the positive correlation between increasing fat content and TAG. The percentages of different lipid classes in all groups and the respective changes are presented in Table 4. When the data were expressed as percentage of total lipids, the proportion of PL seemed to increase in groups C and P, since TAG content decreased. However, when expressed as total amount (mg/100 g^{-1} fillet), PL in group C remained stable until day 28, and decreased rapidly after that, while in group N it remained relatively stable until day 42. Total PL decreased from day 14 in group P. Differences among the groups are evident till day 28; from day 42 there are no significant differences in the amount of PL (Fig. 3).

With prolonged purging time, FA composition in the TAG lipid fraction (Table 5a) showed significant changes in terms of MUFA and PUFA (including n−3 PUFA) in group C. Changes are evident also in group P,

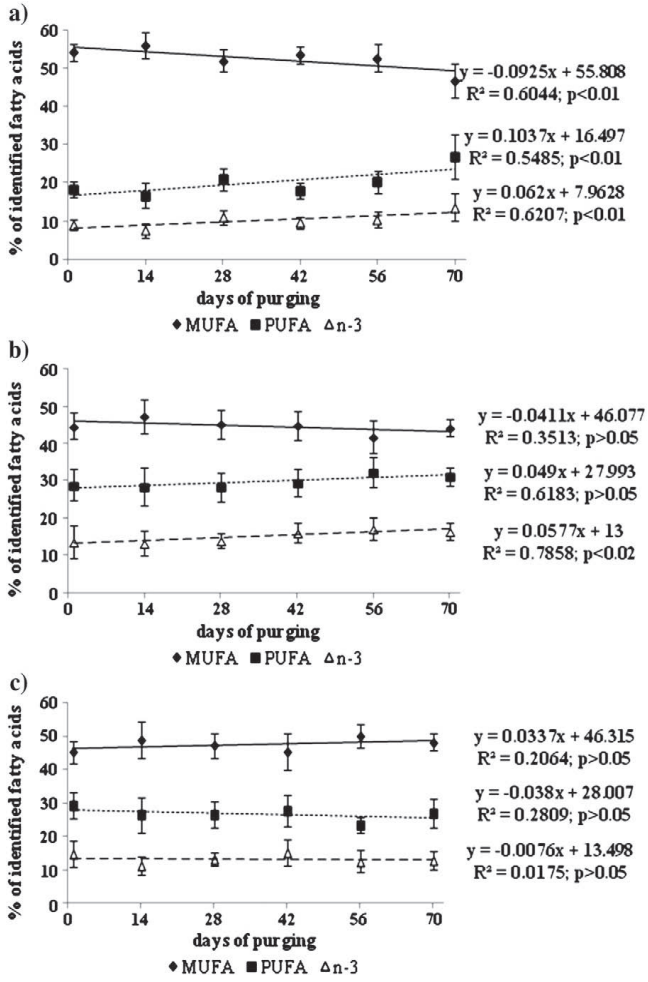

Fig. 2. a, b, c. Percentage of MUFA, PUFA and n−3 PUFA in (a) the group supplemented with cereals; (b) the group supplemented with rapeseed/linseed pellets; and (c) the group fed natural feed only during the purging period; P value indicates regression dependence within tested group during purging period. Abbreviations: MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n−3 PUFA: sum of 18:3 n−3, 18:4 n−3, 20:3 n−3, 20:5 n−3, 22:5 n−3, 22:6 n−3.

Table 4
Lipid class composition (%) in fillets from groups C, P and N carp during the purging period (mean ± standard deviation; n = 6).

Group	Day 1	Day 14	Day 28	Day 42	Day 56	Day 70	*P value*	Lipid class
C	8.69 ± 1.6^{b}	10.6 ± 2.4	12.7 ± 3.9	9.18 ± 1.7^{b}	5.55 ± 1.6^{b}	9.29 ± 2.5	>0.05	PL
	8.54 ± 0.8	7.91 ± 1.6	10.2 ± 1.6	7.14 ± 0.8^{b}	6.47 ± 1.1^{b}	7.93 ± 1.9	>0.05	STEROLS
	3.16 ± 0.2	nd	nd	nd	nd	2.54 ± 0.7	>0.05	FFA
	2.08 ± 0.7	2.04 ± 1.0	2.30 ± 0.9^{a}	2.17 ± 0.2	0.91 ± 0.2^{b}	1.34 ± 0.4	>0.05	DAG
	80.2 ± 3.3^{a}	79.2 ± 5.4	75.3 ± 6.8	81.5 ± 2.5^{a}	85.9 ± 3.0^{a}	80.4 ± 6.2	>0.05	TAG
P	8.79 ± 2.9^{b}	9.40 ± 3.6	14.2 ± 2.4	17.1 ± 6.4^{ab}	14.1 ± 8.1^{a}	12.3 ± 6.2	>0.05	PL
	10.4 ± 4.2	8.47 ± 2.2	9.76 ± 0.6	11.0 ± 3.0^{ab}	10.2 ± 2.8^{a}	10.5 ± 4.4	>0.05	STEROLS
	nd	nd	nd	2.76 ± 0.8	2.15 ± 0.6	3.20 ± 0.7	>0.05	FFA
	2.31 ± 0.9	1.31 ± 0.3	1.27 ± 0.3^{b}	2.15 ± 0.5	1.95 ± 0.8^{a}	1.43 ± 0.7	>0.05	DAG
	79.4 ± 5.5^{ab}	80.7 ± 6.6	74.7 ± 3.1	71.5 ± 12.2^{ab}	73.5 ± 11.1^{b}	74.7 ± 11.3	>0.05	TAG
N	13.7 ± 3.7^{a}	12.8 ± 4.0	12.9 ± 2.1	19.5 ± 6.3^{a}	8.98 ± 2.3^{ab}	9.65 ± 2.3	>0.05	PL
	11.3 ± 2.2	9.91 ± 2.7	9.56 ± 0.7	12.9 ± 3.3^{a}	8.42 ± 1.9^{ab}	8.25 ± 1.8	>0.05	STEROLS
	nd	1.72 ± 0.2	nd	nd	2.42 ± 0.3	2.91 ± 1.2	>0.05	FFA
	2.07 ± 0.8	1.68 ± 0.6	1.23 ± 0.2^{b}	2.54 ± 0.7	1.38 ± 0.4^{ab}	1.28 ± 0.5	>0.05	DAG
	71.9 ± 6.2^{b}	75.0 ± 6.9	76.5 ± 3.1	64.3 ± 10.3^{b}	79.6 ± 3.8^{ab}	78.9 ± 5.0	>0.05	TAG

Mean values with different superscripts within different purging days differ significantly (P < 0.05) among the groups, *P value* indicates regression dependence within fatty acid during purging; Abbreviations: PL: phospholipid; DAG: diacylglycerol; FFA: free fatty acid; TAG: triacylglycerol; nd: not detected; C: cereal fed; P: pellet fed; N: natural fed.

but they are not significant. No changes were observed in group N. The total amount (Fig. 3) and FA composition in the PL fraction (Table 5b) changed mostly in group C, where alterations in percentage content of SFA, PUFA (including n−3 and n−3 long-chain PUFA) and MUFA/PUFA ratio were confirmed. There was a significant decrease in SFA in group P, together with a trend for increasing proportion of PUFA. Unexpectedly, in group N the percentage of PUFA, n−3 PUFA, n−3 long-chain PUFA, EPA and DHA decreased in the first 14 days and then increased back to the initial proportions.

4. Discussion

4.1. Changes in weight, fillet yield and condition factor

For most of the Czech population carp is the traditional Christmas Eve dinner. In order to provide the market with carp within 2 or 3 weeks before Christmas, the harvest time has to be adjusted to the weather conditions and icing of the ponds. This results in some fish being kept in purging conditions for longer than others. In order to evaluate the possible effects of all alternate purging treatments, this experiment was carried out in the period September–December. In the present study purging started at 18.5 °C, which is relatively high, and then the water temperature dropped to 14.6 °C after 4 days and reached 11.9 °C after 18 days. It has been reported that as stocking density is generally high in purging ponds, fish activity will increase (Bauer and Schlott, 2004). However, as the temperature dropped rapidly after the start of our experiment, the fish were not exposed to stressful conditions for long.

In general, weight loss has been observed in purged carp. During normal overwintering, the weight loss has been reported in different studies to be 3% (Bauer and Schlott, 2004), 5–10% (Geldhauser and Gerstner, 2003), and 14–24% (Blasco et al., 1992). In the present study, we found a total weight loss of 9.2%, 14.6% and 19.6% in groups N, P and C, respectively, during purging (Table 1).

Fillet yield is closely related to weight loss. In the present study fillet yield was still >45% at the last sampling point, which was slightly

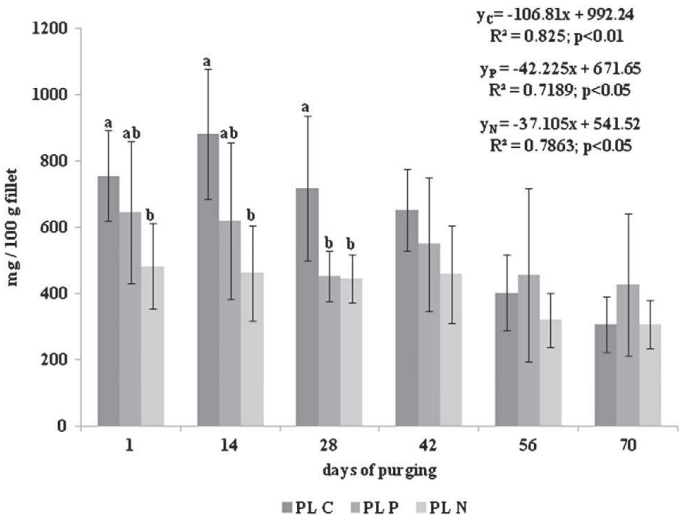

Fig. 3. Calculated total values of phospholipids, with the lines showing changes over time in averages in each group; P value indicates regression dependence within tested group during purging period. Abbreviations: PL: phospholipid; C: cereal group, P: pellet group, N: natural feed group.

Table 5a
Percentage of fatty acid in triacylglycerol fraction of groups C, P and N common carp purged for 70 days (mean ± standard deviation; n = 6); comparison between days 1, 14 and 70.

