Lipids in Common Carp (*Cyprinus Carpio*) and Effects on Human Health

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Abstract

There is evidence that n-3 fatty acids (FA), especially EPA and DHA, are beneficial for human health. This thesis examined factors influencing FA composition in common carp flesh, sought to develop a procedure for long-term sustainable culture of common carp with improved muscle lipid quality (omega 3 carp) and studied the health benefits of eating such carp in the secondary prevention of cardiovascular disease.

An approach using the bioactive compound sesamin to increase biosynthesis of EPA and DHA from alpha linolenic acid showed that addition of sesamin did not alter muscle lipid composition in common carp. Investigations of the response of carp to finishing feeding and prediction of FA changes by a dilution model revealed that fillet FA composition reflected the FA composition of the diet and was correlated to the length of the feeding period. The simple dilution model accurately predicted changes in the fillet FA composition.

A procedure for long-term sustainable culture of omega 3 carp based on supplementation by pellets containing rapeseed cake and extruded linseed as a lipid source was developed and the carp were compared with fish supplemented by cereals and fish kept on natural feed (plankton and benthos) only. Carp supplemented by cereals were characterised by a high level of monounsaturated FA (MUFA) and low level of n-3 FA, whereas carp supplemented by the rapeseed/linseed pellets had a favourable FA profile close to that of fish kept on natural feed only.

Studies on the effects of purging on carp flesh lipid content and composition showed that lipid quality changed during the purging period, with increasing levels of n-3 FA and decreasing levels of MUFA. Supplementation with rapeseed/linseed pellets in the growing period and purging for no longer than 14 days resulted in a nutritionally beneficial FA composition combined with an economically acceptable weight loss.

In studies on the health benefits of carp in the prevention and treatment of cardiovascular disease, consumption of carp improved plasma lipid parameters in patients after major cardiac revascularisation surgery. Overall, these results suggest that culture of carp should be recognised as long-term sustainable and carp should be promoted as a healthy and local product.

Keywords: common carp, fatty acid composition, human health, nutrition, pond culture.

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Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Mráz, J., Schlechtriem, Ch., Olohan, L.A., Fang, Y., Cossins, A.R., Zlabek, V. & Pickova, J. (2010). Sesamin as a potential modulator of fatty acid composition in common carp (*Cyprinus carpio*). *Aquaculture Research* 41, e851-e861.

II Mráz, J., Zajíc, T., Schiller Vestergren, A. & Pickova, J. Prediction of fatty acid composition in common carp (*Cyprinus carpio*) fillet by finishing feeding strategy using the dilution model. (manuscript).

III Zajíc, T., Mráz, J., Sampels, S. & Picková, J. Fillet quality changes as a result of purging of common carp (*Cyprinus carpio* L.) with special regard on weight loss and lipid profile (manuscript).

IV Adámková, V., Kacer, P., Mráz, J., Suchanek, P., Pickova, J., Králová Lesná, I., Skibova, J., Kozak, P. & Maratka, V. (2011). The consumption of the carp meat and plasma lipids in secondary prevention in the heart ischemic disease patients. *Neuroendocrinology Letters* 32(Suppl.2), 101-104.

The contribution of Jan Mráz to the papers included in this thesis was as follows:

I Performed the experiment with fish, collected samples, carried out lipid analyses, processed data and was responsible for compiling the manuscript.

II Planned the study, applied for funding, managed the project, performed the experiment, collected samples, carried out the lipid analyses, processed the data and was responsible for compiling the manuscript.

III Co-worked on the planning, applied for funding, managed the project, co-worked on performing the study, carried out lipid class analyses and co-worked on the manuscript.

IV Co-worked on the planning of the study and application for funding, took care of the fish used for the trial and performed the lipid analyses of the fish samples.

Abbreviations

AA	Arachidonic acid
ACO	Acyl-CoA oxidase
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
C	Carp supplemented by cereals
cDNA	Complementary deoxyribonucleic acid
CHD	Coronary heart disease
CPT	Carnitine palmitoyltransferase
CRP	C-reactive protein
CT	Cycle threshold
CYP	Cytochrome P450
Δ	Delta
DHA	Docosahexaenoic acid
E%	Percentage energy
EFSA	European Food Safety Authority
EF1-α	Elongation factor 1α
Elovl	Elongation of very long chain fatty acids
EPA	Eicosapentaenoic acid
EROD	Ethoxyresorufin O-deethylase
FA	Fatty acids
Fadsd6	Δ-6 fatty acyl desaturase
FAME	Fatty acid methyl esters
FO	Fish oil
GC	Gas chromatography
HDL	High-density lipoprotein
HUFA	Highly unsaturated fatty acids
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography

LA	Linoleic acid
LDL	Low-density lipoprotein
LXR	Liver X receptor
miRNA	Micro ribonucleic acid
mRNA	Messenger ribonucleic acid
MUFA	Monounsaturated fatty acids
N	Carp kept on natural feed only
OO	Olive oil
P	Carp supplemented by rapeseed/linseed pellets
PCR	Polymerase chain reaction
PL	Phospholipids
PPAR	Peroxisome proliferator activated receptors
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
SREBP	Sterol regulatory element binding protein
TAG	Triacylglycerol
TC	Total cholesterol
TLC	Thin layer chromatography
VO	Vegetable oil

1 Introduction

1.1 Fatty acids and aquaculture

The world capture fisheries have been relatively stable in the past decades. It is not possible to further increase the fish capture on a world-wide scale without a risk of overfishing, so aquaculture is the only solution to meet the increasing consumer demand for fish. Aquaculture is the fastest growing animal food producing sector, with a growth rate from 1970 of around 8.3% per year and with 52.5 million tons in 2008 (68.3 million tons including aquatic plants), it accounts for almost half the global food fish supply (SOFIA, 2010).

Fish oil and fish meal have traditionally been used as ingredients in aquafeeds for carnivorous fish culture. Fish oil has a high level of the n-3 highly unsaturated fatty acids (n-3 HUFA; 20 or more carbons and three or more double bonds), especially eicosapentaenoic (EPA; 20:5n-3), docosapentaenoic (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which are natural and nutritious for fish and humans. As aquaculture expands, fish meal and fish oil are becoming more expensive and scarce. Consequently, there is high pressure on the aquafeed producers to replace these ingredients with more sustainable alternatives (Pickova & Morkore, 2007). Generally, vegetable sources of oil and protein are used as the replacement. Vegetable oils can replace a substantial amount of fish oil in the diets of many fish species without affecting growth and feed conversion efficiency. However, the drawback of these alternatives is the lack of n-3 HUFA, which compromises the nutritive value of the farmed fish for consumers. Several alternative oil sources, derived from unicellular algae, pelagic organisms or benthic invertebrates containing high amounts of n-3 HUFA, have been identified and tested in aquafeeds. However, the price of these is still too high for them to be commonly used in aquafeeds (reviewed by Turchini *et al.*, 2009).

1.2 Common carp

Common carp (*Cyprinus carpio*) is one of the most cultured fish species in the world. In 2008, world production was 2 987 433 tons and the European production was 144 747 tons (FAO, 2011). Carp is thus well-established cultured species with a well-known production cycle. It is consumed as a traditional food in central Europe.

Carp is an omnivorous species, eating plankton and benthos (worms, insects, molluscs) as well as detritus in natural conditions (Adamek *et al.*, 2004). The typical carp culture practice is to use artificial shallow earthen ponds in which production is based on plankton and benthos production, supplemented by cereals. The digestive system of carp is better adapted to a diet including more carbohydrates than carnivorous species. The production cycle in Europe usually takes 3-4 years.

There are two sources of n-3 HUFA in carp produced in ponds: i) the natural feed organisms (plankton and benthos), which are rich in n-3 HUFA; and ii) the n-3 HUFA synthesised by carp from alpha linolenic acid (ALA). It has been reported that unlike marine fish, carps are able to bio-convert ALA to n-3 HUFA (Zheng *et al.*, 2004; Tocher, 2003; Olsen *et al.*, 1990; Farkas, 1984). It is therefore of interest to determine and maximise the ability of carp to synthesise n-3 HUFA from ALA in order to preserve the lipid quality of the fish as human food and achieve sustainable utilisation of feed resources. Carp culture could therefore become a net producer of n-3 HUFA if fish with high enzyme activity in FA elongation and desaturation were selected.

Carp also have relatively low requirements for both n-3 and n-6 FA (0.5-1%) which can be met by plant 18-carbon FA (Takeuchi, 1996). The inclusion rate of fish meal in carp culture is low (5%) (Tacon & Metian, 2008), and fish oil can even be omitted. Thus, substitution of these ingredients will be considerably easier for carp than for carnivorous aquaculture species.

Carp culture could therefore be an example of long-term sustainable production without relying on a supply of fish oil and fish meal. In addition, carp culture turns nutrients "lost" to water (especially N and P) into highly valuable nutritious flesh via the natural food chain in carp ponds. The low trophic levels are an especially important source of valuable n-3 HUFA in central parts of continents, where the population has less access to marine fish from capture.

1.3 Health benefits of fish consumption

Fish consumption is steadily increasing world-wide and fish is being promoted as healthy and beneficial for human health. Nutrients in fish and other aquatic organisms that are recognized as characteristic for these foods and that are important for human health are proteins, lipids, especially the n-3 polyunsaturated fatty acids (n-3 PUFA), vitamins D and B12, antioxidants such as astaxanthin and some trace elements, *e.g.* selenium, iodine. Altogether, these compounds make fish consumption an important source of beneficial bioactive compounds in human nutrition.

Studies on the beneficial effects of fish intake are very often directed towards marine fish and shellfish. EPA and DHA are therefore misleadingly called "marine" fatty acids or "fish" fatty acids. These n-3 HUFA are to a large degree synthesized by microalgae, both in freshwater and saltwater, and transported via the food chain in the systems.

Today's Western diet is generally deficient in n-3 fatty acids and excessive in n-6, resulting in a low n-3/n-6 ratio. It has been suggested that humans evolved on a diet with an n-3/n-6 ratio close to 1:1, whereas in the Western diet this ratio exceeds 1:15 (Simopoulos, 2008; Leaf & Weber, 1987). This dietary change is suggested to be associated with many lifestyle diseases. Several studies suggest that the excessive dietary intake of n-6 FA typical of the Western diet also promotes obesity, as reviewed by Ailhaud (2006).

Studies indicate that conversion of ALA to EPA occurs but is limited in humans and that further transformation to DHA is very low (Burdge & Calder, 2005). Therefore it has been proposed that EPA and DHA should be consumed directly to maintain optimal tissue functions. Fish lipids are characterized by a high level of n-3 HUFA, especially EPA, DPA and DHA, which are generally referred to as n-3 long-chain PUFA or n-3 HUFA. They have many different functions and actions in the human body, *e.g.* influencing the physical properties of cell membranes, membrane protein-mediated responses, acting as eicosanoid precursors, cell signalling and gene expression in many different cell types (Calder & Yaqoob, 2009). EPA and DHA have been shown to have beneficial effect on a range of cardiovascular risk factors and can result in primary cardiovascular prevention and reduction in total and cardiovascular mortality (Calder & Yaqoob, 2009).

