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Factors influencing nutritional value of fish

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. Nutritional value of fish and the main factors affecting it

High nutritional value of fish and fish products is due to the high amounts of protein, lipids particularly high content of omega-3 long chain polyunsaturated fatty acids (n-3 LC PUFA) as well as essential micronutrients compared to the land living animals (Tacon and Metian, 2013). Beneficial effects associated with these essential fatty acids (FAs) have been reported to decrease the overall risk of cardiovascular diseases (Chin and Dart, 1995), effects on hypertension (Bonaa et al., 1990), inflammation (Simpoulos, 2002), lowering serum triacylglycerol levels and reducing thrombosis (Von Schacky et al., 1985). The role of docosahexaenoic acid (DHA, C22:6n-3) for the growth and development of infant's brain as well as their importance for the functionality and maintenance of normal brain in adults have been well discussed (Horrocks and Yeo, 1999) which is more detailed and addressed in the review of the nutritional value of fish provided by Khalili Tilami and Sampels (2018). Due to the beneficial effects of consumption of n-3 LC-PUFA, especially eicosapentaenoic acid (EPA, C20:5n-3) and DHA for human health (Mozaffarian and Rimm, 2006; Pourashouri et al., 2014), interest in the intake and enrichment of EPA and DHA of commonly consumed food have been increased (Kaushik et al., 2014). Since farmed fish are raised under controlled conditions from fertilization until slaughter, it is somehow possible to regulate the factors like environment and nutrition throughout their life and thereby also the muscle FA composition. A number of factors can influence the composition of fish flesh. Every step in the history of the fish, from the way of production, fish rearing system and processing can have a great influence on the quality of the final product. In general, factors which can have an effect on lipid content and composition in fish as a great component providing the valuable omega-3 FAs can be divided into the feeding, species, reproductive status, size or (age), water temperature, salinity and season (Henderson and Tocher, 1987; Ackman 1989; Saito et al., 1999; Alasalvar et al., 2002; Khalili Tilami and Sampels, 2018).

1.1.1. Feeding and nutritional value

Under intensive culture, feeding regimen and feed composition have a major influence (Lie, 2001) especially on the lipid content and the FA composition (Henderson and Tocher, 1987; Morris, 2001; Shearer, 2001) which is well-addressed by Khalili Tilami and Sampels (2018). In contrary, as long as fish are fed adequate diets containing all their requirements in the sufficient amounts, the protein content and composition seem to be predetermined regardless of the diet content or the feeding regimen (Morris, 2001; Shearer, 2001). Besides the feeding, handling after the harvest, transport, possible storage or purging of the fish and the slaughter methods (Erikson, 2001; Robb, 2001) are important for the final product quality and can have an effect on lipid content and composition. Fish fed with a diet containing mainly plant protein sources, reduces the protein retention (Daniel, 2018). The reason could be due to the lack of essential amino acids in the plant proteins (Richard et al., 2011; Berge et al., 1998) or deficiency in liver metabolic adaptation to higher levels of plant proteins (Panserat et al., 2009). One other reason could be because plant proteins contain anti-nutritional factors such as protease inhibitors and saponins that reduces the digestion and absorption of nutrients. Utilization of plant proteins without amino acid supplementation (e.g. methionine or lysine) can increase the feed conversion ratio in fish (Berge et al., 1998). Currently, fish meal is still the primary source of protein for farmed fish (Tacon et al., 2011). As a result of fish meal replacement with plant proteins, decline in the protein biosynthesis in fish (e.g. rainbow trout) was observed (Panserat et al., 2008). By developing new feeding strategies, more than 50% of the fat of the feed can be substituted by vegetable oils during

the main period of growth. For instance, replacement of rapeseed oil instead of fish oil (Bell et al., 2003) does not reduce the n-3 HUFA content in farmed fish as compared to the wild fish (Pike and Jackson, 2010). Variation in energy value of different fish species is related to the differences in fat content (Bogard et al., 2015). Carp diet supplemented with cereals which are rich in carbohydrate, has higher lipid content, lower amount of PUFA compared to wild one (Csengeri, 1996). It has been reported that moderate dietary carbohydrate level has positive effect on the growth of carnivorous fish (Hemre et al., 2002) whereas the excess amount of it has adverse effect, resulting in increased level of glycogen, lipid deposition in the liver and higher HIS (Tan et al., 2009, Ren et al., 2011).

1.1.2. Species and nutritional value

Differences in muscle lipid content within fish species is noticeable which can cause differences in FA composition (Fontagné-Dicharry et al., 2010) which is well-addressed by Khalili Tilami and Sampels (2018). The amount or percentage of FAs for instance EPA and DHA are variable among and within a species which can indicate the great role of environment including the rearing condition of fish in the wild or farm (Kris-Etherton et al., 2002) or even between cage-reared and tank-reared fish fed with the same diet (Martelli et al., 2013), in this case differences in FAs might be due to the stocking density (Piccolo et al., 2008) or water and seasonal differences. In bottom dwelling species (as a typical lean fish), fat is stored in the liver. However, migratory species have a higher content of dark muscle rich in fat (Alam et al., 2012).

1.1.3. Reproductive status and nutritional value

During the maturation, along with the accumulation of lipids in the gonads, changes in some FAs can happen (Pérez et al., 2007). In addition to the other functions of PUFA, their important role for the reproductive performance have been investigated. Based on Mazorra et al. (2003) and Jerez et al. (2016) findings, during the reproductive cycle, FAs are metabolized in different way either catabolized for energy or being stored in gonads in the purpose of formation of the membrane or eicosanoid synthesis. In many fish species, arachidonic acid (ARA, 20:4n-6) plays an important role for the successful reproduction (Tocher, 2010). This FA is the main precursor for the 2-series prostaglandins (PG-2), eicosanoids which influence the sexual behavior of female fish in many species in line with stimulating steroid synthesis in the ovary and trigger oocyte maturation (Mercure and Van der Kraak, 1995; Tocher, 2003). Ng and Wang (2011) suggest that high percentage of saturated fatty acid (SFA) in tilapia gonads is essential for the success in their reproduction.

High levels of PUFA in the ovary of both marine and freshwater fish have been reported (Izquierdo et al., 2001). Fish ovaries have the ability to generate eicosanoids from arachidonic acid (ARA, C20:4n-6) (including prostaglandins PGE_2 and $PGF_{2\alpha}$) or from EPA (prostaglandins PGE_1 and PGE_3), which are essential for the metabolism of that tissue in the final maturation phase (Sargent et al., 2002). Based on findings by Sorbera et al. (2001) and Bell and Sargent (2003) on the oocyte of European sea bass (*Dicentrarchus labrax*), eicosanoids generated from ARA (PGE_2 and $PGF2_{\alpha}$) are responsible for regulating oocyte maturation, vitellogenesis and ovulation.

Developmental stage in salmonids, smoltification (parr-smolt transformation):

What makes the salmonids different from the other anadromous species is the smolt age (Stefansson et al., 2008). In salmonids, the juvenile salmon undergo adaptations and changes in morphology, physiology and behavior in fresh water before experiencing the saltwater which is called smoltification. During smoltification, alterations in lipid metabolism, a decrease in the proportion of triacylglyceride (TAG) and also in lipid content in muscle, liver, gut and gills even in the situation of presence of excess food occur (Wendt and Saunders 1973; Sheridan, 1989; Li and Yamada 1992). Desmolt contain more monounsaturated fatty acids (MUFA) and SFA and less n-3 PUFA such as DHA in their tissue lipids compared to the smolts in freshwater (Li and Yamada 1992). Salmonid smolts initiate the alteration in their tissue FA composition and metabolism which includes an increase in the activity of FA elongase and desaturase of isolated hepatocytes beside the increase in the ratio of C20, C22, C18 n-3, C18 n-6 FAs (Tocher et al., 2000). Furthermore, differences in FA composition in freshwater fish from marine fish were observed; while freshwater organisms are rich in 18:2n-6, 18:3n-3, and generally contain less n-3 highly unsaturated fatty acids (HUFA) due to the food chain composition in the environments, marine fish have higher proportions of n-3 HUFA including 20:5n-3, 22:6n-3 which naturally exist in the algae as primary producers of food web (Li and Yamada 1992, Sargent et al., 1999).

1.1.4. Water salinity, temperature and season

Influence of water salinity, temperature and seasonal changes on biochemical contents and FA profile of fish is known (Ackman, 1995; Leger et al., 1977; Bandarra et al., 1997; Farkas, 1984; Fonseca-Madrigal et al., 2012). According to Farkas (1984) and Haliloğlu et al. (2004), at low water temperatures, fish need PUFAs particularly DHA in order to tolerate the condition. Thus the main changes in the FA composition is an increase in DHA percentage at lower temperatures, therefore, higher amount of PUFA are to be expected in the fish living in the cold water. In many poikilotherms, with increase in the temperature, a decrease in the content of unsaturated FA was observed (Farkas, 1984). Also, Jobling and Bendiksen (2003), showed that lower water temperatures in general result in increased proportions of unsaturated FA and lower amount of SFA. More recently, Norambuena et al. (2016) indicated that water temperature obviously affected FA composition in salmon reared at 10 °C versus 20 °C, where fish kept at lower temperature showed higher contents of n-6 FA in fillets. The same author also reported a decreased bioconversion from ALA to EPA and DHA at the increased temperature. Seasonal changes on fish muscle FA composition from temperate water is accompanied by seasonal depletion in MUFA specifically in oleic acid (18:1n-9) somehow associated with mobilization through gonadal development stage (Sargent, 1995; Özyurt and Polat, 2006). During the critical period of winter season in temperate waters, fish may undergo depletion of lipid reserves, increase in the risk of disease due to the low temperature, less availability in food (Wedemeyer et al., 1976; Tort et al., 1998).

1.2. Fish consumption in the Czech republic, the importance of knowing the composition of minor species captured by anglers

Based on the FAO (2016) data, annual fish supply around the world was 20 kg per capita in 2014. Compared to this statistics, in landlocked countries such as in Central Europe much lower average consumption have been shown. For instance in the Czech Republic fish intake was around 5.5 kg per capita in 2008 (MZe, 2009). Simultaneously, in Central Europe, particularly in the Czech Republic, a significant percentage of consumed fish is provided by anglers (catching of fish by traditional angling; angle with one hook attached to the fishing line) and includes fish species, which gain less attention in terms of their nutritional value for human consumption in spite of their importance for a certain part of the population in these countries. Thus, there is an urgent need to evaluate and map the composition of the less noticed species that are caught and consumed by anglers.

1.3. Importance of aquaculture to provide fish and fish products requirements of the growing population

The role of aquaculture as one of the most rapidly growing sector in the world food economy is not neglectable. The importance of fish as major source of animal-based protein for the diet of almost 950 million people all around the world (UNEP 2001) is evident. According to the FAO reports, aquaculture production increased to 8.5% within last 25 years and can cover approximately half of produced fish for human consumption (FAO, 2014). In the 1980s, the origin of most of the feed resources for cultivation of especially carnivorous fish was from the captured pelagic bait fish (Olsen, 2011). Over the last decade, there has been great changes in the usage of pelagic sources with an increasing tendency towards greater use of plant sources from agriculture for aquafeeds, due to the limited availability of marine feed sources and lower production costs in connection with the plant sources (Gatlin et al., 2007; Naylor et al., 2009). In addition, as a result of increasing captures in fisheries, the risk of overfishing and depletion of the stock in world-wide scale increased.

1.3.1. Fish meal and fish oil

The importance of marine fish meal (FM) and fish oil (FO) as major ingredients of the commercial aquafeeds for providing the nutritional requirements for the cultured fish species both regarding protein and lipids is remarkable (FAO, 2007; Turchini et al., 2010; Blomqvist et al., 2018). Annual production of FM and FO has remained stable in the last 20 years around 6 million tons and 1 million tons respectively (Lehane, 2013). Global aquaculture industry cannot continue to rely on FM and FO due to the rapid growth in aquaculture, high demand, decrease in availability and limited supplies (FAO, 2007) and there is an urgent need to find novel, sustainable sources for protein and lipids in fish feeds. For highest sustainability these sources should preferably not compete with the already existing foods and food products e.g. plant oils or plant proteins, that could be used directly for human consumption. Hence, there is a need for novel sources from the non-food sector. Related to this there is also a growing demand for providing the nutritional requirements of farmed fish for human consumption.

1.4. Utilization of the alternative sources for FM and FO in fish diets

Another part of the sustainability question is a sustainable economy. The major costs in aquaculture accounts for the feed (Brett, 1979), as for example in case of carnivorous species high amount of expensive FM is required in their diet. Due to the high price of FM protein beside the obvious decline in the sources of FM (Manzano-Agugliaro et al., 2012) using alternative sources such as plant protein sources like soybean and rapeseed meal (Quartararo et al., 1998; Bureau Harris and Cho, 1999; Gatlin et al., 2007; Hardy, 2010; Médale et al., 2013; Fawole et al., 2016) and animal protein sources including poultry by-products meal, blood and bone meal have been evaluated (Bureau et al., 1999; Rawles et al., 2006). In addition, a great amount of FO is needed for the aquaculture feed to provide required PUFAs including EPA and DHA and the energy for growth, reproduction and metabolism (Blomqvist et al., 2018).

For the above-mentioned reasons, more recently, the use of more sustainable alternatives like insects in the form of live, frozen or meal has received more attention (Henry et al., 2015; Ngoc et al., 2016) since they have potential to feed on bio-waste in addition to their quick growth and high feed conversion (Collavo et al., 2005). Furthermore, they seem advantageous as they are originally consumed as part of natural diet of marine and freshwater fish (Howe et al., 2014) and because they are rich source of amino acids, lipids, vitamins and minerals (Van Huis, 2013). Microbial products, particularly yeast are sustainable ingredient in aquafeeds since these products have the potential to convert low-value biomass from agriculture and forestry into high-value feeds with limited dependence on the changes of climate and water (Øverland et al., 2013). Several studies focused on the use of yeast proteins as an alternative for fish meal due to the high amount of crude protein and good production rate (Sanderson and Jolly, 1994; Tacon, 1994; Ferreira et al., 2010).

1.5. The aim of the thesis

The overall aim of this thesis was to evaluate factors influencing the nutritional value of fish and to map nutritional composition of less known fresh water species including European grayling (*Thymallus thymallus*), common nase (*Chondrostoma nasus*), brown trout (*Salmo trutta morpha fario*), common bream (*Abramis brama*), Prussian carp (*Carassius gibelio*), European perch (*Perca fluviatilis*) and European chub (*Squalius cephalus*).

A secondary aim was to develop and examines the effect of partial replacement of novel feed ingredients for replacement of FM and VO in the feed of carnivorous fish as an important strategy to reach a sustainable aquaculture, reduce the expenses of production and optimize feeding strategies. In one study, we examined the inclusion of oil derived from an oleaginous yeast which was grown on lignocellulose hydrolysate (from wheat straw) to replace VO in fish feed (Blomqvist et al., 2018). In two another works inclusion of insect meal as an alternative protein source instead of FM in the diet of European perch and rainbow trout has been investigated.

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CHAPTER 2

NUTRITIONAL VALUE OF FISH: LIPIDS, PROTEINS, VITAMINS, AND MINERALS

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Nutritional Value of Fish: Lipids, Proteins, Vitamins, and Minerals

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ABSTRACT

The present review aims to give a concise review about important nutrients from fish and their impact on human health. In addition, possible effects of rearing system and feeding on the most vulnerable group of nutrients, the lipids, are summarized.

Fish are considered as nutritionally valuable part of the human diet and consumption two times a week is recommended, mostly due to the content of long chain polyunsaturated n-3 fatty acids. These fatty acids are essential in human nutrition and have proven to be involved in many metabolic functions. Among others, they have anti-inflammatory effects, decrease platelet aggregation and are essential parts in the cell membranes, cardiovascular system, brain, and nervous tissue.

In addition the proteins, peptides and amino acids from fish became more recently known for having positive health effects. Furthermore fish is also a rich source of certain vitamins and minerals as Vitamin D, selenium, phosphorus, and calcium.

It should be highlighted that, when considering nutrition and related health aspects, it is impossible to focus one group of nutrients separately. Most probably the discussed effects of fish on human health are due to the consumption of the fish as a whole and hence the combination of all present nutrients.

KEYWORDS

Calcium; cholecalciferol; n-3 fatty acids; novel feed sources; rearing system

Introduction

Fish and seafood products, have a high nutritional value regarding beneficial amounts of protein, lipids as well as essential micronutrients. Aquatic animal foods are a rich source of protein and have a lower caloric density, and have a high content of omega 3 long chain polyunsaturated fatty acids (n-3 LC PUFA) compared to land living animals (Tacon and Metian, 2013). Strong links between fish and seafood consumption and positive health effects, especially with the decreased risk of coronary heart and cardiovascular diseases, decreased inflammatory disease as arthritis and prevention of cancer have been shown by many researchers (Dyerberg, 1985; Calder, 2004; Rudkowska et al.; 2010; Lund, 2013). Historically the main effects of fish consumption have been attributed to the high content of n-3 LC PUFA. But research is proving more and more, that also other nutrients from fish have positive effects on human health. In addition of being the major source of n-3 LC PUFA, fish and other seafood have also a well-balanced amino acid composition, contain high proportions of taurine and choline, the vitamins D_3 and B_{12} and the minerals calcium, phosphorus, iodine, and selenium. Furthermore, fish and seafood also might provide significant proportions of vitamin A, iron, and zinc to a population if other sources of these nutrients are scarce (Lund, 2013).

Omega-3 fatty acids in fish and lipids in human nutrition

In pre-agricultural times, the foods available to humans were game meat, fish, shellfish, green leafy vegetables, fruits, berries, honey, and nuts (Simopoulos, 2003). This diet, containing higher amounts of n-3 PUFA and lower amounts of n-6 PUFA than modern diets, shaped the genetics of human nutrition. After the agricultural revolution though, intake of cereals increased enormously. Cereals are rich in n-6 PUFA and low in n-3 PUFA and, as a consequence, the n-6/n-3 PUFA balance to which humans are adapted has changed dramatically over the last 10,000 years (Simopoulos, 2002a). Human genetics however could not keep pace with such a fast change in dietary habits, since the spontaneous mutation rate for nuclear DNA is estimated to be 0.5% per million years

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(Simopoulos, 2003). We are therefore (still) adapted to much higher intake of n-3 PUFA in our diet than we actually consume today. In today's Western diets, this ratio is 15 to 20, while it is estimated to have been close to 1 during human evolution (Simopoulos, 2001, 2002b).

A diet rich in PUFA, especially the LC n-3 fatty acids (FA) (≥C20), has been shown to have beneficial effects on human health (Williams, 2000). The n-3 LC PUFA are important for example in the prevention of arteriosclerosis and autoimmune diseases (Kinsella, 1988; Simopoulos, 1999). Eicosanoids synthesized from n-3 PUFA have immunosuppressive properties (Calder, 2001), while the eicosanoids from n-6 PUFA have pro-inflammatory properties and enhance immune reactions like fever and pain (Calder, 2001). A too high intake of n-6 PUFA, is therefore associated with adverse effects on human health, as for example cardiovascular diseases, and diabetes as well as hypertension, depression, neurological dysfunction, and immune disorders (Connor, 2000; Williams, 2000). Also during pregnancy and the neonatal period an optimal diet containing an appropriate amount of the essential LC n-3 PUFA is necessary for neural development of children. The retina and brain of mammals is in general very rich in docosahexaenoic acid, 22:6n-3 (DHA), and the nervous system of newborns has a large demand for it (Lauritzen et al., 2001). It is well established that the maintenance of optimal pre- and postnatal growth and development requires n-3 PUFA (Innis, 1991; Innis et al., 1999).

Mammals are not able to synthesize n-3 or n-6 PUFA in the body (Innis, 1991) but can to a minor amount metabolize the longer chain PUFA from the parental FA α-linolenic acid, 18:3 n-3 (ALA), and linoleic acid, 18:2 n-6 (LA), (Gerster, 1998; Arts et al., 2001). The desaturase and elongase systems for the metabolism of the parental n-3 and n-6 PUFA ALA and LA are the same for both n-3 and n-6 PUFA (De Henauw et al., 2007; Palmquist, 2009). Even if delta 6 desaturase has a higher affinity for ALA than to LA, due to the much higher dietary intake LA has been suggested to limit the conversion of ALA to EPA and DHA (Palmquist, 2009). Considering the metabolic competition between n-6 and n-3 PUFA (Palmquist, 2009) and their opposing properties (Schmitz and Ecker, 2008), it is generally assumed that the intake of n-6 FA is too high in the present diet. An intake ratio of 1 to 4 is generally recommended (Simopoulos, 2001, 2002b). For this reason, a more balanced intake of n-6 and n-3 PUFA is important. Due to this, a daily intake of eicosapentaenoic acid (EPA, 20:5 n-3) and DHA of at least 0.22g each has been suggested as adequate for adults (Simopoulos, 2002b) and many countries have set up their own recommendations for the daily intake of EPA and DHA (Givens and Gibbs, 2008). This makes it important to include sources rich in n-3 PUFA in the daily diet. Oily fish for example, contain high amounts of n-3 LC PUFA, and are therefore a good source for these. Besides, the European Food Safety Authority (EFSA) approved several health claims related to the consumption of fish or EPA and DHA, as for example the maintenance of normal level of blood triacylglycerols, normal brain function and vision, cardiac function and blood pressure (EFSA Panel on Dietetic Products, 2010). European Food Safety Authority has also proposed FA reference labeling intake values for the general population: 250 mg EPA+DHA; 2 g ALA and 10 g of LA per day (EFSA, 2009). Furthermore it was concluded, that a fish consumption of 1 to 2 servings per week could be protective against coronary hearth diseases and ischemic stroke (FAO & WHO, 2011), to reverse the increase in the western world.

Proteins in fish

Fish protein has since long been considered having a high nutritional value (Sargent, 1997). Aquatic animal foods have a higher protein content than most terrestrial meats. In addition aquatic protein is highly digestible and rich in several peptides and essential amino acids that are limited in terrestrial meat proteins, as for example methionine and lysine as suggested by Tacon and Metian (2013).

Nonetheless, only in the last decade, research has also focused on the beneficial health effects of fish protein in human nutrition (Rudkowska et al., 2010; Pilon et al., 2011). Even if this research is still in its beginning, studies related to inflammation, metabolic syndrome, osteoporosis, insulin resistance, obesity-related comorbidity and development of cancer have been executed and fish protein, peptides or hydrolysates have shown of importance in nearly as many areas as fish lipids. For example, a sardine protein diet showed to lower insulin resistance, leptin and TNFα, improved hyperglycemia and decreased adipose tissue oxidative stress in rats with induced metabolic syndrome (Madani et al., 2012). The authors suggested dietary sardine protein as a possible prophylaxis against insulin resistance.

Furthermore, fish protein hydrolysates are considered as superior from a nutritional point of view due to the excellent amino acid composition and easily digestible proteins. But, due to the undesirable fishy odor and flavor they have been earlier mostly used in animal nutrition (Kristinsson and Rasco, 2000; Chalamaiah et al., 2012). It has been shown in human macrophages that fish protein hydrolysates decreased tumor necrosis factor α (TNFα) compared to casein hydrolysates. In the same study the combination of n-3 PUFA with fish protein

hydrolysates synergistically decreased expression levels of (TNFα) compared to fish protein hydrolysates or n-3 treatment only (Rudkowska et al., 2010). The same authors suggested that part of the beneficial effect of fish protein hydrolysates compared to casein hydrolysates could be due to the higher content of arginine in fish protein. Arginine has shown to limit the production of superoxide anions by nitric oxide synthase (iNOS). In addition, the higher content of glycine in the fish protein hydrolysates could be beneficial, as glycine has shown to repress the expression of TNFα and the pro-inflammatory interleukin-6 (IL6) in various cell cultures (Rudkowska et al., 2010). The exact mechanisms are yet unclear, however the authors suggested, it might be by activation of the peroxisome proliferator-activated factor γ (PPAR γ), which is also important in lipid metabolism. The third factor could be taurine, which is an amino acid by-product also highly found in fish, which has shown to also suppress production of TNFα, IL6, interleukin-1β (IL-1β) and iNOS (Rudkowska et al., 2010; Lund, 2013).

In general it seems that these above mentioned amino acids and taurine in fish have similar anti-inflammatory effects as the long chain n-3 PUFA. Moreover, some other amino acids and particularly taurine, may play an important role in the beneficial effects of fish protein especially of oily fish including sardines, by for example, limiting the complications of type 2 diabetes and decreasing glucose, insulin and insulin resistance (Madani et al., 2012). On the other hand, Balfego et al. (2016) showed inclusion of 100 g of sardines 5 days a week into the standard diet for type 2 diabetes in a period of 6 months, did not have effect on glycemic control but had lowering effects on cardiovascular risk.

Furthermore proteins from various fish as bonito, salmon, mackerel, herring and cod have shown anti-inflammatory properties while salmon and cod protein in addition improved insulin sensitivity in rats (Lavigne et al., 2001; Ouellet et al., 2007; Pilon et al., 2011). Dort et al. (2012) found cod protein to better promote growth and regeneration of skeletal muscle after trauma compared to peanut protein and casein and suggested this also to be partly because of the improved resolution of inflammation by cod protein. Salmon calcitonin, a 32-amino acid peptide with blood calcium lowering functions has been used for medical purposes for more than 30 years (Chesnut et al., 2008). Calcitonin preserves bone quality and has been used in the treatment of metabolic bone diseases as osteoporosis and Paget's disease and has also shown potentials for the treatment of osteoarthritis and to reduce postmenopausal osteoporosis (Chesnut et al., 2008). Salmon calcitonin has shown to be 40 to 50 times more potent than human calcitonin (Azria et al., 1995).