Group	Sampling day	Fatty acids							
		EPA + DHA	SFA	MUFA	PUFA	n−3 PUFA	n−3 LC PUFA	n−3/n−6	MUFA/PUFA
C	day 1	1.68 ± 0.3	26.2 ± 1.0	58.1 ± 1.9^{a}	15.7 ± 2.5^{b}	7.00 ± 1.5^{b}	2.23 ± 0.4	0.81 ± 0.2	3.84 ± 0.8^{a}
	day 14	1.71 ± 0.3	26.4 ± 0.9	57.7 ± 2.4^{a}	15.8 ± 2.3^{b}	6.68 ± 1.3^{b}	2.30 ± 0.4	0.72 ± 0.1^{ab}	3.75 ± 0.7^{a}
	day 28	1.71 ± 0.2^{ab}	26.1 ± 1.0	57.1 ± 2.6^{a}	16.8 ± 2.2^{b}	7.06 ± 1.0^{b}	2.35 ± 0.3	0.74 ± 0.1	3.49 ± 0.6^{a}
	day 42	1.75 ± 0.4	27.7 ± 0.5^{a}	57.1 ± 1.5	15.3 ± 1.5^{c}	6.74 ± 1.1^{ab}	2.32 ± 0.5	0.80 ± 0.1	3.78 ± 0.5^{a}
	day 56	1.84 ± 0.7	25.6 ± 1.5	56.8 ± 3.2^{a}	17.6 ± 3.7^{b}	7.53 ± 2.3^{b}	2.43 ± 0.9^{b}	0.73 ± 0.1^{b}	3.41 ± 0.9^{a}
	day 70	2.19 ± 0.6	25.9 ± 1.1	53.8 ± 3.0	20.4 ± 4.0	9.27 ± 2.0	3.05 ± 0.8	0.85 ± 0.2	2.77 ± 0.7
	P value	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	>0.05	<0.05
P	day 1	2.37 ± 1.1	24.4 ± 1.7	47.4 ± 4.4^{b}	28.2 ± 4.3^{a}	13.8 ± 3.6^{a}	3.34 ± 1.4	1.00 ± 0.4	1.75 ± 0.5^{b}
	day 14	2.24 ± 1.0	24.8 ± 1.9	48.4 ± 3.9^{b}	26.8 ± 4.1^{a}	12.1 ± 2.2^{a}	3.13 ± 1.3	0.85 ± 0.2^{a}	1.87 ± 0.4^{b}
	day 28	1.43 ± 0.6^{b}	24.7 ± 1.4	51.9 ± 2.6^{b}	23.4 ± 3.4^{a}	9.44 ± 2.4^{a}	2.07 ± 0.8	0.68 ± 0.2	2.30 ± 0.6^{b}
	day 42	1.46 ± 0.7	24.3 ± 1.4^{b}	53.4 ± 3.5	22.3 ± 2.4^{a}	9.05 ± 2.5^{a}	2.10 ± 0.9	0.70 ± 0.2	2.44 ± 0.4^{b}
	day 56	2.73 ± 0.5	25.2 ± 1.2	44.7 ± 3.1^{b}	30.1 ± 4.0^{a}	14.9 ± 1.9^{a}	3.90 ± 0.6^{a}	1.00 ± 0.1^{a}	1.52 ± 0.3^{b}
	day 70	1.53 ± 0.7	24.6 ± 1.6	54.2 ± 4.5	21.2 ± 4.3	8.62 ± 3.5	2.21 ± 1.0	0.69 ± 0.3	2.72 ± 0.8
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
N	day 1	1.95 ± 1.0	25.0 ± 2.1	53.1 ± 4.4^{a}	21.9 ± 4.4^{ab}	8.84 ± 3.5^{b}	2.69 ± 1.2	0.69 ± 0.3	2.56 ± 0.7^{b}
	day 14	1.50 ± 0.3	24.9 ± 2.1	54.0 ± 2.8^{ab}	21.2 ± 3.8^{ab}	8.06 ± 1.6^{b}	2.08 ± 0.4	0.62 ± 0.1^{b}	2.65 ± 0.6^{b}
	day 28	2.38 ± 0.7^{a}	25.3 ± 2.4	51.6 ± 5.0^{ab}	23.1 ± 5.0^{a}	9.33 ± 2.4^{ab}	3.22 ± 1.0	0.73 ± 0.3	2.36 ± 0.6^{b}
	day 42	1.50 ± 0.4	24.5 ± 1.9^{b}	56.5 ± 2.1	19.0 ± 0.9^{b}	6.49 ± 1.1^{b}	2.26 ± 0.5	0.53 ± 0.1	2.98 ± 0.2^{b}
	day 56	1.82 ± 0.6	24.7 ± 1.1	56.4 ± 1.4^{a}	18.9 ± 1.2^{b}	7.65 ± 1.8^{b}	2.51 ± 0.9^{b}	0.72 ± 0.3^{b}	3.00 ± 0.3^{a}
	day 70	2.04 ± 0.8	24.6 ± 1.0	54.9 ± 2.9	20.6 ± 2.7	8.10 ± 2.5	2.79 ± 1.1	0.66 ± 0.2	2.73 ± 0.5
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Mean values with different superscripts within different purging days differ significantly (P < 0.05) among the groups, *P value* indicates regression dependence within fatty acid during purging; Abbreviations: C: cereal fed; P: pellet fed; N: natural fed, EPA + DHA: eicosapentaenoic and docosahexaenoic acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n−3 long-chain PUFAs are fatty acids with more than 20 carbon atoms and with at least 3 double bonds (20:3 n−3; 20:5 n−3; 22:5 n−3; 22:6 n−3).

higher than the value reported earlier by Kocour et al. (2007) of 41.1% for 3-year-old carp with average weight around 1500 g after harvest. This difference is most likely due to the higher start weight of the 4-year-old carp (body weight 1700–2600 g) used in the present study, resulting in an increased muscle content in these carp. As expected (Einen et al., 1998), percentage fillet yield increased in the first days of purging (days 1–14), after the gut had emptied and thus the relative proportion of muscle increased by 2.5% in groups C and P. No increase in fillet yield was found in group N. This is most probably due to different rearing practices. While the carp in groups C and P had continuous access to feed, resulting in a constantly filled gut, the fish kept in natural conditions in group N had to seek their feed and therefore most likely had a less full gut at the time of harvest. Similar results have been reported by Oberle et al. (1997), who found that carp kept in natural conditions did not reach a similar fillet yield to diet-supplemented carp after short-term purging.

The decrease in the CF value during the purging period reflected the decrease in weight at unchanged BL of purged carp (Fig. 1). Similarly, Bauer and Schlott (2004) reported a decrease in CF in overwintering carp.

Table 5b
Percentage of fatty acid in phospholipid fraction of groups C, P and N common carp purged for 70 days (mean ± standard deviation; n = 6); comparison between days 1, 14 and 70.

Group	Sampling day	Fatty acids							
		EPA + DHA	SFA	MUFA	PUFA	n−3 PUFA	n−3 LC PUFA	n−3/n−6	MUFA/PUFA
C	day 1	14.7 ± 2.2^{b}	32.9 ± 0.3^{ab}	33.9 ± 5.2^{a}	33.3 ± 5.4^{b}	19.7 ± 3.2^{b}	17.8 ± 2.7^{b}	1.46 ± 0.1	1.07 ± 0.3^{a}
	day 14	17.6 ± 2.2	32.7 ± 1.4	28.2 ± 5.4	39.0 ± 4.5	23.4 ± 2.8	21.6 ± 2.5	1.50 ± 0.1^{a}	0.75 ± 0.2
	day 28	19.5 ± 1.2	30.1 ± 2.3	27.3 ± 1.8^{a}	42.6 ± 4.1	25.5 ± 1.9	23.6 ± 1.5	1.52 ± 0.2	0.65 ± 0.1^{a}
	day 42	18.8 ± 2.8	30.6 ± 4.0	29.7 ± 3.3^{a}	39.6 ± 5.9^{b}	24.6 ± 3.8	22.8 ± 3.4	1.65 ± 0.2	0.78 ± 0.2^{a}
	day 56	17.7 ± 3.5	29.5 ± 3.0	31.3 ± 4.8^{a}	39.3 ± 6.0^{b}	23.7 ± 4.5	21.7 ± 4.1	1.51 ± 0.2	0.84 ± 0.3^{a}
	day 70	20.0 ± 1.5	27.4 ± 3.7	27.4 ± 1.8	45.2 ± 5.2	27.1 ± 2.1	24.4 ± 1.7	1.55 ± 0.3	0.62 ± 0.1
	P value	<0.01	<0.001	>0.05	<0.01	<0.01	<0.01	>0.05	<0.05
P	day 1	16.6 ± 5.0^{ab}	34.8 ± 3.7^{a}	24.8 ± 4.4^{b}	39.7 ± 6.8^{ab}	23.6 ± 5.4^{ab}	20.0 ± 5.7^{ab}	1.47 ± 0.3	0.65 ± 0.2^{b}
	day 14	16.5 ± 6.5	34.6 ± 5.0	26.1 ± 5.1	39.4 ± 9.5	23.1 ± 6.6	19.4 ± 7.1	1.41 ± 0.2^{ab}	0.75 ± 0.4
	day 28	20.3 ± 2.2	27.5 ± 2.6	24.3 ± 2.0^{b}	48.2 ± 2.9	27.6 ± 2.6	24.5 ± 2.5	1.36 ± 0.2	0.51 ± 0.1^{b}
	day 42	19.9 ± 1.7	29.4 ± 3.9	23.4 ± 1.1^{b}	47.3 ± 3.5^{a}	27.3 ± 2.3	24.1 ± 2.2	1.39 ± 0.2	0.50 ± 0.1^{b}
	day 56	18.9 ± 1.3	29.5 ± 4.2	23.4 ± 1.4^{b}	47.1 ± 3.5^{a}	26.4 ± 1.8	23.1 ± 1.7	1.29 ± 0.2	0.50 ± 0.1^{b}
	day 70	19.0 ± 3.2	27.5 ± 4.0	26.1 ± 2.7	46.4 ± 4.6	26.1 ± 2.8	23.0 ± 3.4	1.31 ± 0.2	0.57 ± 0.1
	P value	>0.05	<0.01	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05
N	day 1	21.3 ± 3.1^{a}	29.7 ± 2.4^{ab}	22.4 ± 3.1^{b}	47.8 ± 2.4^{a}	28.7 ± 3.8^{a}	26.1 ± 4.0^{a}	1.57 ± 0.5	0.47 ± 0.1^{b}
	day 14	16.3 ± 2.7	32.4 ± 4.3	25.1 ± 3.0	42.5 ± 5.8	23.2 ± 3.2	20.2 ± 2.9	1.20 ± 0.1^{b}	0.61 ± 0.1
	day 28	20.9 ± 2.0	29.6 ± 4.8	23.5 ± 1.3^{b}	46.9 ± 4.2	28.0 ± 2.8	25.7 ± 2.5	1.50 ± 0.2	0.50 ± 0.1^{b}
	day 42	21.9 ± 2.0	26.5 ± 3.9	24.8 ± 3.1^{b}	48.7 ± 3.1^{a}	28.6 ± 2.4	26.5 ± 2.2	1.43 ± 0.1	0.51 ± 0.1^{b}
	day 56	21.7 ± 4.0	27.3 ± 3.9	25.4 ± 2.8^{b}	47.3 ± 4.2^{a}	29.1 ± 5.0	26.9 ± 4.8	1.65 ± 0.5	0.54 ± 0.1^{b}
	day 70	20.5 ± 1.5	28.0 ± 4.1	25.2 ± 2.3	46.8 ± 2.6	27.32.3	25.0 ± 1.9	1.41 ± 0.2	0.54 ± 0.1
	P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Mean values with different superscripts within different purging days differ significantly (P < 0.05) among the groups, *P value* indicates regression dependence within fatty acid during purging; Abbreviations: C: cereal fed; P: pellet fed; N: natural fed, EPA + DHA: eicosapentaenoic and docosahexaenoic acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n−3 long-chain PUFAs are fatty acids with more than 20 carbon atoms and with at least 3 double bonds (20:3 n−3; 20:5 n−3; 22:5 n−3; 22:6 n−3).

4.2. Fat content and fatty acid composition

The muscle fat content decreased significantly in groups C and P, where the highest fat content was found at the beginning of the purging period (8.68 ± 2.8% and 7.32 ± 4.5% respectively; Table 2). Decreased fat stores have also been observed in other studies (Einen et al., 1998; Liu et al., 2011).

The carp from group N, in the natural pond environment, did not show any significant decrease in fat content. The variation in fat content between different fish and sampling dates was high, so the fact that different individuals were measured on each sampling occasion may explain the non-significant weight loss in group N fish. Similarly, Palmeri et al. (2009a) did not find a significant fat loss in low-fat Murray cod during starvation ($P > 0.05$).

Extruded linseed and pressed rapeseed cake (mouldings) are a relatively cheap and easily available source of 18:3 n−3 for fish nutrition (Pickova and Mørkøre, 2007) and were part of the mixture fed to group P in this experiment. The presence of 18:3 n−3 in the pellets was reflected in the FA composition of the carp muscle. At the beginning of purging there was a 1.58-fold higher content of 18:3 n−3 in the muscle of carp from group P compared with group C and a 3.13-fold higher content compared with carp from group N. The effect of diet was also evident in the group C, where a high content of MUFA, especially 18:1 n−9, was observed. The opposite was found in the group N fish, reflecting the FA composition of the prey with high levels of PUFA according to our initial hypothesis and findings by Vacha et al. (2007) and Mraz et al. (2012).

During starvation, free fatty acids (FFAs) are released from TAG as a substrate for β-oxidation in the mitochondria (Reshef et al., 2003). The decrease in TAG and the higher stability of PL found in our study are in line with results reported by Henderson and Tocher (1987). In a study by Kiessling et al. (2001) performed on rainbow trout, excess dietary energy was found to be stored in the form of TAG, as was also the case of the cereal-supplemented group, resulting in the highest percentage of TAG in that group at most sampling dates (Table 4).

When the identified proportion of PL was calculated as absolute amounts per portion of fish, these amounts remained stable until days 14, 28 and 42 of sampling in groups P, C and N, respectively. While these are calculated amounts rather than actual values, they correspond well to the actual values and are suggested to give a good picture of the point when the fish start to use PL. We suggest that this is also the point when the fish start to catabolise not only surplus fat, but also muscle mass to meet energy needs. In addition, we suggest that at this point the fish also start to metabolise PUFA.

During β-oxidation of FA from TAG, in general fish utilise FA selectively in order to save the metabolically essential long-chain PUFA and first use the less important FA as fuel (Kiessling and Kiessling, 1993).

In our study there was a continuous increase in the relative content of n−3 PUFA in the muscle of carp from group C, which can be explained by the gradual degradation of MUFA (Jezierska et al., 1982), especially 18:1 n−9, while PUFAs are protected as described by Csengeri (1996). This could be due to the fluidity of biological membranes being increased and carp surviving better at low water temperatures. Similar results have been reported by Palmeri et al. (2008b) in a study on Murray cod.