The beneficial effects of fish consumption are generally recognised, but, there have been several concerns about the overall safety of eating fish, mainly due to the potential risk of contaminants (mercury, PCB, dioxines). However, Mozaffarian *et al.* (2006) conducted an extensive meta-study and concluded that the benefits of fish intake exceed the potential harmful effects of any pollutants present in the fish.

1.4 Recommendations and situation in the Czech Republic

Many authorities and nutrition and health organisations have developed specific dietary recommendations for intake of fish and fatty acids for different countries around the world.

The **European Food Safety Authority (EFSA)** has approved several health claims related to the consumption of fish or EPA and DHA, *e.g.* maintenance of normal level of blood triacylglycerols, normal brain function and vision, cardiac function and blood pressure (EFSA Panel on Dietetic Products, 2010b).

The EFSA Scientific Panel on Contaminants in the Food Chain stated that: "There is evidence that fish consumption, especially of fatty fish (one to two servings a week) benefits cardiovascular system and is suitable for secondary prevention in manifest coronary heart disease" (CHD) (EFSA, 2005).

The EFSA Panel on Dietetic Products, Nutrition and Allergies proposed reference labelling intake values for fatty acids:

- 250 mg EPA+DHA; 2 g ALA and 10 g of LA per day (EFSA, 2009)

and has set the following Dietary Reference Values for fats (EFSA Panel on Dietetic Products, 2010a):

- Fat: 20-35% energy (E%)
- Saturated FA (SFA) and trans FA: as low as possible

It has also set the following Adequate Intake values (EFSA Panel on Dietetic Products, 2010a):

- LA 4 E%
- ALA 0.5 E%
- EPA+DHA adults 250 mg
- DHA children 6-24 months 100 mg
- Pregnancy and lactation additional 100-200 mg of DHA

The **WHO/FAO** has stated that "regular fish consumption (1-2 servings per week) is protective against coronary heart diseases and ischemic stroke and is recommended. The serving should provide an equivalent of 200-500 mg of EPA and DHA" (WHO/FAO, 2003). The WHO/FAO Expert Consultation recommends that Member States "should emphasise:

- The benefits of fish consumption on reducing coronary heart disease mortality (and the risks of mortality from coronary heart disease associated with not eating fish) for the general adult population

- The net neurodevelopmental benefits to infants of fish consumption by women of childbearing age, particularly pregnant and nursing mothers." (WHO/FAO, 2011).

The **American Heart Association** recommends (Kris-Etherton et al., 2002):

- For the general population: "at least two servings of fish per week (particularly fatty fish)".
- Patients with documented CHD: ≈ 1 g of EPA+DHA/day
- Patients with hypertriglyceridaemia: 2-4 g EPA+DHA/day

The **Czech Society of Nutrition** recommends (Dostálová *et al.*, 2012):

- Fish consumption: 400 g/week
- Fat:
    - adults with low energy expenditure <30 E%
    - adults with high energy expenditure <35 E%
    - children 30-35 E%

- SFA: 10 E% (20 g)
- PUFA: 7 - 10 E%
- n-6/n-3 ratio: max 5:1.
- Trans FA as low as possible, max 1 E% (2.5 g/day)
- Cholesterol: 100 mg/1000 kcal (max. 300 mg/day)

Fish intake is generally very low in the Czech Republic (in 2008 only 5.5 kg of fish or fish products per capita per year) (MZe, 2009), and it is far below current recommendations. Other sources of n-3 FA, including EPA and DHA, are scarce in the diet consumed by the Czech population (Hibbeln *et al.*, 2006). Thus, it would be beneficial to generally increase fish consumption and also to increase the content of the beneficial fatty acids in locally produced fish and associated products.

1.5 Fatty acids and their metabolism

Fatty acid is a carboxylic acid, usually with an aliphatic chain that can be either saturated (without double bonds) or unsaturated (with double bonds). Unsaturated fatty acids are further divided according to number of double bonds into monounsaturated (one double bond) or polyunsaturated (more than one double bond). The double bond can be organised in a cis- or trans-configuration. Another important characteristic is a position of the first double

bond (from the methyl end). The principle of fatty acid nomenclature is shown in Figure 1.

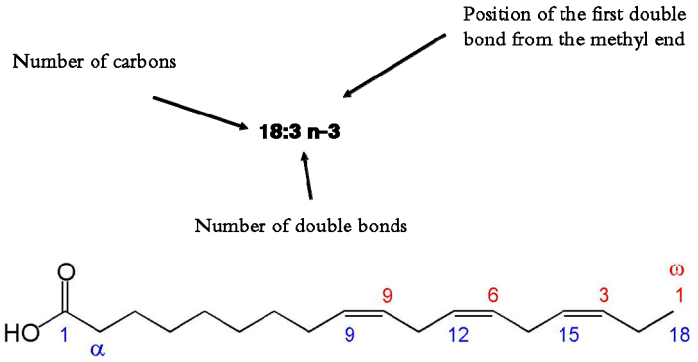

Figure 1. Principle of fatty acid nomenclature

Fatty acids are an important source of energy. In the form of triacylglycerols, they can yield more than twice the amount of energy for the same mass as carbohydrates or proteins. In the form of phospholipids, they serve as a basic building block for all cellular membranes. Fatty acid metabolism consists of catabolic processes, which generate energy and fatty acid metabolites, and anabolic processes, which lead to the creation of fatty acids and other molecules of which they form part.

Fatty acids are predominantly formed in the liver from two-carbon bodies (acetyl-CoA) through the action of a cytosolic multienzyme complex called fatty acid synthetase. All known organisms are able to *de novo* biosynthesise SFA. The SFA can be further modified by inserting a double bond to form mono unsaturated fatty acids (MUFA) by Δ-9 desaturase which is located in the endoplasmatic reticulum. There are two series of PUFA which cannot be formed by any vertebrates (including fish) and are therefore essential. The n-6 family is synthesised from LA (18:2n-6) and the n-3 family is synthesised from ALA (18:3n-3). These two 18-carbon (C18) fatty acids can be further converted to HUFA by desaturases and elongases (Morais *et al.*, 2009; Voss *et al.*, 1991) (Figure 2).

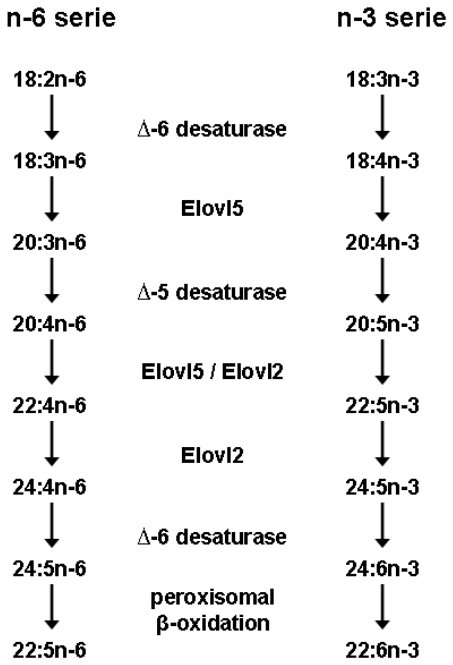

Figure 2. Pathways of HUFA biosynthesis from C18 fatty acids, adapted from Voss *et al.* (1991) and Morais *et al.* (2009)

The rate of conversion varies in different organisms usually depending on the extent to which the species can obtain HUFA from the natural diet. Thus the carnivorous species which can obtain excess HUFA from their natural diet, usually have a lower ability for conversion compared with herbivorous species. In marine fish species this bioconversion occurs poorly, if at all, and therefore they have essential requirements for HUFA in their diet (reviewed by Tocher, 2003). Regulation of the HUFA biosynthetic pathway is described in detail in section 1.7.

1.6 Factors influencing flesh quality with focus on carp

1.6.1 Species

There are differences in muscle lipid content between fish species which also lead to differences in FA composition (Fontagné-Dicharry & Médale, 2010). Some fish species have a low lipid content in the fillet (less than 2 %), *e.g.* pikeperch (*Stizostedion lucioperca*), European perch (*Perca fluviatilis*) and Atlantic cod (*Gadus morhua*). Other fish species have a high lipid content in

the fillet (more than 10%), *e.g.* Atlantic salmon (*Salmo salar*), European eel (*Anguilla anguilla*), while that in common carp is intermediate (Henderson & Tocher, 1987). In general lipids in marine fish species contain more HUFA with a higher n-3/n-6 ratio than those in freshwater fish species (Henderson & Tocher, 1987).

1.6.2 Genetic background

Another important factor affecting lipid content and composition in fish is the genetic background. It has been shown that muscle lipid content is a highly heritable trait (>0.5) in common carp and that there is a relatively high positive genetic correlation between body size (length and body weight) and lipid content (0.71 and 0.59, respectively) (Kocour *et al.*, 2007).

Leaver *et al*. (2011) analysed flesh lipid parameters in 48 families of Atlantic salmon and found that flesh n-3 HUFA composition is a highly heritable trait ($h^2 = 0.77 \pm 0.14$). Specific hepatic mRNA expression patterns were found to be associated with high flesh n-3 HUFA, which indicates a possible mechanism for genotype-dependent deposition in flesh.

There are few data available on the effect of genetic origin on the lipid composition in carp (Fauconneau *et al.*, 1995). In a study with four carp hybrids Buchtova *et al.* (2007) found that FA composition was not affected to any great extent by hybrid type.

1.6.3 Sex, maturation

Kocour *et al.* (2007) reported that females of Hungarian synthetic mirror carp were fattier than males, probably due to later maturation. In a study with four common carp hybrids Buchtova *et al.* (2008) found only minor differences in lipid composition between males and females, possibly caused by different lipid content. Fajmonova *et al.* (2003) did not find sexual dimorphism in lipid content and FA composition in three-year-old carp. This indicates that effects of sex dimorphism on FA composition in carp are probably only minor and observable only in sexually mature stages.