In the more recent research, a decreased risk of metabolic syndrome in adults has been attributed to the consumption of lean fish (Torris et al., 2016). Drotningsvik et al. (2015) indicated that already a low dietary intake of cod protein (25%) compared to a casein only diet, improved lipid metabolism and glucose regulation in obese rats. For humans, Aadland et al. (2015) showed that already 4 weeks of a diet with 60% of proteins from lean-seafood reduced serum triacylglycerol concentrations and prevented elevation in VLDL particle number in comparison to a diet without seafood-proteins. In a follow up study, the lean-seafood intake showed to reduce postprandial C-peptide and lactate concentrations as well as the TG/HDL-cholesterol ratio (Aadland et al., 2016). The authors concluded that the diet with 60% lean seafood protein had an effect on long-term development of insulin resistance, type 2 diabetes, and cardiovascular disease. Furthermore Schmedes et al. (2016) observed higher lipid catabolism after the lean-seafood intake. The results regarding type 2 diabetes are in line with earlier research that has shown that fish protein improved insulin sensitivity and subsequently increased capacity to store glucose as glycogen (FAO & WHO, 2011, Pilon et al., 2011).

These results indicate that fish consumption has a positive effect on human health due to both the lipid and the protein/peptide composition. Many of the mechanisms are not fully explored and more research is still needed to completely understand the effects of fish proteins as well as the synergistic effects from the combined uptake of fish lipids and proteins.

In addition, some amines, such as spermine and spermidine are highly relevant in the newest health discussions and anti-cancer research (Prester 2011; Wang et al. 2017). As these findings are only very premature, we only make this remark.

Vitamin D, selenium, calcium and phosphorus in fish

In addition to its valuable lipid and protein composition, fish is also a significant source of vitamin D (Holick, 2008b). Deficiency of vitamin D leads among others to rickets, osteomalacia, a low bone mineral density (BMD) and thereby to osteoporosis. Also an increased occurrence of cases of falling has been found in people with low vitamin D levels (Cranney et al., 2007). Furthermore, a significant correlation between higher fish intake and a lower risk of hip fractures was found in Chinese elderly (Fan et al., 2013). Beside bone connected issues deficiency of vitamin D has been connected with diabetes (Holick, 2008a), increased aggressiveness of certain cancers and increased occurrence of autoimmune diseases

as well as cardiovascular diseases (Holick, 2008b; Norman, 2008). Norman (2008) found in addition the vitamin D receptor either present or involved in many other body systems, as the adaptive and innate immune system, pancreas and brain. Usually vitamin D can be photochemical produced in the skin by mediation of sunlight. Due to concerns about skin cancer (Norman, 2008) or other reasons for low exposure to the sun, as living on northern altitudes, high rates of vitamin D deficiency have been reported from children and adults all around the world (Holick, 2008b; Norman, 2008). The general recommendation is to ingest at least 1000 IU vitamin D per day, which corresponds to 25 μg (Lu et al., 2007; Holick, 2008b). The form of vitamin D found in fish is vitamin D_3 (cholecalciferol), which is also the form being produced in the skin from 7-dehydrocholesterol when exposed to ultraviolet light and which has recently shown to have more than 3 times higher potency compared to the vitamin D_2 (ergocalciferol) which is found for example in mushrooms (Holick, 2008b; Norman, 2008). The two forms differ by ergocalciferol having one double bond and a methyl group more than cholecalciferol.

Mattila et al. (1995) found a variation of vitamin D content between 0.5 and 30 μg/100 g fish muscle in various species. In addition it was also shown that farmed salmon had a much lower vitamin D content compared with wild salmon and also that the way of preparation might have an influence on the final content (Lu et al., 2007). In the mentioned study, only 50% of the original vitamin D was recovered after frying of salmon (Lu et al., 2007). So clearly these factors have to be considered when predicting the nutritional value of fish.

Selenium is toxic in large doses; but it is essential as a micronutrient in animals and humans. In humans, selenium functions in the form of selenoproteins as cofactor for reduction of diverse antioxidant enzymes, such as glutathione peroxidases and is also responsible for the function of the thyroid gland as a cofactor for the three of the four known types of thyroid hormone deiodinases (Holben and Smith, 1999). Low levels of selenium have been associated with myocardial infarcts and increased death rate from cardiovascular disease. Beside this, low levels of selenium have been correlated with increased risk of cancer and renal disease (Holben and Smith, 1999). Selenium has also shown to decrease the toxicity of methyl mercury (Ralston and Raymond, 2010). Seafood is a good source of selenium and was ranked on place 17 of 25 by the USDA National Nutrient Database according to (Ralston, 2008). In addition, it was found that selenium and selenite from fish was highly bioavailable and had a higher bioavailability than selenium from yeast (Fox et al., 2004). Kehrig et al. (2013) analyzed various fish and seafood from the South Atlantic Ocean and found beside beneficial selenium values also selenium to mercury ratios above the critical value 1:1 which is sufficient to give protection against methyl mercury toxicity. Furthermore, there have been studies on successful supplementations of tilapia with selenium in order to increase selenium content in fish (Molnar et al., 2012). Already earlier Kaneko and Ralston (2007) suggested a so called selenium health benefit value (Se-HBV) based on the absolute amounts and relative proportions of selenium and mercury in seafood as a criteria for seafood safety. More recently, the group updated the Se-HBV value to not only take in account the availability of selenium from fish but also if the selenium status is improved or diminished. This new value is abbreviated HBV_{Se} to distinguish it from the earlier Se-HBV (Ralston et al., 2016).

Calcium is another important mineral in human nutrition being important for bone density. Calcium salts provide rigidity to the skeleton and calcium ions play a role in many if not most metabolic processes (FAO Agriculture and Consumer Protection department, 2002). Nearly 99% of the calcium in the human body is found in the bones (Ghosh and Joshi, 2008). The recommended daily intake of calcium by WHO/FAO is 400 to 500 mg/d for adults. Compared with other minerals, calcium absorbance to the body is relatively inefficient. In general, only about 25% to 30% of dietary calcium is effectively absorbed (FAO Agriculture and Consumer Protection department, 2002). Beside milk and milk products, fish and fish bones are good sources of calcium and it was also shown earlier that calcium absorption from fish is comparable to for example skimmed milk (Hansen et al., 1998). Fish and other aquatic animal food products are rich source of calcium (Martínez-Valverde et al., 2000). An average of 68 to 26 mg/100 g of calcium in crustaceans, molluscs and fish, was documented compared to around 14 mg/100 g in terrestrial meats (Tacon and Metian, 2013). In addition also salmon and cod bones were evaluated as a good source for well absorbable calcium (Malde et al., 2010). The authors suggested these fish bones as a valuable by-product to be used as a natural calcium source in functional foods or food supplements.

Also phosphorus plays an important role in the bones as well as in the cellular membranes as a component of the phospholipids building the membrane lipid bilayer. In addition it is also a component of many intracellular compounds as nucleic acids, nucleoproteins and organic phosphates as for example creatine phosphate and adenosine triphosphate. The total content of phosphorus in the human body is about 700 g of which 80% are bound in the bones, 10.9% in viscera and 9% in the skeletal

muscle tissue (Martínez-Valverde et al., 2000; Ghosh and Joshi, 2008). Deficiency of phosphorus in the body leads to muscle disorder, metabolic acidosis, encephalopathy and alteration in bone mineralization as well as in cardiac, respiratory, neurological and metabolic disorders (Ghosh and Joshi, 2008). In several publications fish and seafood are suggested to be a better source of phosphorus with an average between 204 and 230 mg/100 g phosphorus in fish, mollusks and crustaceans, compared to 176 mg/100 g in terrestrial meats (Martínez-Valverde et al., 2000; Tacon and Metian, 2013).

Factors influencing nutritional value in fish

A number of factors influence the composition of fish flesh. Every step in the history of the fish, for example the way of production and processing influences the quality of the final product. Under intensive culture conditions feed composition and feeding regimen have a major influence (Lie, 2001). Especially the lipid content and the FA composition are easily influenced by feed composition also in addition to feeding regimen and rearing system (Morris, 2001; Shearer, 2001). In contrary, as long as fish are fed adequate diets containing all needed nutrients in sufficient amounts, the protein content and composition seem to be predetermined for each species of fish regardless of the content in the diet or the feeding regimen (Morris, 2001; Shearer, 2001). Ash content and mineral composition are similarly predetermined in fish as the proteins; but some other micronutrients can be influenced and can have some effect on flesh quality (Baker, 2001). Regarding wild fish, the composition cannot be manipulated by the diet, however quality of the fish and later products will be affected by handling and processing (Erikson, 2001). In aquaculture, besides the feeding, handling after the harvest, as transport, possible storage or purging of the fish and the slaughter methods (Erikson, 2001; Robb, 2001) are important for the final product quality. All these steps can have an effect on lipid content and composition.

During processing, FA will be affected due to possible oxidation but especially due to the addition of oils or fat to the products. Last but not least the way of culinary preparation has a significant influence on the FA composition of the finally consumed product. The later aspects have recently been reviewed in separate articles (Sampels, 2015a, 2015b) and will hence be not repeated here.

Effects of feed and rearing system

The FA composition of the feed will be mirrored in the flesh (Robin et al., 2003). Especially in aquaculture, the rearing system and type of feed will have a significant influence as the fish have to feed what they get. This is true for both marine and freshwater intensive aquaculture. In marine aquaculture traditionally fish oil is used in feeds to provide the fish with a sufficient proportion of n-3 PUFA (Watanabe, 1982) and to produce fish with a nutritional valuable FA composition (Steffens, 1997; Torstensen et al., 2005; Steffens and Wirth, 2007). Due to an increasing demand of fish and subsequently an increased aquaculture production, fish oil is getting scarce and since many years, research on good and sustainable substitutes which at the same time preserve the natural, nutritional valuable FA composition of fish (Gatlin et al., 2007; Pickova and Morkore, 2007; Torstensen et al., 2008; Naylor et al., 2009; Thanuthong et al., 2011) is ongoing. Various sources as vegetable oils, algae, krill, insects, single cell oils, plankton, mesopelagic fish, and fungal biomass have been investigated as possible replacers for fish oil (Harel et al., 2002; Pickova and Morkore, 2007; Miller et al., 2010; Olsen et al., 2010; Tocher et al., 2010; Turchini and Mailer, 2010; Berge et al., 2013, Henry et al., 2015; Kousoulaki et al., 2015).

A restricting factor is, that for example vegetable oils do not contain the essential n-3 LC-PUFA EPA and DHA but only the shorter chain precursor ALA. Hence the fish must be able to convert the precursor to the longer metabolites if the diet is only prepared with vegetable oils. Most fish, as mammals including human, are not able to synthesize the n-3 LC PUFA in a sufficient proportion and the change from fish-oil to vegetable oil, in general leads to a decrease in LC PUFA (Steffens, 1997; Trattner et al., 2008b; Turchini et al., 2009). Nevertheless, there are differences between species. Already earlier it has been shown that, fresh water species like carp in contrast to marine fish seem to be able to convert ALA towards the longer chain metabolites in a greater amount (Farkas, 1984; Henderson, 1996; Turchini et al., 2006). It was also discussed that the ability of fish to synthetize n-3 LC-PUFA depends on their particular metabolic and life-history adaptations to varied environments (Leaver et al., 2008). We suggest that predatory species have a lower capacity for the synthetization of n-3 LC-PUFA as these species have these FA available in the diet compared to herbivorous or omnivorous fish, which naturally have less n-3 LC-PUFA in the diet.

There has also been some research to increase the metabolism in fish towards the LC derivatives by adding bioactive compounds to the feed. A promising compound is for example sesamin, that showed to increase n-3 LC PUFA synthesis in rainbow trout (*Oncorhynchus mykiss*) (Trattner et al., 2008a), Atlantic salmon (*Salmo salar*) hepatocytes (Trattner et al., 2008b) and in juvenile barramundi (*Lates calcarifer*) (Alhazzaa et al., 2012). Also Lipoic acid has shown to increase metabolism from

ALA to EPA in South American pacu (*Piaractus mesopotamicus*) (Trattner et al., 2007).

More recently even n-3 LC PUFA rich vegetable oil plants (genetically modified) have been suggested as a sustainable source for n-3 (Kitessa et al., 2014; Napier et al., 2015; Robert, 2006). For example a transgenic *Camelina sativa* has successfully been tested in feeds for Gilthead Sea Bream (*Sparus aurata* L.) (Betancor et al., 2016). In addition, a transgenic canola (*Brassica napus* L.) line has been suggested (Napier et al., 2015) but due to the best of our knowledge until now no results of practical applications have been published. Another example is transgenic *Arabidopsis* producing oil rich in EPA and DHA (Robert et al., 2005; Ruiz-Lopez et al., 2013). Also, a genetically modified yeast (*Yarrowia lipolytica*) has been shown to be applicable for fish oil replacement in fish feeds (Berge et al., 2013).

In addition to novel oil and FA sources also new feeding techniques have been investigated as for example a finishing feeding technique or circadian alteration feeding (Brown et al., 2010; Thanuthong et al., 2011). Other strategies aim to increase the bioavailability of the n-3 FA in the used feed source by different treatments. Berge et al. (2013) for example showed, that the application of a disruption process to yeast cells, increased the digestibility coefficients of EPA and DPA from the yeast biomass for Atlantic salmon significantly.

Another part of the rearing system for some species is the so-called purging. For certain species it is necessary to be starved for some time prior consumption in order to empty the entrails and eliminate rearing odor in the flesh. A very good example for freshwater fish that have to be starved before slaughter, are carp, which are mainly starved to eliminate bad odors and taste (Zajic et al., 2013). From the marine species, salmon are often starved for some time to reduce fat content or to decrease metabolic rate before transport (Erikson, 2001). Another reason to starve fish is to reduce the amount and activity of digestive enzymes in fish that are sold whole without prior evisceration (Rørå et al., 2001). Purging however, if pursued for a longer period also leads to weight loss and storage fat mobilization and hence influences the FA composition (Zajic et al., 2013).

Effects of water temperature and salinity

Besides the feed and rearing system also other factors as water salinity and temperature have shown to influence the FA composition in fish (Farkas, 1984; Fonseca-Madrigal et al., 2012). In many poikilotherms, the content of unsaturated FA decreases with increasing temperature (Farkas, 1984) and vice versa. Also Jobling and Bendiksen, 2003 summarized that lower water temperatures in general result in lower accumulation of SFA and increased proportions of unsaturated FA. More recently, Norambuena et al. (2016) showed that water temperature clearly affected FA composition in salmon reared at 10 °C versus 20°C, where fish kept at 10°C showed higher contents of n-6 FA in fillets. In line with this, also Mellery et al. (2016) found a higher accumulation of C18 n-6 PUFA content in rainbow trout raised at 15°C versus 19°C. The same authors also reported a decreased bioconversion from ALA to EPA and DHA at increased temperatures.

Regarding salinity, Roche et al. (1983) found a lower lipid content in sea dace (*Icentrarchus labrax pisces*) at a salinity of 4 ppt compared to higher values (18, 36, and 40 ppt, respectively). Fish also showed a lower content of MUFA and higher proportion of PUFA at the lowest salinity in this study. In the brackish Baltic Sea, herring (*Clupeus harrengus*) is less fatty compared to the saltier North Sea (National Food Agency Sweden, 2017). In line with this Liu et al. (2017) found a lower fat content at lower salinity in juvenile American shad (*Alosa sapidissima*), but in opposite to sea dace and herring, American shad showed a higher MUFA at the lowest salinity and increasing proportions of PUFA with increasing salinity (Liu et al. 2017). Similar results have been shown earlier for silverside *(Chirostoma estor*), where an increased biosynthetis of long chain n-3 PUFA was found in fish raised at higher salinities (Fonseca-Madrigal et al., 2012). On the other hand resulted a lower salinity in higher biosynthesis of EPA and DHA in red sea bream (*Pagrus major*) (Sarker et al. 2011). Changes in lipid metabolism have also been observed in species that undergo a transfer from freshwater to seawater (anadromous), for example during smoltification in salmonids (Bell et al., 1997; Sargent et al., 1989). During smoltification of juvenile salmonids an increased activity of the long chain PUFA synthesis was found until seawater transfer and a decreased activity during the sea water phase (Bell et al., 1997). In general it is assumed that freshwater fish have a higher ability to elongate and desaturate ALA to DHA compared to marine fish, and it seems that increasing salinity or lower temperatures sometimes can have a stimulating effect in some species (Kheriji et al 2003, Fonseca-Madrigal et al., 2012; Liu et al. 2017). In general, when considering salinity effects, species with a large span of environmental adaptations, have a higher fat content (most likely because of better growth) in their environment of origin. For example herring, being a marine fish species, has a higher fat content in higher salinities, compared to brackish environment (National Food Agency, Sweden, 2017). Salmonids (most species) have a higher fat content when they are on feeding

migration in saltwater compared to the environment where they hatch and smoltify.

Conclusions

When considering fish as food and the nutritional value connected with these products, first of all the n-3 PUFA are in focus. Furthermore, it gets obvious that also the proteins and peptides in fish have not only a high nutritional value but also impact on human health issues. In addition fish can be considered as a good source of several minerals, vitamins and micronutrients.

The most vulnerable nutrients from fish are the FA, as they are significantly influenced by the feed and the processing of the fish, while protein and the minor nutrients seem to be less affected as long as the fish was not starved or wrongly fed or exposed to abusive storage or processing conditions.

In general, it should be highlighted that, when considering human nutrition and related health aspects, it is impossible to focus one group of nutrients separated from all others. Most probably the discussed effects of fish on human health are due to the consumption of the fish as a whole and hence the combination of all present nutrients.

Future work regarding effects of fish consumption on human health should therefore focus on both, a holistic and metabolomic approach, investigating the effects of fish consumption via techniques as NMR, MALDI-TOF, MALDI imaging MS, and HPLC-MS in order to get a more complete picture. When it comes to nutrition studies, metabolomics are developing fast as a powerful tool, enabling a direct insight into metabolism of the diverse nutrients, possible regulation pathways as well as finding markers for disorders (Cornett et al., 2007; Wagner et al., 2014; Cheng et al., 2016; Schmedes et al., 2016).

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CHAPTER 3

NUTRITIONAL VALUE OF SEVERAL COMMERCIALLY IMPORTANT RIVER FISH SPECIES FROM THE CZECH REPUBLIC

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Nutritional value of several commercially important river fish species from the Czech Republic

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ABSTRACT

Proximate and fatty acid (FA) composition of seven freshwater fish species from the Czech Republic were examined. Moreover, the index of atherogenicity (IA) and the index of thrombogenicity (IT) were calculated from the obtained data. These two indices along with the total content of the essential n-3 FAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as the ratio of n-6/n3 FAs, provide good indicators for the nutritional value of the fish. The species had been selected owing to the limited amount of information about their nutritional composition available. Furthermore, they are not typically subject to aquaculture, being almost exclusively obtained by angling. The protein content was relatively stable in all species (17.1 ± 1.55 to 19.2 ± 2.20 g/100 g). The content of carbohydrates ranged from 0.02 ± 0.1 to 0.99 ± 0.0 g/100 g and ash from 1.08 ± 0.20 to 2.54 ± 1.57 g/100 g. As expected, a high variability was observed in the fat content (0.74 ± 0.04 to 4.04 ± 0.81 g/100 g) and the FA composition, as well as the contents of EPA and DHA. IA and IT were close to the values stated for the Eskimo diet, indicating a high nutritional value with a positive effect for human health.

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INTRODUCTION

The consumption of fish as well as fish products has significantly increased during the last two decades (*Food and Agriculture Organization (FAO), 2016*). The popularity of fish is mainly due to the overall high quality and the positive effects on human health. The main health benefits of fish are attributed to their high content of n-3 long-chain polyunsaturated fatty acids (FAs) (n-3 LC-PUFA) (*Kris-Etherton et al., 2002*; *Lund, 2013*; *Khalili Tilami & Sampels, 2018*). The most important n-3 LC-PUFA are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are known to have positive effects on the cardiovascular system as well as the nervous system of children in prenatal development, and to prevent the metabolic syndrome or obesity

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(*Williams, 2000*; *Calder & Yaqoob, 2009*; *Adamkova et al., 2011*; *Torris, Molin & Cvancarova Smastuen, 2016*; *Kanakri et al., 2017*; *Saini & Keum, 2018*). More recently fish proteins, peptides and amino acids have also gained increased attention with similar properties to the n-3 FAs from fish (*Khalili Tilami & Sampels, 2018*).

Moreover, fish proteins are easily digestible and rich in all essential amino acids, particularly methionine, lysine, taurine, which are limited in other kinds of muscle food (*Tacon & Metian, 2013*; *Khalili Tilami & Sampels, 2018*). *Khalili Tilami & Sampels (2018)* provided a depth review of the nutritional value of fish, focusing on lipids and proteins in particular.

While the protein composition is generally very stable in fish, the FA composition is greatly influenced by the diet. The lipid composition of the diet is mirrored in the fillet lipid composition of reared fish following the trend "You are what you eat" (*Chanmugam, Boudreau & Hwang, 1986*; *Sahena et al., 2009*). In addition to the effect of feed composition, other factors including fish feeding habits, fish trophic level and ecosystem trophic status might influence the nutritional composition of fish in natural water bodies via changing the quality of feed sources (*Ahlgren et al., 1996*; *Czesny et al., 2011*; *Gladyshev et al., 2018*). Nutritional composition of various fish species might be influenced by variation in their morphology and physiology (*Rust, 2002*; *Khitouni et al., 2014*).

The differences in nutrient composition between wild and farmed fish of identical species have been reported many times (*Nettleton & Exler, 1992*; *Ahlgren, Carlstein & Gustafsson, 1999*; *Orban et al., 2003*; *Kaushik et al., 2006*; *Hossain, 2011*). The diets for fish in intensive aquaculture consist of complete feeding mixtures based on fish meal and fish oil to meet the fish requirements as well as reaching a nutritionally valuable high content of n-3 FA in the fillet. Nonetheless, due to the increased use in various sectors (aquaculture, pharmaceuticals, cosmetics...), n-3 LC-PUFA rich sources for aquaculture feeds are very limited and must be replaced by sustainable components. For the time being, these replacers are usually plant components, generally causing a decrease in the proportion of n-3 LC-PUFA in the fish. On the contrary, the diet of wild fish consists of natural feed, such as plankton, benthos as well as nekton in case of carnivorous species, which naturally contain the essential n-3 LC-PUFA. The primary producers of n-3 LC-PUFA in freshwater ecosystems are, the same as in the ocean, algae. These compounds are transferred into the fish throughout the feed chain. In addition, fish are able to biosynthesize n-3 LC-PUFA from their 18 carbon precursor (α-linolenic acid; ALA) to a certain degree. This ability is strongly expressed in freshwater non-carnivorous species, compared to marine carnivorous fish, which decreased this ability during evolution (*Tocher, 2003*; *Zajic, Mraz & Pickova, 2016*). Therefore, the consumption of freshwater species from natural habitats should be beneficial not only for human health, but also from sustainability and ecological viewpoints.

While annual fish supply around the world was 20 kg per capita in 2014 (*Food and Agriculture Organization (FAO), 2016*), landlocked countries, like those in Central Europe, have a much lower average consumption; for instance fish intake in the Czech Republic was around 5.5 kg per capita in 2008 (*MZe, 2009*). At the same time, a significant percentage of consumed fish is provided by anglers in these countries, thus consisting of wild fish. The consumed fish also include species that have unjustly gained less attention by experts for

human nutrition. However, they have a relatively high importance for a certain part of the population in Central Europe.

This study aimed to complement the existing information about the nutritional composition and lipid indices of seven less promoted but very interesting freshwater fish species in order to extend an existing knowledge. For some of them no relevant data about proximate composition exist and only fragmentary results have been published regarding the fat content and composition. The list of investigated species includes European grayling (*Thymallus thymallus*), common nase (*Chondrostoma nasus*), brown trout (*Salmo trutta morpha fario*), common bream (*Abramis brama*), Prussian carp (*Carassius gibelio*), European perch (*Perca fluviatilis*) and European chub (*Squalius cephalus*).

MATERIALS AND METHODS

The fish (individuals of a consumerist size) for this study were obtained by anglers from their natural habitat (major river basin of the Dyje, Labe and Vltava rivers in the Czech Republic) during the vegetation season. Samples from each species were caught at different localities. After catching of each fish by traditional angling (angle with one hook attached to the fishing line), fish were separated based on their weight. Then, the individuals with the marketable-size were selected. Immediately after capture, the selected fish were killed by a blow to the head, weighted (Table 1) and transported on ice (0 °C) to the processing facilities of the Institute of Aquaculture and Protection of Waters, Faculty of Fisheries and Protection of Waters, University of South Bohemia, Ceske Budejovice, Czech Republic. The temperature was monitored during the transport. Fish were filleted and processed as skin-on and scale-less. Fillet with skin were used in order to include all the flesh and FA deposits which contain n-3 LC-PUFA. Then the whole remaining fillet was homogenized in a table blender so that the taken sample was sufficiently representative, while containing all the edible parts.