The FA composition of the TAG and PL fractions of purged carp from group C showed a clear increase in the proportion of PUFA, including n−3 PUFA, EPA and DHA, to the detriment of MUFA (Tables 5a and 5b). These changes were pronounced in PL, combined with a significant decrease in the MUFA/PUFA ratio. This suggests that carp preferentially metabolise MUFA, while PUFAs are protected as suggested by Kiessling and Kiessling (1993) for rainbow trout. At the point when the carp started to metabolise the PUFA, they seemed to metabolise n−6 and n−3 equally. Again, this is in line with Kiessling and Kiessling (1993), who found similar oxidation rates for 18:2 n−6 and 18:3 n−3 in the mitochondria of rainbow trout.

In addition, we suggest that fish have a certain metabolically defined composition of FA that is necessary for functionality and that once that level is reached, fish start to catabolise all FA equally to preserve the relative composition. This theory is supported by the fact that after the longer purging period, the FA composition did not differ more between the groups, despite the significant differences at the beginning of the experiment. With decreasing water temperature, prolonged starvation and metabolism of fat stores, the FA composition gradually equalised in the PL fraction and after 70 days of purging, all groups showed almost identical values of SFA, MUFA, PUFA (including n−3, n−3 long-chain PUFA, EPA and DHA).

In relation to human nutrition, it is well known that fish are an important source of beneficial n−3 FA (Simopoulos, 2002). The European Food Safety Authority (EFSA, 2009) recommends a daily intake of n−3 PUFA for the general population of 2 g and an EPA + DHA intake of 250 mg. One portion (200 g) of carp from groups C, P or N purged for 14 days, which time we concluded as one with the best nutritional values, contained 1.06 g n−3 PUFA and 326 mg EPA + DHA, 1.72 g PUFA and 453 mg EPA + DHA, or 0.75 g PUFA and 354 mg EPA + DHA, respectively. This means that the best choice in terms of human nutrition seems to be carp from group P purged no longer than 14 days.

5. Conclusions

Lipid analyses of purged common carp reared in three different production systems showed that the type of diet prior to purging significantly affected the flesh quality of the fish. Supplementation with rapeseed/linseed pellets in the growing period resulted in a nutritionally beneficial FA flesh composition. The purged carp were able to selectively metabolise FA for energy needs early in the purging period, and when the carp were supplemented with cereals or rapeseed/linseed pellets, which had higher CF, they mainly used MUFA as their energy source. However with prolonged purging and loss of surplus fat, the fish from all groups started to metabolise long-chain PUFA, leading to a decrease in nutritionally valuable n−3 PUFA. Therefore a purging period should be long enough to eliminate possible unpleasant odours and flavours, but as short as possible from a practical handling point of view to preserve the beneficial FA composition of n−3 enriched carp. For this reason, we recommend that carp supplemented with linseed/rapeseed pellets (group P) should not be purged no longer than 14 days. More studies are needed to identify the mechanisms behind the selective FA metabolism in purged carp. This study provided valuable information about FA metabolism in carp that can be used in the further development of feeds and rearing systems.

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CHAPTER 4

EFFECT OF FRYING FAT AND PREPARATION ON CARP (*CYPRINUS CARPIO*) FILLET LIPID COMPOSITION AND OXIDATION

Paper III:

Sampels, S., Zajic, T., Mraz, J., 2013. Effect of frying fat and preparation on carp (Cyprinus carpio) fillet lipid composition and oxidation. (manuscript)

EFFECT OF FRYING FAT AND PREPARATION ON CARP (*CYPRINUS CARPIO*) FILLET LIPID COMPOSITION AND OXIDATION

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Abstract

We investigated the changes caused by frying in fat uptake, fatty acid (FA) composition and oxidation in omega-3 enriched carp fillets. Four different fats were used and fillets were fried plain or battered. Fillet fat content increased during frying and FA composition in the fillets mirrored the composition of the frying fat. Frying with sunflower oil negatively influenced the nutritional valuable composition decreasing the n-3/n-6 ratio in the fillets. Frying with rapeseed oil preserved the favorable n-3/n-6 ratio without increasing the saturated fatty acids (SFA). Frying with lard and butter preserved the n-3/n-6 ratio but increased the SFA content. There was no increased oxidation with the use of rapeseed oil. We recommend using rapeseed oil to fry fish to preserve the nutritional valuable composition. The dilution model seems to be a good tool to predict changes in fish FA composition due to frying, but needs to be further confirmed.

Keywords: Dilution model, DHA, EPA, n-3, pan-frying, TBARS

Introduction

The inclusion of fish and fish products in the human diet at least twice a week is recommended from a nutritional point of view, due to the high content of omega-3 polyunsaturated fatty acids (n-3 PUFA) in marine and fresh water fish (Steffens, 1997). The n-3 PUFA are well known to have positive effects on different health aspects as for example metabolic syndrome, obesity, diabetes, arteriosclerosis, neural and brain development (Calder and Grimble, 2002; Connor, 2000; Richardson, 2006; Storlien et al., 1997; Williams, 2000). However, today's Western diet contains an increasing content of omega-6 (n-6) PUFA (Ailhaud et al., 2006; Simopoulos, 1999), leading to increased incidence of cardiovascular and atherosclerotic diseases, type 2 diabetes and obesity (Simopoulos, 1999).

Both n-6 and n-3 PUFA are precursors for a variety of divers chemical messengers, regulating factors and eicosanoids as prostaglandins, leukotriens and related substances, which have important roles in inflammation and the regulation of immunity (Calder, 1997; Calder, 2001; Horrobin, 1995; Kinsella, 1988). Since metabolites of n-3 and n-6 PUFA have different, often opposing biological actions and potencies (Calder, 2001; Schmitz and Ecker, 2008), the intake ratio between n-3 and n-6

PUFA is important. Values for n-6/n-3 in the diet of 1 to 4 have been recommended (Simopoulos, 2002a) while in today's Western diets this ratio is often 15 to 20.

It is known from earlier studies, that the type of preparation has some influence on the final fatty acid (FA) composition of the product and that especially during frying the used fat will have some influence on the FA composition of the final product as shown for meat and fish by Ågren and Hänninen (1993); Ramirez et al. (2005); Sioen et al. (2006); Weber et al. (2008). Effects are partly due to the heat, which can cause oxidation but also due to the fact that during frying an exchange will take part where some water from the surface layer of the fried object is exchanged into fat from the frying (Candela et al., 1998; Dobarganes et al., 2000). In addition, when dealing with an object like fatty fish, containing fat with a very low melting point and a higher liquidity than the frying fat due to its high unsaturation, there might be a loss of the original fat due to leakage as well, however leakage might also depend on fish fat content as shown by Sioen et al. (2006).

Most of the work until now has been done in deep-fat frying whereas only few researchers looked at pan frying, as for example Al-Saghir et al. (2004); Haak et al. (2007); Mai et al. (1978); and Sioen et al. (2006) have done on different low and high fat species. Pan-frying is however one of the most used ways to prepare fish or fish products at home. Therefore knowledge about what happens during this process is important when giving nutritional recommendations to consumers.

Many investigators have fried fish in sunflower oil and found increased proportions of 18:2 n-6 influencing the n-3/n-6 ratio enormously (Gladyshev et al., 2006; Gladyshev et al., 2007), however in a study on catfish by Weber et al. (2008) fried in canola oil 18:3 n-3 was increased and thereby the n-3/n-6 ratio leading to a nutritional more valuable composition.

In Czech Republic and central Europe carp is a quite commonly consumed fish species and recently an omega-3 rich carp has been patented (Mraz et al., 2011) and is sold on the marked as a product with positive effects for health based on results from a clinical study (Adamkova et al., 2011). Adamkova et al. (2011) showed that the intake of two 200g portions of this carp rich in n-3 per week improved the blood lipid values of patients recovering from ischemic heart disease.

On carp there are only very few papers concerning the preparation before consumption at home. De Castro et al. (2007) and Naseri et al. (2010) investigated cooking methods without adding any oil or fat and only one reference was found, which investigated deep fat frying of whole carp fillets (Tothmarkus and Sasskiss, 1993). A very recent publication investigated for the first time also shallow fat frying of silver carp with olive, sunflower and canola oil (Rahimabadi and Dad, 2012).

Especially with the new developed n-3 rich carp it is important to know what types of changes occur in the fish FA composition when using different types of frying fat, so we chose butter and lard beside sunflower and rapeseed oil as the most common used oils in Czech homes. In addition we fried the fillets plain and battered, which is a quite usual way of preparation in Czech homes. Beside FA composition we also monitored the oxidation due to frying with different fats.

The aim was to see the combined effects of fat exchange and oxidation in order to be able to make suggestions on the preferable way to prepare carp in order to keep the nutritional value. As the effects might be similar in other oily fish species the results might also have a more general use. In order to test if the changes in FA composition are predictable we tried to apply a simple dilution model on one group.

Material and methods

Fish samples

Twelve 4-year-old market scaly carp (*Cyprinus carpio*) reared for one season (April–September) with a rapeseed/linseed pellet supplementation were used. After harvesting and purging, the fish were killed and filleted. Fillets were frozen in -20 °C until the frying experiment.

Lard, butter, rapeseed oil and sunflower oil obtained in the local supermarket were used for frying. Before frying the fish fillets were cut in three parts. Front and back part of the fillets were cut to be about 100 g similar sized pieces to be fried, while the minor middle part was used as raw control for each fillet. From each corresponding fillet one back and one front part where used to be fried in the same fat source, either with or without a batter. The batter was prepared according to a traditional recipe by first turning the filet piece in wheat flower, then in mixed egg and finally in breadcrumbs. No spices or salt were added. For frying 50 ml of the fat (butter and lard were carefully melted before use) were used to fry the two pieces of the same fish and the same treatment (plain or battered) in a Teflon pan with 24 cm in diameter. The amount of fat was chosen so that the bottom of the pan was covered with the liquid fat. The fat was heated to approximately 130 °C and then the pieces were fried for 4+4 min on each side, and then for additional 2 min on each side until a core temperature of 65 °C, measured with a meat thermometer, was reached. After frying and cooling the whole pieces were separately minced in a table mixer to assure all eatable parts were included equally in the sample. The 2 stripes of raw samples from one fish were combined and minced together. For each fat type three fish were used resulting in 3 duplicate plain fried pieces, 3 duplicate battered fried pieces and 3 raw samples. Minced samples were frozen at -80 °C until further analysis.

Total fat content and FA composition

The lipid extraction was performed according to Hara and Radin (1978), with slight modifications. The samples were semi-thawed, and a sub-sample of ca. 1 g was taken for extraction from fish and 200 mg from the fats. The samples were homogenised for 3 x 30 s in 10 ml HIP (hexane:isopropanol (3:2), v/v) using an Ultra Turrax (T25, Janke and Kunkel, IKA Werke, Germany), and 6.5 ml Na_2SO_4 solution (0.47 M) were added. The homogenate was left to separate in 4 °C for 20 min and the upper phase was then transferred to a new tube and evaporated under N_2. The lipid content of the fillet pieces was determined gravimetrically from this total extracted lipid, which was then dissolved in 1 ml hexane. The samples were stored at -80 °C in normal atmosphere until further analyses.

FA from the total lipids were methylated with BF3 according to Appelqvist (1968) (method b). To each sample, 2 ml of a 0.01 M solution of NaOH in dry methanol was added, and the samples were then heated for 10 min at 60 °C. Next 3 ml of BF_3 reagent (boron triflouoride-methanol complex) were added and the samples were reheated at 60 °C for 10 min. Thereafter the tubes were cooled in ice water and 2 ml of a 3.42 M NaCl solution in water was added to all tubes. The FA methyl esters (FAME) were extracted with 2 ml hexane, the upper layer was transferred to a new tube and evaporated under nitrogen gas to dryness. The lipids were dissolved in 0.5 ml hexane and stored under normal atmosphere at -80 °C until gas chromatography analysis.

The FAME then were analysed with a gas chromatograph (Trace Ultra FID, Thermo Scientific) equipped with a flame ionisation detector and PVT injector, using a BPX 70 column (SGE, Austin, Texas), length 50 m, id 0.22 mm, and film thickness 0.25 µm. The GC was programmed with a constant gas flow of 1.2ml/min and a temperature program which started at 70 °C for 0.5min, followed by a ramp of 30 °C/min up to 150 °C and a second ramp with a rate of 2 °C min^{-1} until 220 °C and a final constant time of 11 min. at 220 °C. Injector and detector temperature were programmed at 150 °C and 250 °C respectively. The injector was programmed in splitless mode, with a splitless time of 0.8 min and a split flow 25 ml/min. The peaks were identified by comparing their retention times with those of the standard mixture GLC-68D (Nu-Chek Prep, Elysian, USA) and other authentic standards.

Oxidation

Analysis of thiobarbituric acid reactive substances (TBARS) was conducted in the fresh fillets, the fried fillets and the fresh fats and oils according to a method described by (Miller, 1998) and slightly modified by Sampels (2005). The fats and oils were solved in methanol instead of TCA solution. After reaction in darkness for 15–20 hrs (overnight) at room temperature (20 °C), the reaction complex was detected at a wavelength of 530 nm against the sample blank using a UV-visual spectrophotometer (Specord 210, Analytik Jena, Germany).