1.6.4 Tissue

Fish fillet is heterogeneous and is composed of white muscle, pink and red muscle, adipose tissue and skin (Nathanailides *et al.*, 1995). The tissues differ greatly in lipid content and therefore the lipids are not equally distributed in the fillet. Mráz & Pickova (2009) reported that the lipid content in carp white muscle, red muscle and abdominal wall was around 1, 17 and 30 % respectively. The lipid class and FA composition in the three tissues was highly influenced by the lipid content. Abdominal wall had the highest

proportion of triacylglycerols with a high level of MUFA and low level of n-3 FA, whereas white muscle was the leanest and had a high proportion of phospholipids and a high level of n-3 FA including EPA and DHA. The effect of fattiness on the FA composition can be explained to a large extent by differences in the FA composition of the major lipid fractions and the relative contribution of these fractions to total lipids. Phospholipids are particularly rich in PUFA, whereas triacylglycerols contain high levels of MUFA and much lower levels of PUFA (Kiessling *et al.*, 2001). Thus PUFA are negatively correlated to fattiness and MUFA are positively correlated (reviewed by Henderson & Tocher, 1987).

1.6.5 Nutrition

"You are what you eat" - nutrition has a major impact on the lipid content and composition in fish. Thus good pond management to maintain a sufficient amount and appropriate structure of the planktonic and benthic community in ponds is of great importance when seeking to improve carp FA composition.

Cereals are usually used as a supplemental feeding for carp. Since they are rich in carbohydrates and have very low levels of n-3 FA, the flesh of the farmed carps generally contains a high level of oleic acid and a low level of favourable n-3 HUFA (Csengeri, 1996; Mráz *et al.*, 2012). A supplementary feedstuff which is rich in ALA could be an alternative way to increase the n-3 HUFA content in carp flesh. Feedstuffs with a high level of ALA that are cheap and easily available include rapeseed, linseed and hempseed. Rapeseed or rapeseed cake is becoming an important part of pellets for carp nutrition in the Czech Republic for its low price and availability. Rapeseed and linseed oil have a moderate / high level of ALA (13% and 60%, respectively) and a favourable n-3/n-6 ratio (around 1:2 and 5:1, respectively) (Pickova & Morkore, 2007). They are commonly used in feed for salmonids as a replacement for fish oil (Bell *et al.*, 2001) to different proportions. Mráz *et al.* (2012) studied lipid content and FA composition in carp supplemented by rapeseed cake pellets in comparison to carp supplemented by cereals and carp fed only on natural feed (plankton and benthos) available in ponds. The supplementation by rapeseed cake pellets resulted in higher levels of PUFA and HUFA compared with the cereal-supplemented group. Those authors suggested that part of the rapeseed in the feed mixture should be replaced by linseed to further improve carp FA composition.

1.6.6 Bioactive compounds

An alternative approach to influence muscle lipid composition might be to use biologically active compounds which modulate the fish metabolism to synthesize or deposit more n-3 HUFA.

One such biologically active compound could be sesamin. In the first study investigating sesamin effects in fish Trattner *et al*. (2008a) found that sesamin/episesamin supplementation increased the level of DHA up to 37% in white muscle of rainbow trout (*Oncorhynchus mykiss*) fed high ALA vegetable oil. An *in vitro* study with Atlantic salmon hepatocytes showed that sesamin/episesamin exposure led to increased elongation and desaturation of ALA to DHA, indicating that sesamin has modulatory effects on lipid metabolism leading to increased levels of DHA and higher β oxidation activity (Trattner *et al.*, 2008b). However, there are still many questions about the use of sesamin in fish feed, especially whether effects similar to those observed in some studies on salmonids can also be observed across different fish species, including cyprinids.

Another potential bioactive compound is lipoic acid. Lipoic acid acts as an antioxidant both in the hydrophilic and hydrophobic phases (Navari-Izzo *et al.*, 2002). It is reported to have several effects on lipid metabolism in chickens (Hamano, 2006) and rats (Mythili *et al.*, 2006). Trattner *et al*. (2007) studied the effect of lipoic acid on FA composition in brain and muscle of South American pacu (*Piaractus mesopotamicus*) and found that lipoic acid increased the level of EPA in muscle polar lipids.

Conjugated linoleic acid and tetradecylthioacetic acid are also reported to have stimulatory effects on DHA synthesis in salmonids (Kennedy *et al.*, 2007; Moya-Falcón *et al.*, 2004).

1.6.7 Purging, starvation

Purging of fish before slaughter or delivery to market is a common practice in aquaculture to remove possible off-flavours and eliminate undigested food from the intestine. It usually involves transferring the fish to clean water and keeping them without feeding for a period lasting from a few days to many weeks. Purging can also improve the nutritional quality of farmed fish by reducing excessive fat and increasing the n-3 HUFA percentage (Palmeri *et al.*, 2008b; Einen *et al.*, 1998). Einen *et al.* (1998) studied the effect of starvation prior to slaughter in Atlantic salmon and found significant but rather marginal effects of starvation on FA composition in muscle, belly flap and liver. However, the fish used in the study had quite a high muscle lipid content (16%) and much larger effects would probably be seen in fish with a lower muscle lipid content.

Csengeri (1996) studied the effects of starvation on lipid content and composition in common carp and observed a consistent decrease in the oleic acid levels in both muscle and liver, whereas PUFA were partly protected. He also concluded that the effect of starvation was dependent on the previous feeding regime. Vacha *et al.* (2007) studied the effects of long-term starvation on FA in common carp fed either on cereals or natural feed only. The carp supplemented by cereals had a high lipid content (>10%), whereas in the carp fed on natural feed was only 1.8%. The largest differences in FA composition were seen in the fish fed on natural feed only, where mainly decreased levels of PUFA were observed.

1.6.8 Preparation of fish as food

The last but by no means least important factor influencing lipid content and composition is processing and cooking. It has been shown that in particular, the quality of fats and oils added during processing has a very strong influence on lipid composition (Sampels *et al.*, 2009; Ansorena & Astiasaran, 2004). Sampels *et al*. (2009) found high variation in the n-3/n-6 ratio in fish products, with it being up to 400 fold lower than in raw fish. They concluded that fat sources used during fish processing and preparation have the largest impact on the food the FA content and composition in the table-ready food and proposed that this be stated on the product label. The lipid composition in fish may change further when it is fried before consumption (Ramirez *et al.*, 2005).

1.7 HUFA biosynthesis

1.7.1 HUFA biosynthesis in common carp

The HUFA biosynthetic pathway in fish was established using rainbow trout hepatocytes (Buzzi *et al.*, 1996). It is anticipated, and also suggested by the cumulative evidence, that the same pathway exists in other fish species, including common carp (Tocher, 2003). Common carp has been shown to be able to elongate and desaturate n-3 and n-6 HUFA from its C18 precursors. This was first demonstrated by Farkas *et al.* (1978) using injection of radiolabelled sodium acetate into common carp juveniles, followed by detection of incorporated radioactivity in different FA. In later experiments, Farkas (1984) used slices from common carp liver incubated with radiolabelled FA. Tocher *et al.* (1999) demonstrated the ability of common carp to convert ALA to HUFA in cell lines. Furthermore, feeding studies have shown that the essential FA requirements of common carp can be satisfied by C18 PUFA, which suggests that common carp are able to convert these to HUFA

(RadunzNeto *et al.*, 1996; Takeuchi, 1996). Despite the large number of studies investigating the ability of common carp to produce HUFA from C18 precursors, the molecular mechanisms behind HUFA biosynthesis are not fully understood and the genes coding the enzymes responsible remain to be identified and characterised in common carp.

1.7.2 Genes related to HUFA biosynthesis and their regulation

The first desaturase to be characterised in fish was from the model species zebrafish (*Danio rerio*). This work was done by Hastings *et al*. (2001), who used heterologous expression in the yeast *Saccharomyces cerevisiae*. They found that zebrafish has a bifunctional Δ-5/Δ-6 enzyme which possesses the capacity to desaturate both types of substrate. In contrast, the other fish desaturases characterised to date are either unifunctional or have major activity towards one substrate and only residual capacity to desaturate other substrates (Monroig *et al.*, 2010b; Zheng *et al.*, 2005a; Hastings *et al.*, 2004; Zheng *et al.*, 2004). In Atlantic salmon, three different desaturases with Δ-6 activity have been identified and characterised (Monroig *et al.*, 2010b) (Table 1). The only HUFA-related gene characterised so far for common carp is Δ-6 desaturase (GenBank accession no. AF309557) (Zheng *et al.*, 2004) (Table 2). It includes an open reading frame of 1335 base pairs specifying protein of 444 amino acids. It is more specific towards the n-3 substrate and shows low Δ-5 desaturase activity. Thus the specific Δ-5 desaturase probably remains to be identified for common carp. Recently, Ren *et al.* (2012) cloned two Δ-6 desaturase-like cDNAs (Fad6-a and Fad6-b) and studied their expression in liver related to dietary FA in common carp (Table 2). They found that there was almost no effect on expression of Fad6-b, whereas diet had a strong effect on expression of Fad6-a.

Several elongases have been identified in mammals with three involved in elongation of PUFA (ELOVL2, ELOVL4 and ELOVL5) (Jakobsson *et al.*, 2006). ELOVL2 predominantly elongates C20 and C22 whereas ELOVL5 primarily elongates C18 and C20 (Leonard *et al.*, 2000). Agaba *et al*. (2004) characterised a multifunctional elongase from zebrafish with ability to elongate C18, C20, C22 as well as MUFA and PUFA. Hastings *et al*. (2004) characterised ELOVL5 in Atlantic salmon with activity to elongate mainly C18 and C20. Morais *et al*. (2009) characterised ELOVL2 and a second ELOVL5b from Atlantic salmon and showed that the elongase ELOVL5b primarily elongates C18 and C20 and ELOVL2 elongase predominantly elongates C20 and C22. ELOVL4 has been characterised in zebrafish (Monroig *et al.*, 2010a) and in Atlantic salmon (Carmona-Antoñanzas *et al.*, 2011) with ability to convert C20 and C22 up to C36 (Table 1). Recently, Ren

et al. (2012) cloned two Elovl5-like elongase (Elovl5-a and Elovl5-b) cDNAs and studied their expression in liver related to dietary FA in common carp (Table 2). They found that there was almost no effect on expression of Elovl5-b whereas diet had strong effect on expression of Elovl5-a.