Proximate composition

The chemical composition of fish samples was analyzed following standardized AOAC (*Association of Official Analytical Chemists (AOAC), 2000*) methods. For dry matter analysis, 12 individuals of common bream, seven European perch, eight Prussian carp, seven common nase, 12 brown trout, nine European grayling, and five European chub were used. To determine dry matter, five g of homogenized sample was mixed with some sea sand in a pre-dried porcelain dish and then dried in the oven at a temperature of 105 °C to the constant weight. A total of 12 individuals of common bream, 10 European perch, nine Prussian carp, nine common nase, 12 brown trout, 13 European grayling, and 11 European chub were taken for ash analysis. Ash was analyzed by incinerating five g homogenized muscle at 550 °C in a muffle furnace for 12 h. Carbohydrates were calculated using the following formula:

$$\text{Carbohydrates } (\%) = 100 - (\text{moisture} + \text{lipids} + \text{proteins} + \text{ash})$$

For protein analysis, 12 individuals of common bream, ten European perch, nine Prussian carp, nine common nase, nine brown trout, 12 European grayling,

Table 1 **List of seven analyzed freshwater fish species from major river basin of Dyje, Labe and Vltava river, the Czech Republic, with weight (average ± standard deviation) and captured fish number (*N*).**

Common name	Latin name	Average weight (g)	*N*
Freshwater bream	*Abramis brama*	761 ± 158	16
European perch	*Perca fluviatilis*	142 ± 29	10
Prussian carp	*Carassius gibelio*	483 ± 96	13
Common nase	*Chondrostoma nasus*	510 ± 115	10
Brown trout	*Salmo trutta morpha fario*	140 ± 48	12
Grayling	*Thymallus thymallus*	315 ± 44	13
Chub	*Squalius cephalus*	243 ± 35	11

Note:
N-captured fish number.

and 10 European chub were used. Total nitrogen was analyzed in a certified laboratory (ALS Czech Republic, Prague) by Dumas combustion, the protein content being subsequently calculated using 6.25 as a conversion factor. The energy value was calculated assuming conversion factors of 23.6, 39.5, and 17.2 kJ/100 g for proteins, lipids, and carbohydrates, respectively (*NRC, 1993*).

Fat content and fatty acid composition

A total of 15 individuals of common bream, 10 European perch, 13 Prussian carp, nine common nase, 12 brown trout, 10 European grayling, and 10 European chub were used for analysis. One g of the homogenized fillet was taken for analysis. Lipids were extracted in HIP (hexane-isopropanol 3:2 *v:v*) following the method of *Hara & Radin (1978)* with modifications described by *Mraz & Pickova (2009)* and the fat content was determined gravimetrically. Subsequently fatty acid methyl esters (FAME) were prepared according to *Appelqvist (1968)* with NaOH in dry methanol and boron trifluoride–methanol complex (BF_3). Obtained FAMEs were analyzed using the gas chromatograph Trace Ultra (ThermoScientific, Waltham, MA, USA) equipped with a flame ionization detector and capillary column BPX 70 (AGE, Austin, TX, USA) with 50 m length × 0.22 mm i.d. × 0.25 µm film thickness. FA were identified by comparing to the standard mixture GLC-68D (Nu-Check Prep, Elysian, MN, USA) and other individual standards. For calculations of the absolute amount of individual FA, an internal standard (21:0) (Nu-check Prep, Elysian, MN, USA) was used.

Lipid health indices

The obtained data were used to calculate both the index of atherogenicity (IA) and the index of thrombogenicity (IT) according to *Ulbricht & Southgate (1991)*. The IA refers to the ratio between the main saturated FA (SFA) and the sum of monounsaturated FA (MUFA), and polyunsaturated FA (PUFA). The result of this index is a number indicating the risk of formation i.e., atherosclerosis. The higher the IA is, the higher risk it constitutes. The IT is defined as the ratio between pro-thrombogenic (myristic, palmitic, and stearic) and anti-thrombogenic (MUFA, n-6 PUFA and n-3 PUFA) FA.

An increasing IT indicates a risk of developing a blood clot (*Garaffo et al., 2011*; *Ulbricht & Southgate, 1991*). The following equations were applied:

$$\mathrm{IA} = (12{:}0 + 4 \times 14{:}0 + 16{:}0)/[\Sigma\ \mathrm{MUFA} + \Sigma\ \mathrm{PUFA}]$$
$$\mathrm{IT} = [14{:}0 + 16{:}0 + 18{:}0]/[(0.5 \times \mathrm{MUFA}) + (0.5 \times \text{n-6}) + (3 \times \text{n-3}) + (\text{n-3}/\text{n-6})]$$

Statistical analysis

Statistical evaluation was performed using one-way analysis of variance (ANOVA) with subsequent post hoc comparisons using Tukey's honest significant difference test to determine the effects of different localities on the changes of FAs, lipids, proteins, dry matter, and carbohydrates within species. Probability values of $p \leq 0.05$ were considered as significant. These statistical analyses were performed using the STATISTICA software (Version 13; StatSoft, Inc., Tulsa, OK, USA) for MS Windows. The relation between FAs and lipid content of each species were evaluated using linear regression. Kruskal Wallis one-way ANOVA was performed in order to determine differences in FAs, dry matter, proteins, lipids, and ash content among fish species. In case of significant differences Dunn post hoc test were performed. These analyses were done with rcompanion (*Magniafico, 2018*) and FSA (*Ogle, 2018*) packages in R version 3.4.4 (*R Development Core Team, 2018*).

RESULTS

The present study analyzed the fillet composition of seven wild freshwater fish species. The fish species presented in this study are normally solely captured from open waters with exception of European perch (*Mairesse et al., 2006*) and to a limited extent brown trout (*Arzel et al., 1994*). The obtained samples originated exclusively from the natural conditions of the species studied, from the Dyje, Labe, and Vltava river basins. The purpose was to only take the fish that had reached the consumable size (Table 1), as the nutrient composition with lipids in particular can vary with the growth of the fish (*Mraz & Pickova, 2011*). It has to be considered that beside growth and the already earlier mentioned feed composition many other factors can influence nutrient composition in fish. For example, ecological factors including the trophic status of the water body (eutrophic ecosystem enriched with phytoplankton as the main producers of feed chain versus oligotrophic ecosystem) (biotic factor) (*Ahlgren et al., 1996*; *Czesny et al., 2011*; *Vasconi et al., 2015*, *Gladyshev et al., 2018*), temperature (*Arts et al., 2012*) as well as lightning conditions (abiotic factors) (*Boujard & Leatherland, 1992*) were reported to have influence on FA and lipid composition of the fish by changing the quality of their feed. Other factors like fish feeding habits, their preference for eating, presence and threat of predation (*Daan, 1981*) which can change the fish preferred time of feeding in spite of their fixed feeding rhythms as diurnal or nocturnal feeders, are also important. The role of phylogenetic factor is more discussed for carnivorous species (*Gladyshev et al., 2018*). However, in the present study the aim was to investigate the natural composition and possible diversity in order to be able to give better

Table 2 **Proximate composition of seven freshwater fish species from major river basin of Dyje, Labe and Vltava river, the Czech Republic.**

	Dry matter g/100 g	Protein g/100 g	Lipids g/100 g	Ash g/100 g	Carbohydrate g/100 g	Energy value kJ/100 g	Energy value kcal/100 g
Common bream	22.5 ± 1.85^{a}	18.0 ± 1.24^{ab}	2.17 ± 0.19^{a}	1.35 ± 0.18^{bc}	0.99 ± 0.0^{a}	528 ± 18^{ab}	126 ± 4^{ab}
European perch	20.9 ± 2.67^{c}	17.6 ± 1.85^{bc}	0.74 ± 0.04^{c}	2.54 ± 1.57^{ab}	0.02 ± 0.1^{e}	500 ± 31^{b}	114 ± 2^{b}
Prussian carp	20.8 ± 1.41^{ac}	17.1 ± 1.55^{c}	1.94 ± 1.13^{a}	1.08 ± 0.20^{c}	0.68 ± 0.0^{ab}	518 ± 4^{ab}	124 ± 1^{ab}
Common nase	23.4 ± 1.47^{bd}	17.6 ± 0.98^{bc}	4.04 ± 0.81^{b}	1.25 ± 0.08^{bc}	0.51 ± 0.1^{bc}	604 ± 58^{ac}	144 ± 14^{ac}
Brown trout	24.3 ± 1.50^{b}	19.2 ± 1.50^{a}	3.32 ± 0.1^{ab}	1.56 ± 0.20^{abc}	0.30 ± 0.1^{df}	619 ± 56^{c}	148 ± 13^{c}
European grayling	21.6 ± 1.94^{ad}	17.4 ± 0.52^{bc}	2.77 ± 0.92^{ab}	2.35 ± 1.05^{a}	0.18 ± 0.1^{ef}	536 ± 33^{ac}	128 ± 8^{ac}
European chub	24.9 ± 0.2^{b}	19.2 ± 2.20^{ab}	3.49 ± 0.53^{b}	1.86 ± 0.51^{ab}	0.37 ± 0.0^{cd}	611 ± 76^{ac}	146 ± 18^{ac}

Notes:
Different letters indicated significant differences ($p \leq 0.05$) for the respective parameter among different species.
Data are mean ± standard deviation.

information about nutritional composition to the consumers. Therefore, these factors were not in the focus of the work.

Proximate composition

The proximate composition of the analyzed fish is listed in Table 2. Fat content varied from 0.74% in European perch to 4.04% in common nase. All studied species showed a similar protein content (17.1 ± 1.55 to 19.2 ± 2.20 g/100 g fillet). The carbohydrate content was varying from 0.02 g/100 g (European perch) to 0.9 g/100 g (common bream). Like proteins and carbohydrates, the ash content in the fillet with skin was comparable among all the analyzed species (1.08 ± 0.20 to 2.54 ± 1.57 g/100 g).

Fatty acid composition

Fatty acid composition of the chosen species is presented in Table 3. FA composition varied between species. The nutritional valuable n-3 FA, EPA, and DHA showed values between 2.03% in brown trout and 8.15% in common bream for EPA and 7.33 in common nase to 27.60% in European perch for DHA. Total content of EPA plus DHA was calculated to range from 190 mg/100 g in European perch to 471 mg/100 g in common nase (Fig. 1).

Lipid health indices

One of the sub-objectives of this study was to determine the lipid health indices (IA and IT) of the analyzed species. In this study, the IA reached a maximum of 0.39 (common nase) and the highest IT (0.26) was calculated for brown trout (Table 3).

DISCUSSION

Protein content results varied at the predictable levels and corresponded to the indicated values for fish flesh (*Lazos, Aggelousis & Alexakis, 1989*; *Puwastien et al., 1999*; *Tuomisto & Froyland, 2008*; *Gjedrem, Robinson & Rye, 2012*; *Zotos & Vouzanidou, 2012*). Carbohydrate content in fish is usually lower than 0.5 g/100 g flesh (*Gjedrem, Robinson & Rye, 2012*). Ash content varied from 1.08 to 2.54, this might be due to the variation in feed intake, species, physiology, and sex (*Khitouni et al., 2014*). Similar

Table 3 **Fatty acid composition (% of total identified), atherogenicity and thrombogenicity indices of seven freshwater fish species caught from the major river basin of Dyje, Labe and Vltava river, the Czech Republic.**

	Common bream	European perch	Prussian carp	Common nase	Brown trout	European grayling	European chub
14:0	2.02 ± 0.8^{a}	0.99 ± 0.22^{b}	2.15 ± 0.46^{a}	2.69 ± 0.88^{a}	1.95 ± 0.58^{a}	1.94 ± 0.57^{a}	1.93 ± 0.29^{a}
14:1	0.62 ± 0.45^{a}	0.32 ± 0.35^{c}	1.26 ± 2.19^{ab}	0.22 ± 0.09^{c}	0.32 ± 0.15^{abc}	0.06 ± 0.08^{bc}	0.58 ± 0.15^{a}
16:0	14.3 ± 6.82^{ab}	22.38 ± 3.35^{c}	17.31 ± 2.83^{ab}	16.19 ± 2.82^{bd}	18.57 ± 4.20^{ac}	12.89 ± 2.79^{d}	17.09 ± 1.11^{ab}
16:1	9.78 ± 5.33^{bc}	3.85 ± 1.33^{a}	7.81 ± 2.10^{ac}	15.00 ± 4.73^{b}	7.92 ± 2.72^{ac}	3.57 ± 1.31^{a}	9.99 ± 2.05^{bc}
18:0	5.98 ± 2.13^{a}	4.47 ± 2.35^{ac}	2.83 ± 2.54^{bc}	3.09 ± 0.36^{bc}	4.67 ± 1.07^{b}	2.87 ± 0.58^{ab}	3.26 ± 0.42^{bc}
18:1n-9	17.9 ± 9.19^{a}	7.93 ± 2.93^{b}	7.33 ± 5.67^{b}	17.12 ± 1.95^{a}	22.94 ± 15.9^{a}	27.30 ± 9.39^{c}	21.23 ± 2.47^{ac}
18:1n-7	6.53 ± 1.65^{a}	3.85 ± 0.33^{bc}	4.99 ± 0.49^{ab}	4.81 ± 0.07^{ab}	3.97 ± 1.27^{abc}	2.83 ± 0.30^{c}	6.01 ± 0.13^{a}
18:2n-6	7.63 ± 2.51^{a}	3.22 ± 0.39^{b}	7.12 ± 3.00^{a}	5.14 ± 1.45^{a}	5.91 ± 2.47^{a}	16.29 ± 2.87^{c}	8.23 ± 4.16^{a}
18:3n-3	3.61 ± 1.61^{ab}	1.94 ± 1.12^{c}	5.35 ± 2.63^{a}	2.29 ± 1.14^{bc}	6.75 ± 4.37^{ab}	2.54 ± 0.70^{bc}	4.99 ± 0.50^{a}
20:0	0.46 ± 0.22^{a}	0.15 ± 0.06^{c}	0.28 ± 0.1^{abc}	0.20 ± 0.0^{abc}	0.38 ± 0.1^{abc}	0.14 ± 0.1^{bc}	0.35 ± 0.1^{ab}
20:1n-9	0.70 ± 0.28^{ab}	0.81 ± 0.91^{b}	1.41 ± 0.32^{ac}	2.09 ± 2.85^{cd}	0.80 ± 0.56^{ac}	2.63 ± 0.61^{d}	0.92 ± 0.11^{c}
20:2n-6	1.16 ± 0.37^{ab}	0.43 ± 0.37^{c}	3.79 ± 2.43^{a}	0.40 ± 0.10^{cd}	0.68 ± 0.76^{cd}	1.12 ± 1.69^{bd}	1.04 ± 0.93^{bcd}
20:4n-6	6.12 ± 3.65^{a}	7.89 ± 2.97^{a}	2.41 ± 3.83^{bd}	2.41 ± 3.83^{bd}	2.73 ± 1.38^{bc}	1.10 ± 0.57^{d}	3.87 ± 0.63^{ac}
20:3n-3	0.62 ± 0.22^{ab}	0.35 ± 0.19^{d}	3.31 ± 2.10^{c}	0.40 ± 0.20^{ad}	0.61 ± 0.22^{ab}	0.19 ± 0.04^{d}	0.82 ± 0.17^{bc}
22:0	0.09 ± 0.09^{a}	0.03 ± 0.04^{c}	0.08 ± 0.04^{abc}	0.07 ± 0.04^{abc}	0.20 ± 0.06^{abc}	0.00 ± 0.00^{bc}	0.01 ± 0.02^{ab}
22:1	0.56 ± 0.98^{ab}	1.04 ± 1.29^{b}	0.75 ± 0.54^{a}	0.60 ± 0.45^{ab}	0.88 ± 0.59^{a}	0.35 ± 0.09^{ab}	0.81 ± 1.20^{ab}
20:5n-3	8.15 ± 11.6^{a}	4.45 ± 1.75^{a}	3.85 ± 1.79^{ac}	6.82 ± 5.02^{a}	2.03 ± 0.92^{b}	2.32 ± 1.54^{bc}	3.39 ± 1.48^{abc}
24:1	0.52 ± 0.66^{a}	1.04 ± 1.29^{a}	0.87 ± 0.78^{a}	2.15 ± 1.68^{a}	1.25 ± 0.91^{a}	0.40 ± 0.32^{a}	0.49 ± 0.57^{a}
22:5n-3	2.85 ± 1.26^{a}	2.48 ± 0.26^{a}	2.72 ± 0.68^{a}	3.02 ± 0.68^{a}	1.60 ± 0.43^{b}	1.08 ± 0.36^{b}	1.89 ± 0.31^{b}
22:6n-3	7.68 ± 4.43^{a}	27.60 ± 3.61^{d}	13.19 ± 5.00^{b}	7.33 ± 2.37^{ac}	12.83 ± 8.76^{abc}	12.95 ± 7.33^{bc}	10.48 ± 4.23^{a}
24:0	6.31 ± 2.23^{a}	1.01 ± 0.33^{abc}	0.70 ± 0.25^{bc}	0.24 ± 0.04^{b}	0.53 ± 0.40^{b}	2.06 ± 0.41^{ac}	1.53 ± 1.70^{bc}
SFA	25.7 ± 5.51^{ab}	28.30 ± 5.05^{b}	22.88 ± 4.41^{ac}	22.24 ± 3.65^{ac}	25.72 ± 5.18^{ab}	17.75 ± 4.34^{c}	23.23 ± 1.26^{ab}
MUFA	37.9 ± 10.6^{a}	21.77 ± 7.01^{c}	35.59 ± 11.11^{ab}	42.17 ± 5.53^{b}	38.71 ± 13.35^{ab}	45.75 ± 8.63^{b}	40.62 ± 3.03^{ab}
PUFA	37.8 ± 11.4^{a}	48.34 ± 4.31^{c}	41.33 ± 6.10^{ac}	27.80 ± 5.91^{b}	33.15 ± 8.72^{ab}	37.80 ± 7.07^{a}	34.71 ± 2.80^{ab}
n-3 PUFA	22.9 ± 11.3^{a}	36.80 ± 3.80^{c}	28.42 ± 4.55^{bc}	19.86 ± 5.13^{a}	23.83 ± 8.79^{ab}	19.07 ± 8.40^{a}	21.57 ± 3.09^{ab}
n-6 PUFA	14.9 ± 3.93^{ab}	11.54 ± 2.68^{c}	12.91 ± 2.89^{ac}	7.94 ± 4.93^{d}	9.32 ± 3.74^{cd}	18.73 ± 3.09^{b}	13.14 ± 3.98^{ac}
n-3 HUFA	19.3 ± 11.8^{a}	34.86 ± 3.64^{c}	23.07 ± 6.27^{bc}	17.57 ± 4.49^{a}	17.08 ± 8.72^{ab}	16.53 ± 8.95^{a}	7.21 ± 6. 88^{ab}
n-3/n-6	1.74 ± 1.61^{ab}	3.40 ± 1.10^{ce}	2.29 ± 0.54^{ade}	3.19 ± 1.84^{cde}	2.95 ± 1.39^{c}	1.10 ± 0.76^{b}	1.88 ± 0.81^{ad}
IA	0.30 ± 0.11^{a}	0.38 ± 0.06^{a}	0.35 ± 0.07^{a}	0.39 ± 0.09^{a}	0.37 ± 0.10^{a}	0.07 ± 0.30^{b}	0.35 ± 0.02^{ab}
IT	0.25 ± 0.10^{ab}	0.22 ± 0.04^{ab}	0.20 ± 0.05^{ab}	0.25 ± 0.02^{a}	0.26 ± 0.07^{a}	0.06 ± 0.22^{b}	0.25 ± 0.03^{ab}

Notes:
Data are presented as mean ± standard deviation.
Different letters indicate significant differences ($p \leq 0.05$) for the respective FA among different species.
IA, index of atherogenicity; IT, index of thrombogenicity; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

variations have been observed in the results of previous studies (*Puwastien et al., 1999*; *Zotos & Vouzanidou, 2012*; *Zivkovic et al., 2013*). As protein and carbohydrate contents are known to be very stable in fish, these results had been expected (*Morris, 2001*; *Shearer, 2001*). Significant differences occurred only earlier when the whole-body composition (with bones, fins, and scales) of fish was analyzed (*Van Pelt et al., 1997*).

Common bream is a lean fish with approximately one g of lipids per 100 g fillet (*Lazos, Aggelousis & Alexakis, 1989*; *Aggelousis & Lazos, 1991*). In our study we found fat contents up to 2.17 ± 0.19, which is rather comparable with the North European

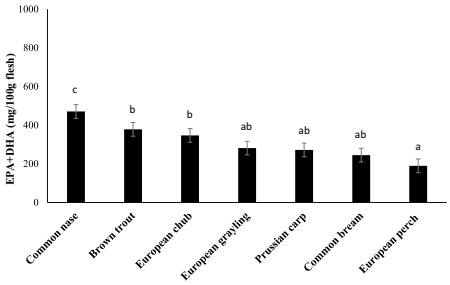

Figure 1 The content (mg/100 g flesh) of eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids in the fillet of seven freshwater fish species from the major river basin of Dyje, Labe and Vltava river, the Czech Republic. Data are the mean ± standard deviation. Different letters indicate significant differences among species ($p \leq 0.05$).

Full-size DOI: 10.7717/peerj.5729/fig-1

populations of this species with 1.8 g/100 g (*Puustinen, Punnonen & Uotila, 1985*). Even higher fat levels (3.63–5.51 g/100 g) are published by *Zmijewski et al. (2006)* and *Zivkovic et al. (2013)*. This variability in fat content is consequently accompanied by differences in FA composition, as the relative content of n-3 LC-PUFA generally decreases with an increasing fat content, as storage fat is built by triacylglycerols (TAG), which are usually higher in SFA and MUFA (*Henderson & Tocher, 1987*). Common bream showed to have a nutritionally very favorable FA composition, with high proportions of n-3 LC-PUFA (Table 3). This most probably reflected the composition of the natural diet, as diet FA composition was shown earlier to be the most important factor influencing the fish muscle composition (*Robin et al., 2003*; *Pickova, Sampels & Berntsen, 2010*). Common bream as a benthos- and plankton feeders species with the nocturnal feeding habits has a vast feeding spectrum which can feed on detritus, mollusks and macrophytes (*Adamek & Marsalek, 2012*; *Zapletal et al., 2012*; *Golovanova et al., 2014*). They belong to the higher trophic levels therefore, digestion for them is not as easy as for herbivorous species. This might influence the metabolic apparatuses and fish FA composition (*Rodrigues et al., 2017*). Considering the differences between the intestine length and morphology of various fish species and their consequent effects on the intestine absorptive surface and digestibility of the feed (*Rust, 2002*), nutritional composition of fish species might be influenced.

According to the fillet fat content, the proportion of n-3 LC-PUFA in similar studies varies widely from 4.7 up to 31.8%. The n-3/n-6 ratio could be close to one (*Zivkovic et al., 2013*), around 1.7 (present study) or up to 2.9 (*Aggelousis & Lazos, 1991*) also indicating the effect of feed composition as the natural feed composition most probably varies in different water bodies. However, all values are within the recommended values of a n-6/n-3 ratio of 1–4 (*Simopoulos, 2008*). When discussing the nutritional value of fish for human, it must be observed that normally the ratio between n-6 and n-3 FAs in food items and in nutrition is expressed as n-6/n-3, while in fish the ratio

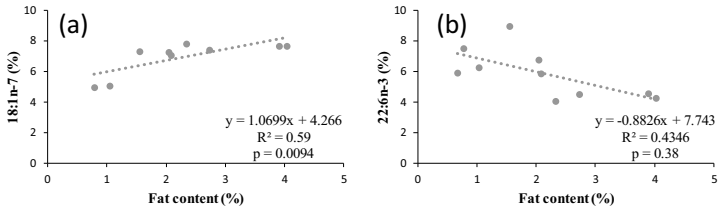

Figure 2 Examples of regression between lipid content and two FAs in common bream: (A) 18:1n-7; (B) 22:n-3. Full-size DOI: 10.7717/peerj.5729/fig-2

between is often expressed as n-3/n-6, since the opposite ratio would lead to very low values below 0. For example, the n-3/n-6 ratio of 1.7 for common bream in the present study corresponds to n-6/n-3 ratio of 0.65. The regression between the FAs and lipid content of common bream was investigated. In terms of individual FA, in case of MUFA with an increase in the percentage of the fat content, the percentage of some MUFAs including 14:1 ($p = 0.02$, $R_2 = 0.49$); 16:1 ($p = 0.02$, $R_2 = 0.49$); 18:1n-7 ($p = 0.009$, $R_2 = 0.59$) significantly increased (positive correlation) (Fig. 2A as an example for one of the MUFAs) whereas in 22:6n-3 ($p = 0.03$, $R_2 = 0.43$), with an increase in the percentage of the fat content, a significant decrease in this PUFAs percentage was observed (inverse correlation) (Fig. 2B). This confirms the earlier mentioned fact that a higher fat content also corresponds to a lower LC-PUFA percentage. In general, the fat is stored in TAG, resulting in an increase proportion of this lipid fraction in fatty fish, while in general LC-PUFA are stored in the phospholipids, which are mainly the constituents of biological membranes (*Sargent et al., 1999*). As a higher proportion of TAG automatically results in a (relative) lower proportion of phospholipids, this will also lead to a relatively lower proportion of LC-PUFA (*Henderson & Tocher, 1987*).

Similarly as for common bream, lower fat contents compared to the present work were stated also for European chub by *Lazos, Aggelousis & Alexakis (1989)* and *Aggelousis & Lazos (1991)* (average of 1.5 g/100 g compared to 3.49 ± 0.5 g/100 g in the present study). On the other hand, *Donmez (2009)* found similar values to 3.5 ± 0.4 g/100 g. Based on the available data, the n-3/n-6 ratio in European chub showed to vary from 1.7 (*Aggelousis & Lazos, 1991*) through 1.9 (present study) up to 2.7 (*Donmez, 2009*). The differences are most likely caused by feed available in the habitat, as the fish, being an omnivorous species with their shallow water preferences, can eat everything from fallen fruit to small fish (*Piria et al., 2005*), which subsequently influences the fillet fat content and FA composition.