Dilution model

The data obtained from lipid analyses of fillet tissues before and after frying were compared against predicted data calculated according the dilution model designed by Robin et al. (2003) and shown in Equation 1.

PT = PR + [(P0 – PR) / (QT/Q0)] (Equation 1)

With:
PT = Predicted percentage of a FA in a fillet after frying with rapeseed oil
PR = Percentage of a FA in the oil
P0 = Percentage of a FA in the raw fillet
QT = Quantity of total FA in the fillet after frying
Q0 = Quantity of total FA in the raw fillet.

The predicted percentage of specific FA, e.g. EPA after the frying), (PT) was calculated by taking the percentage of the specific FA measured in the oil and the corresponding FA percentage in the raw fillet. Q0 was taken as the average total FA content of the raw fillet and QT as the final total FA content of fish from the corresponding fried fillet. All values represent the mean of three replicates.

Statistics and calculation

Averages and standard deviations were calculated in Excel and statistical evaluation was performed using the Mixed Procedure in SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA) using. Changes in FA percentages were calculated in Excel.

Results

Total fat content

Fat content is presented in Table 1. Fat content increased significantly due to frying and even more when the fillet was battered before frying. The increase between plain fried and battered fried fillet was significant for lard.

Fatty acid composition

FA composition in raw and fried samples is presented in Table 1. The main FA of the fats are found in table 2.

When using butter for frying the SFA significantly increased from raw to plain fried and to fried in batter, while MUFA and PUFA decreased however the difference was significant only when the fillets were fried battered. The FA that increased most were C14:0 and C16:0 (Figure 1).

Frying with lard resulted in significant increase of SFA and significant decrease of PUFA and n-3, while n-6 was stable. The main FA increasing were C16:0 and C18:0 while all unsaturated FA decreased more or less significantly. The decrease in n-3 also caused a significant decrease in the n-3/n-6 ratio.

Frying with rapeseed led to significantly decreased SFA and significant increase of MUFA. In the battered fillets after frying even the total n-3 FA were significantly decreased compared to the raw fillets. However, that decrease was not significant in the plain fried fillets. The main increased FA was 18:1n-9 (Figure 1).

Fillets fried in sunflower oil showed a significant increase in total PUFA and n-6 FA, while SFA and n-3 FA decreased significantly. The FA that increased significantly were 18:2n-6 and 18:1n-9. The significant increase in n-6 also caused a significant decrease in the n-3/n-6 ratio. 20:5n-3 (eicosapentaenoic acid, EPA), 22:5n-3 (docosapentaenoic acid, DPA) and 22:6n-3 (docosahexaenoic acid, DHA) decreased significantly in all frying treatments independent from the used fat. 20:4n-6 (arachidonic acid, AA) decreased significantly due to frying with lard, rapeseed oil and sunflower oil, however not when butter was used.

Oxidation

TBARS between the raw fish samples were similar. Frying increased TBARS in the fish fried with lard and sunflower significantly, while butter did not increase TBARS and frying with rapeseed oil only showed a tendency towards increased TBARS values ($p = 0.0674$). TBARS in the battered fillets were not analyzed as the batter interfered with the TBA solution leading to a yellowish color making a comparison with the standard impossible.

The TBARS measurement in the fats and oils was affected by the high background absorbance; hence the values are not comparable to those from the fish and are not presented. However it was possible to estimate at least the order of oxidation which showed to be: lard > butter > sunflower oil > rapeseed oil (highest to lowest oxidized).

Discussion

In the present study the main aim was to investigate effects of pan frying on the nutritional value of carp fillets. Especially as the carp fillets had an enriched content of n-3 PUFA compared to fillets from normal production using cereal supplementation, which created an additional necessity to investigate the effect of frying on this special product.

Total lipid content

During the frying the lipid content in the fillets increased to approximately the double amount when the fillets were fried plain and to up to 3 times when the fillets were battered. Increase of lipid content in fish fillets has been reported earlier by various researchers (Gladyshev et al., 2006; Gladyshev et al., 2007; Mai et al., 1978; Sioen et al., 2006; Weber et al., 2008) in salmon, cod, herring and other fish species. (Mai et al., 1978) found even higher increase of lipid content during frying with or without a breading in bluegill (*Lepomis macrochirus*) and white sucker (*Catostomus commersoni*) fillets, were the lipid content increased nearly 10 times when fried with a breading and 4 times in the white sucker when fried without a breading. In lake trout (*Savelinus namaycush*) however, the increase of lipid content was not as drastic (from 8.8 to 9.5%), which probably can be ascribed to the higher lipid content in the trout fillets.

However the increase of lipid content in the fillets in our study was independent from the fat source. This is in line with results from (Weber et al., 2008), who also did find a significantly increase in lipid content in silver catfish (*Rhamdia quelen*) due to panfrying but similar lipid content in fillets fried with different oils. When comparing the different species it is clearly visible that in general the lipid content in lean fish like cod increases to a much higher percentage than in fatty fish.

So the lipid uptake in fish fillets during pan-frying seems to be negatively correlated to the raw fish fat content. Also (Mai et al., 1978) concluded that lean fish take up more fat and lipid changes are higher compared to more fatty fish. This hypothesis is strengthened by the fact that the fat content in the catfish fillets increased much more in study by (Weber et al., 2008) compared to our carp fillets. In our trial the plain fried carps had fat contents from 9.5–11.9% while (Weber et al., 2008) reported fat contents of 13–14%.

Fatty acid composition

After frying the fillets showed an increase in the major FA of the different fats and oils used for frying (Table 1 and 2). Butter and lard were rich in SFA and resulted in line with this to increased proportions of 14:0; 16:0 and 18:0. The fillets fried in butter showed the highest increase in 16:0 which also was the major FA in butter (38.6%). Frying with rapeseed oil resulted in an increase of 18:1n-9 up to 50% and up to 8% of 18:3n-3, reflecting the proportions of these FA in the oil. In the oil used, the proportions of these FA were 60% and 8.5% respectively.

In addition it was discussed that 18:1n-9 is a FA being easily taken up as it has a higher viscosity compared to the more unsaturated FA, leading to a stronger adherence of the FA to the meat. Similar effects have been discussed by (Kalogeropoulos et al., 2007) in pan-fried potatoes. Also (Weber et al., 2008) found an increased uptake of

18:1n-9 in catfish from canola oil, which is in line with our findings. However, in the fillets fried with lard it can be observed that the SFA seem to have even a stronger effect, as lard we used had 18:1n-9 as major FA but the main increasing FA was 18:0, which was only 17.4 % compared to 35.7% of 18:1n-9.

In addition the increase in 18:0 in the fillets fried with lard was relatively higher than the increase in 16:0 despite the higher content of 16:0 in lard. This could be either due to the higher liquidity of 18:0 and hence an easier uptake into the fillet or due to the higher content of 16:0 in the raw fillets, as the ratio of these FA in the fillets was approaching to the ratio in lard (16:0/18:0 from 4.3 to 2.0 in the raw and fried fillets respectively compared to 0.8 in lard). Furthermore we found a significant increase of 14:0 and a minor increase in 18:0 in the samples fried with butter, despite the fact that the proportion of 18:1n-9 was higher in butter than those SFA. This would strengthen the hypothesis that the FA are taken up into the fish fillets depending both on the FA composition of the used fat and of the raw fillet in a way that the final FA composition in the fried fish reflects the FA proportion of the used oil. This might in addition be influenced by the lipid content of the raw fillets with lean fish facilitating higher uptake of lipid than fatty fish.

This hypothesis is supported by results from (Weber et al., 2008) showing an increase in 18:3n-3 in catfish when frying in canola oil in opposite to our results in carp. This can be explained with the fact that the raw catfish fillets had a lower total fat content and lower percentage of 18:3n-3 (2.5% and 1.4% respectively) compared to our carp (3.1–5.1% and 5.9–8.5% respectively) leading hence to an increased uptake of fat overall and also a relatively increased proportion of 18:3n-3 in reflectance of the greater discrepancy in these FA between oil and fish.

Also in the study on shallow- and deep-fat frying of silver carp from Iran (Rahimabadi and Dad, 2012) after frying the FA composition in the fillets reflected the FA composition of the frying fats to a great extent. However as these authors did not report the total fat content before and after frying it is difficult to estimate the influence of the fat content.

The significant decrease of EPA, DPA and DHA in all frying treatments independent from the used fat depends most probably on the fact that these FA were not present in the frying fats and hence could not be taken up. As our results are expressed in proportions of FA and not the absolute amounts, the decrease is most probably only relatively and consequentially from the increase of the major FA from the frying fats.

The decrease in AA in the fillets was significant after frying with lard, rapeseed oil and sunflower oil, however not when butter was used. However, there was a tendency towards lower AA in the samples fried with a batter ($p = 0.055$). Most probably that was due to the lower proportion of AA in the raw fillets before the frying with butter, so the proportional difference was not significant.

Concerning the nutritional value of fish in general and especially our n-3 enriched carp fillets in the present study, it can be stated frying with lard and sunflower oil resulted in a significant decrease of the n-3/n-6 ratio, while the ratio was stable when the fillets were fried with butter or rapeseed oil. The total proportion of n-3 was significantly decreased due to the frying process in all samples, however in the samples fried with rapeseed oil this decrease was the lowest in percentage (36.5% compared to 48.1%; 74.2% and 77.4% in battered samples fried with rapeseed oil, butter, lard and sunflower oil respectively). At the same time the proportion of total n-6 increased slightly in the samples fried with butter and lard and significantly in

the samples fried with sunflower oil. So especially the use of lard and sunflower oil decreased the nutritional value of the fish by changing the n-3/n-6 ratio, as the metabolic pathways for n-3 and n-6 FA will be shifted more towards the n-6 products, which are connected to increased inflammation, and platelet aggregation (Simopoulos, 2002b). This makes butter and rapeseed oil better candidates as frying fats for fish. However as frying with butter resulted in increased SFA in the fillets this the use of butter will also decrease the nutritional value of fish. SFA are also well-known for their negative effects on human health, as for example increasing blood cholesterol and prevalence of coronary heart disease (Williams, 2000). Related to final FA composition of the fried fish fillets, rapeseed oil showed to be the best choice, preserving the n-3/n-6 ratio in the fillets without increasing the content of SFA at the same time.

With the above discussed results we decided to test if the final FA composition in a fillet fried with an oil of a known FA composition could be predicted. According to our hypothesis that the FA are taken up into the fish fillets depending both on the FA composition of the used fat, the FA composition of the raw fillet and the lipid content of the raw and fried fillets we used a simple dilution model, which has earlier been used to predict the changes in fillet FA composition achieved by a finishing feeding strategy (Robin et al., 2003). As the sample number in our trial was quite small and the fat content in the fish was highly varying we only used the fillets fried in rapeseed as the fish from this group had the lowest variation in fat content. The prediction plot for the fillets fried plain in rapeseed oil is shown in Figure 2. The slope of the regression line was close to 1 (0.999) showing a very good prediction of the observed values. This indicates that indeed the changes in FA composition during frying are only an effect of dilution, independent from the viscosity or melting points of the respective FA. This would give a simple tool to consumers, dietitians, nutritionist and medical professionals to calculate final FA acid composition of a fried fish fillet. The results need to be confirmed in a study with more replicates and fish with different levels of fat contents. A next step then should be to test the applicability of this model on various animal products containing added oil or fat. It has been shown earlier that the FA composition of many products, especially fish products showed considerable differences compared the values shown in an official database, most probably, because the composition of the added fat was not taken into consideration (Sampels et al., 2009). If the dilution model could be used to calculate the effect of frying and added fat it would facilitate the correct calculation of nutritional FA composition of animal food products.

Oxidation

In general oxidation of oil is positively correlated to the number of double bonds from 18:2n-6 and 18:3n-3 and negatively correlated with 18:1n-9 (Kamal-Eldin, 2006). Therefore the question arises if the use of an oil rich in 18:3n-3 will lead to increased oxidation in the final product. In our present study we could not find an increased oxidation with rapeseed oil compared to sunflower oil (Table 3). Considering the conclusions drawn by (Kamal-Eldin, 2006), this could be explained by the high proportion of 18:1n-9 in the rapeseed oil used in the present study. As this was 60% it could have protected the rapeseed oil during frying as (Romero and Morton, 1977) showed that 18:1n-9 could act as an inert. Furthermore Petukhov et al. (1999); Okeefe et al. (1993) and Martí-Polvillo et al. (1996) showed an increased

stability of canola; peanut and sunflower oils respectively with increased proportions of 18:1n-9. So we hypothesize that the high percentage of 18:1n-6 in the rapeseed oil compared to the used sunflower oil and the other fats lead to the good oxidative stability in comparison to the other fats.