Table 1. *Overview of HUFA biosynthetic genes characterized in Atlantic salmon (Salmo salar)*

Gene name	GenBank accession no.	Product	Characterised by
D6fad_a	AY458652	delta-6 fatty acyl desaturase D6fad_a	(Zheng *et al.*, 2005a)
D6fad_b	GU207400	delta-6 fatty acyl desaturase	(Monroig *et al.*, 2010b)
D6fad_c	GU207401	delta-6 fatty acyl desaturase	(Monroig *et al.*, 2010b)
Fadsd5	AF478472	delta-5 fatty acyl desaturase	(Hastings *et al.*, 2004)
Elov12	FJ237532	Polyunsaturated fatty acid elongase Elov12	(Morais *et al.*, 2009)
Elvol5a	AY170327	Polyunsaturated fatty acid elongase Elvol5a	(Hastings *et al.*, 2004)
Elovl5b	FJ237531	Polyunsaturated fatty acid elongase Elovl5b	(Morais *et al.*, 2009)
Elovl4	HM208347	Elongation of very long chain fatty acid-like 4	(Carmona-Antoñanzas *et al.*, 2011)

Table 2. Characterised/cloned HUFA biosynthetic genes in common carp (Cyprinus carpio) and primers used for rea- time PCR

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
Fadsd6†	-	-
Fad6-a*	ATCGGACACCTGAAGGGAGCG	CATGTTGAGCATGTTGACATCCG
Fad6-b*	GTACCAATGGGAGGTTCGGCAC	GAGTTGAAGGTTTGGATGAAATGCATG
Elovl5-a*	GTCCTGACCATGTTCCAGACATCTTG	CTGTAAGCGGACGAGGTGTCGTC
Elovl5-b*	GTCCTGACCATGTTCCAGACATCTTG	CATGAAGCTCCTCTACTGCGCTG

† GenBank accession no. AF309557, characterised by Zheng *et al.* (2004)

* Cloned and primers designed by Ren *et al.* (2012)

Nutritional factors such as content of FA (Jump & Clarke, 1999) and bioactive compounds in the diet affect the expression of genes involved in lipid metabolism (Leaver *et al.*, 2008; Trattner *et al.*, 2008a; Trattner *et al.*, 2008b; Monroig *et al.*, 2010b; Ren *et al.*, 2012; Schiller Vestergren *et al.*,

2012). In general, both desaturases and elongases are up-regulated in fish fed a diet with C18 precursors (vegetable oils) compared with fish fed a diet containing HUFA (fish oil) (Morais *et al.*, 2009; Leaver *et al.*, 2008; Zheng *et al.*, 2005b; Tocher *et al.*, 2001). Environmental factors have also been shown to influence the expression of Δ6 desaturase in fish (Zheng *et al.*, 2005b). For more detailed information, see the review by Vagner & Santigosa (2011).

Hepatic Δ-6 and Δ-5 desaturases are coordinately regulated. The rate of transcription of Δ-6 desaturase is positively correlated with the nuclear concentration of sterol regulatory element binding protein 1 (SREBP-1) and is stimulated by peroxisome proliferator-activated receptor α (PPARα) activators. The nuclear content of SREBP-1 is increased by insulin and glucose, and back-regulated by n-6 and n-3 PUFA. Different PPARα activators, *e.g.* fibrates, induce Δ-6 desaturase enzymatic activity by an increased rate of Δ-6 desaturase gene transcription caused by enhanced binding of activated-PPARα to the Δ-6 desaturase promoter (He *et al.*, 2002). Elongases are regulated by the nuclear receptor liver X receptor α (LXR) and the transcription factor SREBP-1c (Qin *et al.*, 2009), which in turn are both influenced by the concentration of PUFAs. PUFAs repress expression of SREBP-1c and Elovl5, but when combined with LXR ligand stimulation, which increases SREBP-1c mRNA and nuclear SREBP-1c, Elovl5b mRNA levels are restored to normal. Elovl5 is also the target for several miRNAs in zebrafish (zebrafish miRNA database, http://cbio.mskcc.org/cgi-bin/mirnaviewer/mirnaviewer4.pl).

Dreesen *et al.* (2006) found in the human genome a protein non-coding antisense RNA gene that functions as a naturally occurring ‘‘cis-antisense’’ regulator of Δ-5 desaturase gene expression. Anti-sense transcripts may regulate gene expression by interfering with gene transcription and mRNA processing, accelerating mRNA decay and slowing translation of mRNA into protein. Dreesen *et al*. (2006) provided evidence that co-expression of reverse Δ-5 desaturase non-coding RNA (RevΔ5ase ncRNA) decreased the Δ-5 desaturase enzymatic activity by >70%. RevΔ5ase ncRNA most likely exerts its influence through the binding of complementary regions of the Δ-5 desaturase transcript. The resulting double-stranded RNA complex could accelerate Δ-5 desaturase mRNA decay, and/or interfere with Δ-5 desaturase mRNA translation. However, it is not known whether the RevΔ5ase ncRNA exists in fish.

2 Objectives

The overall aims of this work were to identify factors influencing FA composition in common carp flesh; develop a long-term sustainable culture of common carp with improved muscle lipid quality; and determine the health benefits of meals from such fish in the prevention and treatment of cardiovascular disease.

Specific objectives were to:

Study the effects of sesamin on fish performance, lipid content, FA composition, CYP content, EROD activity and global gene expression in common carp (Paper I).

Test the response of common carp to finishing feeding technology and attempt to predict of FA changes by a dilution model (Paper II).

Examine the effects of purging period on lipid content and quality in fillet of common carp (Paper III).

Develop a long-term sustainable culture of common carp with improved muscle lipid quality (Paper III).

Study the health benefits of meals from common carp in the prevention and treatment of cardiovascular disease (Paper IV).

3 Materials and Methods

The materials and methods used in the studies carried out within the framework of this thesis are described briefly in this section. For a more detailed description of each method, see Papers I-IV. An overview of the materials and methods used is given in Table 3.

3.1 Study design

3.1.1 Study I

Two-year-old common carp (*Cyprinus carpio*) individuals (mean weight 830 g) were reared in six 1 m^3 tanks (6 fish per tank) connected to a recirculation system. The fish were fed diets with or without sesamin addition (0.58 g/100 g feed) for 9 weeks. Survival, specific growth rate and feed conversion ratio were calculated for each treatment. Samples of white dorsal muscle and hepatopancreas were taken from all fish. The white muscle samples were analysed for lipid content and FA composition (in total lipids, phospholipids and triacylglycerols). The samples from hepatopancreas were analysed for total content of cytochrome P450, EROD activity and global gene expression profiling.

3.1.2 Study II

Study II examined the response of common carp to a fish oil finishing feeding treatment and sought to predict FA changes in fillet using a dilution model. During the 110-day experiment, market-size fish kept in cages connected to a recirculation system were fed either vegetable oil diet (rapeseed/linseed; VO) and olive oil diet (OO) only or with a subsequent fish oil (FO) finishing treatment (30 or 60 days), with each treatment carried out in duplicate.

Table 3. *Overview of study design for papers I-IV*

Study	I	II	IIIa	IIIb	IV
Aim	Effects of dietary sesamin	Finishing feeding and dilution model	Culture of omega 3 carp	Effects of purging on carp flesh	Clinical trial focusing on effects of carp consumption
Species	Common carp	Common carp	Common carp	Common carp	Human - patients after cardiac revascularisation
Size/age	Start: 0.73 kg	Start: 0.78 kg	Start: 1.1 kg	Start: 1.7-2.7 kg	41-82 years
	End: 1.7 kg	End: 1.8 kg	End: 1.7-2.7 kg	End: 1.6-2.2 kg	56 C/ 87 T individuals
Type of culture/ Location	Cages with recirculation system	Cages with recirculation system	Ponds	Storage pond with water flow	Spa Podebrady
Treatment	Pelleted feed +/- 5.8 g of sesamin per feed	Vegetable oil diet (VO) Olive oil diet (OO) Fish oil diet (FO) VO/FO OO/FO	Natural food (N) N + cereals (C) N + rapeseed/linseed pellets (P)	Carp previously fed N, C and P purged for 1, 14, 28, 42, 56 and 70 days	Control: Standard spa diet Treatment: Standard spa diet + omega 3 carp 2 x 200 g serving per week for 4 weeks
Trial length	9 weeks	110 days	5 months	70 days	4 weeks
Analyses	Production data	Production data	Production data	Production data	Body mass index (kg/m^2)
	Lipid content	Lipid content	Lipid content	Lipid content	Blood pressure
	Fatty acids	Fatty acids	Fatty acids	Lipid classes	Plasma total, HDL and LDL cholesterol
	CYP content	Gene expression		Fatty acids	
	EROD activity				Plasma triglycerides
	Gene expression				Plasma C-reactive protein

The scheme of the experimental design used in study II is shown in Figure 3. Three individuals were randomly sampled from each tank (3 x 2 tanks = 6 per treatment) after 50, 80 and 110 days for lipid analysis. At the end of the trial, survival, growth and feed conversion were calculated for each treatment.

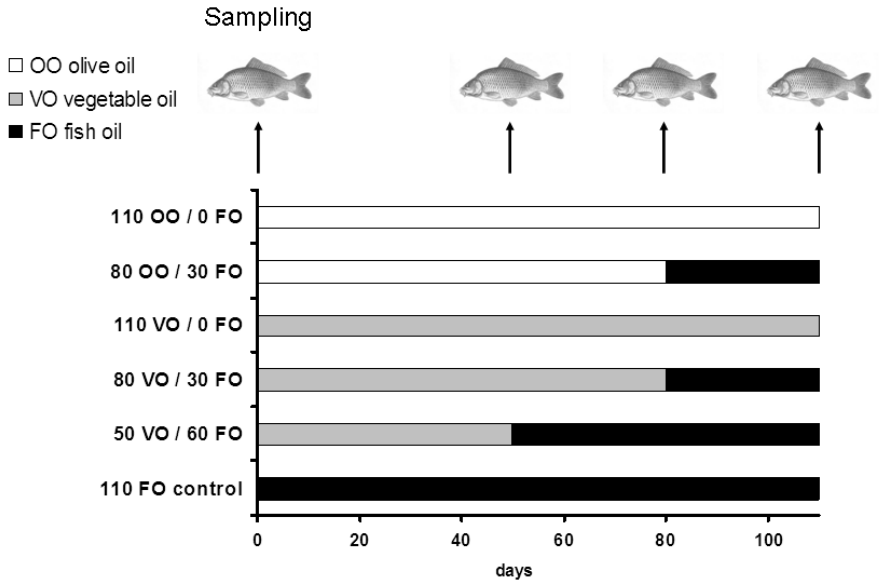

Figure 3. Experimental design for the 110-day feeding trial. Fish (10 per tank) were fed one of three diets or combinations of diets. The different dietary regimens were: 110 OO/0 FO; 80 OO/30 FO; 80 OO/30 FO; 110 VO/0 FO; 110 VO/0 FO; 80 VO/30 FO; 50 VO/60 FO and 110 FO control. Treatments were carried out in duplicate. Fish were sampled at 0, 50, 80 and 110 days.