In addition, the effect of different localities on the changes in the proximate composition in European chub was tested. No significant changes in the parameters were observed. The regression between the lipid content and several FAs including 14:0 ($p = 0.002$, $R_2 = 0.69$); 16:0 ($p = 0.002$, $R_2 = 0.70$); 18:1n-9 ($p = 0.020$, $R_2 = 0.50$); 18:2n-6 ($p = 0.029$, $R_2 = 0.46$); 18:3n-3 ($p = 0.01$, $R_2 = 0.57$); 20:1n-9 ($p = 0.005$, $R_2 = 0.63$); 20:5n-3 ($p = 0,09$, $R_2 = 0.30$) showed positive correlation, whereas in 14:1 ($p = 0.0005$,

R_2 = 0.79); 20:3n-3 (p = 0.07, R_2 = 0.33); 24:1 (p = 0.003, R_2 = 0.67); 22:5n-3 (p = 0.07, R_2 = 0.34); 22:6n-3 (p = 0.00009, R_2 = 0.86) negative correlation was observed, again confirming the correlation between a higher fat content and increased SFA content as mentioned before.

The feed of common nase, which is herbivorous benthopelagic species (*Junger, Kotrschal & Goldschmid, 1988*; *Riede, 2004*), may consist of indigestible material in a high portion which requires longer gut (*Hofer, 1982*). Previously, very low amounts of lipids (~1 g/100 g) were found in the fillet of nase (*Lazos, Aggelousis & Alexakis, 1989*). A slightly higher content (1.3 ± 0.46 and 2.7 ± 0.4 g/100 g) was shown by *Aggelousis & Lazos (1991)* and *Lazos (1997)*, respectively. On the other hand, our result showed much higher values with an average fat content of 4.04 ± 0.81 g/100 g. The differences could be attributed to the fact that the fish in the studies reporting a lower fillet fat content originated from Southern Europe with a higher average year-round water temperature. Subsequently, the fish do not need to store as much lipid as in the areas with a cold winter. Other noticeable findings for this species in the present study were high proportions of EPA and DHA (6.82 ± 5.02 and 7.33 ± 2.32%, respectively), which is, given the relatively low fat content, comparable with some marine species (*Usydus et al., 2011*). This is probably due to an exceptionally good nutritional environment of the investigated fish in combination with the fact that freshwater fish are able to convert 18 carbon precursors into their longer LC-PUFA derivatives (*Tocher, 2003*). Furthermore, *Aggelousis & Lazos (1991)* stated quite high values of these important FA (6% EPA and 9% DHA) in nase together with a very low-fat content as mentioned above. The effect of different localities on the proximate composition in nase showed significant differences in the ash content and moisture, whereas no significant changes were observed in the other parameters; this might be related to the feed and environment. The PUFA percentage tended to decrease significantly when total lipid content increased, such as in 20:4n-6 (p = 0.003, R_2 = 0.68); 22:5n-3 (p = 0.013, R_2 = 0.51); 22:6n-3 (p = 0.01, R_2 = 0.53) inverse correlation were observed. Similar conclusions were obtained by *Belling et al. (1997)* and *Zhang et al. (2014)*.

The only representative of a species which is captured as well as farmed in our study, is European perch. Our results confirm that wild European perch contains a minimum (0.3–1.5%) of fat in the fillet (*Puustinen, Punnonen & Uotila, 1985*; *Mairesse et al., 2006*; *Orban et al., 2007*), in our case (0.74 ± 0.04 g/100 g). Subsequently, due to the low-fat content, the relative percentage of n-3 LC-PUFA rises, which is confirmed by the highest percentage of these FA (36.80 ± 3.80%) of all species analyzed in this study. *Olsson et al. (2007)* found that the length of the gastrointestinal tract of European perch has an adaptive plasticity based on the different feed type they consume. This means that any changes in their diet leads to an individual specialization in the morphology of their digestive organs, particularly alteration in the relative length of the gastrointestinal tract can take place (*Olsson et al., 2007*). This is relevant as the natural habitat of the European perch (the littoral and pelagic habitats) has a high variation in the feed sources and in consequence results in differences in gastrointestinal tract length. The size of the

digestive organs is connected with a more efficient use of the feed source (*Sibly, 1981*; *Magnan & Stevens, 1993*; *Olsson et al., 2007*).

European perch as an ichthyofagous/optional benthofagous species (*Golovanova et al., 2014*) is very popular among anglers in Central Europe and its high and appreciated flesh quality resulted in the beginning of farmed perch production. However, fish kept in recirculation systems usually have a higher fillet fat content, as the feeding intensity and the fat content of the feed can be higher than under natural conditions (*Xu et al., 2001*). Significant changes were observed in all parameters except dry matter, due to the effect of different localities indicating a high variation of feed composition and availability at the different localities. It could be also due to the sensitivity of European perch to the feed effects in general, since lipid is a major concern in European perch, which is greatly influenced by n-3 and n-6 FAs in the diet (*Xu & Kestemont, 2002*). Some FAs, including 14:0 (p = 0.09, R_2 = 0.20); 16:1 (p = 0.0006, R_2 = 0.63); 18:2n-6 (p = 0.042, R_2 = 0.29) showed positive correlation and in case of 20:1n-9 (p = 0.09, R_2 = 0.22) negative correlation to the fat content with significant changes was observed consequently due to the changes in fat content as a result of the effect of different localities.

European grayling is among the rarely consumed species, with an importance primarily in sport fishing. Additionally, its population is currently threatened in the region of Central Europe (*Turek et al., 2014*). Hence, European grayling is little known from a nutritional point of view. The fillet of European grayling showed to have a relatively low-fat content in line with earlier 2.3–2.6 g/100 g (*Renaville et al., 2013*); 2.77 ± 0.92 g/100 g in this study. According to our findings, there is a high proportion of n-3 PUFA (19.07 ± 8.40%) including EPA and DHA with relatively high variability, most probably again due to the respective available diet. Interestingly, the only similar study focused on the nutritional composition of wild and farmed European grayling brought substantially different results of FA composition compared to our results. While we found n-3/n-6 ratio of 1.10 ± 0.76, *Ahlgren, Carlstein & Gustafsson (1999)* described a ratio at 4–6 for wild and even 7–13 for farmed fish. The main difference was the high content of n-6 linoleic acid (16.29 ± 2.87%) in the fillet of European grayling from Central Europe. Comparable values were only presented in studies on Arctic grayling (*Thymallus arcticus*) published by *Sushchik et al. (2006)* and *Gladyshev et al. (2012)*. Significant changes were observed for all parameters except protein content in connection with the changes in localities. Some FAs, including 16:0 (p = 0.059, R_2 = 0.26); 18:0 (p = 0.01, R_2 = 0.4); 20:4n-6 (p = 0.001, R_2 = 0.56); 22:5n-3 (p = 0.0006, R_2 = 0.63); 22:6n-3 (p = 0.0008, R_2 = 0.62) showed an inverse correlation, to total fat content whereas a positive correlation was observed in FAs, including 16:1 (p = 0.06, R_2 = 0.25); 18:1n-9 (p = 0.004, R_2 = 0.5); 18:3n-3 (p = 0.006, R_2 = 0.47). ALA is preferably stored in neutral lipid fraction, which is mainly consisting of TAG, hence it can increase with an increasing fat content (*Enser et al., 2000*).

In Central Europe, Prussian carp is an invasive carp-like species. We found a lower fat content (1.94 ± 1.13 g/100 g flesh) compared to *Zivkovic et al. (2013)*, who presented values between 3.3 and 3.7 g/100 g flesh. A similar variability (1.2–4.5 g/100 g flesh) can be found in related—but better nutritionally mapped-crucian carp (*Carassius carassius*)

(*Lazos, Aggelousis & Alexakis, 1989*; *Aggelousis & Lazos, 1991*; *Donmez, 2009*). The fat content and FA composition found in the present study is comparable with Prussian carp analyzed by *Ozparlak (2013)* with a low lipid content and a relatively high proportion of n-3 PUFA. Significant changes in lipid content, ash and moisture were noted, whereas no changes were seen in protein and dry matter. In some FAs including 14:0 ($p = 0.03$, $R_2 = 0.35$); 18:3n-3 ($p = 0.03$, $R_2 = 0.36$); 22:1 ($p = 0.02$, $R_2 = 0.43$) a positive correlation to the fat content was observed, while in the 18:1n-9 ($p = 0.02$, $R_2 = 0.39$); 20:4n-6 ($p = 0.01$, $R_2 = 0.46$); 20:5n-3 ($p = 0.01$, $R_2 = 0.48$); 22:6n-3 ($p = 0.006$, $R_2 = 0.53$) a negative correlation was observed. *Kaya & Erdem (2009)* observed comparable values to our findings for a fillet fat content in wild brown trout throughout the year (1.85 ± 0.1 g/100 g of flesh in January to 3.57 ± 0.2 g/100 g in June). Very similar results were also published by *Kaushik et al. (2006)*, confirming that 2.5–3.5 g/100 g flesh is most likely a normal average fat content of wild brown trout across Europe. Also the n-3/n-6 ratio, which is 2.95 ± 1.39 in this study (Table 3), was similar to the values found by *Kaya & Erdem (2009)* in the same season (spring) showing a ratio 3–4. Meanwhile *Kaushik et al. (2006)* found lower values of 1.5–2. Again, this indicates a different composition of feed and confirms a generally high variability in the fillet FA composition within the same fish species. Significant changes in the ash content were observed as a result of changes in the localities. Brown trout with specific intestinal characteristics, including about 45 pyloric caeca, can digest the feed enzymatically through proteolytic activity (*Burnstock, 1959*) which facilitates in the absorption of the digested feed. However, most of the carnivorous species have shorter and thicker intestines compared to the herbivorous species (*Smith, 1980*; *Rust, 2002*), as well as increased enzymatic activity of for example, proteases and peptidases, which facilitates the absorption of the peptides and amino acids for the carnivorous species like common bream and rainbow trout (*Rodrigues et al., 2017*).

A positive correlation of FAs to fat content including 14:0 ($p = 0.07$, $R_2 = 0.28$); 16:1 ($p = 0.05$, $R_2 = 0.33$) was observed, whereas in 22:6n-3 ($p = 0.09$, $R_2 = 0.33$) a negative correlation was observed.

Another aspect is the FA content in absolute amounts. Although some leaner fish (here European perch) may contain a high percentage of EPA and DHA compared to fatter species, the absolute amounts logically increase with an increasing fat content. The European Food Safety Authority (EFSA) recommends a minimum daily intake of EPA + DHA of 250 mg for normal population (*EFSA, 2009*). This means, considering 150 g fish as an average portion, all fish fulfil more than the minimal recommended intake of EPA and DHA and hence they can contribute to a much healthier diet. 300 g of nase would even fulfil the intake for a whole week. However, since fish consumption is low in Central Europe, it needs to be promoted and increased.

The lipid health indices are described in detail by *Ulbricht & Southgate (1991)*, who stated that the values of IA and IT in food are good indicators for the risk of atherogenic and thromobogenic effects of foods, and subsequently the risk for the development of cardiovascular diseases (CVDs). The higher those values are, the higher the atherogenicity and thrombogenicity of the food items respectively is. *Ulbricht & Southgate (1991)* also provide IA and IT values for pork, beef and chicken (0.6, 0.7, and 0.5,

respectively for IA and 1.4, 1.3, and 0.95 for IT, respectively). All found values of IA and IT are very close to the values stated for the so-called Eskimo diet, which is related to very low incidences of the coronary heart disease (IA 0.39 and IT 0.28) (*Ulbricht & Southgate, 1991*). In 1970s, Bang and Dyerberg, have investigated the low risk of CVD in the Greenland Eskimos population. They found reduced risks of CVD in connection with the consumption of high amount of fish and marine mammals in the Eskimos diet. At that time, n-3 PUFA consumption of the Eskimos was five times higher than Danish people intake. Subsequently, the so-called Eskimo diet has very low incidences of the coronary heart disease (IA 0.39 and IT 0.28) (*Ulbricht & Southgate, 1991*).

Moreover, the values of these two indices in our study are in agreement with the results of *Rodrigues et al. (2017)* and *Monterio et al. (2017)*. Our results confirm that wild fish is clearly favorable for human nutrition.

CONCLUSIONS

In this study the proximate and FA composition of seven wild freshwater fish species from the Czech Republic was analyzed. According to our findings we conclude that the chosen species have a standard protein content, minimum carbohydrates and relatively low contents of fat, which can, however, vary to some degree in various localities, most probably related to the availability and composition of feed. In addition, factors such as fish physiology and feeding habits as well as ecological factors including water body trophic status, could have an influence on the variation of nutritional composition of the different species.

As expected, we showed that there can be some variation of FA composition in the same species, depending on natural habitat and availability of feed. Simultaneously, we observed a very favorable FA composition with high proportions of n-3 PUFA, including EPA and DHA in all analyzed species. Consequently, the values of both IA and IT were low and close to the values of the so-called Eskimo diet.

The obtained data increased the nutritional information about the chosen species for experts as well as consumers. It would be beneficial to provide the local fishermen and anglers association with this information to promote consumption of fish in general, especially of yet underutilized species. Regarding the effects of the fat content, in some MUFA, PUFA, and SFAs there were correlations with the lipid content. The dynamic interaction between them needs more investigation, which then could partly explain the differences among the localities.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Sarvenaz Khalili Tilami conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Sabine Sampels conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Tomáš Zajíc conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Jakub Krejsa performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, approved the final draft.
- Jan Másílko conceived and designed the experiments, contributed reagents/materials/analysis tools, approved the final draft.
- Jan Mráz conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The following information was supplied regarding data availability:
The raw data are provided in the Supplemental Files.

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CHAPTER 4

OLEAGINOUS YEAST AS A COMPONENT IN FISH FEED

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Oleaginous yeast as a component in fish feed

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This study investigates the replacement of vegetable oil (VO) in aquaculture feed for Arctic char (*Salvelinus alpinus*) with oil produced by the oleaginous yeast *Lipomyces starkeyi* grown in lignocellulose (wheat straw) hydrolysate. VO is extensively used to partially replace fish oil in aquaculture feed, which can be seen as non-sustainable. VO itself is becoming a limited resource. Plant oils are used in many different applications, including food, feed and biodiesel. Its replacement in non-food applications is desirable. For this purpose, yeast cells containing 43% lipids per g dry weight were mechanically disrupted and incorporated into the fish feed. There were no significant differences in this pilot study, regarding weight and length gain, feed conversion ratio, specific growth rate, condition factor and hepatosomatic index between the control and the yeast oil fed group. Fatty and amino acid composition of diet from both groups was comparable. Our results in fish demonstrate that it is possible to replace VO by yeast oil produced from lignocellulose, which may broaden the range of raw materials for food production and add value to residual products of agriculture and forestry.

Fish is one of the most traded food commodities and has great potential to contribute to food security for a growing population[1]. Fish is already the major source of protein in many cultures[2]. Moreover, it is also the major resource of n-3 long chain polyunsaturated fatty acids (LCPUFA)[3]. Hence, aquaculture is a rapidly growing industry and is an important source of animal-based foods. This growth generates an increased demand for feed for farmed fish. Currently, fish meal and fish oil are still the primary resources to meet the demand for protein and lipids of farmed fish[2]. Aquaculture consumes about 70% of the globally produced fish oil (FO), and 90% of this oil is derived from reduction fisheries[3]. Thus, a sustainable further expansion of the aquaculture industry can only happen when alternative resources/replacements for FO can be found. Those alternatives can be both vegetable oil (VO) and terrestrial animal oil[3–6]. Although both VOs and animal fat do not provide a good supply of n-3 long chain polyunsaturated FA (LCPUFA), they are metabolised by the fish in beta-oxidation, to provide energy. It has been shown that FO can be replaced by VO or animal fat without negatively impacting fish health or growth[4–8]. In Europe, VO is the most common partial substitute for fish oil, whereas in other parts of the world, terrestrial animal fats are also incorporated into aquafeeds. Nevertheless, VOs have a broad range of applications, including direct food production and biodiesel production. Especially with a view towards the latter, discussions about the sustainability of VO production have been raised. Finding alternatives to VO may lessen the push towards monocultures, with risk for land use changes and rainforest cutting, and in general, reduce the food carbon print of aquaculture[9–13].

Microbial oils or single cell oils have been regarded as a potential replacement for VO in biodiesel production, and in some cases even for food purposes. Oleaginous yeasts, *i.e.* yeasts that can accumulate 20% and more of their biomass as lipids, can form single cell oils from a variety of low value substrates, including lignocellulose hydrolysate[14–16].

While there are a number of reports on utilising yeasts as a protein source in fish feed (e.g.[17,18]), only little is known about utilising yeast-derived oil in fish feed. Several oleaginous yeast species can utilise lignocellulosic hydrolysates and convert them to lipids. We have recently demonstrated that the oleaginous yeast *Lipomyces starkeyi* can efficiently synthesise lipids from the hemicellulose fraction of birch wood and the cellulose fraction of wheat straw[19,20], and other studies have also used lignocellulose hydrolysate as a substrate for oil production with this yeast[21–24]. The lipid composition of *L. starkeyi* was shown to be similar to that of saturated fatty acids (SFA) rich VO, for example olive oil or palm oil[19,20,25]. In this pilot study, the aim was to test whether it is possible

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Fatty acid	Proportion in VO (control) feed [% of total fatty acids]	Proportion in yeast oil feed [% of total fatty acids]
C14:0	3.5	4.5
C16:0	15.9	21.3
C18:0	4.0	3.6
C18:1, tot	30.2	26.0
C20:1, tot	1.5	2.0
C22:1, tot	4.2	4.2
C18:2n-6	3.8	2.8
C20:4n-6	0.45	0.53
C18:3n-3	1.4	1.7
C20:5n-3	8.5	9.5
C22:5n-3	1.0	1.0
C22:6n-3	6.8	8.1

Table 1. Fatty acid composition (% of total FA) of the two experimental diets (duplicate analyses, the deviation of the single measurements was below 1.5%).

to replace VO in the feed for Arctic char (*Salvelinus alpinus*) with single cell oils derived from *L. starkeyi* grown on lignocellulose hydrolysate from wheat straw, *i.e.* a non-edible, residual material.

Results

Hydrolysate analysis. The cellulosic hydrolysate from wheat straw (*i.e.* the enzymatically hydrolysed solid phase after steam explosion (see method part)) contained glucose 87.3 g/l, xylose 22.2 g/l and acetic acid 3.8 g/l. Due to the high acetic acid concentration we started the fermentation with 50% hydrolysate and then pumped in 100% hydrolysate in the feeding phase of the cultivation.

Yeast cultivation. At harvest, *L. starkeyi* cells had consumed all carbon sources, and the total yeast dry weight of 575 g (cells from four fermentors) was produced from a total amount of 23.2 l hydrolysate, *i.e.* 2628.6 g carbon sources (glucose, xylose, and acetic acid). The final intracellular lipid content of the yeast was determined to be 43 ± 0.8%, thus the total amount of yeast lipids produced was 247.25 g, corresponding to a lipid yield of 0.09. Yeast growth and carbon source consumption are illustrated in Supplementary Fig. S1. Cells were disrupted by French press, as described in Methods; successful disruption was confirmed by microscopic inspection of the cell lysate. No further oil extraction was performed, to avoid contamination with toxic solvents and to retain the yeast proteins and polysaccharides in the hydrolysate.

Fish performance. Fish were fed with a standard experimental diet[26] (see Methods) containing standard ingredients and either VO and casein (control diet) or disrupted *L. starkeyi* cells instead of VO and casein. The fatty acid composition of the feeds is shown in Table 1. In both feeds, the main source of amino acids was fish meal. Accordingly, the amino acid profiles of the control- and yeast-based feeds did not differ significantly (Table 2). Initial and final weight and length, liver weight and the calculated performance factors are presented in Table 3. Initial weight of fishes was 148.2 g (control) and 149.8 g (yeast oil feeding), and the final weight was 265 g in both cases. There were no significant differences between the control and yeast fed fish regarding feed conversion rate (FCR), specific growth rate (SGR), condition factor (CF) and hepatosomatic index (HSI), indicating that both feeds were metabolised in a similar way and the addition of yeast in the feed did not negatively impact growth.

There was a large standard deviation of the individual fish weight, both in the yeast-fed treatment and the control towards the end of the experiment. This effect was most likely due to the small number of fish, which enabled a few dominant individuals to consume a major proportion of the provided feed, at the costs of other, minor individuals, which hardly showed any growth. The number of fishes was adjusted to the size of the 3 tanks and 2 months feeding to ensure appropriate water parameters such as NH_4^+ and oxygen tension when fish biomass increases. However, the total mass of the fish did not significantly differ, in spite of the dominant individuals. Consequently, sampling was carried out on fishes representing all sizes from all units. This growth effect does not hinder the evaluation of the fatty acid composition of the tissue samples, as the individual fish reflected the feed fatty acid profile, which is a common result in experiments performed on salmonids.

Lipid content and fatty acid composition. The total weight (whole fish), fat content and fatty acid profile of the muscle tissue of six yeast fed fishes and six control fed fishes are shown in Table 4 (two from each tank).

Overall, no significant differences between the two different feeds were observed, except for linoleic acid (C18:2 n-6) where the fish fed with control feed had slightly higher levels compared to yeast fed fish.

Discussion

In this study, we investigated whether it is possible to replace VO with oil produced by an oleaginous yeast, *L. starkeyi*, grown on lignocellulose (wheat straw) hydrolysate. Inclusion of yeast oil into fish feed has been tested previously but in the context of replacing fish oil in the feed, using oil from genetically engineered *Yarrowia lipolytica* cultivated on first generation substrate (glucose)[27].

Amino acid	Proportion of total determined amino acids [%] in VO feed[a]	Proportion of total determined amino acids [%] in yeast oil feed[a]
Alanine	6.4	6.8
Arginine	6.6	6.7
Aspartic acid	9.4	9.6
Cysteine + Cystine	1.0	1.0
Glutamic acid	16.7	16.2
Glycine	6.6	7.1
Histidine	2.1	2.1
Isoleucine	4.2	4.2
Leucine	8.0	8.0
Lysine	7.7	7.5
Methionine	2.8	3.0
Phenylalanine	4.3	4.3
Proline	5.6	5.5
Serine	4.8	4.8
Threonine	4.4	4.5
Tyrosine	3.7	3.7
Valine	5.1	5.1

Table 2. Amino acid composition of the two experimental diets. [a]Amino acid analyses were performed by Eurofins Food & Feed Testing Sweden AB. The confidence interval of all values is 15%.

	Control (n = 24)	Yeast (n = 24)
Initial length (cm)	23.63 ± 0.05	23.58 ± 0.30
Initial weight (g)	148.2 ± 3.9	149.8 ± 4.4
Final length (cm)	27.79 ± 0.76	27.91 ± 0.79
Final weight (g)	265.1 ± 34.7	265.0 ± 29.8
Liver weight (g)	4.15 ± 0.88	4.00 ± 0.59
FCR* (%)	1.86 ± 0.55	1.69 ± 0.38
SGR* (%)	0.95 ± 0.18	1.00 ± 0.14
CF* (%)	1.17 ± 0.06	1.16 ± 0.02
HSI* (%)	1.47 ± 0.11	1.42 ± 0.06

Table 3. Performance factors for fish fed with either control feed or feed with yeast as a substitute for VO. Data are presented as means ± standard deviation. Feeding trial was conducted in triplicates with n = 8 in each tank (n total = 24 fish in each treatment). No significant differences between the feeds were identified. *Abbreviations: FCR- feed conversion rate, SGR- specific growth rate, CF- condition factor, HSI- hepatosomatic index.

Our study shows that it is possible to convert second generation substrate (lignocellulose) to a feed component, enabling the replacement of feed oil (mostly VO) as an energy source in aquaculture. VOs are listed among the products causing the largest environmental impacts. They are also regarded as the fastest growing food commodities worldwide[28]. Some vegetable oils have a high greenhouse gas potential associated with their production: for instance palm- and soybean oil are estimated to emit more than 2000 kg CO_2 equivalents per ton produced, and considerable areas of arable land are used for producing vegetable oils[29]. Since biodiesel is also produced from vegetable oils, their consumption in the EU greatly exceeds local production, and thus, a major proportion of the utilised plant oil has to be imported[30]. There are reports of rainforest clearing due to palm- and soya oil production and there are moves in the EU to reduce the use of imported vegetable oils, especially palm oil (http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//TEXT+REPORT+A8-2017-0066+0+DOC+XML+V0//EN).