The significant increase of oxidation after frying with lard was somehow unexpected as the lard was quite rich in SFA's that are relatively stable during oxidation. One reason could be that the lard was more oxidized from the beginning due to the processing procedure, which includes heating. Our results of MDA contents suggest that lard was the most oxidized from the fats used in the present trial. However, as described above the measurement was heavily influenced by the high background absorbance, which we did not manage to extinguish. Further investigation is needed in this area.

Conclusions

We conclude that frying with rapeseed oil preserved the favorable n-3/n-6 ratio without increasing saturated fats in n-3 rich carp fillets. It seems that FA uptake in fish is negatively correlated to the raw fish's fat content and positively correlated to the FA composition in the used frying fat. Comparing our results with earlier studies in the literature, we expect similar changes of FA composition in other fish species with similar fat content. However, the concrete mechanisms for uptake and leakage of FA in and from fish fillets needs to be investigated additionally, in order to be able to give recommendations for the appropriate frying fat for different species to the public and official institutions dealing with human nutrition. The dilution model seems to be a good tool to predict changes in fish FA composition due to frying, but needs to be further confirmed. The frying with rapeseed oil did not increase the oxidation compared to frying with sunflower oil, which confirms the suitability of rapeseed oil for pan frying of fish. Therefore we can recommend using rapeseed oil for the frying of fish and other n-3 rich food.

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Table 1. *Fat content (%) and fatty acid composition of raw and fried fillets of carp (n=3 in each treatment) (% of total identified), fried in different fats, plain or covered with a bread-batter (averages and standard deviation) with each fish as their own raw control.*

	Butter			Lard			Rapeseed oil			sunflower oil		
	raw	fried plain	fried with batter	raw	fried plain	fried with batter	raw	fried plain	fried with batter	raw	fried plain	fried with batter
Total fat	4.69±2.53^{D}	10.5±3.16^{E}	14.1±2.65^{E}	3.07±2.65^{D}	9.82±1.76^{E}	14.0±4.08^{F}	3.74±1.05^{D}	9.47±1.39^{E}	13.1±1.34^{E}	5.07±4.31^{D}	11.9±5.05^{E}	13.9±3.32^{E}
C14:0	1.19±0.09^{D}	5.31±1.08^{Ea}	8.11±1.02^{Fa}	1.04±0.26	1.48±0.08^{b}	1.43±0.05^{b}	1.04±0.17	0.54±0.09^{c}	0.40±0.21^{c}	1.33±0.51^{D}	0.60±0.17^{cDE}	0.42±0.24^{Ec}
C16:0	19.0±1.88^{D}	25.3±1.08^{Ea}	30.3±1.87^{Fa}	18.7±0.22^{D}	23.95±1.52^{Ea}	25.21±0.84^{Eb}	16.8±0.79^{D}	10.4±0.90^{Eb}	10.1±3.30^{Ec}	18.12±1.50^{D}	12.8±1.90^{Eb}	12.3±4.45^{Ec}
C16:1tr	1.14±0.10^{Dab}	0.83±0.07^{D}	0.65±0.11^{Eab}	1.35±0.49^{Dabc}	0.87±0.26^{E}	0.66±0.23^{Ea}	1.01±0.29^{Db}	0.59±0.05^{E}	0.42±0.23^{Eb}	1.59±0.45^{Dc}	0.63±0.27^{E}	0.41±0.29^{Eab}
C16:1	7.72±1.88^{Da}	6.12±1.73^{DEa}	4.66±1.24^{Ea}	4.03±2.60^{b}	3.72±1.03^{b}	3.14±0.85^{ab}	4.53±1.42^{b}	2.87±0.48^{b}	2.18±0.9^{b}	6.75±5.67^{Dab}	3.15±1.80^{Eb}	2.14±2.04^{Fb}
C18:0	5.12±0.27	6.21±1.44^{a}	7.41±1.80^{a}	4.33±1.95^{D}	11.9±2.44^{Eb}	12.3±3.62^{Eb}	5.12±0.51	3.17±0.32^{c}	3.10±0.84^{c}	6.51±2.16^{D}	4.47±0.74^{DEac}	3.84±1.00^{Ec}
C18:1n-9	37.2±4.05^{a}	33.7±4.08^{a}	31.2±2.23^{a}	29.5±3.83^{ab}	33.9±0.93^{a}	35.9±0.60^{b}	36.4±3.20^{Da}	50.5±1.46^{Eb}	49.7±8.3^{Ec}	34.3±8.58^{Db}	34.0±4.45^{Ea}	35.6±10.2^{Eab}
C18:1n–7	3.42±0.15^{D}	2.58±0.31^{DEab}	2.02±0.35^{acE}	2.40±2.08	2.85±0.22^{ab}	2.72±0.21^{a}	3.37±0.33^{D}	3.35±0.12^{aDE}	3.71±1.17^{Db}	2.30±2.01^{D}	2.06±0.47^{Deb}	1.71±0.65^{Ec}
C18:2n-6	9.0±2.16	6.8±1.02^{a}	6.3±1.41^{a}	11.2±1.28	10.5±0.71^{ab}	10.9±0.4^{a}	11.4±1.19	15.6±1.00^{b}	19.2±5.65^{b}	12.2±0.78^{D}	32.8±10.48^{Ec}	40.6±14.18^{Fc}
C18:3n-3	5.92±2.30^{Da}	4.17±1.22^{DEa}	2.96±1.25^{Ea}	8.21±2.07^{Dab}	4.34±1.84^{Ea}	3.08±1.60^{Ea}	6.74±0.81^{ab}	7.79±0.34^{b}	6.99±1.40^{b}	8.46±2.18^{Db}	3.61±1.35^{Ea}	2.41±1.18^{Ea}
C20:1n-9	2.00±0.10^{Dab}	1.39±0.15^{DE}	0.90±0.25^{Eab}	1.23±1.06^{a}	1.17±0.23	0.92±0.16^{ab}	2.53±0.39^{Db}	0.99±0.81^{E}	1.40±0.29^{Ea}	2.46±1.58^{Db}	1.08±0.48^{E}	0.59±0.17^{Eb}
C20:3n-6	0.28±0.08^{Da}	0.21±0.06^{DE}	0.11±0.04^{E}	0.58±0.23^{Db}	0.18±0.07^{E}	0.09±0.08^{E}	0.48±0.07^{Db}	0.11±0.08^{E}	0.05±0.08^{E}	0.40±0.22^{Da}	0.13±0.05^{E}	0.06±0.07^{E}
C20:4n-6	1.89±0.50	1.10±0.18	0.73±0.14	4.80±0.96^{D}	1.13±0.17^{E}	0.88±0.21^{D}	2.29±0.63^{D}	0.68±0.28^{E}	0.42±0.13^{E}	2.96±2.07^{D}	0.73±0.30^{E}	0.45±0.20^{E}
C20:3n-3	0.28±0.12^{a}	0.16±0.07	0.05±0.04	1.13±1.12^{Db}	0.18±0.13^{E}	0.25±0.29^{E}	0.37±0.10^{a}	0.15±0.13	0.06±0.11	0.29±0.25^{a}	0.36±0.19	0.08±0.08
C22:1	0.32±0.17^{ab}	0.27±0.10	0.16±0.07	0.48±0.07^{Db}	0.29±0.18^{DE}	0.15±0.20^{E}	0.15±0.01^{a}	0.15±0.05	0.10±0.06	0.55±0.38^{Db}	0.21±0.08^{E}	0.08±0.09^{E}
C20:5n-3	2.21±0.80^{Da}	1.33±0.29^{E}	0.75±0.28^{E}	4.70±1.23^{Db}	1.51±0.51^{E}	0.90±0.47^{E}	2.63±0.54^{Da}	1.12±0.11^{E}	0.67±0.36^{E}	3.79±1.34^{Dc}	1.30±0.37^{E}	0.65±0.22^{E}
C22:5n-3	0.95±0.29^{Da}	0.54±0.09^{DE}	0.34±0.11^{E}	1.94±0.78^{Db}	0.56±0.08^{E}	0.32±0.09^{E}	1.09±0.27^{Da}	0.38±0.06^{E}	0.21±0.10^{E}	1.63±0.88^{Db}	0.49±0.22^{E}	0.20±0.07^{E}
C22:6n-3	2.03±0.80^{Da}	0.98±0.33^{E}	0.58±0.21^{E}	3.79±1.26^{Db}	0.99±0.24^{E}	0.56±0.23^{E}	2.31±0.70^{Da}	0.83±0.14^{E}	0.38±0.17^{E}	2.41±2.35^{Da}	0.86±0.49^{E}	0.40±0.11^{E}
SFA	25.6±2.03^{Dab}	40.8±2.34^{Ea}	51.3±3.83^{Fa}	24.7±1.74^{Dab}	37.4±3.97^{Ea}	39.1±4.12^{Eb}	23.4±0.60^{Da}	14.6±1.08^{Eb}	14.2±4.62^{Ec}	31.1±10.75^{Db}	18.4±2.66^{Eb}	16.5±3.43^{Ec}
MUFA	52.0±5.82^{Da}	44.8±6.46^{DEa}	39.0±4.09^{Ea}	40.3±4.95^{bc}	42.8±1.17^{a}	43.7±1.57^{a}	48.5±3.78^{Dab}	58.5±0.79^{Eb}	57.6±9.58^{DEb}	35.6±8.46^{c}	40.9±7.38^{a}	38.4±9.37^{a}
PUFA	23.3±7.12^{Da}	15.8±2.79^{DEa}	12.1±3.33^{Ea}	34.9±3.20^{Db}	19.7±2.91^{Eab}	17.2±2.62^{Ea}	28.2±3.20^{ab}	26.9±1.17^{b}	28.3±5.12^{b}	33.4±3.94^{Dab}	40.6±9.88^{DEc}	45.1±12.64^{Ec}
n3	11.4±4.29^{Da}	7.18±1.70^{Ea}	4.69±1.84^{Fa}	19.8±1.30^{Db}	7.59±2.72^{Ea}	5.10±2.40^{Fa}	13.1±1.53^{Da}	10.3±0.35^{DEb}	8.32±0.71^{Eb}	16.6±2.63^{Db}	6.61±1.65^{Ea}	3.75±1.53^{Fa}
n6	11.9±2.90	8.50±1.18^{a}	7.29±1.55^{a}	15.1±2.28	12.1±0.79^{ab}	12.1±0.43^{a}	15.0±1.73	16.7±0.83^{b}	19.9±5.79^{b}	16.7±1.79^{D}	33.9±10.14^{Ec}	41.2±13.90^{Ec}
n-3/n-6	0.94±0.15	0.83±0.10	0.62±0.11	1.32±0.15^{D}	0.63±0.23^{E}	0.42±0.19^{E}	0.88±0.03	0.62±0.01	0.46±0.08	0.99±0.13^{D}	0.41±0.54^{E}	0.43±0.85^{E}

Different capital letters in a row indicate significant difference ($p<0.05$) between raw, plain fried and fried in batter in the same fat, different small letters in a row indicate significant differences ($p<0.05$) between the raw fish or same frying type across different fats. Abbreviations: n-6 = omega-6; n-3 = omega-3, SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids; PUFA = sum of polyunsaturated fatty acids; n-3/n-6 = ratio between the sum of omega-3 and omega-6 fatty acids

Table 2. FA composition in the fats used for frying (%).

	butter	lard	rapeseed oil	sunflower oil
C10:0	3.11	n.d.	n.d.	n.d.
C12:0	4.35	0.11	n.d.	n.d.
C14:0	13.8	1.56	0.07	0.09
C16:0	38.6	27.8	4.58	6.60
C18:0	10.3	17.4	1.59	3.25
C18:1n-9	22.6	35.7	60.5	26.5
C18:1n-7	0.74	2.42	3.32	0.73
C18:2n-6	2.44	10.2	19.0	60.8
C18:3n-3	0.57	0.84	8.46	0.36

Table 3. TBARS in raw and plain fried pieces from carp fillets fried in different oils µg malondioaldehyd / g fish (average and stdev).

	butter	lard	rapeseed oil	sunflower oil
raw	0.37±0.05	0.61 ± 0.49^{A}	0.44±0.21	0.42 ± 0.31^{A}
fried plain	0.58 ± 0.22^{a}	2.03 ± 0.55^{Bb}	0.97 ± 0.23^{ac}	1.42 ± 0.40^{Bc}

Different capital letters in a column indicate significant differences ($p<0.5$) between raw and fried fish. Different small letters in a row indicate significant difference between fillets fried in different fats.