The data obtained from the lipid analyses for groups 80 OO/30 FO, 80 VO/30 FO and 50 VO/60 FO were compared against predicted data calculated according to the dilution model designed by Robin *et al*. (2003) and verified by Jobling (2004):

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

P_T = Predicted percentage of a particular FA at time T
P_R = Percentage of a particular FA measured at time T in the fillet of control fish continuously fed the reference/finishing diet

P_0 = Percentage of a particular FA in the fillet of test fish at the beginning of the finishing feeding period
Q_T = Quantity of total FA in the test fish at time T
Q_0 = Quantity of total FA in the test fish at the beginning of the finishing feeding period

The predicted percentage of an individual FA, *e.g.* EPA, at a specific point in time (PT) (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=110 FO control 110 days) and the corresponding percentage in test fish (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 the average total FA content (lipid content x body mass) of the test fish before the finishing feeding period and Q_T the final total FA content in fish from the corresponding group at the end of the experimental trial.

3.1.3 Study III

Study III investigated lipids in the flesh of common carp reared in different production systems and the effects of a long-lasting purging period on carp flesh lipid content and composition.

The study used 4-year-old market carp (average weight 1700-2600 g) previously reared for five months in on of three different production systems (natural food only, N; supplemented by cereals, C; supplemented by rapeseed/linseed pellets, P) (Figure 4). Fish were labeled by groups and placed into a storage pond with continuous inflow of clear freshwater (experimental design is shown in Figure 4 and 5). During the 70-day experiment, water temperature decreased continuously from 18.5°C at the start to 2.5°C at the end of the experiment. Every 14 days, 10 fish were weighed, fillet yield was determined and samples for lipid analyses were taken from each group.

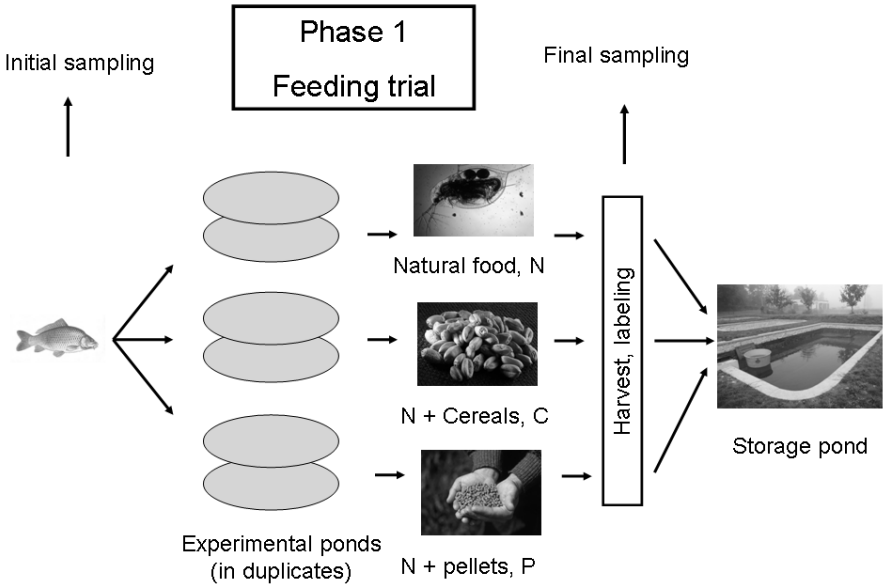

Figure 4. Experimental design of the purging experiment, phase 1 feeding trial. Fish placed in 6 experimental ponds were cultured in three production systems for 5 months. Ponds were harvested and the fish labelled by groups and placed into a storage pond (duplicates)

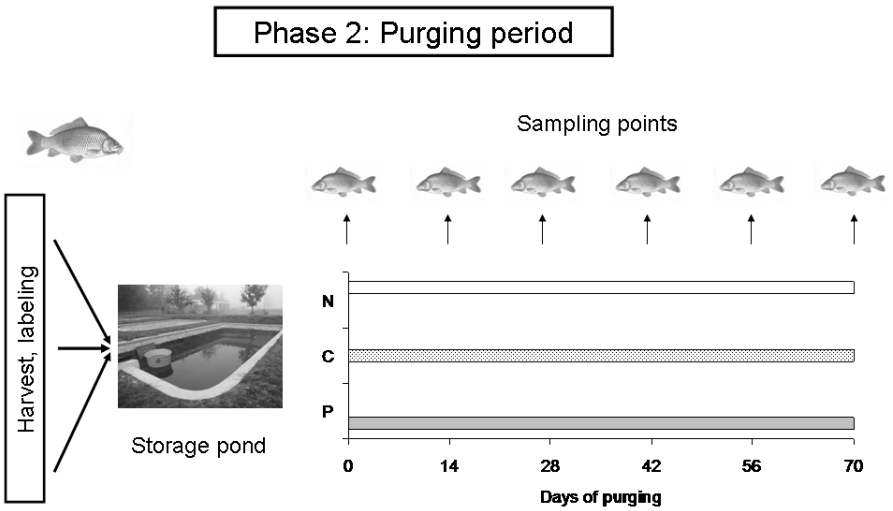

Figure 5. Experimental design of the purging experiment, phase 2 purging period. N, fish previously fed natural food only; C, fish previously supplemented by cereals; P, fish previously supplemented by the rapeseed/linseed pellets. Fish were sampled on days 1, 14, 28, 42, 56 and 70

3.1.4 Study IV

Influence of carp (carp with increased content of omega 3 FA) consumption on subjects recovering from cardiac revascularisation surgery during a follow-up spa treatment was studied.

After the surgery the subjects were randomly allocated into two groups and underwent a four-week follow-up spa treatment in Spa Podebrady, Czech Republic. During the experiment the subjects consumed either the standard spa diet (Control; 56 individuals, 41 males, 15 females, age 41-80 years) or a diet enriched with two 200 g servings of carp (Treated; 87 individuals, 64 males, 23 females, age 50-83 years). The carp fillets used in the trial originated from carp reared in a pond on a diet supplemented by pellets containing rapeseed as the lipid source. One 200 g carp serving contained on average 878 mg of n-3 PUFA. The energy intake was equal in both groups of subjects. Body mass index, blood pressure, plasma lipids and C-reactive protein (CRP) were measured in subjects at the beginning of the spa treatment and after 4 weeks.

3.2 Lipid analyses

3.2.1 Lipid extraction and fatty acid composition analyses

Lipid analyses were performed as already described in detail by Mráz & Pickova (2009). Lipids from tissues and diets were extracted by the hexane-isopropanol method (Hara & Radin, 1978). Total lipids were fractionated by thin layer chromatography for separation of lipid classes (Pickova *et al.*, 1997). FA were methylated (Appelqvist, 1968) and analysed with a Varian CP3800 gas chromatograph (Stockholm, Sweden) equipped with flame ionisation detector and split injector and fitted with a 50 m length x 0.22 mm i.d. x 0.25 μm film thickness BPX 70 fused-silica capillary column (SGE, Austin, TX, USA) (Fredriksson Eriksson & Pickova, 2007). FA were identified by comparison with the standard mixture GLC-461 (Nu-check Prep, Elysian, MN, USA) using retention time. Peak areas were integrated by means of Varian Galaxy chromatography workstation software (Varian AB, Stockholm, Sweden). FA were quantified by use of the internal standard 15-methylheptadecanoate (Larodan Fine Chemicals AB, Malmo, Sweden).

3.2.2 Composition of lipid classes

Analyses of lipid classes composition was performed according to Olsen & Henderson (1989) with minor modifications. Extracted lipid samples dissolved in hexane (conc. 1 μg/μL) were applied by a Camag ATS 4 automatic TLC sampler (Camag, Muttenz, Switzerland) in 4 mm lines on pre-developed and

activated 60 HPTLC plates (20x10, 0.20 mm layer; Merck, Darmstadt, Germany). The lipid classes were separated by automatic developing chamber AMD2 (Camag, Muttenz, Switzerland) using hexane-diethyl ether-acetic acid (85:15:2, v/v) as mobile phase. The separated lipid classes were derivated by dipping the plate into the phosphoric acid/ethanol with subsequent heating treatment in the oven (150°C, 10 minutes). Quantitative analyses of the lipid classes were performed densitometrically by use of the Camag TLC scanner 3 (Camag, Muttenz, Switzerland). The lipid classes were identified and quantified by comparison against an external standard (TLC 18-4A; Nu-Check Prep, Elysian, Minnesota, USA).

3.3 Sesamin analyses

Sesamin was analysed from extracted lipid samples with HPLC according to Moazzami & Kamal-Eldin (2006). Separation was performed on a silica column using hexane/1,4-dioxane (94:4, v/v) as mobile phase and detection was using a fluorescence detector (excitation wavelength 296 nm and emission wavelength 324 nm). External standards were used for identification and quantification.

3.4 Total content of cytochrome P450 and ethoxyresorufin O-deethylation

Microsomal fraction was prepared from the hepatopancreatic homogenate by means of Ca-aggregation method as described by Zamaratskaia *et al.* (2009). The total cytochrome P450 content was determined using the spectrophotometric method of Omura & Sato (1964), measuring the differences in the spectra (dithionite+carbon monoxide) – dithionite. The activitiy of 7-ethoxyresorufin O-deethylase was estimated using an HPLC-based method according to Zamaratskaia & Zlabek (2009).

3.5 Global gene expression profiling

Global gene expression analysis was performed using a cDNA common carp microarray with 26K gene probes (carp ARRAY ver 5.; Williams *et al.*, 2008). Total RNA was extracted from hepatopancreas using the TRIzol Plus RNA Purification Kit (Invitrogen 12183-555, Paisley, UK). Total RNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The RNA was reverse-transcribed to cDNA and labeled using the SuperScript Plus Indirect cDNA Labeling System

(Invitrogen L1014-04, 05 and -06). The labelled cDNA was hybridised on the microarrays using the Maui hybridisation system (BioMicro Systems, Salt Lake City, UT, USA). The microarrays were scanned using the Agilent DNA microarray scanner and the data obtained were processed by BLUEFUSE software (BlueGnome, Great Shelford, Cambridge, UK).

3.6 Total RNA extraction, cDNA synthesis and real time PCR

In addition to study II, gene expression study was performed to examine effects of dietary oils on the HUFA biosynthetic pathway in common carp (not included in the manuscript). For that purpose samples of hepatopancreas from groups 110 OO/0FO, 110 VO/0FO and FO control were used (6 fish/treatment).

Total RNA was isolated from 30-50 mg of carp hepatopancreas using the RNeasy® Mini Kit with on-column Rnase free DNase set (Qiagen, MD, USA). All protocols were performed according to the manufacturer's instructions. Purity and density were measured by optical density (NanoVue, Spectrophotometer, GE Healthcare Life Sciences, Uppsala, Sweden) and samples were stored in RNase-free water (Eppendorf, Hamburg, Germany) at -80˚C. The cDNA was synthesised following the protocol from the ImProm-IITM Reverse Transcription System (Promega, MD, USA). A mixture of the Oligo d(T)15 and random primers were used. The reactions were performed by incubating the samples at 25˚C for 5 min, 42˚C for 60 min, 75˚C for 15 min.