The yeast cells contain, apart from oil, also proteins and other components that can be utilised by the fish. The first implication of this is that the yeast cells contributed to protein biomass in the feed; this was adjusted by removing the casein from the yeast feed, whereas it was the standard protein additive in the control feed, as commonly used in other fish feeding trials[26]. The second implication is that it was not necessary to extract the oil from the mechanically disrupted yeast cells. This is advantageous compared to for instance microbial biodiesel production, where extraction is regarded as one of the most crucial steps in obtaining a sustainable process[31]. Analyses of growth parameters and composition of the final fish demonstrated that there was no negative impact of replacing VO and casein by *L. starkeyi*-biomass. The amino acid profile of the yeast-based feed did not change compared to the control. There was a slight but significant decrease in the total amount of n-6 fatty acids in the yeast fed fish. A low n-6/n-3 ratio is advantageous, as in most modern diets this ratio is too high, leading to a variety of diseases[32]. Our experiment demonstrates that it is possible to replace terrestrial plant- and animal based lipid and protein sources by yeast biomass. The fatty acid composition of yeast strains varies with both strain and

	Control, n = 6	Yeast, n = 6
Weight, g	271 ± 51.0	295 ± 36.1
Fat content %	4.70 ± 0.64	7.07 ± 2.81
Fatty acid composition		
C14:0	4.10 ± 0.20	4.08 ± 0.23
C15:0	0.29 ± 0	0.30 ± 0.01
C16:0	16.0 ± 0.41	17.6 ± 0.64
C17:0	0.36 ± 0.03	0.33 ± 0.04
C18:0	2.51 ± 0.24	2.43 ± 0.15
C20:0	1.23 ± 0.04	1.30 ± 0.16
C16:1n-7	6.04 ± 0.35	6.76 ± 0.61
C18:1n-9	31.9 ± 0.33	31.0 ± 0.81
C20:1n-9	3.19 ± 0.03	3.13 ± 0.31
C22:1n-9	2.29 ± 0.07	2.29 ± 0.20
C18:2n-6	5.82 ± 0.08[a]	4.78 ± 0.50[b]
C20:2n-6	0.16 ± 0.02	0.16 ± 0.04
C20:4n-6	0.39 ± 0.01	0.40 ± 0.05
C18:3n-3	1.24 ± 0.08	1.09 ± 0.16
C20:5n-3	6.98 ± 0.37	7.15 ± 0.44
C22:5n-3	1.38 ± 0.06	1.46 ± 0.13
C22:6n-3	14.3 ± 1.02	13.8 ± 2.25
SFA*	24.5 ± 0.85	26.0 ± 0.58
MUFA*	41.3 ± 0.41	41.1 ± 1.67
PUFA*	30.4 ± 0.93	29.0 ± 2.04
n-3	23.9 ± 0.87	23.5 ± 2.53
n-6	6.48 ± 0.08[a]	5.48 ± 0.50[b]
n-6/n-3	0.27 ± 0.01	0.24 ± 0.05

Table 4. Weight, fat content and fatty acid profile in the fillet (dark and light muscle tissues) from Arctic char fed with either control feed or feed with yeast as a substitute for VO. The different letters above the numbers represents values with significant differences; without letters represents values with no significant differences. (n = 6, Mean ± standard deviation). *SFA = saturated fatty acids, MUFA = mono unsaturated fatty acids, PUFA = poly unsaturated fatty acids.

cultivation conditions[19,20,33]. Selecting appropriate yeast strains and culture conditions may thus represent a possibility to positively influence the fatty acid composition and thereby the n-6/n-3 ratio.

From the lignocellulose substrate, 0.09 g lipids were produced per g consumed carbon source. This is within the range of values previously reported in similar cultivations[19,20,34]. In this study, yeast cultivation was performed to generate biomass for the fish trial; optimisation of the yeast fermentation conditions was not within the scope of the study. Nevertheless, rapid and efficient lipid production from the substrate can greatly improve the overall energy output and greenhouse gas impacts of any single cell oil production process[31,35], and therefore, optimisation of fermentation conditions and strains for lipid production is one of the major topics of our ongoing research.

This pilot study, to the best of our knowledge, investigates for the first time the utilisation of lignocellulose-derived yeast oil in fish feed. The results demonstrate that it is possible to completely replace VO and partially replace protein (casein in the control feed, in the present study) with the yeast biomass, without any significant effects on fish growth and final quality. Previous studies have shown that there is a limit to including yeast-based protein into fish diets[17,27]. On the other hand, there are also studies indicating a positive effect of yeast cell wall β-glucan on the immune system of fish[36] and a barrier function of yeasts against prions[37]. Moreover, utilising different yeast strains and different lignocellulose substrates may have some impact on the final quality of the fish. All these possible effects require further investigation and will be the subject of future studies.

Methods

Strains and media. *L. starkeyi* CBS 1807 (Centraalbureau vor Schimmelcultures, Utrecht, The Netherlands) was maintained on YM-agar plates (glucose 10 g/l, yeast extract 3 g/l, peptone 5 g/l, malt extract 3 g/l, Agar 16 g/l). The pre-culture medium was YPD (glucose 20 g/l, yeast extract 10 g/l, peptone 20 g/l).

Preparation of hydrolysate. The steam explosion and enzymatic hydrolysis was performed at the Department of Chemical Engineering, Lund University, Sweden. Wheat straw was soaked with 1% acetic acid over night, and fluid removed by pressing. The acid soaked biomass was then steam exploded at 190 °C for 10 min in a 10 L steam pretreatment reactor. The liquid fraction (mainly hemicellulose) was separated from the solid fraction and the latter was enzymatically hydrolysed. The hydrolysis was performed at 45 °C and pH 4.8. Cellic CTec3 enzyme cocktail (Novozyme A/S, Bagsværd, Denmark) was added at 10 FPU/g substrate. After hydrolysis, the suspension was centrifuged to separate the solid residues (mainly lignin) and repeatedly filtered, using filters

Feed ingredients	Control feed		Yeast feed	
	(g)	%	(g)	%
Fish meal	550	49.4	550	50.3
Fish oil	130	11.7	130	11.9
VO (Olive oil)	55	4.94	0	0
Mineral and vitamin mix	4	0.36	4	0.37
Wheat meal	295	26.5	245	22.4
Casein	55	4.94	0	0
Ca_2SO_4	25	2.24	25	2.29
Yeast DM	0	0	140	12.8
Total	1114	100	1094	100

Table 5. Ingredients in the two types of fish feed: the control feed and the feed with yeast as a substitute for VO.

with decreasing pore size in each step. The last filtration step was performed with a 0.45 μm sterile filter. The sugar and acetic acid concentration was determined by HPLC as described previously[19].

Pre cultures. Before inoculation in fermentors, *L. starkeyi* was cultivated in two steps with increasing medium volumes. For the first pre-culture, a loopful of *L. starkeyi* cells was inoculated from a YM-agar plate into 100 ml YPD-medium in 500 ml baffled shake flasks and incubated at 25 °C and 150 rpm in a rotatory shaker. After 48 h, the 100 ml culture was transferred to 400 ml YPD medium in a 3 l shake flask and incubated at 25 °C and 150 rpm for 72 h. The cells were harvested by centrifugation (4000 g, 10 min) and washed twice with saline solution (NaCl, 9 g/l). After washing, the pellet was resuspended in 50 ml saline and inoculated into the fermentor.

Fed-batch cultivation in fermentors. *L. starkeyi* was cultivated in four Dolly fermentors (Belach Bioteknik, Stockholm, Sweden, working volume 8 l) at 25 °C. A volume of 1.5 l of sterile filtered cellulose hydrolysate was added to each fermentor containing 1.5 l sterile deionised water, representing a starting volume of 3 l comprised of 50% cellulose hydrolysate. The pH was set at 5 and automatically controlled by addition of NaOH (25% w/w) or 3 M H_3PO_4. The aeration was initially 1 l/min; during the experiment it was continuously increased up to 5 l/min. The dissolved oxygen tension (pO2) was controlled by a DO-electrode, set to 20% and maintained by changing the stirring speed. One ml of polypropylene glycol 2000 (Alfa Aesar, Karlsruhe, Germany) was added to prevent foaming. *L. starkeyi* was first cultivated in a batch phase for 48 h, then the fed-batch phase started with pumping cellulosic hydrolysate at a speed of approx 24 ml/h in 7.5 days, *i.e.* 4.3 l of hydrolysate was added to each fermenter during the feeding phase; the total amount of hydrolysate was thus 5.8 l per fermenter.

***L. starkeyi* harvesting.** Cells were centrifuged at 5400 g for 10 min, washed with deionised water and then disrupted in a French press (Constant systems LTD, Daventry, UK) at 40 psi. Dry weight of the disrupted cells was determined by drying a portion of the cell-lysate in a Precisa xm 60 oven (Precisa Instruments LTD, Dietikon, Switzerland) and the disrupted cells were stored at −20 °C until incorporation into the fish feed[18].

Fish feed preparation. The composition of the fish feed is shown in Table 5 [26]. The ingredients were mixed by hand to a homogeneous consistency and pressed through a kitchen meat grinder. The feed was dried at room temperature for 48 h before vacuum packing into air tight plastic bags. Total amino acids were quantified in the prepared feeds (Eurofins Food & Feed Testing Sweden AB, Lidköping, Sweden, Method: SS-EN ISO 13903:2005).

Feeding trial. The experiment was carried out in accordance with EU legislation (*i.e.*, Directive 2010/63/EU), and received the approval of the Ethical Committee for Animal Experiments in Umeå, Sweden.

Arctic char was kept in flow through system with natural photo period at Kälarne Aquaculture North, Sweden. The water temperature was ambient, approx 12 °C and the water system was always fully aerated from the inlet. Inlet water quality was always assured. The tanks were 1 × 1 m and water depth 20 cm. Tanks were randomly assigned to the two diets with randomly selected fish (n = 8/tank). Prior to the trial all fish were fed a commercial diet suitable for Arctic char juveniles. The feed was distributed by band feeders 4 times a day[26]. Fish was anaestesised before handling[38].

The Arctic char were measured for weight and length and then divided into six tanks (n = 8): three were fed with the control feed and three with yeast feed. Feeding ratio was 2% of the actual biomass in the tanks.

After 2 months, the fish were weighed and measured again after a 24 h starvation period and liver weight was registered. After filleting, the muscle tissue was frozen on dry ice and then stored at −80 °C until lipid extraction.

Fish performance. Based on the measurements and the consumed feed, feed conversion ratio (FCR), specific growth rate (SGR), condition factor (CF) and hepatosomatic index (HSI) were calculated as follows:

$$FCR = F/(Wt - W0)$$

$$SGR = [(\ln Wt - \ln W0)/t] \times 100$$

$$CF = Wt/Tl^3 \times 100$$

$$HSI = (Wl/Tw) \times 100$$

where Wt = final weight of fish in g; W0 = initial weight of fish in g; F = amount of dry feed fed in g; t = time (days); TL = total length in cm, Wl = weight of liver in g; Tw = total weight of fish without liver in g.

Lipid extraction from yeast, feed and fish. The lipid content of yeast cells was determined as previously described[19,20]. For the fish and feed, total lipid analysis was performed according to Pettersson, *et al.*[26]. Lipids were extracted from six muscle samples from each treatment (sourced from two fish from each replicate) and from the feeds. A subsample of 1 g of fish feed or muscle (light and dark) of individual Arctic char was used for lipid extraction (in duplicate). The sample was homogenised in hexane:isopropanol (HIP; 3:2, v-v) with an Ultra-Turrax (Janke and Kunkel, IKA Werke, Staufen, Germany). For lipid and non-lipid phase separation, 6.67% of Na_2SO_4 was added to the homogenate and it was centrifuged. After gravimetrical identification of the total lipid content from dried samples, the lipids were stored in hexane at −80 °C for further analysis. All chemicals and solvents (reagent grade) were purchased from Merck (Darmstadt, Germany) except chloroform (Sigma Chemicals Co. St. Louis, MO, USA). The solvents were used without further purification.

Determination of fatty acid profiles. Fatty acid methyl esters (FAME) from total lipids in muscle and feeds were prepared with BF_3 methanol according to the method described by Appelqvist[39]. FAME were stored in hexane at −80 °C for further analysis.

FAME were analysed by GC using a CP 3800 instrument (Varian AB, Stockholm, Sweden) equipped with a flame ionization detector and a split injector, and separated on a 50 m fused silica capillary column BPX 70 (SGE, Austin, Tex) (0.22 mm i.d × 0.25 µm film thickness)[40]. The injector temperature was 230 °C and the detector temperature 250 °C. Helium was the carrier gas, at a flow rate of 0.8 mL/min, and nitrogen was used as make-up gas. Peaks were identified by comparing their retention times with those of the standard mixture GLC 68A (Nu-check Prep, Elysian, USA) and quantified using an internal standard (methyl-15-methylheptadecanoate; Larodan Fine Chemicals AB, Malmö, Sweden). Peak areas were integrated using Galaxie chromatography data system software version 1.9 (Varian AB, Stockholm, Sweden).

Statistics and calculations. Mean values, standard deviations and FA percentages were calculated in Excel and statistical analyses were performed using the Statistica CZ 12 software package. One-way analysis of variance (ANOVA) and Tukey's HSD test were performed to characterise the differences between control and experimental group. The performance factors data were treated by One-way ANOVA in Excel.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author upon request.

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Author Contributions

J.Bl. was involved in study design, and performed a major part of laboratory work and writing the manuscript. J.P. provided a major contribution for study design, data analysis and final manuscript writing. S.K.T. performed parts of the laboratory work and data analysis. S.S. performed chemical analysis, data evaluation and was involved in manuscript writing. N.M. and J.Br. performed major parts of laboratory work, M.S. and V.P. coordinated the project and provided major contributions to study design, data analysis and final manuscript writing. All authors reviewed the final manuscript.

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CHAPTER 5

INSECT MEAL AS A PARTIAL REPLACEMENT FOR FISH MEAL IN A FORMULATED DIET FOR PERCH (*PERCA FLUVIATILIS*)

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My share on this work was 50%.

INSECT MEAL AS A PARTIAL REPLACEMENT FOR FISH MEAL IN A FORMULATED DIET FOR PERCH *PERCA FLUVIATILIS*

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ABSTRACT

The replacement of 25% fishmeal by a mixture of house cricket *Acheta domesticus* and superworm *Zophobas morio* meal as an alternative protein source in feed for Eurasian perch *Perca fluviatilis* was evaluated. Our results show that the replacement of 25% of fish meal in the perch diet with an insect mixture and an adjustment of the amino acid composition does not negatively affect survival but did decrease growth and increase feed conversion. The hepatosomatic index, as well as hepatic EROD and BFCOD activity, did not differ between the control and experimental feeding groups. Feeding with insect pellets resulted in significantly increased 18:2 n-6 and total n-6 percentage compared to the control group. The change in FA composition, is only minor and does not affect the nutritional value for human consumption of the fish. Further evaluation should be conducted with different ratios or different insect species.

Introduction

Eurasian perch *Perca fluviatilis* is a valuable fish species, especially in Europe, and is also considered as a game fish. However, there is generally little information regarding the feeding and dietary requirements of perch. Since there is no specific feed for perch, formulated commercial diets that have been used for salmonids are used for perch (Brown et al. 1996). The major operating expense in aquaculture accounts for the cost of the feed (Brett 1979), which is approximately 40-70% of the cost for production in aquaculture. Feed costs are especially high for the culture of carnivorous fish, which require great amounts of fishmeal (FM) in their diet (Manzano-Agugliaro et al. 2012). Therefore, it is important to optimize the feeding strategy in a way that also reduces the costs of production (Schnaittacher et al. 2005) and increases growth as well as feed conversion. Among the required nutrients for fish, protein is an expensive ingredient. This is due to the combination of the drastic increase in the need for aquaculture feed as well as a decline in the sources of FM due to the over-fishing of

pelagic species and a subsequent decrease in the reliance on marine sources of protein from FM (FAO 2014). Therefore, using alternative sources of protein in the diet instead of FM, such as plant proteins, have been evaluated (Quartararo et al. 1998; Gatlin et al. 2007; Medale et al. 2013; Fawole et al. 2016). In addition, various animal protein sources have been considered as a replacement of FM (Bureau et al. 1999; Rawles et al. 2006). More recently, the use of insects as a protein source has been investigated (Henry et al. 2015; Ngoc et al. 2015). Insects represent an attractive alternative to traditional sources of proteins due to their high feed conversion, quick growth, and their potential to feed on bio-waste (Collavo et al. 2005), which makes their production highly sustainable. In addition, they are a rich source of amino acids, lipids, vitamins and minerals (van Huis 2013). Because some species are consumed as part of the natural diet of fish (Howe et al. 2014), they seem to be an appropriate replacement for FM. The amount of protein varies between 50-82% in different insect species, which reflect good nutritional value (Rumpold and Schluter 2013).

Eurasian perch is a carnivorous species with a high protein requirement, and in their juvenile stage in the wild, they feed mostly on insects before starting diets based on fish (Riddick 2013). Therefore, insects seem a very good candidate for FM replacement of this species.

When choosing alternative feed components, including proteins, it is highly important to consider the needs of the fish species for which the feed is intended. In deciding which proteins and raw products are the most appropriate as feed ingredients for each species, priority must be given to the metabolic demands of the fish species. Factors, such as survival, growth and feed conversion need to be determined before a new feed component can be considered adequate.

Cytochrome P450 (CYP) enzymes play important roles in the metabolism of many xenobiotic and endogenous compounds. The first three families of CYP are involved in biotransformation of xenobiotics. Fish that consumed different components than their usual feeding habit may metabolize some bioactive compounds as xenobiotics. The measurement of enzyme activity CYP 1A and 3A might provide information about the xenobiotic nature of selected insects.

Many researchers have investigated the use of black soldier fly *Hermetica illucens* (Bondari and Sheppard 1981), common housefly maggot *Musca domestica* (Ossey et al. 2012), mealworm *Tenebrio molitor* (Ng et al. 2001) and grasshopper *Locusta migratoria* (Johri et al. 2010) meal as FM replacement, but house cricket and superworm meal have received less attention. However, these species have great potential for future use, since they are frequently cultivated and used for pet nutrition, and the nutritional needs of these insects are already known. Furthermore, their production systems are well established.

From a human nutritional point of view, a high content of long chain n-3 fatty acids (FA) in fish is desired. Strong links between fish and seafood consumption as well as positive health effects, especially with a decreased risk of coronary heart and cardiovascular diseases, decreased inflammatory diseases, such as arthritis, and prevention of cancer have been demonstrated by Lund (2013). In the present study, meal from whole insects was used, which corresponded to 5% of the total fat content in the feed, and it was also necessary to evaluate the effects on fish muscle FA composition.

The aim of the present study was to investigate the effects of a replacement of 25% FM by a mixture of house cricket *Acheta domesticus* and superworm *Zophobas morio* meal in the diet of perch on survival, growth, feed conversion and the hepatosomatic index (HSI) as indicators for the well-being of fish as well as microsomal ethoxyresorufin O-deethylase (EROD, CYP1A) and 7-benzyloxy-4-trifluouromethylcoumarin O-debenzylase (BFCOD, CYP3A) activity as markers for exposure to xenobiotic compounds and metabolic detoxification in fish. In addition, FA composition as an indicator for the nutritional value of fish for human consumption was evaluated.

Materials and Methods

Fish, feeding trial and experimental design

Two isoenergetic and isoproteic diets were formulated to provide both the protein and lipid requirements of the perch, and the diets contained 52% protein and 15% total fat (Table 2). The control diet (CONT) was based on FM only, and in the experimental diet (INS), 25% of the original amount of FM was replaced with superworm and house cricket meals (10% in total 5% of each species). Insects were obtained from a local producer (Vladimír Šefl, Bušanovice) then sacrificed through liquid nitrogen freeze-drying and delivered to the feed production company (EXOT HOBBY s.r.o., Černá v Pošumaví) where the insects were milled and then processed into pelleted feed. Analyses of the proximate composition of crickets and superworms (Table 1) and of the pelleted feed (Table 2) were carried out by an accredited laboratory (Státní veterinární ústav Praha, Testing laboratory No. 1176). From the fat content, 9% and 8.5% came from salmon oil made from fish by-products in the CONT and INS, respectively, while the rest originated from the other ingredients including the FM and insects. Threonine and Methionine were added to both feeds in different ratios to adjust for an adequate amino acid composition (Table 2).

After adaptation of fish to the system and feeding conditions, the feeding experiment was performed in a recirculation system for 12 weeks at the experimental facility of the Faculty of Fisheries and Protection of Waters in Vodňany.

A total of 1440 perch were randomly divided into two groups of 720 juveniles each (control and insect group) and assigned to a dietary treatment with three replicates. In each replicate, 240 fish (mean weight of 23.1 ± 0.6 g) were held in 600 L round fibreglass tanks. The fish were fed continuously by an automatic feeder with a daily feed rate of 1.5% of the total weight of the stock. During the experiment, water parameters were checked twice a day (temp. 19.5 ± 0.1°C, dissolved oxygen 9.2 ± 0.6 mg L^{-1}).

Sampling and growth performance

Body weight and total length were recorded 5 times during the experiment at the beginning and then at a three-week interval using a total of 300 fish at each time point (150 per treatment, CONT and INS group). Finally, the total stock of each tank was weighed at the end of the experiment after a 24 h starvation period.

Survival was determined by observing the number of dead fish during the trial.

Based on the measurements, the feed conversation ratio (FCR), specific growth rate (SGR), condition factor (CF), survival rate (SR) and hepatosomatic index (HSI) were calculated as follows:

$FCR = F / (Wt - W0)$

$SGR = [(\ln Wt - \ln W0) / t] \times 100$

$CF = Wt/T_L^3 \times 100$

$SR (\%) = [Nt / N0] \times 100$

$HSI = (Wl / Tw) \times 100$

Where Wt = final weight of fish in g; W0 = initial weight of fish in g;
F = amount of dry feed fed in g; t = time (days); T_L= total length in cm; N0= initial number of fish; Nt = final number of fish; Wl= weight of liver in g; Tw= total weight in g.

At the beginning and at the end of experiment, 10 fish from each group were killed and filleted for analyses of lipid content and composition. Total weight and liver weight were used for calculating the HSI. Liver samples were immediately frozen in liquid nitrogen and stored at -80°C for further biochemical assays.

Fatty acid and lipid content analysis

The lipid extraction of feeds and the skinless fillets were performed in duplicate based on the methods of Hara and Radin (1978), and the lipid content was quantified gravimetrically. For FA analyses, methylation of total lipids was performed according to the methods of Appelqvist (1968). FA composition was analysed by gas chromatography (GC) (Trace Ultra FID; Thermo Scientific, Milan, Italy) using a BPX-70 50 m fused silica capillary column (id. 0.22 mm, 0.25 μm film thickness, SGE, USA). The peaks were identified by comparing sample retention times to retention times of the standard mixture GLC-68-A (Nu-Chek Prep, Elysian, USA).

Microsomal fraction preparation and protein analysis

Fish hepatic microsomes were prepared by differential centrifugation (Li et al. 2011). All steps were carried out on ice. Microsomal fractions were immediately frozen and stored at -80°C for further analysis. The protein levels were estimated spectrophotometrically through the method described by Smith et al. 1985) using bovine serum albumin as the standard. The microsomes were diluted to obtain a protein concentration of 5 mg/mL.

Measurements of catalytic activities of EROD and BFCOD

The catalytic activity of EROD was measured as the rate in the formation of resorufin from 7-ethoxyresorufin (Kennedy and Jones 1994). The incubation mixtures contained 0.2 mg microsomal protein in an incubation medium of 0.5 mM potassium phosphate buffer (pH 7.4) with 1.0 mM nicotinamide adenine dinucleotide phosphate (NADPH) and 2 μM of 7-ethoxyresorufin.

The catalytic activity of BFCOD was measured as the rate in formation of 7-hydroxy-4-trifluoromethylcoumarin (HFC) from Resorufin, 7-ethoxyresorufin, 7-Benzyloxy-4-trifluoromethylcoumarin (BFC) (Burkina et al. 2016). Briefly, the reaction incubations contain 0.2 mg of microsomal protein in an incubation medium of 0.5 mM potassium phosphate buffer (pH 7.4) with 0.5 mM NADPH and 12.5 μM of BFC.

A fluorescence detector (Infinite 200 – Photometer TECAN) was used for detection of resorufin (excitation/emission 544/590 nm) and HFC (excitation/emission 410/538 nm). Enzymatic activities were expressed as pmol of resorufin or HFC formed per min and per mg of microsomal proteins (limits of detection were 2 and 1 pmol/min for resorufin and HFC, respectively).

Statistical analysis

Statistical analyses (T-test and one-way analysis of variance (ANOVA)) were performed using the Statistica CZ 12 software package.

Results

Growth parameters and survival

The average weight gain and final weight of the fish fed INS was significantly lower and FCR was significantly increased compared to the CONT group ($p \leq 0.05$), SGR and CF decreased significantly in the fish fed the experimental diet. Survival and HIS did not differ between the groups (Table 3).

Fat content and fatty acid composition in the fish

Fillet fat content was similar in both groups and did not change throughout the experiment. The feeding with INS affected the FA composition only slightly compared to the CONT group, mirroring the FA composition of the feeds. Feeding with insect pellets resulted in significantly increased 18:2 n-6 and subsequently increased total n-6 compared to the CONT group (Table 4). In addition, 20:1 n-9 was decreased in the INS group. Fatty acid composition in the insects and the feed are shown in Tables 5a and 5b, respectively.

EROD and BFCOD activity

Hepatic EROD and BFCOD activity did not differ ($p>0.05$) between group fed with the experimental diet compared to the group fed with the control diet (Figure 1).

Discussion

The aim of the experiment was to investigate whether the partial replacement of FM by 25% insects in the feed for perch affected survival, growth parameters, HSI, and FA composition, as well as whether the substitution caused alterations in enzyme activity of EROD and BFCOD, which are responsible for xenobiotic detoxification.

The lower growth parameters in the experimental group indicate lower nutritional value and digestibility of the feed. Another reason might have been the taste of the feed because we observed a tendency towards lower feed consumption in the experimental group ($p=0.06$). Lower palatability of feeds containing insects due to unpalatable compounds or anti nutritional factors in the insects were discussed earlier (Finke 2002). In perch, replacement of protein has been scarcely investigated until recently. However, similar results of a lower performance were found in channel catfish *Ictalurus punctatus* with a partial substitution of 10% FM by dried larvae of the soldier fly *Hermetia illucens* L., which showed a slower growth rate when reared in cages during a 15-week period. However, catfish reared in culture tanks still showed a slower but no significant decrease in growth, indicating that besides feed, the rearing system also had some influence on performance (Bondari and Sheppard 1981).