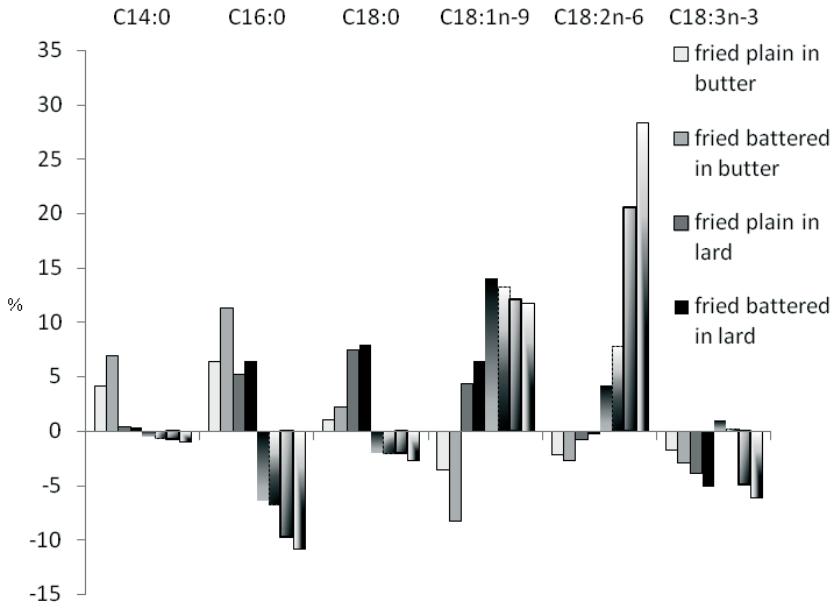

Figure 1. *Changes in fatty acid percentage in plain or battered carp fillets, due to frying in different fats.*

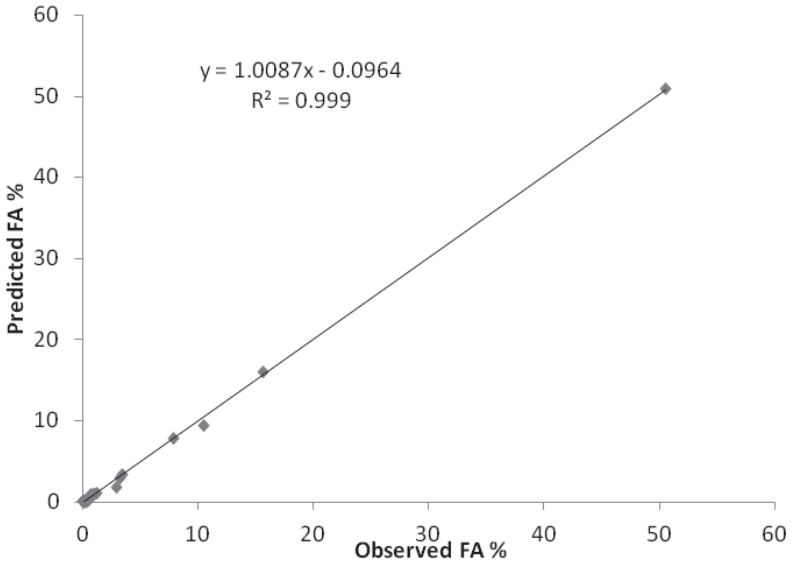

Figure 2. *Prediction plot of fillet fatty acid composition (%) according to the dilution model; showing observed versus predicted percentage of fatty acids.*
Abbr.: FA = fatty acids

CHAPTER 5

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

Paper IV:

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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%).

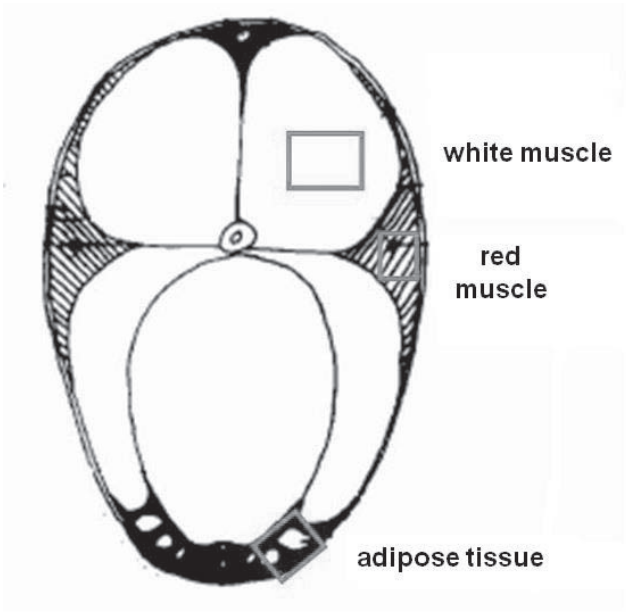

Figure 2. Distribution of major types of muscle in edible parts of fish (adapted from Kiessling et al., 1991).

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):

$$P_T = P_K + [(P_0 - P_K) / (Q_T / Q_0)]$$

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:

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

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

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.

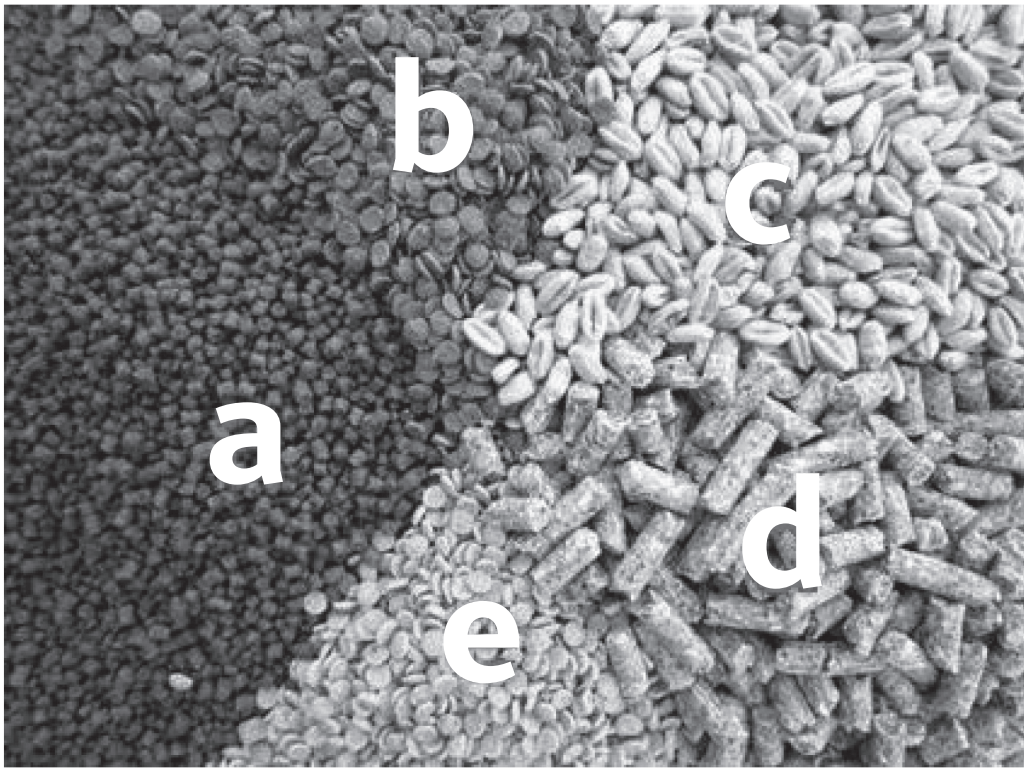

Figure 3. Examples of aquaculture feeds applicable in the rearing of freshwater fish species. a) Complete, commercially available pellets with fish oil; b) complete extruded mixture with green algae addition (10%); c) wheat; d) supplementary rapeseed/linseed pellets; e) complete extruded diet with rapeseed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

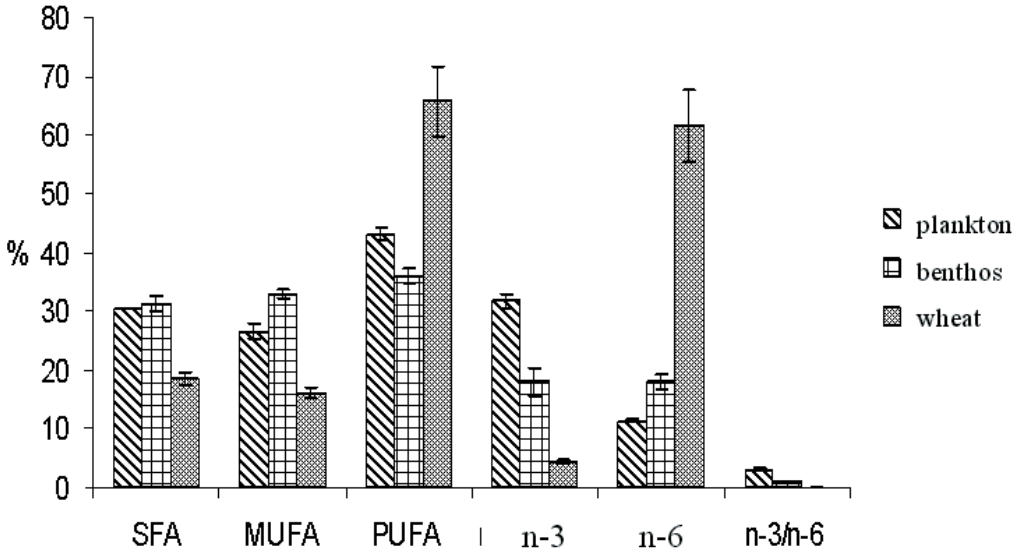

Figure 4. Comparison of fatty acid composition in plankton, benthos and wheat.

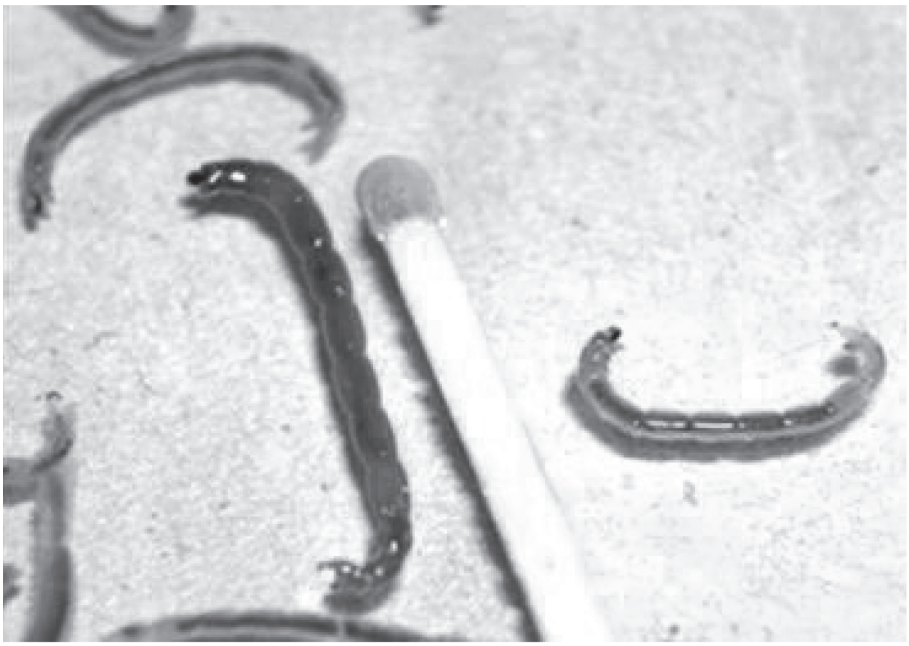

Figure 5. A representative of pond benthos: Chironomus plumosus larvae (photo: M. Bláha).

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.

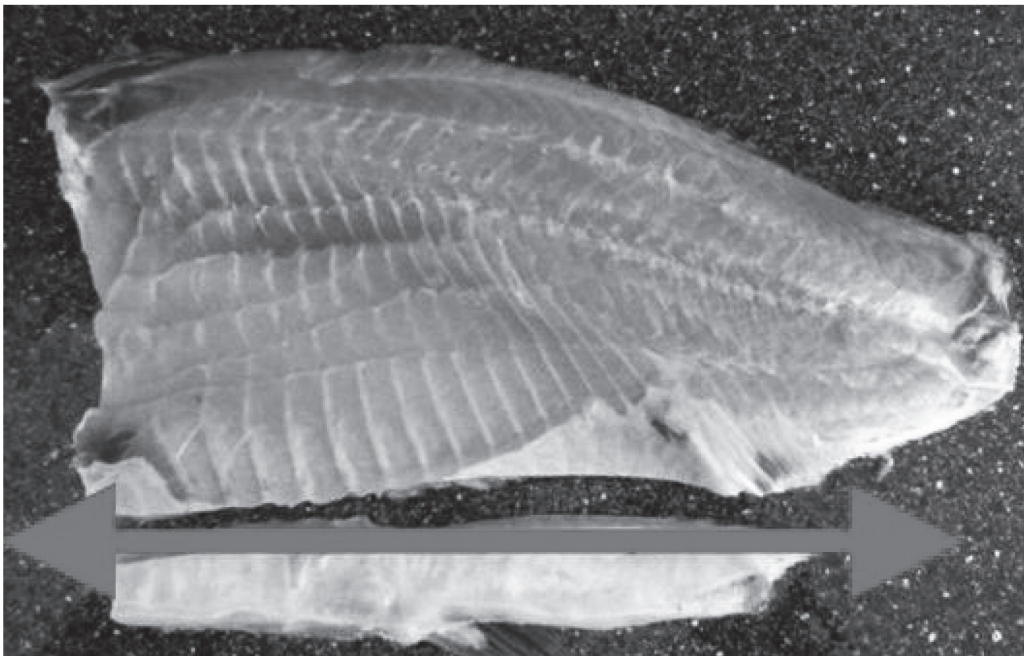

Figure 7. Carp fillet with removed belly (adipose) part.