Primers for Real-Time PCR analysis (Table 4) were designed using Primer Express® software version 3.0 (Applied Biosystems, Foster City, CA, USA), ordered from Invitrogen (Carlsbad, CA, USA) and were validated by melting curve. Sequences of genes were found in common carp EST database in GenBank® by sequence similarity to zebra fish (*Danio rerio*) sequences using BLAST.

Real-time PCR was performed in a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with gene-specific primers for elongation factor 1α (EF1-α), protein 13-40S = (40S), peroxisome proliferator-activated receptor α and γ (PPARα, PPARγ), liver X receptor (LXR), sterol regulatory element binding protein 1 (SREBP-1), Δ-6 fatty acyl desaturase (Fadsd6), elongation of very long chain fatty acid-like 2 and 5 (ELOVL2, ELOVL5), acyl-CoA oxidase (ACO), carnitine palmitoyl transferase I (CPT1). A 2xPower SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was used in the PCR reaction mix of 20 µL with 4 µL primers (final concentration 0.5 µM), and 10 µL cDNA. All samples were analysed in triplicate with a non-template control on each plate. The reactions were

incubated at 95˚C for 5 min, 45 cycles of 95˚C for 10s, 60˚C for 15s and 72˚C for 15s.

3.7 Statistical analyses

The data were processed using the data analysis software STATISTICA CZ, v. 9 (StatSoft, Inc., Prague, Czech Republic) or Microsoft Office Excel v. 2003 (Microsoft Corporation). All values were expressed as mean ± standard deviation. For statistical analyses, the two-tail student's t-test and one-way ANOVA with following Tukey's post-hoc test with statistical level of significance α=0.05 were used.

The microarray data were first normalised (Huber *et al.*, 2002) and corrected (Cleveland & Devlin, 1988). The differentially expressed genes were extracted using Q-values (Storey, 2002) and a control false discovery rate at a level of 10 % (Benjamini & Hochberg, 2000). The up and down regulated genes were associated with the gene ontology terms to examine which metabolic pathways were influenced by sesamin addition.

The ΔCT was calculated and the reference genes were evaluated using the DataAssist software version 2.0 (Applied Biosystems, Foster City, CA, USA). S40 was chosen as reference gene, having the best stability over all samples and treatments. The relative expression was then calculated by comparing the ΔCT values for fish fed VO, OO and FO diets using the term $2^{-\Delta\Delta CT}$ and reported as arbitrary fold change units (Livak & Schmittgen, 2001).

Table 4. *Sequences of primers and GenBank accession numbers used*

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	GenBank acc. no
EF1-α*	GGAGCCCAGCACAAACATG	TTACCCTCCTTGCGCTCAAT	AF485331.1
40S*	GCAGCCCACCATCTTCCA	CCTTGCCACTTCCTTCAACAG	AB012087
PPARα	AAAGAACCGCAACAAATGTCAA	GCCCACTGCAAGGCACTT	FJ849065.1
PPARγ	GGCGTCATCGAAGTCCTCAT	GGGTGCCGTCTTTGTTCATG	FJ849064.1
LXR	ACTGGTGGCCATGCAGAAA	TGGGCCGGTCAAGAAAAG	FJ919778.1
Fadsd6	TCGCCACTTCCAGCATCAC	TGTTGACGTCCGGGTCCTT	AF309557.1
ELOVL2	TTGACAAACAGGGACGACAAGT	CCCCTGGGTTGTTTGAAATTT	DW720345.1
ELOVL5	ACCATCACGCCACAATGCT	CACACGGCACCCAGTTCA	JF836160.1
ACO	TGGTGAAGCTGTTTGCTGCTA	GCACAGAGTGGACAGCCGTAT	CF660510.2
CPT1	CCTGCGGTGGAGGATTTG	TGATGTATGACACGCCGTAAC C	EX821713.1
SREBP-1	GCTGGAGAAACTAGGCGACAA	CCGCTGCCCAGTTTGATG	CA965744.2

Abbreviations: * housekeeping genes; EF1-α = Elongation factor 1α, 40S = protein 13 – 40S, PPAR = peroxisome proliferator-activated receptor, LXR = liver X receptor, Fadsd6 = delta-6 fatty acyl desaturase, ELOVL = elongation of very long chain fatty acids-like, ACO = acyl-CoA oxidase, CPT1 = carnitine palmitoyl transferase I, SREBP-1 = sterol regulatory element binding protein-1.

4 Summary of results

4.1 Study I

There were no significant differences in fish survival, specific growth rate, food conversion ratio and lipid content in white muscle between the fish supplemented with sesamin and the control fish. Sesamin supplementation did not alter FA composition in carp white dorsal muscle. Sesamin increased total cytochrome P450 content in hepatopancreatic microsomes, as well as 7-ethoxyresorufin O-deethylase activity. Transcriptomic analysis, using microarray with 26K gene probes, revealed that expression of 662 genes was altered by sesamin in carp liver. However, it failed to establish any significant pattern of transcriptional response, including lipid biosynthetic genes. In conclusion, sesamin proved not to be effective in increasing n-3 HUFA biosynthesis in common carp muscle.

4.2 Study II

The fillet lipid content in common carp was not affected by any dietary treatment over the course of the feeding trial and varied between 9 and 10% at the end of the trial. Replacing the OO or VO by FO finishing feeding resulted in fillets with clearly different FA profiles. The longer the fish were fed the finishing FO diet, the higher the levels of SFA, MUFA, n-3 PUFA and the lower the level of n-6 PUFA and the n-6/n-3 ratio in the group previously fed the VO diet. In the group previously fed the OO diet, the FO finishing treatment caused a lower level of MUFA, lower n-6/n-3 ratio and higher level of n-3 PUFA. PUFA composition showed differences in the content of EPA and DHA that were directly correlated to the length of the FO finishing feeding period. The percentage of EPA and that of DHA increased linearly with an

increasing time of fish oil consumption. The percentages of EPA and DHA were up to six-fold higher in FO groups compared with VO-only groups.
Even though the fillet FA composition changed significantly as a result of the dietary FA composition, the FA levels in the fillet did not match the levels in the feed supplied. The most obvious differences were seen in MUFA and PUFA levels. The levels of MUFA in the VO- and FO-only diets were 31% and 35%, respectively. The MUFA level was considerably higher in the fillet and varied between 47% and 54%. The PUFA contents in the VO- and FO-only diets were 57% and 38%, respectively, but the corresponding values in the fillet were significantly lower and varied from 20% to 34%.
At the end of the experiment, the measured 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 with the predicted values obtained using the dilution model designed by Robin *et al*. (2003). It was found that the dilution model gave very good predictions for the 10 most important FA or FA groups in common carp, with a slope of the regression line close to 1 (0.97, 0.99 and 1.00, respectively) and with R^2 values of 0.996, 0.993 and 0.992, respectively. Similar regression data were obtained for all the FA identified (data not shown).

4.3 Study III

After phase I, the fish reared in the three production systems had clearly distinct FA profiles. Fish supplemented by cereals (group 'C') had the highest fat content (8.7%) and were characterised by a high level of MUFA, especially oleic acid, and a low level of n-3 PUFA. Fish kept on natural feed only (group 'N') had a lower fat content (3.5%) and were characterised by a low level of MUFA and a high level of n-3 PUFA. Fish supplemented by rapeseed/linseed pellets (group 'P') had a similar fat content (7.3%) to group C and their FA profile was much closer to that of the fish kept on natural feed only (Figure 6).

During phase II, fish body weight decreased 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). There was a distinct reduction in fat content within all groups during the experiment. The largest decrease was measured in group C (-62%), followed by group P (-48%) and the lowest was in group N (-9%).

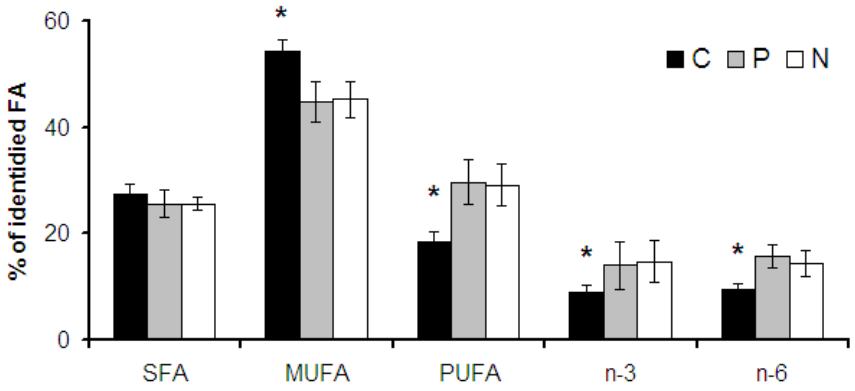

Figure 6. Fillet fatty acid in carp from three production systems. C, supplemented by cereals; P, supplemented by the rapeseed/linseed pellets; N, fed natural food (plankton benthos) only. (mean±SD; n=6; * indicates significant difference ($p < 0.05$) between the groups).

FA composition also changed with the length of the purging period. There were almost no changes in the proportion of SFA within the groups depending on time. MUFA decreased continuously in group C, from the initial 54.2±2.19 % to 46.9±4.49 % at the end. A decrease was also observed in group P, but was not significant. Almost no changes were observed in group N, where the proportion of MUFA varied between 45.2±3.41 % and 48.2±2.44 % during the whole purging period. The proportion of PUFA increased linearly in groups C and P, where the significantly highest proportion of PUFA was found at day 70 (26.9±5.72 % and 31±2.46 %, respectively). In contrast to the other groups, a trend of slightly decreasing PUFA was observed in group N (29.1±3.97 % at the beginning; 26.9±4.23 % at the end). The n-3 PUFA content followed the same trend as total PUFA. Its proportion increased continuously within groups C and P, while it remained unchanged in group N. Interestingly, there were almost no significant differences between the groups after 70 days of purging.

4.4 Study IV

Plasma lipids improved significantly in the group of human subjects in the secondary prevention study receiving carp compared with the control group. In brief, total cholesterol decreased by 27% in the treated group compared with 2% in the control group ($p<0.001$), LDL cholesterol decreased by 26% compared with 4% ($p<0.001$), plasma triglycerols decreased by 26% compared with 3% ($p<0.001$) and plasma HDL cholesterol increased by 30% compared with 10% in the control group ($p<0.001$) (Figure 7). The carp group also had significantly decreased glucose ($p<0.05$) and CRP ($p<0.01$) compared with the

control group. There were no differences in body mass index between the groups.