In contrast to our results, channel catfish fed fully or partially with the larvae of the soldier fly showed the same performance in total weight and length compared to the control group (Bondari and Sheppard 1981). This shows that species react differently to different insect replacement and underlines the importance of testing each insect species on the fish species in question before use. Also, the proportion of replacement needs a thorough evaluation. For example, juvenile Nile Tilapia *Oreochromis niloticus* fed with diets that included different proportions of superworm meal (0, 25, 50, 75, 100%) showed higher weight gain and SGR for fish fed with diets of 25 and 50% superworm meal compared to fish fed with a higher degree of replacement (Jabir et al. 2012). In the group supplemented with 100% superworm meal, a

decreased growth performance compared to the groups fed lower ratios was observed. These results indicate that there is an upper level for inclusion of insects as a protein source. In our study, 10% insect inclusion already resulted in slightly decreased growth rate, which indicates either that the chosen insect mixture was not the most suitable or that the proportion was too high already. The final FA composition of the fish was only affected to a minor extent. Therefore, we conclude that the chosen proportion and mixture of insects is not negatively affecting FA composition.

The similar HSI and EROD and BFCOD activities in CONT and INS group indicate no toxic or negative effects of the insects on metabolism. HSI in our study was also comparable to earlier results in wild and cultivated perch by Jankowska et al. (2007).

As we added the complete insects, to the diet, it was necessary to investigate the effects on fish FA composition. In general, the FA composition of the diet is reflected in the fish flesh (Menoyo et al. 2004). The significant increase of 18:2 n-6 in the INS group (Table 4) is due to the higher content of this FA in both the crickets and superworms and subsequently in the experimental diet (Tables 5a and 5b). However, from a nutritional point of view, we consider this change negligible.

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Table 1. *Proximate composition of house cricket Acheta domesticus and superworm Zophobas morio analysed by Státní veterinární ústav Praha, Testing laboratory No. 1176.*

Parameter (g/100 g)	House cricket	Superworm
Protein	21.7	19
Total carbohydrate	4.1	4.7
Ash	1.9	1.8
Dry matter	32.2	43.8
Fat	4.6	18.3
Energy value (kJ/100g)	606	1080

Table 2. *Ingredients and proximate composition of control (CONT) and experimental diet containing house cricket and superworm (INS) (%). Information from the producer (EXOT HOBBY s.r.o., Černá v Pošumaví), analyses by Státní veterinární ústav Praha, Testing laboratory No. 1176 and AGRO-LA, spol.s.r.o, Středisko laboratory.*

Ingredients (%)	CONT	INS
Fish meal[a]	39	29
Wheat meal[b]	27	24
Wheat gluten[c]	20	22
Insects	-	10 (5% cricket, 5% superworm)
Salmon oil[d]	9.0	8.5
Brewer's yeast[e]	3	-
Bolifor[f]	0.4	1.0
Premix Vit	0.35	0.35
Limstone	0.30	0.75
Proximate composition (percentage of dry matter basis)		
Crude Protein	52.2	47.2
Crude Fat	18.4	17.9
Crude Fiber	2.45	3.21
Ash	7.07	7.09
Mineral mixtures[g]	2.2	3.53
Amino acid composition (percentage of dietary protein)[h]		
Histidine	0.77	0.75
Isolucine	1.59	1.44
Lucine	3.03	3.02
Phenylalanine	1.69	1.61
Valine	1.82	1.73
Lysine	2.81	3.08
Methionine	0.66	0.69
Cystine	0.42	0.42
Threonine	1.62	1.79
Tryptophan	0.79	0.76
Arginine	1.91	1.77

Ingredients (%)	CONT	INS
Non-essential amino acids		
Aspartate	3.06	2.86
Serin	1.97	1.79
Glutamate	12.5	11.6
Glycine	2.85	2.60
Alanine	2.90	2.74
Tyrosine	1.28	1.24
Proline	3.17	3.02

[a]Hanstholm Prime (FF Skagen), Denmark; Protein 70-72%, Fat max 12%, Water max 10%, Salt max 4.5%, Ash 10-16%, Antioxidant min 150 ppm ethoxyquin were added.
[b]Vesco, Veselí nad Lužnicí, Czech Republic
[c]Krnovská škrobárna, Czech Republic
[d]Vfcux, Bioceval, Cuxhaven, Germany
[e]Brewer's yeast, Mráz Agro CZ, s.r.o., Blatná, Czech Republic
[f]Bolifor, Bioferm CZ, s. r.o., Brno, Czech Republic
[g]Each 1,000 g of mineral premix of CONT diet contained: 162 mg Fe; 7 mg Cu; 52 mg Mn; 1 mg Se; 88 mg Zn; 10 g K and INS diet contained: 129 mg Fe; 7 mg Cu; 49 mg Mn; 1 mg Se; 91 mg Zn; 8 g K.
[h]Amino acid analyses according to the standard method of commission regulation (EC) No 152 (2009).

Table 3. *Growth factors for fish fed with control (CONT) and experimental diet containing house cricket and superworm (INS); (mean±standard deviation)*

	CONT (n=150)	INS(n=150)
Survival (%)	94.0±40	94.6±1.9
Initial weight (g)	23.4±0.10	22.8±0.90
Final weight (g)	56.9±0.70[a]	48.6±2.10[b]
Weight gain (g)	33.5±0.61[a]	25.7±2.23[b]
FCR (%)	1.44±0.08[a]	1.75±0.15[b]
SGR (%/day)	1.06±0.01[a]	0.90± 0.07[b]
CF (%)	1.49 ± 0.11[a]	1.43 ± 0.13[b]
HSI (%) (n=10)	3.60± 0.90	3.80± 0.60

HSI was calculated for 10 randomly chosen fish in each group. Different superscripts indicate significant differences between the groups ($p \leq 0.05$)

Table 4. *Total identified fatty acids (%) in skinless fillet of perch (higher than 0.5%) at the beginning and at the end of the experiment fed with control (CONT) or experimental diet containing house cricket and superworm (INS); (mean±standard deviation)*

	Start	CONT final	INS final
Fat content	1.53±1.3	1.08±0.32	1.21±0.25
C14:0	0.78±0.10	1.23±0.40	1.14±0.27
C16:0	22.1±1.02	22.6±1.26	23.1±0.79
C16:1trans	1.15±0.23	1.16±0.16	1.28±0.17

C16:01	2.30±0.49	3.04±1.45	3.00±1.19
C18:00	3.68±0.32	3.82±0.66	4.10±0.47
C18:1n-9	15.1±1.88	18.4±4.64	18.3±3.08
C18:1n-7	2.23±0.14	2.28±0.22	2.18±0.10
C18:1n-5	0.63±0.03	0.47±0.12	0.45±0.08
C18:2n-6	9.34±1.15	9.45±0.85[a]	10.9±0.70[b]
C18:3n-3	1.24±0.14	1.82±0.28	1.78±0.21
C20:1n-9	1.25±0.16	1.52±0.18	1.30±0.24[b]
C20:4n-6	0.99±0.14	0.81±0.18	0.71±0.10
C20:5n-3	3.46±0.74	4.66±0.6	4.13±0.64
C22:5n-3	1.37±0.25	1.73±0.37	1.57±0.21
C22:6n-3	34.3±3.00	25.3±5.17	24.6±4.20
SFA	26.8±1.53	27.9±1.47	28.6±0.96
MUFA	23.2±3.00	27.93±6.34	27.5±4.55
PUFA	50.8±5.46	44.21±5.15	43.9±4.03
n-3	40.5±4.17	33.9±5.76	32.4±4.56
n-6	10.3±1.29	10.3±0.75[a]	11.6±0.68[b]
n-6/n-3	0.26±0.31	0.32±0.00	0.37±0.08

Different small letters indicate significant differences between the treatment groups (CONT and INS) (p≤0.05)

[1]SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Table 5a. *Fatty acid composition (%, higher than 0.5%) of house cricket Acheta domesticus and superworm Zophobas morio analysed in duplicate (mean ±standard deviation).*

	House ricket	**Superworm**
Fat content	5.57±1.11[a]	17.6±0.21[b]
C14:0	0.68±0.06[a]	1.09±0.06[b]
C16:0	24.1±0.61[a]	28.4±0.00[b]
C16:1trans	0.48±0.03[a]	1.52±0.20[b]
C16:01	0.92±0.08	0.91±0.06
C18:00	9.78±0.23[a]	7.86±0.15[b]
C18:1n-9	27.9±1.17[a]	38.7±0.29[b]
C18:1n-7	0.54±0.03	0.73±0.05
C18:2n-6	33.5±0.12[a]	18.8±0.17[b]
C18:3n-3	1.19±0.07	1.15±0.05
SFA	34.8±0.91	37.5±0.12
MUFA	30.1±1.12[a]	42.2±0.37[b]
PUFA	35.2±0.21[a]	20.3±0.25[b]
n-3	1.67±0.32	1.51±0.09
n-6	33.6±0.15[a]	18.8±0.16[b]
n-6/n-3	20.6±4.05	12.4±0.66

Different small letters indicate significant differences between the house cricket and superworm (p≤0.05)

Table 5b. *Fatty acid composition (%, higher than 0.5%) of control diet (CONT) and the experimental diet containing 10% house cricket and superworm (INS); (mean ±standard deviation).*

	CONT	INS
Fat content	18.4±0.64	18.0±0.40
C14:0	2.83±0.02[a]	2.55±0.04[b]
C16:0	13.2±0.14[a]	14.8±0.37[b]
C16:1trans	0.25±0.01[a]	0.31±0.00[b]
C16:01	3.15±0.03[a]	2.80±0.02[b]
C18:00	2.75±0.04[a]	3.20±0.07[b]
C18:1n-9	36.2±0.27	35.3±0.28
C18:1n-7	3.04±0.09[a]	2.64±0.02[b]
C18:1n-5	0.01±0.01	0.01±0.01
C18:2n-6	16.6±0.03[a]	17.0±0.12[b]
C18:3n-3	4.94±0.10[a]	4.53±0.04[b]
C20:1n-9	2.30±1.68	3.00±0.01
C22:1	2.24±1.15	2.45±0.01
C20:5n-3	3.99±0.09[a]	3.25±0.04[b]
C24:1	0.56±0.01[a]	0.49±0.01[b]
C22:5n-3	1.07±0.02	1.02±0.17
C22:6n-3	5.77±0.17[a]	4.73±0.00 [b]
SFA	19.2±0.29[a]	20.9±0.37[b]
MUFA	47.7±0.15	47.0±0.32
PUFA	33.1±0.44	32.1±0.05
n-3	16.2±0.32[a]	13.9±0.08[b]
n-6	22.7±0.29[a]	22.9±0.13[b]
n-6/n-3	1.40±0.01[a]	1.64±0.02[b]

Different small letters indicate significant differences between the CONT and INS pellets ($p \leq 0.05$)

[2]SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

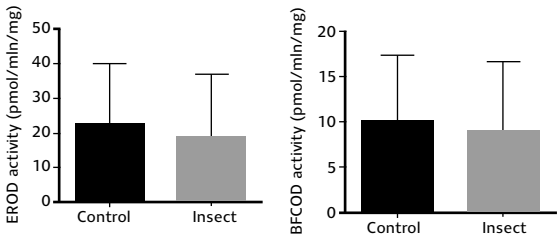

Figure 1. *EROD and BECOD activity in hepatic microsomes og perch (n = 10 in each group), fed with control diet or with experimental diet containing 10% house cricket and superworm; (mean activity ± standard deviation).*

[3]EROD, ethoxyresorufin O-deethylase; BFCOD, 7-benzolyoxy-4-trifluoromethylcoumarin O-debenzylase

CHAPTER 6

INSECTS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FEED: EFFECT ON GROWTH, FATTY ACID COMPOSITION AND SENSORY ATTRIBUTES

Turek, J., Sampels, S., Khalili Tilami, S., Červený, D., Kolářová, J., Randak, T., Mráz, J., Másílko, J., Steinbach, C., Burkina, V., Kozak, P., Zlabek, V., 2019. Insects in rainbow trout (*Oncorhynchus mykiss*) feed: effect on growth, fatty acid composition and sensory attributes. (manuscript)

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INSECTS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FEED: EFFECT ON GROWTH, FATTY ACID COMPOSITION AND SENSORY ATTRIBUTES

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ABSTRACT

Five isocaloric diets containing commercial pellets and live insects were evaluated in rainbow trout *Oncorhynchus mykiss* in a 60-day feeding trial. The control group (K) was fed commercial pellets only. In other groups, 25% gross energy of pellets was replaced by live adult house cricket *Acheta domestica* (Group C), live superworm *Zophobas morio* larva (Group L), or a combination of 12.5% crude energy of each (Group LC). A final group (I) was fed live cricket and superworm only (50/50 crude energy). No significant differences were found in growth, survival, feed conversion ratio (dry basis), or energy utilization among groups. Protein efficiency ratio was highest in Group K. Insect inclusion was associated with lower content of nutritionally valuable n-3 fatty acid in fish muscle. Muscle of fish fed insects only showed EPA and DHA content approximately 45% and 63%, respectively, of that in the Group K, a significant difference. EPA content was significantly lower in other insect fed groups, while DHA was comparable to control group. Subjective sensory evaluation of cooked fillets revealed significantly less acceptable taste, aroma, and aftertaste in Group I than for Groups K, L, and LC. Groups LC and I showed significantly whiter fillet colour than K Group. Redness of Group LC was significantly less intense than in Group C. The control group had significantly lower firmness compared to those receiving the insect diets. No gross morphological or histological anomalies were observed in any group. No significant differences were observed in EROD activity.

Keywords: *Rainbow trout, fillet quality, insect feed, growth performance, fatty acids, alternative feeds*

Introduction

Rainbow trout *Oncorhynchus mykiss* is widespread in temperate regions, and represents a large share of worldwide salmonid production. Aquaculture is a rapidly growing industry with production increasing at an average annual rate of 5.8% percent to 73.8 million tonnes in 2014. Salmon and trout represented about 17% of the total value of internationally traded fish products in 2014 (FAO, 2016). Rising production has led to increased demand for quality feed, the biggest component of production costs. Despite their declining proportion in aquafeeds, fish meal (FM) and fish oil (FO) remain major dietary components, especially for carnivorous finfish, including salmonids (Tacon and Metian, 2008). Decreasing availability, rising prices, and the negative environmental impact of FM and FO use have intensified the search for alternative protein and lipid sources.

Insects at various life stages constitute the major part of the natural salmonid diet either throughout life or in the juvenile stage, and show potential for inclusion in formulated feeds. They have been evaluated for potential FM replacement in aquafeeds with varying results (Makkar et al., 2014; Henry et al., 2015). In temperate regions, large quantities of crickets, mealworms, locusts, and housefly maggots are commercially produced for pet food and fish bait (van Huis et al., 2013). Insects can be cultured on food manufacturing by-products, and their nutritional composition can be altered through diet (Oonincx et al., 2015; St Hilaire et al., 2007a), making them a sustainable, environmentally sound feed source. Determining the effects of insects in the diet on fish performance and health, nutritional content, and attractiveness to consumers is a prerequisite to wider use of insects as feed for salmonids.

Inclusion of the black soldier fly *Hermetia illucens* (BSF) larvae and mealworm *Tenebrio molitor* has been assessed in salmonid diets. Replacement of 25% to 50% of the FM with BSF meal in a rainbow trout diet showed no significant effects on weight gain and feed conversion ratio, but resulted in lower levels of omega-3 fatty acids in fillets (St-Hilaire et al. 2007b; Stamer et al., 2014). Sealey et al. (2011) reported satisfactory growth of rainbow trout fed a diet replacing 50% of FM with BSF reared on manure enriched with trout offal, while a diet containing BSF reared on manure only was associated with significantly slower growth compared to the commercial diet. No sensory differences among fish fed the two BSF and control diets were found. Lock et al. (2014) found 100% FM replacement by BSF meal in the diet of Atlantic salmon to have no detrimental effects on growth, histology, or sensory aspects, with the caveat that the method of insect meal preparation had considerable impact on its usability. Replacement of 25% and 50% of FM with mealworm larva meal in a rainbow trout diet did not significantly affect growth and reduced the hepatosomatic index compared to fish fed a control diet (Gasco et al., 2014a).

The house cricket *Acheta domestica* (Gryllidae) and superworm *Zophobas morio* (Tenebrionidae) are commonly-produced insects that can be successfully cultured on organic by-products (Fuah et al., 2015; Oonincx et al., 2015; van Broekhoven et al., 2015). Cytochrome P450 (CYP) enzymes are a group of hem-containing enzymes playing a key role in the metabolism of many xenobiotic including food components (Anric et al., 2015). Replacement of natural food of fish diet might affect the activity of metabolizing enzymes in fish. CYP1A is the most studied isoform in fish due to its important role in the metabolism of xenobiotic compounds. The measurement catalytic activity of CYP1A may provide information of xenobiotic nature of selected insects and introduced to fish diet.

The aim of this study was to evaluate the effect of partial to full replacement of commercial FM-based diets with live insects on growth and health parameters of rainbow trout, as well as on sensory and texture attributes and fatty acid composition in fish muscle that can influence its nutritional value and palatability.

Materials and methods

Experimental fish and rearing conditions

One-hundred-forty juvenile rainbow trout *Oncorhynchus mykiss* weighed 264.3 ± 6.6 g (mean weight ± standard deviation) were reared in a recirculation system at the Faculty of Fisheries and Protection of Waters in Vodňany, Czech Republic. Fish were fed commercial pellets only (EFICO Enviro, 4.5 mm, Biomar) before the start of the experiment. Prior to beginning the experiment, 10 randomly chosen fish were measured, weighed, sacrificed, and filleted for baseline analysis of lipid content and composition. Remaining fish were separated into groups of 10, bulk-weighed, and stocked into thirteen 400 L aerated glass aquaria. The mean initial stock weight per aquarium was 2643 ± 66 g. Aquaria were filled with tap water filtered through an active carbon filter. Each aquarium was connected to an individual external filter (Eheim professional 4+, EHEIM GmbH, Germany). Fish excrement and other sediment was drained daily at approximately 12:00 h, and ~200 L water was exchanged. Water temperature was 14.3 ± 1.2 °C, oxygen content 10.1 ± 1 mg L^{-1}, and pH 7.2 ± 0.7. The duration of the experiment was 60 days.

Diets

Five isocaloric diets were formulated using commercial pellets and live insects. Prior to experimentation, nutrient composition of the feeds was analysed (Table 1) by an accredited laboratory (Státní veterinární ústav Praha, Testing laboratory No. 1176). Four experimental diets were tested with three replicates. A control group (K) was fed commercial pellets (EFICO Enviro 4.5 mm, Biomar) only. For other groups, 25% of the crude energy of pellets was replaced with live adult house crickets (C), live superworm larvae (L), or a combination of 12.5% crude/gross energy each of the insect species (LC). A final group (I) was fed live crickets and superworms only (50/50 crude energy). For economic reasons, this group was not replicated, therefore, they were not included in the statistical evaluation for survival, weight gain and feed efficiency.

The insects were purchased fresh from a local producer (Vladimír Šefl, Bušanovice, Czech Republic) twice per week. The crickets were held at 6 °C to ensure that they remained inactive, and superworms were kept in barley bran at 22 °C according to producer recommendations.

After three days acclimatisation, feeding was initiated in all tanks. Pellets were fed at 1.5% of stock weight daily and at 1% after monitoring on day 26. For Groups C, L, and LC, the pellets were decreased to 75% and supplemented with the appropriate proportion of insects. In Group I, pelleted feed was replaced with insects. The required quantity of insects was calculated based on weight necessary to provide energy content similar to pellets:

1 g pellets = 4 g house crickets = 2.4 g superworms

The feed adaptation phase was carried out for five days, during which Group K was fed 25% of its allocated daily ration, and other groups were fed the insect portion only. From day 8, each group received the full feed ration. Fish were fed manually four times per day, and all feed provided was consumed. Expected weight gain was calculated with respect to a feed conversion ratio (FCR) of 1:1 (based on full pellet portion weight). Fish were not fed on the monitoring and final sampling days (26, 48, and 60) or on the preceding day.

Growth monitoring, including individual weight and biometric measurements, was carried out in all aquaria on days 26 and 48. At the conclusion of the trial, all fish were individually

weighed. For each aquarium, feed conversion ratio (FCR), protein efficiency ratio (PER), and gross energy (GE) utilization was calculated.

FCR = weight feed (dry; g)/weight gained (g)

PER = wet weight gain (g)/protein intake (g).

GE utilization = wet weight gain (g)/GE intake (MJ)

Ten fish per group were sacrificed, bled out, and hand-filleted. Individual weight (g) standard length (mm), and total length (mm) were measured. Condition factor (CF) was calculated for each fish:

$CF = 100 \cdot (W \cdot TL^3)$, (TL in cm, W in g)

Viscera and liver were weighed for determination of viscerosomatic (VSI) and hepatosomatic (HSI) indices.

VSI = (weight of viscera/total weight) · 100

HSI = (weight of liver/total weight) · 100

Samples of raw, skinned fillets were used for analysis of fatty acid (FA) composition and for sensory evaluation, while liver tissue were used for further preparation of microsomal fractions and measuring of CYP mediated reaction.

Fatty acid and lipid content analysis

Lipid extraction was performed in duplicate according to Hara and Radin (1978) and lipid content was quantified gravimetrically. For FA analyses, methylation of total lipids was conducted according to Appelqvist (1968). FA composition was analysed by gas chromatography (Trace Ultra FID; Thermo Scientific, Milan, Italy) using a BPX-70 50 m fused silica capillary column (id 0.22 mm, 0.25 μm film thickness, SGE, USA). The peaks were identified by comparing sample retention times to those of the standard mixture GLC-68-A (Nu-Chek Prep, Elysian, MN, USA).

Sensory analyses

The sensory quality of fillets was evaluated with respect to attributes such as aroma, taste, aftertaste, and consistency (Martinsdóttir et al., 2009). One-hundred 30 g samples (five groups, 10 fish from each, in duplicate) were prepared for a panel of 10 members of a trained jury from the Faculty of Fisheries and Protections of Waters. Tasting samples were composed of six small pieces of flesh, each from a different fish of the appropriate group (ISO 6658, 2005). Samples were taken from corresponding areas of the fish body, stored on ice for 2 h, and cooked separately in code-labelled 0.15 L glass jars for 15 min at 150 °C in an electric oven. To conform to ISO 6658 (2005) and ISO 8589 (2007) criteria, no salt, oil, or spices were added. Panellists were separated from one another in individual cubicles (ISO 8589, 2007). Each panellist was provided with still water, distilled spirits, and bread to cleanse the palate. Samples were rated on a hedonic consumer scale (Martinsdóttir et al., 2009) modified according to Kříž et al. (2007). Panellists were asked to evaluate the intensity of

aroma, taste, aftertaste, and consistency and to indicate a rating by assigning a point on a 100 mm unstructured abscissa (0 mm = very good quality; 100 mm = unacceptable).

Instrumental analysis of colour and texture

Flesh colour was assessed at three locations above the lateral line (anterior, middle, and caudal) of each fillet (n = 7/group) using a colour spectrophotometer CM-600d (Konica Minolta Inc., Japan). Colorimetric data were represented according CIE (1976) as L* = whiteness, a* = the red-green axis, and b* = the yellow-blue axis were measured directly on the fillet with each spot evaluated in duplicate. Measurements were performed within 1 h post-mortem.

Samples (n = 10/group) for texture analysis were taken from dorsal area of fillets between the end of the dorsal fin and the beginning of the anal fin. Firmness, defined as the maximum force detected during initial compression, was measured using a TPA-meter (TA.XTPlus, Stable Micro Systems, Godalming, Surrey, U.K.). A 10 mm diameter cylindrical probe (sms p/10) was set at pretest speed of 5 mm/s and test speed of 2 mm/s until the fillet was compressed to 50% of its original thickness.

Histology

During dissection, gross examination of intestines, liver, gills, and heart was performed. For histological examination, samples of liver, heart, stomach, and intestine (mid-section) were fixed in 10% natural buffered formalin, paraffin-embedded, and routinely processed as described by Bancroft and Gamble (2002). Sections (4 μm) were stained with Mayer's haematoxylin-eosin. Slides were examined at magnification of 10-40x using an Olympus SZ9 microscope.

Ethoxyresorufin O-deethylase activity (EROD)

Resorufin, 7-ethoxyresorufin, and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma-Aldrich (Steinheim, Germany).

Microsomal fraction preparation and protein analysis: Fish hepatic microsomes were obtained by differential centrifugation. Briefly, liver (~1 g) was homogenized in three volumes of Tris-sucrose buffer (10 mM Tris–HCl, 250 mM sucrose, and 0,1 mM EDTA, pH 7.4) with subsequent centrifugation (Beckman Coulter Optima™ L-90 K) at 30,000 rpm for 15 min at 4 °C. The supernatant was further centrifuged at 100,000 g rpm for 60 min at 4 °C. As a final step, the microsomal fraction was diluted in glycerol buffer (0.1 mM EDTA, 20% glycerol, 50 mM Tris and 10 mM potassium phosphate, pH 7.4) and homogenized (UltraTurrax; Ika, Germany). All steps were carried out on ice. Microsomal fractions were immediately frozen and stored at -80 °C for 7-ethoxyresorufin-O-deethylase (EROD) analysis. The protein levels were estimated spectrophotometrically as described by Smith et al. (1985) using bovine serum albumin as standard. The microsomes were diluted to obtain a protein concentration of 10 mg/mL.