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.

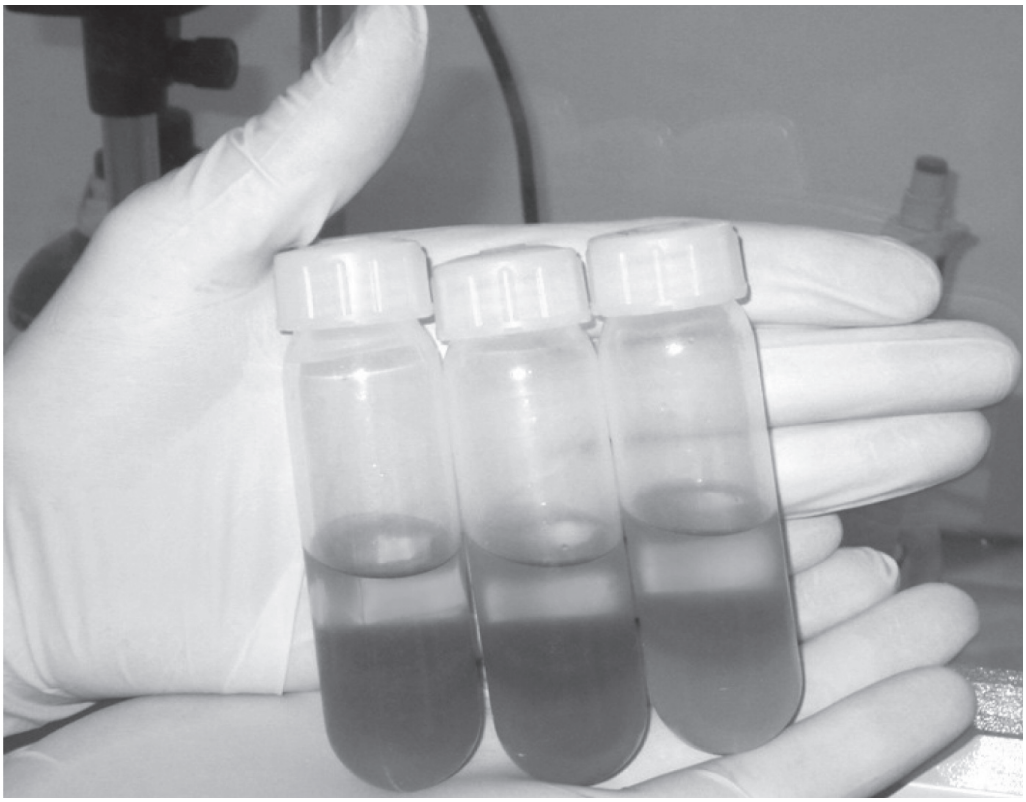

Figure 8. *Lipids extracted from samples in Teflon tubes.*

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.

V. REFERENCES

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CHAPTER 6

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLEDGEMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING STUDY
CURRICULUM VITAE

GENERAL DISCUSSION

As a result of the increasing proportion of plant components in the feed supplied to fish produced in aquaculture, there has been increased interest in the development of rearing technologies for new fish species, often freshwater fish (Gozlan, 2008). For the future, a promising strategy is to focus on domesticated freshwater fish species with a high growth potential, many of which have the ability to biosynthesise n-3 HUFA *de novo*. In addition, many freshwater species are being included in some genetic breeding programmes to improve growth and quality. One of the species which meets these conditions is common carp.

The overall aim of this thesis work was to investigate culture of common carp and fish quality. More specifically, Paper I focused on the alternative system of carp production in the controlled conditions of recirculation aquaculture system. Paper II focused on a very important part of carp rearing technology, the purging period, with special emphasis on fillet lipid changes and composition, while Paper III examined changes in the total amount and composition of carp lipids during kitchen preparation. Finally, in Paper IV the findings available so far on quality of carp flesh were summarised and the possibilities to affect the flesh quality of farmed fish were considered. Thus the four papers on which the thesis is based focus on different stages of carp rearing 'from farm to plate'.

Paper I. Culture of common carp (*Cyprinus carpio*) with defined flesh quality for prevention of cardiovascular diseases using finishing feeding strategy

The aim of this study was to increase the quality of farmed carp using a finishing diet containing n-3 fatty acids (fish oil, rapeseed oil, linseed oil, etc.). The method used was based on results obtained in different fish species, such as turbot (*Psetta maxima*) by Robin et al. (2003), Atlantic salmon (*Salmo salar*) by Jobling (2003) and the Asian cyprinid species rohu (*Labeo rohita*) by Karanth et al. (2009), and was concluded to be promising also for carp. The overall aim was to produce carp flesh with defined quality for prevention and treatment of cardiovascular disease and to examine the applicability of the finishing feeding strategy and dilution model in common carp production.

It was found that the fillet fatty acid composition reflected the composition of the diet and was significantly correlated to the length of the feeding period. These findings are consistent with those of previous studies on common carp (Steffens and Wirth, 2007; Karanth et al., 2009) and on other fish species (Thanuthong et al., 2011; Lane et al., 2006). The dilution model designed by Robin et al. (2003) for carnivorous species proved reliable in the case of common carp. Its simplicity and accuracy make it suitable for application by fish farmers to predict the fatty acid quality of fish in practice. It also proved suitable for brook char (*Salvelinus fontinalis*) in the conditions of the Czech Republic (unpublished results).

Production of carp by this method is relatively more expensive than pond farming. In addition, fish oil is used in the finishing diet, causing problems with sustainability (Pike and Jackson, 2010; Tacon et al., 2011). However, the method allows fish farmers

to control the final flesh quality of the carp more easily and to produce fish with standardised and tailored quality. Using this information, fish farmers could declare the content of n-3 fatty acids and EPA+DHA on the product label, as recommended by Pickova (2009), which could increase the market value of carp and support consumption of this locally produced fish. For future use, this rearing procedure could also be considered to achieve limited production of carp flesh intended for specific conditions, such as treatment spas, hospitals etc. The recommended daily intake of EPA+DHA is 250 mg (EFSA, 2009). According to the predictions by the dilution model and experimentally obtained values, we concluded that the finishing feeding treatment needs to be applied for 70 days to achieve the recommended daily value of 250 mg EPA+DHA in two 200 g servings a week (250 mg × 7 days = 1750 mg/2 servings = 875 mg/serving).

Paper II. Fillet quality changes as a result of purging of common carp (*Cyprinus carpio L.*) with special regard to weight loss and lipid profile

A very important production step included in carp culture in the Czech region is the process of purging. In Paper II, a number of factors influencing weight losses and changes in the lipid profile of carp from three different production systems were examined. A number of studies have been published on starvation (or purging) of common carp (Blasco et al., 1992; Csengeri, 1996; Bauer and Schlott, 2004; Vacha et al., 2007). However, no previous study has focused on changes in the lipid composition of carp with an intentionally increased content of n-3 fatty acids. Lipid analyses of purged common carp reared in three different production systems showed that the type of diet prior to purging significantly affected the flesh quality of the fish even after the purging period. While carp reared extensively, with access only to natural feed (plankton and benthos), usually achieve a fat content of 5% (Steffens and Wirth, 2007), carp supplemented with cereals reach a much higher fat content (between 8–15%) (Urbanek et al., 2010). In Paper II we found a value of 3.51±0.8% for market carp kept in natural conditions and 8.68±2.8% for fish supplemented with cereals. Moreover, it was found that carp supplemented with rapeseed/linseed pellets achieved 7.32±4.5% fat, which was confirmed on other experiments (unpublished results).

With prolonged purging and loss of surplus fat (Einen et al., 1998), the fish from all groups started to metabolise HUFA too, leading to a decrease in nutritionally valuable n-3 fatty acids. It is known that phospholipids serve as components of cell membranes and structures, while triacylglycerols serve as a reserve of energy and are mainly stored in adipose tissue (Sargent et al., 1995). Thus, with a higher level of fat, a higher content of triacylglycerols can be observed. This was confirmed in Paper II, where carp supplemented with cereals showed a higher fat content and therefore higher content of triacylglycerols than carp reared on natural feed only. Moreover, during the purging period these differences gradually decreased with decreasing fat content and there were no significant differences in the representation of triacylglycerols and phospholipids among the test groups after 70 days of purging.

We suggest that carp are able to metabolise fatty acids selectively for their energy needs and defer metabolism of nutritionally valuable n-3 fatty acids if they are in good condition. This means that as long as purged carp have enough MUFA in fat stores, these 'less unsaturated' acids are primarily metabolised to cover energy needs. The reason could be the continually decreasing water temperature, because unsaturated fatty acids have a lower solidification point and therefore ensure cell membrane fluidity at low temperatures. Only after energy reserves are exhausted do carp begin to metabolise HUFA too.

A purging period should be long enough to eliminate possible unpleasant odours and flavours, but as short as possible from a practical handling point of view to preserve the beneficial fatty acid composition of n-3 enriched carp. With such a strategy the nutritional qualities of edible flesh are improved, while the unavoidable body weight loss is limited. Therefore, we recommend a purging period of no longer than 14 days for carp, which is consistent with findings published for other fish species (Einen et al., 1998; Palmeri et al., 2008).

Paper III. Effect of frying fat and preparation on carp (*Cyprinus carpio*) fillet lipid composition and oxidation

To complete the whole process of production of carp with elevated content of n-3 fatty acids, it was necessary to clarify the effect of kitchen preparation on the final quality of fat in served carp intended for human consumption. If the preparation of food from fish is inappropriate (e.g. inappropriate choice of frying fat), then the health benefits resulting from the fish and fish products consumption are degraded. This is a huge nutritional problem in Western countries, as reported by Pickova (2009) and confirmed by Sampels et al. (2009). Paper III concluded that the best way to fry n-3 enriched carp is to use rapeseed oil, because the use of lard and sunflower oil decreases the nutritional value of the fish by negatively affecting the n-3/n-6 ratio. The use of butter increases the saturated fatty acid content in the portion, which has also negative impacts on human health by increasing blood cholesterol (Williams, 2000). Furthermore, Paper III showed that the changes in the proportion and representation of different fatty acids in a portion of fish flesh before and after frying can be predicted using a simple dilution model developed for a finishing feeding strategy (Paper I). This simple tool can be used by consumers or nutritionists to calculate the final fatty acid composition of a fried fish portion.

Lipids of common carp usually contain oleic acid (18:1n-9) as one of main fatty acids, regardless of the type of production system used (Mráz et al., 2012; Aprodu et al., 2012). Therefore, the risk of oxidation of fat in prepared carp fillet is considerably lower than in some other species, because Paper III confirmed that the degree of oxidation is negatively correlated with the content of 18:1n-9 (Kamal-Eldin, 2006). The same effect was reflected in our study. The lowest oxidation was found in samples fried on rapeseed oil, which contained the highest amount of 18:1n-9.

Paper IV. Potential of freshwater fish production with increased content of omega-3 fatty acids

Paper IV summarised existing knowledge about the metabolism of fatty acids and factors influencing fatty acid composition in the lipids of freshwater species and described several ways to increase the n-3 fatty acid content in fish flesh, mainly in carp. This certified methodology was specifically designed for fish farmers in the Czech Republic. The following ways to improve the fatty acid composition in fish lipids were presented:

1) A finishing feeding strategy represents a viable way for farmers to reduce the use of fish oil in feeds, while the lipid quality of farmed fish is maintained at a high level. A dilution model was presented for carp. The reliability and applicability of this model for various fish species has been verified by many authors (Jobling, 2004; Izquierdo et al., 2005; Torstensen et al., 2005) and also by our own studies on common carp (Paper I) and in the practical conditions of Czech fisheries for brook char (*Salvelinus fontinalis*) (Zajic, unpublished results).