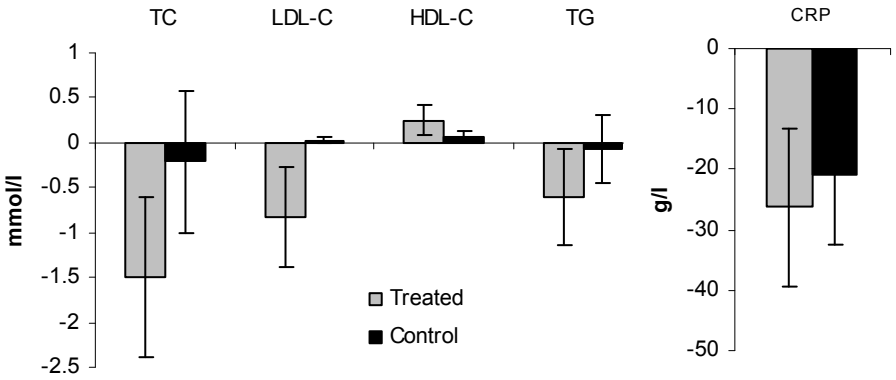

Figure 7. Changes of the plasma lipids and C-reactive protein after 4-week intervention with carp (difference between baseline and final values). TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerols; CRP, C-reactive protein.

5 General discussion

This thesis examined factors influencing lipid content and composition in common carp flesh and health effects of carp consumption on subjects after cardiovascular surgery.

5.1 Factors influencing lipid content and quality

Lipid content and composition of carp flesh are influenced by several environmental and internal factors. The environmental factors are *e.g.* diet, starvation and water temperature, and the internal factors are *e.g.* genetic background, size, age, sex, maturation. When the carp flesh is prepared as a meal for consumers, other factors further influence the final composition, such as the part of the fish used, processing and preparation (cooking, frying, use of additional fat).

As the results presented in this thesis show, there are several possibilities to further improve the lipid composition of cultured carp. High amounts of plankton in the pond, a good supplemental diet containing high levels of ALA and suitable processing and cooking with healthy ingredients were identified as the most important measures. Further improvements might be achieved in future by better understanding of HUFA biosynthesis, effects of genetic background and consequent selection of carp with higher ability for n-3 HUFA biosynthesis, as well as advances in the area of bioactive compounds.

5.2 Effects of dietary sesamin

The aim of the study I was to investigate whether a diet with sesamin had a positive effect on FA composition in common carp muscle, as previously reported for salmonids (Trattner *et al.*, 2008a; Trattner *et al.*, 2008b). It was found that dietary sesamin did not alter the FA composition of carp muscle.

Four possible explanations for this difference between salmonids and carp were proposed in Paper I:
i) There may be an evolutionary aspect. Salmonids, being predators, do not naturally consume this type of vegetable sources, whereas cyprinids as omnivores usually include such compounds in their diet. It has also been shown in mammals that there are species-dependent differences in the physiological response to dietary lignans (Kushiro *et al.*, 2004).
ii) HUFA biosynthesis may be suppressed by the n-3 HUFA content in experimental diets.
iii) Pure sesamin was used in Paper III, instead of an equimixture of sesamin/episesamin as used by Trattner *et al*. (2008a). Therefore, episesamin and sesamin might have different actions.
iiii) Rate of HUFA biosynthesis may differ depending on fish age and size, since sesamin can affect different life stages depending on their natural capacity.

In Paper I there was higher EROD activity and a higher content of total CYP P450 in carp with sesamin supplementation, similar to that reported in rainbow trout liver (Trattner *et al*. 2008a). This indicates that carp also recognise sesamin as a xenobiotic compound in contrary to that suggested above in i).

Transcriptomic profiling with a cDNA microarray showed that sesamin supplementation altered expression of 662 genes in carp liver. However, there was no viable pattern to the responding genes that might signal changes in lipid metabolism.

In conclusion, there were no alterations in HUFA biosynthesis in the experiment with carp described in Paper I. It would be interesting to investigate in more depth the mechanism of sesamin action and metabolism in salmonids, in which sesamin has positive effects, before attempting to study it in other fish species. In the future, it would also be interesting to identify and investigate other bioactive compounds which possibly influence n-3 HUFA biosynthesis.

5.3 Response to finishing feeding and prediction of FA changes

Paper II evaluated the response of common carp flesh lipids to a high fish oil finishing diet after a growth period in which different vegetable oils were used. It was found that the fillet FA composition reflected the FA composition of the diet and was significantly correlated to the length of the feeding period. This agrees with previous findings showing that it is possible to boost the content of

beneficial EPA and DHA in fish fillet by n-3 HUFA supplementation prior to harvest of common carp, Atlantic salmon and other species (Benedito-Palos *et al.*, 2009; Steffens & Wirth, 2007; Turchini *et al.*, 2006; Torstensen *et al.*, 2005; Bell *et al.*, 2004; Steffens, 1997).

The results in Paper II revealed that the simple dilution model proposed by Robin *et al*. (2003) gives 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 proposed by Robin *et al*. (2003) has its advantages and disadvantages depending on application. However, the model is clear in its simplicity and is therefore likely to be applicable for fish farmers, enabling production of high quality fish as well as minimising the use of expensive finishing feed.

The EFSA recommends a daily intake of 250 mg EPA+DHA per person (EFSA, 2009), or two servings of 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 in Paper II, 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. Reducing FO feeding to this shorter period would significantly reduce fish production costs and lead to more sustainable use of limited FO resources.

5.4 Effects of purging on lipid content and quality

The process of purging is characteristic and crucial in carp aquaculture in the conditions of Central Europe for two reasons. First, at least several days of purging are necessary to empty the gut and eliminate possible unpleasant odour and taste. Second, market carp in the Czech Republic are harvested from ponds during the autumn and then purged for several weeks before reaching the market in time for Christmas, as carp is the traditional Christmas Eve meal for most of the Czech population. It would be impossible to harvest all the market carp within 2 or 3 weeks before Christmas due to frosty weather and other practical problems.

In general, weight losses are observed in purged carp as a result of prolonged starvation. During the purging, fat content also decreases. This might be a positive effect in fish with excessive fat (>10%), which is common for fish produced on a low amount of natural feed in the pond and supplemented with cereals. On the other hand, too low a fat content (<5 %) has

a negative impact on sensory properties and lowers the amount of beneficial fish FA. In addition, the length of the purging period should be limited for economic reasons.

The FA composition also changes during purging. This change is caused by several factors including:

i) The change in fat content, which is connected to changes in the proportion of phospholipids and triacylglycerols. Phospholipids are rich in n-3 PUFA and n-3 HUFA, whereas triacylglycerols are rich in MUFA (Mráz & Pickova, 2009). Thus decreasing fat content causes a higher proportion of phospholipids and consequently higher levels of n-3 PUFA and n-3 HUFA and a lower level of MUFA in the flesh when expressed as a percentage of total FA.

ii) Selective utilisation of FA for energy needs. Fish selectively utilise dietary FA for β-oxidation. Kiessling & Kiessling (1993) showed that SAFA and MUFA are preferentially utilised by red muscle mitochondria in rainbow trout compared with PUFA. Thus MUFA are most likely preferentially utilised during purging also in common carp and the levels of n-3 PUFA and n-3 HUFA are consequently higher in the flesh when expressed as a percentage of total FA.

iii) Fish adjust their FA composition according to water temperature to ensure suitable fluidity of biological membranes. Paper III showed that until day 56 there was a significant increase in the relative content of stearidonic acid (18:4n-3) in all test groups, with 18:4n-3 being the first desaturation step product of long-chain PUFA synthesis from 18:3n-3. Between days 1 and 56, the proportion of 18:4n-3 increased about five-fold in all groups. It appears that Δ-6 desaturase is activated by decreasing the temperature and stimulating the synthesis of 18:4n-3 in carp. This metabolic addition of PUFA may help enhance the fluidity of biological membranes at low water temperatures. Similar results have been observed by Palmeri *et al.* (2008a) in a study with Murray cod (*Maccullochella peelii peelii*).

It is important to note that although the relative content of n-3 PUFA increased continuously during purging in Paper III and the flesh composition was therefore relatively improved, the real weight and fat content of the purged fish decreased. In addition, with prolonged purging and loss of surplus fat, the fish from all groups started to metabolise n-3 PUFA, leading to a decrease in their nutritional value. Therefore, is important for both economic and nutritional reasons to terminate purging at the right time in order to optimise the combined benefits. These results indicate that a purging period should be long enough to allow the elimination of possible unpleasant odour and taste,

but short enough from a practical handling point of view to preserve the beneficial FA composition of the n-3 enriched carp.

In Paper III the best compromise was achieved after 14 days, which corresponded to 220 °D (day degrees = sum of the average daily water temperature). One portion (200 g) of carp purged for 14 days contained 1.13 g n-3 PUFA and 316 mg EPA+DHA (group C); 1.54 g and 453 mg (group P); 0.95 g and 320 mg (group N).

5.5 Production of omega 3 carp

In this thesis, a system for production of so-called omega 3 carp (carp with increased content of n-3 FA) was developed and successfully tested. The rearing technology is based on semi-intensive culture of carp in ponds with maximum use of natural feed (plankton, benthos), in combination with affordable, environmentally sustainable and FA enhancement of the feed. The system consists of the following components:

i) The ponds chosen for omega 3 carp production are suitable in terms of productivity (at least 300 kg/ha; natural productivity of ponds in the Czech Republic is usually 200-500 kg/ha), dry overwintering and fertilisation with nutrients to support growth of natural plankton and benthos.
ii) In April, carp with approximate weight 1 kg are stocked in the pond in an appropriate stocking density to obtain 50% of yield from natural production (2-2.3-fold the natural productivity of the pond).
iii) The carp diet is supplemented by pellets based on rapeseed cake and extruded linseed as a lipid source (Mráz *et al.*, 2011a).
iv) The ponds are harvested in the period October-November and the carp are purged for 2-4 weeks according to water temperature in order to empty the gut, eliminate possible unpleasant odour and taste and improve the lipid profile.
v) The fish are filleted and, if necessary, excess fat on the abdominal wall is removed.

Fillets of omega 3 carp are characterised by a lower level of MUFA, higher level of n-3 PUFA and n-3 HUFA and lower n-6/n-3 ratio than fillets from carp supplemented by cereals (Mráz & Pickova, 2011). One serving (200 g) of fillet from omega 3 carp contains approximately 300 mg EPA+DHA and 1 g n-3 FA, which is very close to the recommended values.

The feeding formula developed here is now protected by utility pattern no. 21926 (Mráz *et al.*, 2011a) and the rearing technology by Czech national patent no. 302744 (Mráz *et al.*, 2011b). The omega 3 carp are already available on the market in the Czech Republic under a specific trademark (Figure 8).

Figure 8. Trademark used in Czech Republic for carp with an increased content of n-3 FA.