The catalytic activity of CYP1A was measured as the rate of formation of resorufin from 7-ethoxyresorufin (Kennedy and Jones, 1994). The incubation mixtures contained 0.5 mg microsomal protein in an incubation medium of 50 mM potassium phosphate buffer (pH 7.4) with 1.0 mM NADPH and 2 μM of 7-ethoxyresorufin. The fluorescence detector (Infinite 200 – Photometer TECAN) was used for detection of resorufin (excitation/emission 544/590 nm). Enzyme activity was expressed as pmol resorufin/mg protein/min (detection limit was 1 pmol/min).

Statistical analysis

Sensory attributes, colour and texture analyses, biometric data, and FA profile were subjected to one-way ANOVA. The differences among means were tested by post-hoc Tukey's honest significant difference test. Differences among means for instrument-based colour and texture analyses were assessed by Fisher's LSD test. Data of percentage of FA was arcsin transformed. Homogeneity of variance was tested using the Cochran-Hartley-Bartlett test. Survival of fish was compared with the Pearson and maximum likelihood $\chi 2$ test. All analysis was done using Statistica 12.0 (StatSoft CR, Prague, Czech Republic). Differences were considered significant when $P < 0.05$.

Results

Growth and biometric parameters

After the feed adaptation phase, all fish in experimental groups consumed insects actively and preferentially consumed insects over pellets. No differences were found in mean fish weight among groups at monitoring days or at the end of the experiment (Fig. 1). Feeding regime was not associated with survival, total weight gain, FCR, or utilization of feed gross energy. Protein efficiency ratio (PER) in the co-fed groups was significantly lower than in the control group, with the exception of Group L (Table 3). No dietary effects were found in mean final condition factor (K) or VSI in fish sampled for FA analysis (n = 10/group). Fish from Group I displayed significantly higher ($P = 0.022$) HSI values than did Group C. Mean final K in Group I was significantly ($P = 0.009$) higher than in fish sampled at beginning of trial (Table 4).

Fat content and fatty acid composition

Values of lipid content and FA composition are given in Table 5. Total fat content did not differ significantly among groups, but was slightly higher in group K compared to the experimental groups. Significant among-group differences were found in all selected FAs as well as in relative content of saturated FA (SFA), mono-unsaturated FA (MUFA), and poly-unsaturated FA (PUFA) (Table 5).

Palmitic acid (16:0) was the predominant SFA, and stearic acid (18:0) constituted >1% of total lipid in all groups. Their relative content was significantly lower in group K compared to the other groups, with the highest values found in Group I. Other SFAs made up less than 1% of total FA. The quantity of SFA was significantly higher in group I than in C, L, and LC groups and was lowest in group K.

The level of total MUFA observed in Group K was significantly higher than in other groups, intermediate levels were seen in C, L, and LC, and lowest in group I. A similar pattern was observed in levels of oleic acid (18:1 n-9) (the predominant MUFA in all groups), vaccenic acid (18:1 n-7), and erucic acid (22:1 n-11).

Linoleic acid (LA, 18:2 n-6) was the predominant PUFA in all groups, and showed the highest relative level in Group I. Significant differences among groups were found in relative levels of docosahexaenoic acid (DHA, 22:6 n-3), alpha-linoleic acid (ALA, 18:3 n-3), eicosapentaenoic acid (EPA, 20:5, n-3), eicosadienoic acid (20:2, n-6), and other PUFAs, each of which represented <1% of total FA (Table 5). In general n-3 FA showed lower proportions in the insect fed groups (C,L,CL and I), while n-6FA were higher in those groups, reflecting the FA composition of the diet. No significant differences in relative PUFA proportion were found among Groups K, C, L, and LC or between Groups LC and I. Group I showed significantly lower Σn-3 FA proportion than

observed in all other groups. In contrast, Σn-6 FA content was highest in Group I, intermediate in Groups C and LC, and lowest in K and L. The Σn-6:Σn-3 ratio was significantly affected by diet, with the lowest value in Group K and the highest in Group I (Table 5).

Sensory analyses

The results of sensory evaluation showed significantly lower acceptability of Group I fillets with respect to aroma and taste in comparison with fillets from Groups K, L, and LC (Fig. 2). Fillets from Group C did not show differences from the other groups in these attributes. Presence of an aftertaste was significantly higher for Group I compared to other groups. No effect of diet was observed in consistency scores.

Instrument-based colour and texture analyses

Inclusion of insects in the feed formulation (group LC and I) significantly increased L* whiteness of fillets ($P < 0.05$). Redness a* was only slightly influenced by dietary regime, with only group LC exhibiting significantly lower redness value from Group C. There was no difference in yellowness b* among groups (Fig. 3).

The control group showed significantly lower firmness compared to the insect diets ($p < 0.05$) (Fig. 4).

Histology

There was no gross morphological alteration, but there was a large quantity of fat around the intestines in all groups. Hepatocytes were characterized by a moderate to high number of fat vacuoles in all fish. There were no signs of pathology in liver or histopathological aberrations in heart, stomach, or intestine of any group.

Ethoxyresorufin O-deethylase activity (EROD)

No effect of diet treatment on EROD activity was detected. Slightly higher values were seen in Group L fish compared to other groups (Fig. 5).

Discussion

Growth and biometric parameters

Results indicated that house cricket and superworm can be used as partial or total isocaloric replacement of commercial diet for rainbow trout without negative effects on growth, survival, FCR, or gross energy utilization. The observed growth rate in Group I fish demonstrated that a combination of raw crickets and superworm larvae is nutritionally adequate for growth compared to commercial feed of similar energy value. This is not surprising, as insects are an important component of natural prey of salmonids (Groot, 1996), including rainbow trout (Raleigh et al., 1984). Nevertheless, most studies have reported total replacement of FM with insect meal to be unsuccessful, generally due to nutritional imbalances or deficiencies (Henry et al., 2015), for example in calcium (Makkar et al., 2014) and amino acids including histidine, lysine, and tryptophan (Sánchez-Muros et al., 2014). Partial fish meal replacement with processed insect meal without negative effect on growth of salmonids was reported Gasco et al. (2014), who successfully replaced up to 50% of fish meal with mealworm larva meal, and Stamer et al. (2014), who used BSF meal in the rainbow trout diet. Fish meal

replacement with insect meal ≥50% was associated with significantly reduced growth in most studies (Sealey et al., 2011; Stamer et al., 2014; St-Hilaire et al., 2007), although total FM replacement with BSF meal without deterioration of growth parameters was reported by Lock et al. (2014) in Atlantic salmon. Potential utilization of live/raw insects in salmonid mass culture is problematic; however, collaboration of fish and insect farming may be possible with local producers. The readiness of fish to eat inactive crickets in our study showed the potential of using frozen insects for rainbow trout without influencing palatability and/or digestibility, which can be a problem with methods of processing insect meal (Lock et al. 2014).

A major obstacle to the use of insects in commercial farms is their high cost. In the present study, the cost of 1 MJ gross energy from crickets was 25-fold and, from superworms, 8-fold that of the commercial pellets. Nevertheless, local insect producers may be able to less expensively provide overproduced or dead insects to fish farms. The price of insects may be reduced by using organic by-products (Oonincx et al., 2015) including remains from fish processing (Vladimir Šefl, personal communication) for insect production.

Fatty acid composition

From a human nutrition point of view, fish are a good source of long-chain n-3 FA (Tacon and Metian, 2013). These n-3 fatty acids, especially 20:5 n-3 (EPA) and 22:6 n-3 (DHA), have multiple functions in metabolism and are associated with prevention of cardiovascular and inflammatory diseases as well as certain forms of cancer (Simopoulos, 2002a; Rudowska, 2010; Calder, 2014). Therefore, it is important to maintain a high content of these FA in fish. This is usually obtained by the use of fish oil as a fat source in the feed. If the fat is replaced by other sources, FA composition of the diet is generally mirrored in the fish muscle (Sargent et al., 1999; Morris, 2001; Shearer, 2001). In the present study, an increased proportion of insects corresponded with increasing proportions of the FA dominant in the insects. This was especially notable in the significantly higher proportion of 16:0 (palmitic acid) and significantly lower proportions of EPA and DHA in the group fed insects only. However, in the groups with 25% insect replacement, the proportion of DHA was comparable to the control fish. In contrast, EPA was significantly decreased with insect replacement of 25%. Addition of crickets only, but not superworm, to the feed resulted in increased proportions of 18:2 n-6 (LA) and a consequent increase in the n-6/n-3 ratio. As n-6 and n-3 FA are metabolised via the same enzyme system (Palmquist, 2009), but have opposing effects (Schmitz and Ecker, 2008), it is important to keep the n-6:n-3 ratio as low as possible. The recommendation is 1:4 (Simopoulos, 2002b). The fish with the 100% insect diet were in that range, but the ratio as well as the content of the long chain n-3 PUFA EPA and DHA should be monitored carefully if insects are used in a bigger scale in fish feeds.

The 100% insect diet resulted in EPA and DHA of approximately 45% and 63%, respectively, respectively, of that in fish fed the control diet. This indicates a decrease in nutritional value of these fish for human nutrion and needs to be addressed. One solution to restore the level of n-3 FA after feeding an insect based diet could be a so called finishing feeding strategy, where a relatively short final feeding period with diets containing a rich blend of fishmeal and fish oil (Parés-Sierra et al., 2014). In fish fed 25% of the energy as insects, EPA was reduced to 75-80% that of controls, while DHA was comparable, demonstrating that partial replacement does not have a great effect on the fish nutritional value.

Sensory analyses and instrument-based colour and texture analyses

Higher cricket content resulted in lower sensory scores for aroma, taste, and aftertaste by the majority of panellists (Fig. 2). This is contradictory to studies replacing FM with BSF meal (Sealy et al., 2011) and soybean meal (D'Souza et al., 2006), in which consumers were unable to differentiate between fish fed the control and experimental diets. This may limit wide use of crickets, and probably other *Orthoptera* sp., for salmonid production. Despite this, two panellist evaluated taste as good (<10) and did not detect aftertaste (0) in fillets from Group I in both testing replicates. This could indicate that the distinct taste of fish fed insects may be acceptable to some consumers. Instrumental colour analyses showed trout flesh to be lighter in colour in groups fed the insect combination. Since colour is an important trait to consumers when buying fresh fish, when replacing commercial feed with insects, it is important to use a finishing feeding strategy to obtain a desired flesh colour. All insect-fed groups showed higher firmness compared to controls, probably related to the slightly higher lipid content of control fish, also observed by Hardy and Lee (2010). Firm texture of fillets is an important trait for consumers, indicating fresh fish.

Ethoxyresorufin O-deethylase activity (EROD), histology and pathology

A potential increase in EROD activity with alternative feeds has been reported in some studies (Mráz et al., 2010; Trattner et al., 2011). Similar EROD activity among groups in the present study indicated that the raw insects used were unlikely to contain xenobiotic compounds. Fish showed no pathological and morphological abnormalities, thus house cricket and superworm are considered safe alternatives to commercial pellets from a fish health standpoint. However, there is a risk of insect toxicity related to rearing on biological (especially plant) waste or by-products (Sword, 2001).

Conclusions

Uncooked superworm larvae and house crickets are sustainable as feed for rainbow trout. Fish fed the diet containing insects at 25% and 100% of gross energy showed similar growth and feed efficiency as those fed a commercial diet with the same calories, except in PER.

Neither partial nor total replacement of commercial diet with raw insects showed a detrimental effect on fish health.

The insect-containing diet resulted in lower n-3 FA content of fillets. Fillet EPA and DHA of fish fed insects only was significantly reduced and may indicate the necessity of a final feeding period with an FM/FO rich diet.

Changes in sensory attributes, texture, and colour of flesh from insect-fed trout, particularly those fed a high proportion of house crickets, may decrease their acceptability to consumers.

The high cost of "pet quality" insects represents a significant limitation to the wider use of insects in trout production. It may be possible to use overproduced or lower quality insects from local producers.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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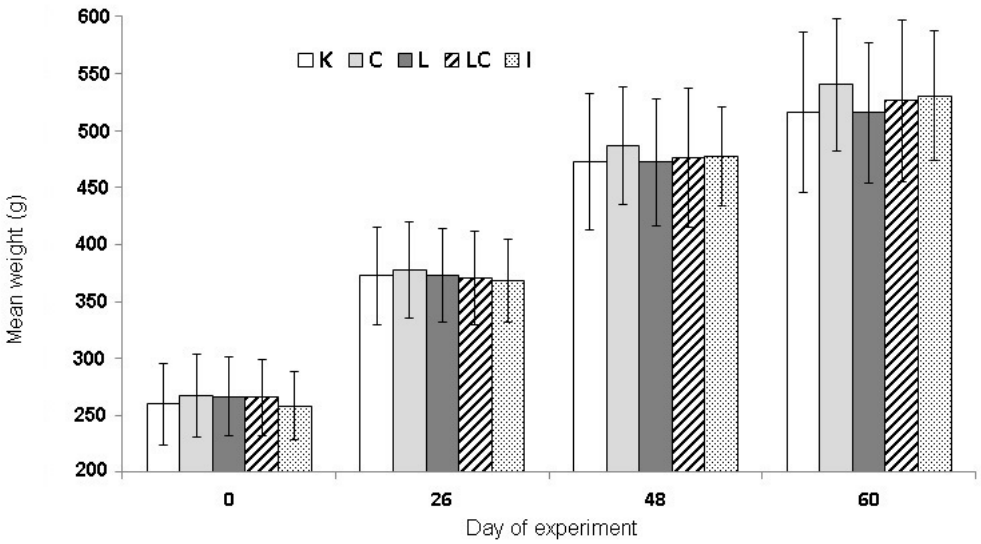

Figure 1 *Weight (mean ± S.D.) of rainbow trout fed four different diets during a 60-day feeding trial: K = control; C = 25% live house crickets, L = 25% live superworm larvae; LC = 25% of an equal combination of insect species; I = 100% combination of insect species.*

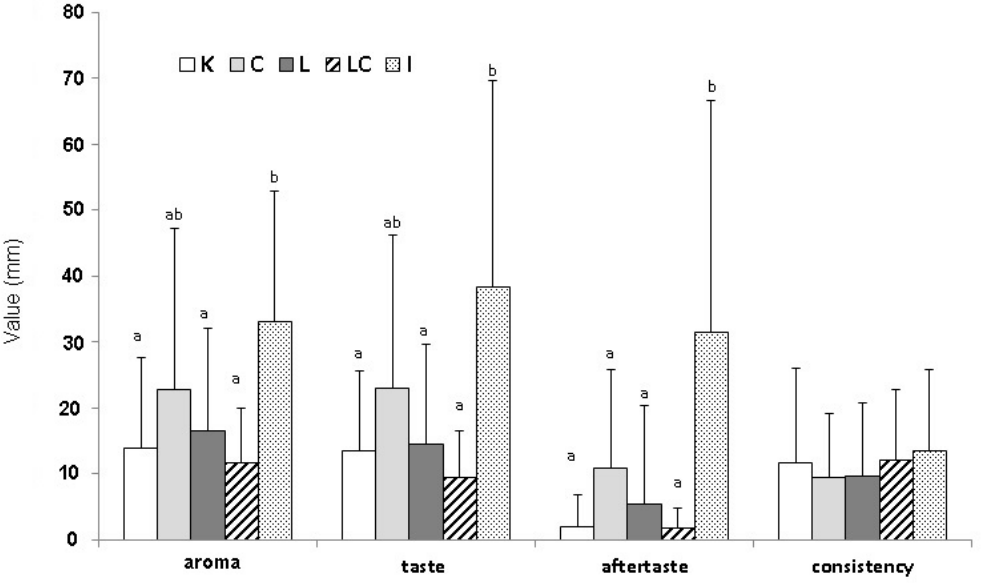

Figure 2 *Sensory attributes of rainbow trout fillets fed different diets during a 60-day feeding trial: K = control; C = 25% live house crickets, L = 25% live superworm larvae; LC = 25% of an equal combination of insect species; I = 100% combination of insect species. Sensory evaluation (mm) is presented as mean (bars) ± S.D. (whiskers). Different letters indicate significant differences (P < 0.05) among groups according to ANOVA, post-hoc Tukey HSD test.*

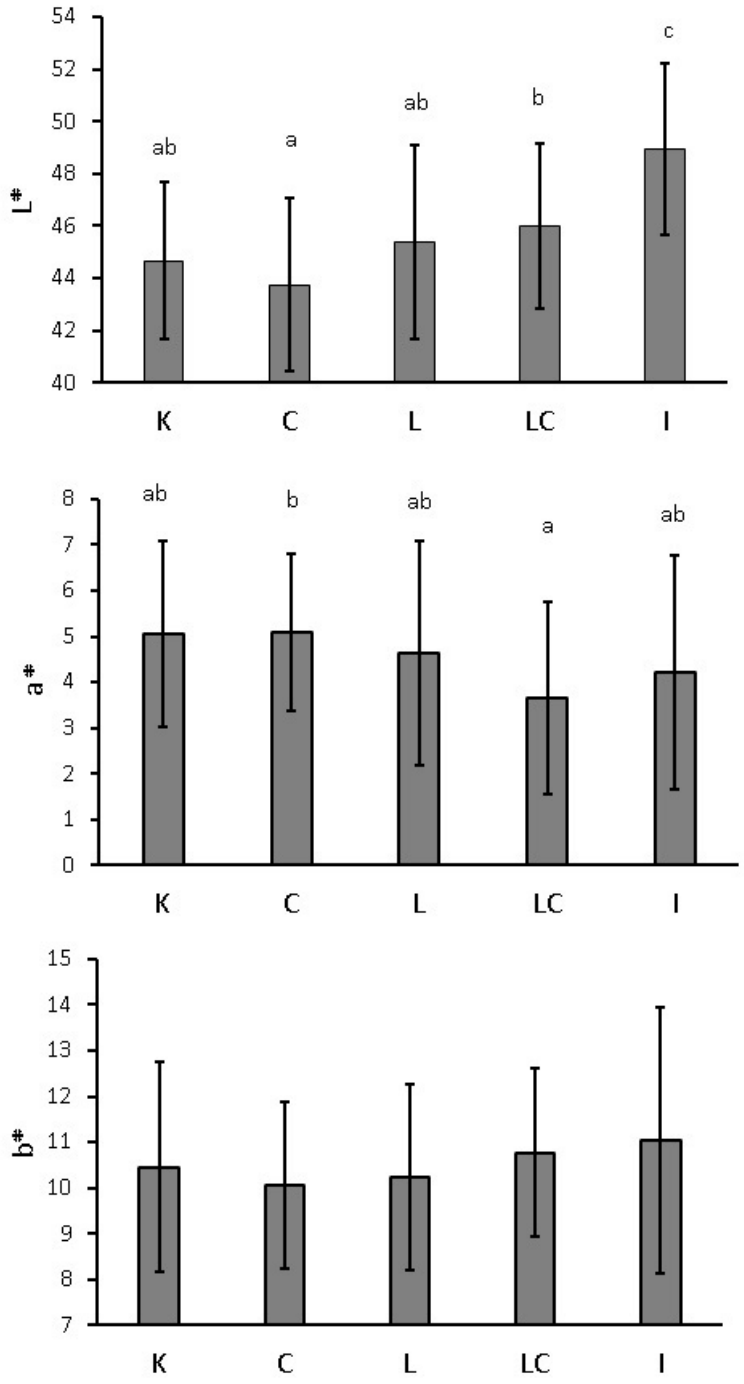

Figure 3 *Fillet colour parameters of rainbow trout fed different diets during a 60-day feeding trial: K = control; C = 25% live house crickets, L = 25% live superworm larvae; LC = 25% of an equal combination of insect species; I = 100% combination of insect species represented as L* - whiteness, a* - redness and b* - yellowness (mean ± S.D.; n = 7). Different letters indicate significant (P < 0.05) differences among groups according to ANOVA, Fisher's LSD test.*

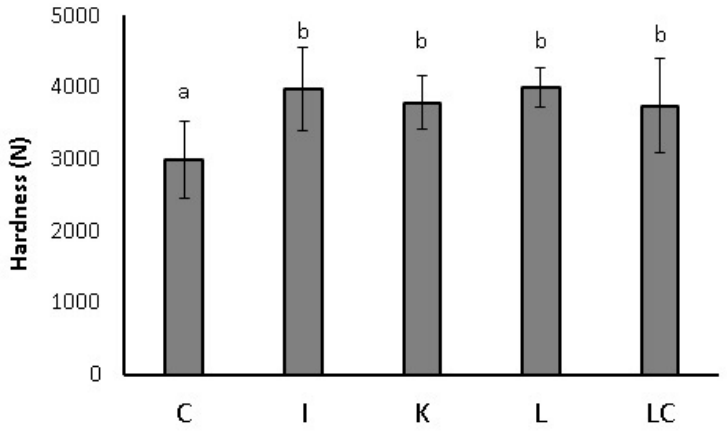

Figure 4 *Fillet firmness (N) of rainbow trout fed different diets during a 60-day feeding trial: K = control; C = 25% live house crickets, L = 25% live superworm larvae; LC = 25% of an equal combination of insect species; I = 100% combination of insect species (mean ± S.D.; n = 10). Different letters indicate significant (P < 0.05) differences among groups according to ANOVA, Fisher's LSD test.*

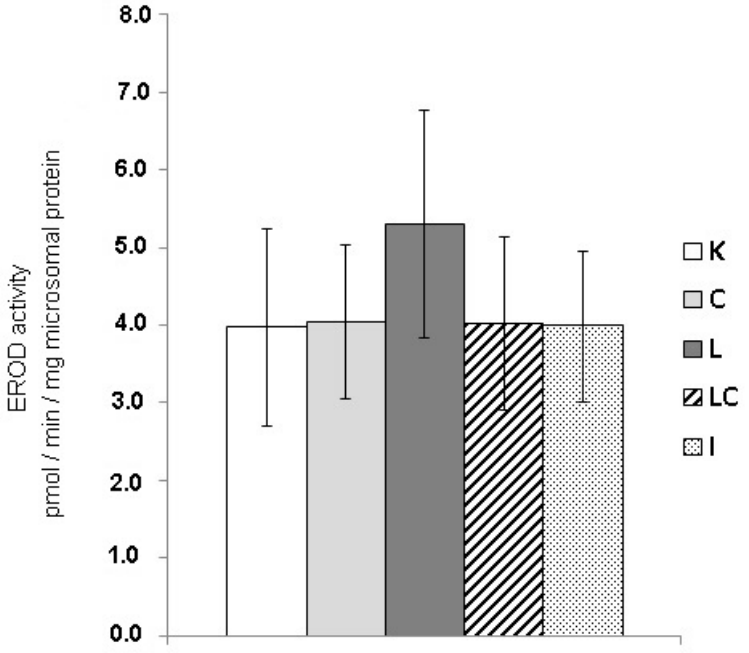

Figure 5 *Ethoxyresorufin O-deethylase activity, EROD (pmol /mg protein/min; mean ± S.D.; n = 10) in liver of rainbow trout fed different diets during a 60-day feeding trial: K = control; C = 25% live house crickets, L = 25% live superworm larvae; LC = 25% of an equal combination of insect species; I = 100% combination of insect species.*

Table 1. *Approximate composition of feed sources analysed by State Veterinary Institute Prague, Testing laboratory No. 1176.*

Composition (as-is basis)	Feed		
	Pellets	Crickets	Superworm larvae
Crude protein (%)	42.9	21.7	19.0
Crude fat (%)	30.1	5.6	18.3
Carbohydrates (%)	15.2	4.1	4.7
Ash (%)	5.8	1.9	1.8
Moisture (%)	4.9	68.8	56.2
Gross energy (MJ/kg)	24.4	6.1	10.1

Table 2. *Fatty acid composition of pellets, house crickets (Acheta domestica) and superworm (Zophobas morio) larvae used in feed experiment (mean ± S.D.; n = 3). Data are expressed as percent of total fatty acids, fat content as percent weight on as-is basis.*

Fatty acid	Feed		
	Pellets	House crickets	Superworm larvae
Fat content	27.26 ± 0.01	6.68 ± 0.70	18.21 ± 2.27
14:0	1.96 ± 0.00	0.86 ± 0.05	1.06 ± 0.06
16:0	9.92 ± 0.06	24.98 ± 0.63	32.36 ± 1.76
16:1	2.22 ± 0.00	1.17 ± 0.06	0.80 ± 0.15
18:0	3.35 ± 0.01	7.50 ± 0.33	7.53 ± 0.73
18:1 n-9	44.63 ± 0.05	21.51 ± 0.19	33.89 ± 3.63
18:1 n-7	3.12 ± 0.01	0.70 ± 0.01	0.31 ± 0.04
18:2 n-6	15.20 ± 0.07	39.59 ± 0.53	22.53 ± 1.95
18:3 n-3	6.76 ± 0.03	1.31 ± 0.02	0.92 ± 0.13
20:0	0.40 ± 0.01	0.38 ± 0.00	0.17 ± 0.02
20:1 n-9	2.51 ± 0.01	0.44 ± 0.03	0.16 ± 0.04
20:2 n-6	0.49 ± 0.01	0.08 ± 0.00	0.07 ± 0.01
20:4 n-6	0.21 ± 0.00	0.27 ± 0.05	0.02 ± 0.03
20:4 n-3	1.84 ± 0.02	0.04 ± 0.00	0.03 ± 0.01
22:0	0.25 ± 0.01	0.11 ± 0.02	0.05 ± 0.03
20:5 n-3	2.68 ± 0.00	0.76 ± 0.01	0.05 ± 0.07
22:5 n-3	0.60 ± 0.02	0.03 ± 0.01	0.23 ± 0.03
22:6 n-3	2.88 ± 0.01	0.21 ± 0.02	0.05 ± 0.08
ΣSFA	15.64 ± 0.06	33.85 ± 0.33	41.18 ± 1.98
ΣMUFA	52.48 ± 0.08	23.89 ± 0.28	35.17 ± 3.78
ΣPUFA	30.47 ± 0.04	42.26 ± 0.55	23.65 ± 1.92
Σn-3	15.70 ± 0.06	2.29 ± 0.03	1.03 ± 0.13
Σn-6	14.77 ± 0.03	39.94 ± 0.58	22.62 ± 1.93
Σn-6/Σn-3	1.06 ± 0.01	17.44 ± 0.43	22.39 ± 3.71

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, poly unsaturated fatty acids;

Table 3. *Survival, weight gain, and feed efficiency of rainbow trout groups fed different diets in a 60 day feeding trial. Dietary treatment: K = control; C = 25% live house crickets, L = 25% live superworm larvae; LC = 25% of an equal combination of insect species (n = 3). Value of I group (100% combination of insect species, n = 1) was not included in statistical analysis.*

	Dietary treatment				
	K	**C**	**L**	**LC**	***I***
Survival (%)	100	96.7	90.0	100	*100*
Weight gain (g/tank)	2562 ± 69	2676± 202	2342 ± 83	2602 ± 131	*2726*
Feed intake (g/tank)					
- Pellets[1]	2015 ± 37	1476 ± 57	1399 ± 55	1491 ± 43	---
- Crickets[1]	---	2179 ± 79	---	1097 ± 32	*4023*
- Superworms[1]	---	---	1243± 46	658 ± 19	*2414*
FCR	0.75 ± 0.02	0.79 ± 0.03	0.80 ± 0.01	0.79 ± 0.04	*0.86*
PER	2.96 ± 0.06^{a}	2.42 ± 0.11^{c}	2.80 ± 0.05^{ab}	2.60 ± 0.14^{bc}	*2.05*
GE utilization	52.1 ± 1.1	54.2 ± 2.3	50.2 ± 0.9	52.3 ± 2.8	*55.7*

[1]Weight of feed expressed on an as-is basis.