2) Feeding carp with a mixture containing HUFA precursors is probably the most promising way to achieve a high n-3 fatty acid content together with good economic results for the fish farmer. Part of the cereal in the diet is replaced with plant components that naturally contain precursors of HUFA (rapeseed and linseed). Extruded linseed and pressed rapeseed cake (mouldings) are a relatively cheap and easily available source of 18:3n-3 for fish nutrition (Pickova and Mørkøre, 2007). Carp supplemented with rapeseed/linseed pellets contained elevated levels of 18:3n-3, as well as n-3 HUFA, which confirms the ability of carp to biosynthesise n-3 HUFA *de novo* (Farkas, 1984; Tocher, 2003).

3) Use of natural plankton/benthos during production in carp ponds is the cheapest way to obtain high quality carp with regard to beneficial n-3 fatty acids, because the natural feed in the pond environment contains the majority of these (Adamek et al., 2004). The n-3/n-6 ratio is favourable in the carp produced on this type of diet. As shown by our results (Paper II), utilisation of natural feed can result in an increased percentage of EPA and DHA, but these fish generally contain less fat, which reduces the absolute values of these fatty acids in an individual portion.

Beside the ways to alter the fatty acid composition in freshwater fish discussed above, there are a numbers of other alternative sources of n-3 HUFA that are potentially applicable in carp culture. One is targeted culture of specific single-cell organisms, such as algae, bacteria and fungi, that naturally contain high levels of n-3 HUFA in dry matter (Gupta et al., 2012; Jermsuntiea et al., 2011). This has been successfully tested in experimental conditions using a variety of different sources and is very promising for the future. However, the price is still too high for them to be commonly used in aquaculture feeds (Turchini et al., 2009).

Until now, a published prescriptive method for fish farmers has been lacking in the Czech Republic. The methodology presented in Paper IV could be a tool for large-scale and small-scale fish farming enterprises and the food processing industry. The list of internal and external factors influencing the fat content and composition of fish and the possibilities described for improving the quality of farmed fish could

serve as guidelines to help farmers and fish processors to produce high quality fish and fish products for human consumption.

Conclusions and future prospects

The results presented in this thesis extend existing knowledge and bring some new findings to the field of common carp culture and flesh quality. They confirm that it is possible to introduce new perspectives to the otherwise fairly conservative approach taken to carp production in the Czech Republic. Carp with an increased content of healthy beneficial n-3 fatty acids have the potential to be an interesting commodity on the Czech market, thus making carp a more attractive fish in the eyes of Czech consumers. By application of novel production methods, the content of n-3 fatty acids in flesh lipids can be significantly increased. In an era of a continually growing aquaculture industry and a rising interest in freshwater fish species, existing rearing technologies should be constantly developed and improved. Fish production in the future must be sustainable in the long-term and environmentally friendly, with outputs of the highest quality.

The thesis verified that carp can achieve a substantial increase in n-3 HUFA content through production using environmental friendly methods with renewable, local sources of raw materials for feed production. The positive effects of carp consumption were demonstrated by a clinical study focused on subjects after heart surgery. The whole system (from 'farm to plate') of carp production with increased content of n-3 fatty acids was practically validated and the results are very promising (Mráz and Pickova, 2009; Mráz et al., 2010; Adamkova et al., 2011; Mráz et al., 2011a; 2011b; Mráz et al., 2012)+ Papers I–IV). Carp has the potential to be a desirable, high quality commodity in the field of 'functional food'.

Ongoing and future research will seek to identify the principles of n-3 HUFA biosynthesis in carp and will include the development and validation of novel methods for production of freshwater fish species ensuring the high quality and sustainability. Promising strategies, such are bioflocs and aquaponics, will be tested in the conditions of Czech aquaculture. Another important area for future research will be the development of novel freshwater fish products with respect to high quality.

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ENGLISH SUMMARY

Impact of production systems on lipid quality of common carp (*Cyprinus carpio*)

Tomáš Zajíc

It is the sad fact, that in the Czech Republic, consumption of fish is very low compared with the EU average, but, at the same time, it belongs to the countries with the highest incidence of cardiovascular diseases. Sufficient intake of n-3 poly-unsaturated and highly-unsaturated fatty acids (n-3 PUFA and HUFA) is important in preventing and treating cardiovascular disease. Fish is the main source of these compounds in the human diet. This thesis examined factors influencing n-3 PUFA and HUFA levels in fish lipids and ways of increasing the n-3 fatty acid (FA) content in freshwater farmed.

Production of common carp (*Cyprinus carpio* L.) with defined flesh quality using a finishing feeding strategy and prediction of fillet FA by a dilution model were investigated in a 110*day experiment. Fillet FA composition was measured in fish fed diets with two different vegetable oils (rapeseed/linseed blend, VO; olive oil, OO) only, or with a fish oil (FO) finishing treatment for 30/60 days, and compared with values predicted by the dilution model. The FO finishing treatment resulted in a higher percentage of saturated (SFA), monounsaturated (MUFA) and n-3 PUFA and a lower percentage n-6 PUFA and n-6/n-3 ratio ($p<0.001$) in groups previously fed the VO diet and a lower MUFA percentage and n-6/n-3 ratio and higher n-3 PUFA percentage ($p<0.001$) in groups previously fed the OO diet. The dilution model accurately predicted changes in fillet fatty acid content (R^2=0.992–0.996). A finishing feeding strategy is thus suggested for production of common carp with the required flesh FA composition for special nutritional purposes, especially primary and secondary prevention of cardiovascular disease.

The effects of purging on lipid content and fillet quality of common carp in Central Europe were examined in fish kept for up to 70 days in clear water without feeding. Purging empties the gut, decreases the entrail proportion and eliminates possible off-flavours, but leads to weight loss and storage fat mobilisation. Analysis of the 4-year-old carp (weight 1700–2600 g) in the three different production systems tested (C: cereal supplemented; P: linseed/rapeseed pellet supplemented; N: natural feed) showed that fillet yield was highest after 14 days and decreased thereafter. Throughout the experiment, fillet fat content decreased continuously in groups C and P, but remained stable in group N. Carp from groups C and P mainly metabolised MUFA initially, but with prolonged starvation fish from all groups started to metabolise more PUFA. After 70 days of purging, all groups showed almost identical saturated SFA, MUFA and PUFA values. Thus carp can metabolise selected FA for their energy needs when they are in good condition and have surplus fat stores. However, when body fat content is low, they may metabolise all fatty acid types equally to sustain metabolic functions.

Changes caused by frying on fat uptake, fatty acid composition and oxidation in the fillet of carp with increased n-3 fatty acid content were examined using four different fats to fry either plain or battered fillet. Fillet fat content increased during frying and fatty acid composition in the fillets mirrored the composition of the

frying fat. Frying with sunflower oil negatively influenced the nutritionally valuable composition, decreasing the n-3/n-6 ratio in the fillets. Frying with rapeseed oil preserved the favourable n-3/n-6 ratio without increasing SFA content. Frying with lard and butter preserved the n-3/n-6 ratio but increased the SFA content. Oxidation did not increase when rapeseed oil was used, so rapeseed oil should be used to fry fish to preserve the nutritional valuable composition.

The possibilities described for improving the quality of farmed fish can serve as guidelines to help farmers and fish processors produce high-quality fish and fish products for human consumption.

CZECH SUMMARY

Vliv chovu na kvalitu tuku kapra obecného
(*Cyprinus carpio*)

Tomáš Zajíc

Je smutným faktem, že konzumace rybího masa v České republice je v porovnání se státy EU velice nízká. Zároveň patří k zemím s nejvyšším výskytem kardiovaskulárních onemocnění. Dostatečný příjem n-3 polynenasycených a vysocenenasycených mastných kyselin (PUFA a HUFA) je prokazatelně účinný jednak v prevenci proti těmto chorobám a dále při rehabilitaci pacientů postižených těmito chorobami. Nejvýznamnějším zdrojem PUFA a HUFA jsou ryby. Je známo několik hlavních faktorů, které ovlivňují zastoupení těchto kyselin v rybích lipidech a zároveň existují způsoby, jak obsah n-3 mastných kyselin v rybách účinně zvyšovat. Tyto faktory a příklady technologických postupů pro zvýšení obsahu n-3 mastných kyselin v tuku sladkovodních ryb jsou sumarizovány ve Studii IV.

Studie I popisuje způsob chovu kapra s předem definovatelnou kvalitou masa s využitím strategie *finishing feeding* a propočtem obsahu mastných kyselin pomocí ředícího modelu. Během experimentu trvajícího 110 dní byly ryby krmeny dietou tvořenou pouze rostlinnými oleji (směs řepkový/lněný olej, VO; olivový olej, OO) nebo s finálním krmením (30 a 60 dnů) krmivem s rybím olejem (FO). Byla provedena analýza kompozice mastných kyselin ve filetu a výsledky byly porovnány s daty vypočítanými ředícím modelem. Výsledkem aplikace *finishing feeding* bylo vyšší zastoupení SFA, MUFA a n-3 PUFA a nižší podíl n-6 PUFA a poměr n-6/n-3 ($p<0{,}001$) ve skupině VO a zároveň nižší podíl MUFA a poměr n-6/n-3 a vyšší procentické zastoupení n-3 PUFA ($p<0{,}001$) ve skupině OO. Ředící model dává spolehlivou předpověď změny v obsahu jednotlivých mastných kyselin (sklon regresní přímky 0,97–1; R_2 0,992–0,996). Strategie finishing feeding je tedy doporučena pro produkci kapřího masa o požadovaném obsahu n-3 mastných kyselin pro účely speciálních diet, hlavně v primární a sekundární prevenci kardiovaskulárních chorob.

Studie II objasňuje vliv procesu sádkování na kvalitu masa kapra. Při sádkování jsou ryby drženy v čisté vodě bez krmení, za účelem vyprázdnění zažívadel a eliminace nežádoucích pachutí. Ryby zároveň ztrácí část své hmotnosti vlivem mobilizace tukových zásob. Tato studie sledovala vliv dlouhodobého sádkování (70 dnů) na obsah a kvalitu tuku kapra obecného. Byly použity 4 roky staré tržní ryby (hmotnost 1 700–2 600g) ze třech produkčních systémů (C: přikrmování obilovinami; P: přikrmování řepkovo-lněnými peletami; N: přirozená potrava). Odběr vzorků svaloviny probíhal každých 14 dní, byly zjišťovány váhové úbytky a výtěžnost filet a byly prováděny analýzy množství a složení lipidů. Během experimentu byl zaznamenán trvalý pokles obsahu tuku ve skupinách C a P, zatímco ve skupině byl téměř neměnný. Ryby ze skupin C a P metabolizovaly zpočátku především MUFA, ale s prodlužující se dobou sádkování docházelo rovněž k metabolizování PUFA rybami ze všech skupin. Po 70 dnech sádkování vykazovaly všechny skupiny téměř identické zastoupení SFA, MUFA i PUFA. Na základě výsledků bylo konstatováno, že kapr, pokud je v dobré kondici a s dostatkem zásobního tuku, je schopný selektivně metabolizovat jednotlivé mastné kyseliny k pokrytí energetických potřeb. Teprve při významném poklesu

obsahu zásobního tuku jsou metabolizovány mastné kyseliny všech typů k udržení metabolických pochodů.

Studie III zkoumá změny v obsahu tuku, kompozici mastných kyselin a oxidaci, ke kterým dochází při smažení filetu kapra se zvýšeným obsahem n-3 mastných kyselin. Byly použity 4 různé smažicí tuky a porce kapra byly smaženy samotné nebo obalené v trojobalu. Obsah tuku byl během smažení významně zvýšen a kompozice mastných kyselin spolehlivě odráží kompozici použitého smažícího tuku (oleje). Smažení na slunečnicovém oleji negativně ovlivnilo nutričně hodnotné složení tuku kapra snížením poměru n-3/n-6 mastných kyselin. Smažení na řepkovém oleji zachovává vhodný poměr n-3/n-6, ale zvyšuje obsah SFA. Použití řepkového oleje nezvýšilo oxidační pochody ve zkoumaných vzorcích. Na základě výsledků je doporučeno použití řepkového oleje ke smažení masa kapra, neboť nejlépe zachovává kompozici zdravotně prospěšných n-3 mastných kyselin a zároveň nezvyšuje intenzitu oxidace.

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Sampels, S., **Zajíc, T.,** Mráz, J., 2013. Effect of frying fat and preparation on carp (*Cyprinus carpio*) fillet lipid composition and oxidation. (*manuscript*)

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Sampels, S, Mraz, J., **Zajic, T.,** 2012. Influence of frying fat on fatty acid composition of omega-3 rich carp fillets, Proceedings of the Euro Fed Lipid meeting Novel Sources for Omega-3 for Food and Feed, 14–15 November 2012, Copenhagen, Danmark.

Sampels, S., Mraz, J., **Zajic, T.,** 2012. The influence of frying fat on fatty acid composition of omega-3 rich carp, Proceedings of the AQUA 2012 Global meeting, 1–5 September 2012. Conference Center Prague, Czech Republic, p. 977.

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