5.6 Effects of carp consumption on human health

To the best of my knowledge, Paper IV was the first study to investigate the effects of common carp consumption on human health. The results showed that consumption of common carp flesh with increased content of n-3 FA had positive effects on plasma lipids and inflammation markers (CRP) in subjects recovering from heart surgery. This indicates in turn that carp consumption is beneficial for the central European population in general.

The improvement of plasma lipid parameters (decrease in total cholesterol, LDL cholesterol and triglycerols and increase in HDL cholesterol) after consumption of carp flesh observed in Paper IV is in agreement with published studies on sea fish (Balk *et al.*, 2006). In Paper IV there was a significant decrease in CRP in the blood of recovering heart patients, which might have resulted from the anti-inflammatory effects of omega 3 FA (Al-Khalifa *et al.*, 2007). The carp flesh used in Paper IV had a relatively low amount of n-3 HUFA in relation to the high effects observed on plasma lipid parameters.

It is possible that it was not only lipid quality that was responsible for the change in lifestyle-related parameters observed in Paper IV. The subjects replaced meat with fish, which might be one contributing factor, as many studies have shown that fish protein has some beneficial properties with the same type of effects (Wergedahl *et al.*, 2004).

Fish proteins have been shown to have several positive effects on different disorders and parameters of metabolic syndrome. Among the most important findings observed are increased insulin sensitivity, anti-inflammatory effects, and prevention of type 2 diabetes and obesity (Lavigne *et al.*, 2001).

Cod protein has been shown to protect against development of obesity-linked insulin resistance and glucose intolerance in mice (Lavigne *et al.*, 2001; Lavigne *et al.*, 2000). Similar effects have been observed in human trials (Ouellet *et al.*, 2008; Ouellet *et al.*, 2007; von Post-Skagegård *et al.*, 2006). In addition, a decrease in the level of CRP, which is increased in insulin resistance (Chou *et al.*, 2010), has been reported (Ouellet *et al.*, 2008; Ouellet *et al.*, 2007).

The mechanisms underlying these effects remain to be investigated. High levels of specific amino acids with a low level of branched amino acids, such as taurine and arginine, might be one possible explanation. Salmon calcitonin, the bioactive part of fish protein, has been shown to protect against osteoporosis (Chesnut Iii *et al.*, 2008) and is a homologue of amylin, a hormone involved in regulation of satiation and energy expenditure (Osaka *et al.*, 2008).

In a recent study, Pilon *et al.* (2011) compared the effects of proteins from different fish species (bonito, herring, mackerel and salmon) included in a high fat, high sucrose diet fed to rats. They found that proteins from all fish species showed anti-inflammatory action through tumour necrosis factor-α and interleukin-6 compared with a casein diet. In addition, the group fed salmon protein had lower weight gain and reduced fat in epididymal white adipose tissue. This suggests that there might be specific effects linked to proteins from different fish species.

This leads to the conclusion that carp can be of major importance in combating metabolic disorders in many populations in central continental regions without access to the sea, as carp can be produced world-wide in large quantities. However, more studies are needed with both animal studies and intervention studies on human subjects to confirm this.

5.7 Ongoing work related to carp n-3 HUFA biosynthesis

We believe that by understanding the regulation of HUFA biosynthesis in carp, it would be possible to identify tools for fully exploiting the internal ability of carp to convert ALA to n-3 HUFA by means of a balanced diet, inclusion of bioactive compounds in the diet, selection of more efficient lines, *etc*. The genome of common carp has not yet been sequenced/published and the majority of genes related to n-3 HUFA biosynthesis have not yet been

annotated/characterised. Most of the molecular work related to HUFA biosynthesis in fish so far been has been performed on Atlantic salmon and zebrafish.

We aimed to find carp HUFA biosynthetic genes in the available expressed sequence tags in GenBank by sequence similarity to zebra fish and to study how the expression of these genes is affected by dietary FA. Therefore trial described in study II was used to analyse gene expression related to different oils in the diet.

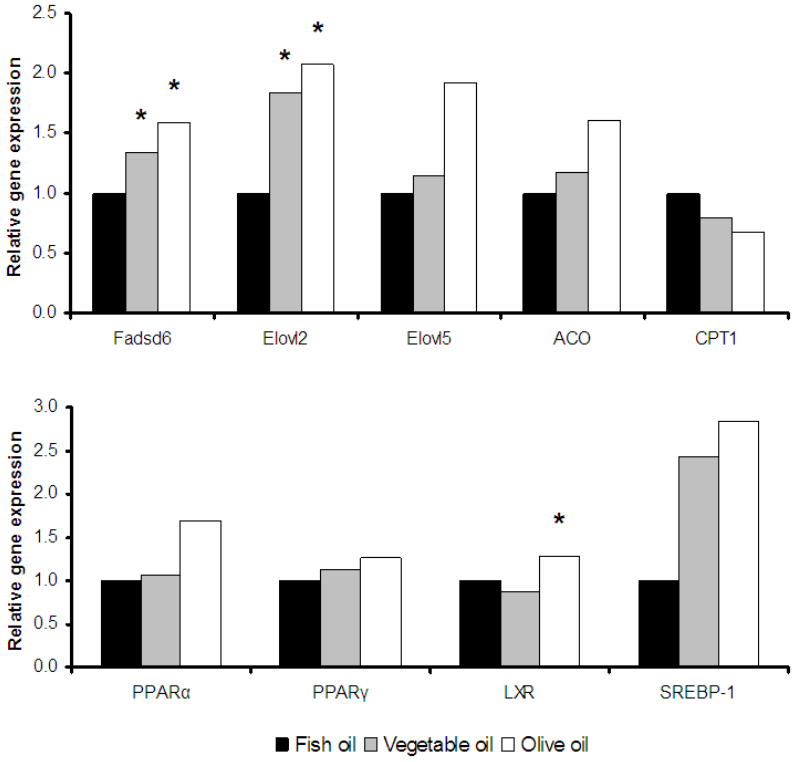

Figure 9. Gene expression in hepatopancreas of common carp fed one of three diets with different lipid sources (fish oil, vegetable oil (rapeseed/linseed blend), olive oil). Relative gene expression was calculated according to the $2^{-\Delta\Delta Ct}$ method using S40 as reference gene and normalised against the fish oil group. *significant *p*-value in comparison with the fish oil group (n=6). Abbreviations: Fadsd6 = delta-6 fatty acyl desaturase, ELOVL = elongation of very long chain fatty acid-like, ACO = acyl-CoA oxidase, CPT1 = carnitine palmitoyl transferase I, PPAR = peroxisome proliferator-activated receptor, LXR = liver X receptor, SREBP-1 = sterol regulatory element binding protein-1.

Melting curve analyses confirmed that all primers were specific for one target only. The results showed that replacement of fish oil by a mixture of vegetable oils (rapeseed/linseed) and olive oil, respectively, significantly increased expression of Fadsd6 and Elovl2, which are genes involved in HUFA biosynthesis (Figure 9). A similar trend for Elovl5 was observed in the olive oil group, but was not statistically significant. Expression of liver X receptor was slightly but significantly increased in the olive oil group compared with the fish oil group. There was a trend for increased expression of SREBP-1 in the vegetable and olive oil groups compared with the fish oil group, but it was not statistically significant. In conclusion, excessive amounts of HUFA (fish oil group) in the diet of carp decreased expression of genes involved in HUFA biosynthesis, confirming findings in other fish species.

These results are promising, although some uncertainty exists in relation to gene sequences in common carp and thus further verification is required.

The unknown factors behind the different responses and discrepancies between gene expression and FA synthesis in studies by Trattner *et al.* (2008a, 2008b), Schiller Vestergren *et al.* (2011, 2012) and Vestergren *et al.* (2012) and those presented above for carp need further examination. In a first step, we aim to study whether small RNAs are part of the unknown regulation. It is likely that miRNAs and other small RNAs play a similar critical role in more or less all stages of uptake, transport, elongation, desaturation, β-oxidation and composition of n-3 and n-6 lipids in freshwater fish. However, to the best of our knowledge, no studies have been conducted to date on miRNA regulation of HUFA biosynthesis in carp.

6 Conclusions

Sesamin supplementation had no impact on fish weight, specific growth rate, food conversion ratio and lipid content. In addition, sesamin did not positively alter the FA composition of carp muscle. Sesamin increased total cytochrome P450 content in preparation of hepatopancreatic microsomes, as well as EROD activity. The transcriptomic profiling with cDNA microarray showed that sesamin supplementation altered expression of 662 genes in carp liver. However, there was no viable pattern in the responding genes that might signal changes in lipid metabolism. Overall, sesamin was ineffective as a means of changing the tissue lipid composition in common carp.

In general, common carp muscle has a favourable FA composition and should be regarded as a healthy product. There are several ways in which the lipid composition of cultured carp can be further improved, the most important being high amounts of natural food, a good supplemental fish diet containing high levels of ALA and suitable processing and cooking with healthy ingredients. Further improvement might be achieved in future through better understanding of HUFA biosynthesis and effects of genetic background and consequently selection of carp with higher ability for n-3 HUFA biosynthesis, and also through development of bioactive feed compounds.

A simple dilution model can provide an excellent description of changes in the fillet FA composition of common carp fed mainly vegetable oil with a finishing period of fish oil. Therefore the model can be used for calculating the finishing feeding period required to achieve a specific fillet FA composition in terms of *e.g.* EPA and DHA content, while saving fish oil resources.

Lipid analyses on purged common carp reared in three different production systems showed that the type of diet prior to purging significantly affected carp flesh quality. Purging time was also important, mainly for economic reasons. Supplementation with rapeseed/linseed pellets in the growing period and purging for no longer than 14 days (or 220 °D) resulted in a nutritionally beneficial FA composition combined with an economically acceptable weight loss. The physiological processes in lipids of carp during purging need to be investigated in terms of amount and composition of FA in individual lipid classes, especially triacylglycerols and phospholipids.

Consumption of common carp with an increased content of n-3 FA significantly improved plasma lipid parameters in patients recovering from major cardiac revascularisation surgery. Carp lipid quality was responsible for part of the change observed in lifestyle related parameters, and replacing meat with fish was probably another contributing factor. Culture of common carp should therefore be recognised as long-term sustainable and carp should be promoted as a healthy local product.

7 Future research

This thesis evaluated factors influencing lipid content and composition in common carp muscle. The results presented here form the first step to developing long-term sustainable culture of common carp with improved flesh lipid quality, which can be important in the prevention and treatment of cardiovascular disease. Some specific areas for future research are:

- Fatty acid metabolism in common carp
- The effects of minor lipid soluble bioactive compounds on fish welfare, metabolism and quality
- The effects of genetics and heritability on lipid quality in common carp
- The storage stability of carp flesh with increased content of n-3 FA
- Other health-beneficial compounds such as specific peptides, proteins and amines and other small compounds in fish flesh, especially that of freshwater fish.

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