FCR (feed conversion ratio) = g feed (dry basis)/g weight gained.

PER (protein efficiency ratio) = wet weight gain (g)/protein intake (g).

GE (gross energy) utilization = wet weight gain (g)/GE intake (MJ)

Different lower case superscripts indicate significant (P < 0.05) differences among groups at the end of the experiment according to ANOVA, post-hoc Tukey HSD test.

Table 4. *The effect of four diets on standard length (SL), total length (TL), weight (W), condition factor (CF), viscerosomatic index (VSI), and hepatosomatic index (HSI) of fish. Data present as mean ± standard deviation, n= 10 in each group. Dietary treatment: K = control; C = 25% live house crickets, L = 25% live superworm larvae; LC = 25% of an equal combination of insect species; I = 100% combination of insect species.*

	Day 0	**Day 60**				
		Dietary treatment				
	Stocking	**K**	**C**	**L**	**LC**	**I**
SL (mm)	249.5 ± 12.6	304.5 ± 9.6	308.0 ± 12.3	299.5 ± 15.6	307.5 ± 11.9	300.6 ± 9.9
TL (mm)	279.5 ± 12.6	339.0 ± 10.9	344.0 ± 13.2	335.0 ± 16.0	341.5 ± 12.7	333.1 ± 8.0
W (g)	271.6 ± 41.2	509.7 ± 76.1	540.6 ± 66.2	505.0 ± 82.5	534.4 ± 55.3	530.7 ± 50.8
K	1.74 ± 0.18	1.79 ± 0.16	1.84 ± 0.10	1.87 ± 0.11	1.84 ± 0.11	1.95 ± 0.13*
VSI (%)	12.86 ± 0.75	12.76 ± 1.05	12.78 ± 0.70	12.92 ± 1.26	12.75 ± 0.66	13.96 ±2.11
HSI (%)	1.20 ± 0.18	1.32 ± 0.20^{a}	1.14 ± 0.09^{a}	1.21 ± 0.12^{a}	1.22 ± 0.13^{a}	1.35 ± 0.14^{b}

Different superscripts indicate significant (P < 0.05) differences among groups at the end of the experiment according to ANOVA, post-hoc Tukey HSD test.

Asterisk indicates significant (*P* < 0.05) differences in CF, VSI and HSI between day 0 and at end of experiment for each group according to ANOVA, post-hoc Tukey HSD test.

CF = 100 x W/TL^3; TL in cm, W in g

VSI = (weight of viscera/total weight) x 100

HSI = (weight of liver/total weight) x 100

Table 5. *The effect of four diets on fatty acid composition of fish fillets in a 60-day feeding trial. Data are expressed as percent of total fatty acids, fat content as weight percent on as-is basis (mean ± SD; n = 10). Dietary treatment: K = control; C = 25% live house crickets, L = 25% live superworm larvae; LC = 25% of an equal combination of insect species; I = 100% combination of insect species.*

Fatty acid	Stocking (Day 0)	Dietary treatment (Day 60)				
		K	C	L	LC	I
16:0	11.96 ± 0.51	11.79 ± 0.30^{a}	14.40 ± 1.38^{b*}	15.21 ± 2.48^{b*}	14.43 ± 0.99^{b*}	21.25 ± 0.78^{c*}
16:1	2.35 ± 0.18	2.39 ± 0.09^{a}	2.28 ± 0.17^{a}	2.20 ± 0.20^{ab}	2.14 ± 0.19^{b*}	2.01 ± 0.18^{b*}
18:0	3.07 ± 0.14	2.87 ± 0.15^{a*}	4.03 ± 0.53^{b*}	3.80 ± 0.68^{b*}	3.78 ± 0.35^{b*}	6.42 ± 0.54^{c*}
18:1n-9	44.06 ± 1.52	44.59 ± 0.52^{a}	41.54 ± 1.85^{b*}	42.79 ± 1.49^{b}	41.87 ± 1.10^{b*}	37.09 ± 1.21^{c*}
18:1n-7	3.19 ± 0.05	3.29 ± 0.05^{a*}	2.91 ± 0.16^{b*}	2.73 ± 0.33^{b*}	2.78 ± 0.13^{b*}	1.65 ± 0.15^{c*}
18:2n-6	13.87 ± 0.51	14.50± 0.27^{a*}	15.99 ± 0.59^{b*}	14.72 ± 0.26^{a*}	15.62 ± 0.54^{b*}	17.73 ± 0.72^{c*}
18:3n-3	4.67 ± 0.24	5.09 ± 0.17^{a*}	4.21 ± 0.32^{b*}	3.95 ± 0.56^{b*}	4.16 ± 0.26^{b*}	2.09 ± 0.28^{c*}
20:0	0.28 ± 0.02	0.28 ± 0.02^{ab}	0.30 ± 0.02^{b}	0.23 ± 0.03^{c*}	0.26 ± 0.02^{ac}	0.25 ± 0.04^{ac*}
20:1n-9	2.70 ± 0.33	2.63 ± 0.09^{a}	2.59 ± 0.19^{a}	2.41 ± 0.23^{a*}	2.44 ± 0.11^{a*}	1.81 ± 0.21^{b*}
20:2n-6	0.81 ± 0.10	0.98 ± 0.07^{a*}	1.19 ± 0.13^{cd*}	1.02 ± 0.10^{ab*}	1.14 ± 0.09^{bc*}	1.29 ± 0.12^{d*}
20:3n-3	0.29 ± 0.04	0.40 ± 0.03^{a*}	0.49 ± 0.16^{a*}	0.51 ± 0.10^{a*}	0.50 ± 0.07^{a*}	1.13 ± 0.22^{b*}
20:4n-6	0.41 ± 0.07	0.31 ± 0.03^{a*}	0.39 ± 0.11^{a}	0.41 ± 0.08^{a}	0.40 ± 0.06^{a}	0.97 ± 0.19^{b*}
20:4n-3	0.23 ± 0.02	0.25 ± 0.03^{a*}	0.24 ± 0.04^{a}	0.21 ± 0.03^{b}	0.24 ± 0.03^{a}	0.09 ± 0.02^{c*}
22:0	0.20 ± 0.10	0.25 ± 0.02^{a}	0.22 ± 0.01^{b}	0.20 ± 0.01^{bc}	0.20 ± 0.01^{c}	0.17 ± 0.01^{d}
22:1	1.11 ± 0.52	0.98 ± 0.08^{a}	0.88 ± 0.07^{b}	0.80 ± 0.11^{b}	0.86 ± 0.03^{b}	0.49 ± 0.06^{c*}
20:5n-3	1.84 ± 0.36	1.90 ± 0.11^{a}	1.43 ± 0.25^{b*}	1.53± 0.33^{b}	1.50 ±0.24^{b*}	0.86 ± 0.13^{c*}
24:1	0.46 ± 0.07	0.45 ± 0.06^{a}	0.42 ± 0.05^{ab}	0.37 ± 0.05^{b*}	0.36 ± 0.11^{b*}	0.25 ± 0.03^{c*}
22:5n-3	0.62 ± 0.36	0.58 ± 0.03^{a}	0.48 ± 0.09^{b*}	0.52 ± 0.11^{ab*}	0.52 ± 0.06^{ab*}	0.30 ± 0.06^{c*}
22:6n-3	6.62 ± 0.92	6.30 ± 0.68^{a}	5.89 ± 1.46^{a}	6.26 ± 1.29^{a}	6.71 ± 0.79^{a}	3.95 ± 0.58^{b*}
Fat content	6.52 ± 2.42	8.48 ± 1.70	6.43 ± 2.31	7.22 ± 2.20	6.96 ± 1.33	6.86 ± 1.10
Σ SFA	17.40 ± 1.00	15.31 ± 0.40^{a*}	19.05 ± 1.84^{b*}	19.52 ± 3.12^{b}	18.75 ± 1.32^{b*}	28.14 ± 1.23^{c*}
Σ MUFA	54.23 ± 1.55	54.33 ± 0.52^{a}	50.63 ± 2.17^{b*}	51.30 ± 2.12^{b*}	51.41 ± 1.41^{b*}	43.30 ± 1.51^{c*}
Σ PUFA	28.36 ± 1.25	32.21 ± 0.66^{a*}	31.73 ± 2.03^{a*}	30.67 ± 2.22^{ab*}	32.30 ± 0.96^{a*}	29.28 ± 1.01^{b}
Σ n-3	14.04 ± 1.24	14.53 ± 0.66^{a}	12.74 ± 1.91^{a}	12.98 ± 2.08^{a}	13.63 ± 1.05^{a}	8.43 ± 0.90^{b*}
Σ n-6	14.32 ± 0.51	15.79 ± 0.25^{a*}	17.57 ± 0.62^{b*}	16.15 ± 0.33^{a*}	17.16 ± 0.56^{b*}	19.99 ± 0.88^{c*}
Σn-6/Σn-3	1.03 ± 0.11	1.09 ± 0.05^{a}	1.41 ± 0.25^{b*}	1.27 ± 0.21^{ab*}	1.27 ± 0.12^{ab*}	2.40 ± 0.28^{c*}

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, poly unsaturated fatty acids.

Different superscripts indicate significant ($P < 0.05$) differences among groups according to ANOVA, post-hoc Tukey HSD test.

Asterisk indicates significant ($P < 0.05$) differences between values at day 0 and at end of experiment for each group according to ANOVA, post-hoc Tukey HSD test.

CHAPTER 7

GENERAL DISCUSSION

Strong links between fish and seafood consumption and positive health effects on human have been found (Dyerberg, 1985; Calder, 2004; Rudkowska et al., 2010; Lund, 2013). Given that fish is rich source of n-3 LC-FAs including EPA and DHA (Tacon and Metian, 2013) which are deficient in European diet especially for the Czech people. FAs as the most vulnerable nutritive components of fish is remarkably influenced by the feed regimen and composition, handling and processing, whereas, protein and the minor nutrients seem to be less affected as long as the fish was not starved or wrongly fed or exposed to abusive storage or processing conditions. As a result of the limited sources of FM and FO in the feed of fish in aquaculture, substitution of the novel feed ingredients for replacement received more attention (Quartararo et al., 1998; Gatlin et al., 2007; Henry et al., 2015). Wild fish feed is composed of natural organisms, including plankton, benthos as well as nekton in case of carnivorous species, which naturally contain the essential n-3 LC-PUFA. The primary producers of n-3 LC-PUFA in freshwater ecosystems are, the same as in the ocean, algae. These compounds are transferred into the fish throughout the feed chain. In addition, fish are able to biosynthesize n-3 LC-PUFA from their 18-carbon precursor (α-linolenic acid; ALA) to a certain degree. This ability is strongly expressed in freshwater non-carnivorous species, compared to marine carnivorous fish, which decreased this ability during evolution (Tocher, 2003). Therefore, the consumption of freshwater species from natural habitats is beneficial not only for human health, but also from sustainability and ecological viewpoints.

The overall aim of this thesis was to highlight the high value of natural feed for fish, also to highlight different aspects that affect nutritional quality of fish and how to assure a high nutritional quality of fish reared in aquaculture with a high sustainability of production. More specifically, in paper I, a number of factors influencing the nutritional value of fish in relation to the lipids, proteins, vitamins, and minerals were examined. Paper II focused on the proximate and FA composition (nutritional aspects) of seven species frequently caught by anglers in the Czech Republic. Paper III investigated the effects of VO replacement by the oil from oleaginous yeast grown on a second-generation substrate (lignocellulose hydrolysate from wheat straw) in the feed of Arctic char. In the paper IV and V the inclusion of insects as a replacement for FM in the feed and their effects on different quality aspects of two carnivorous species was investigated. Thus, the five papers on which the thesis is based focused on the different aspects of general nutritional value of fish and examined different alternatives to replace FO and FM in the feed of carnivorous.

Paper I – Nutritional Value of Fish: Lipids, Proteins, Vitamins, and Minerals

This work summarized and discussed the valuable constituents in fish, the effects of dietary FA, protein and peptides on human nutrition and health as well as factors which have great contribution and influence on the fish flesh composition and nutritional value. The risk of obesity, overweight, the metabolic syndrome, cardiovascular diseases, cancer and inflammatory diseases are growing in the Western population. The n-6 and n-3 FA influence the metabolism of eicosanoids and gene expression (Simpoulos, 2009) and the ratio of them is important for the further transformation of the essential FA, linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, ALA) to PUFA and their derivatives. This ratio in the diet has been increasing in the modern times and there is a large body of evidence that this changed balance have been connected with increased risk of disease (Simpoulos, 2006). There is a close relation between the dietary habits and changing the pattern of lipid intake and composition. The importance of n-3 LC PUFA are associated with the prevention of arteriosclerosis, neurological

dysfunction, insulin resistance and autoimmune diseases (Kinsella, 1988; Simopoulos, 1999; Connor, 2000; Calder and Grimble, 2002). Eicosanoids synthesized from n-3 PUFA have immunosuppressive properties (Calder, 2001), while the eicosanoids from n-6 PUFA have pro-inflammatory properties and enhance immune reactions like fever and pain (Calder, 2001). A high intake of n-6 PUFA, is therefore associated with adverse effects on human health, as for example cardiovascular diseases, and diabetes as well as hypertension, depression, neurological dysfunction, and immune disorders (Connor, 2000; Williams, 2000). An optimal diet containing an appropriate amount of the essential LC n-3 PUFA is necessary for neural development of children during the pregnancy and the neonatal period. It is well established that the maintenance of optimal pre-and postnatal growth and development requires n-3 PUFA (Innis, 1991; Innis et al., 1999). Moreover, proteins, peptides and amino acids from fish have been considered to have a high nutritional value (Sargent, 1997) and positive health effects (Rudkowska et al., 2010; Pilon et al., 2011). Studies related to inflammation, metabolic syndrome, osteoporosis, insulin resistance, obesity-related comorbidity and development of cancer have been executed and fish protein, peptides or hydrolysates have shown of importance in nearly as many areas as fish lipids (Madani et al., 2012; Chalamaiah et al., 2012).

Paper II – Nutritional value of some commercially important river fish species from the Czech Republic

The study was performed in order to complete and extend the existing information regarding nutritional value and lipid indices of the less gained attention species (European grayling, common nase, brown trout, common bream, Prussian carp, European perch and European chub) caught by anglers in the Czech Republic. Fish consumption is rather low in the Czech Republic therefore, the aim was to emphasize the importance of the species consumed by anglers and less known for the consumers diet. According to our results, there was some variation of FA composition in the species, depending on the natural habitat and differences in feed and its availability. Simultaneously, we observed a very favourable FA composition with good proportions of n-3 PUFA, including EPA and DHA in all analyzed species which reflects the composition of the natural diet (Robin et al., 2003). Consequently, the values of both IA and IT were low and close to the values of the so-called Eskimo diet, which is related to very low incidences of the coronary heart disease (Ulbricht and Southgate, 1991). According to our findings we conclude that the chosen species have a standard protein content, minimum carbohydrates and relatively low contents of fat, which can, however, vary to some degree in various localities, most probably related to the availability and composition of the feed.

Paper III – Oleaginous yeast as a component in fish feed

Fish oil (FO) represent the major source for the required lipids of the cultured species (Tacon et al., 2011). Vegetable oil (VO) is extensively used to partially replace FO in the aquaculture feed. Currently, alternative replacements of FO like VO turn to the limited sources and seems to be as non-sustainable. In replacing feed constituents, it is important to consider their effects on fish growth, health, welfare, and final product quality as well as the subsequent impact on human health. Many studies showed no significant effect of partial replacement of FO by VO on the fish growth fed by the replaced feed (e.g. Bell et al., 2001; Torstensen et al., 2005). However, few studies demonstrated effects on the welfare of fish fed VO. Some estrogenic effects in addition to the effects on immune function have been discussed (Mourente et al., 2005; Pickova and Morkore, 2007). Another important issue in case of replacement is the effect of substitution on changing the FA composition of fish for human consumption due

to the fact that VOs contain more n-6 PUFA (Orsavova et al., 2015) and do not contain the essential n-3 PUFA, EPA and DHA. However, some contain the shorter chain precursor ALA. In addition from a sustainability point of view VO can be used directly for human consumption and its replacement with underutilized novel sources is therefor favourable.

The aim of this study was to evaluate the possibility of VO replacement in the feed of Arctic char (*Salvelinus alpinus*) by single cell oils (oils derived from the oleaginous yeast) *Lipomyces starkeyi* grown on the second-generation substrate; namely lignocellulose hydrolysate (from wheat straw) which seems to be more sustainable compared to the VO with regards that cell oils do not rely on the arable land and that a waste product (wheat straw) is used in the production. Previous researches were focused on the usage of oil derived from genetically engineered *Yarrowia lipolytica* cultivated on first-generation substrate (glucose) (Hatlen et al., 2012; Katre et al., 2012; Zhu and Ethel, 2015). In addition, in this study we used not only the oil but the whole cells, replaceing also part of the protein in the feed and making the whole process easier and economically more feasible as no oil extraction step was needed.

In our study, the replacement indicated no significant changes between yeast fed fish and control feed, in terms of feed conversion rate and condition factor showing similar metabolism pathway of the feeds. In the yeast fed fish, slight significant decrease in the total n-6 FAs which results in low n-6/n-3 ratio was noticeable result since n-6 FAs is associated with adverse health effect for human including heart disease, promotion of inflammation, diabetes and cancer (Simopoulos, 2006; Libby, 2007). Proper yeast strains and culture conditions can have a positive influence on FA composition and the n-6/n-3 ratio. Our study for the first time indicates, the possibility to convert second generation substrate to a feed component therefore, it is acceptable to substitute terrestrial plant and animal based lipid and protein sources by yeast biomass. In conclusion, based on the potential of single cell oil production form the second-generation substrate, lignocellulose hydrolysate can be utilized as the basis to industrialize the production of single cell oil which can be considered to replace VO by yeast oil in the feed of carnivorous species.

Paper IV – Insect meal as a partial replacement for fish meal in a formulated diet for perch (*Perca fluviatilis*)

Due to the urgency to find potential substitute for traditional protein source FM in the feed, there has been increased interest in the utilization of insect as highly nutritious feed. The aim of this study was to investigate the effects of a replacement of 25% FM by a mixture of insect meal including house cricket- (*Acheta domesticus*) and superworm- (*Zophobas morio*) meal (with an amino acid adjustment) in the diet of perch, on survival, growth, feed conversion with special emphasis on lipid changes and composition in addition to the determination of the hepatosomatic index (HSI) as well as microsomal ethoxyresorufin O-deethylase (EROD, CYP1A) and 7-benzyloxy-4-trifluouromethylcoumarin O-debenzylase (BFCOD, CYP3A) activity as markers for exposure to xenobiotic compounds and metabolic detoxification in fish. Many studies have been focused on the utilization of other types of insect meal including black soldier fly (Bondari and Sheppard, 1981), common housefly maggot (Ossey et al., 2012), mealworm (Ng et al., 2001) and grasshopper meal (Johri et al., 2010) but house cricket and superworm meal have received less attention. Due to their frequently cultivation, well-established production system, usage for pet nutrition, beside the existed information about the nutritional requirements of these insects, these insects seem to have a great potential. Therefore, we wanted to see the possibilities of the partial replacement by this mixture. The lower growth performance in the fish group fed by insect indicated lower nutritional value and digestibility of the feed along with a possible bad taste of the feed. Interestingly FA composition of the fish fillet which reflects the composition of the diet was only affected to

a minor extent. The significant increase of 18:2 n-6 in the group fed by insect was due to the higher content of this FA in both insects and subsequently in the experimental diet. However, this change was so small that from a nutritional point of view this was neglectable. Beside our pilot study, further evaluations with the graded level of above mentioned insect meal or different insect species in the feed of carnivorous fish is needed.

Paper V – Insects in rainbow trout (*Oncorhynchus mykiss*) feed: effect on growth, fatty acid composition and sensory attributes

According to our result, partial (25% of pellet replaced live house cricket, 25% of pellet replaced by superworm, combination of 12.5% crude energy of each group) or total replacement (50% of each) of house cricket and superworm for FM in the commercial diet of rainbow trout indicated no negative effects on growth, survival, FCR and gross energy utilization. Inclusion of insect was connected with lower content of nutritionally valuable n-3 FAs (EPA and DHA). However, many studies have been reported the unsuccessful substitution of FM by total replacement with insect meal as a result of deficiencies or nutritional imbalances (Makkar et al., 2014; Sánchez-Muros et al., 2014; Henry et al., 2015), in our study total replacement with the mixture of insects, resulted in a better growth performance compared to the commercial feed of similar energy value. Most probably as insects are good live food for salmonids (Groot, 1996). However with increased proportion of insect's in the feed of fish, negative changes in the sensory properties, texture and colour of fish flesh occured resulting in less acceptability and preference by consumers.

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ENGLISH SUMMARY

When considering fish as food, first of all the n-3 LC-PUFA, particularly EPA and DHA are in focus. Furthermore, it gets obvious that the proteins and peptides in fish have not only a high nutritional value but also impact on human health issues. In addition, fish can be considered as a good source of several minerals, vitamins and micronutrients. In general, it should be highlighted that, when considering human nutrition and the related health aspects, it is impossible to focus one group of nutrients separated from all others. The overall aim of the thesis was to highlight different factors which influence nutritional quality of fish and to focus on the nutritional value of some commercially important river fish species from the Czech Republic. Moreover, to examine different sustainable alternatives to replace FO and FM in the feed of carnivorous. According to our results, there were some variation of FA composition in the selected seven freshwater fish species from the Czech Republic, depending on the natural habitat and differences in feed and its availability. Simultaneously, we observed a very favourable FA composition with good proportions of n-3 PUFA, including EPA and DHA in all analyzed species which reflects the composition of the natural diet. Consequently, the values of both index of atherogenicity (IA) and index of thrombogenicity (IT) were low and close to the values of the so-called Eskimo diet, which is related to very low incidences of the coronary heart disease. According to our findings we concluded that the chosen species have a standard protein content, minimum carbohydrates and relatively low contents of fat, which can, however, vary to some degree in various localities, most probably related to the availability and composition of the feed.

Due to the combination of the drastic increase in the need for aquaculture feed as well as decline in the sources of FM and FO, utilization of alternative sources received more attention. Based on our result, it is possible to replace VO by yeast oil produced from lingocellulose in the feed of Arctic char (*Salvelinus alpinus*). There were no significant differences in the study, regarding weight and length gain, feed conversation ratio, specific growth rate, condition factor and hepatosomatic index between the control and the yeast oil fed group. According to the results of another study, partial (25% of pellet replaced live house cricket, 25% of pellet replaced by superworm, combination of 12.5% crude energy of each group) or total replacement (50% of each) of house cricket and superworm for FM in the commercial diet of rainbow trout indicated no negative effects on growth, survival, FCR and gross energy utilization. Inclusion of insect was connected with lower content of nutritionally valuable n-3 FAs (EPA and DHA). In our study total replacement showed the mixture of insects, caused the better growth performance compared to the commercial feed of similar energy value as insects are good live food for salmonids. With increase in the proportion of insect's inclusion in the feed of fish, changes in the sensory properties, texture and colour of fish flesh was in a way that showed less acceptability and preference by consumers. Replacement of 25% FM by a mixture of insect meal including house cricket- (*Acheta domesticus*) and superworm- (*Zophobas morio*) meal (with an amino acid adjustment) in the diet of perch, on survival, growth, feed conversion with special emphasis on lipid changes and composition showed FA composition of the fish fillet was only affected to a minor extent. However, the lower growth performance in the fish group fed by insect indicated lower nutritional value and digestibility of the feed along with the taste of the feed. Interestingly, the significant increase of 18:2 n-6 in the group fed by insect was due to the higher content of this FA in both insects and subsequently in the experimental diet which from the nutritional point of view this change was neglectable. Beside our pilot study, further evaluations with the graded level of above mentioned insect meal or different insect species in the feed of carnivorous fish is needed.

CZECH SUMMARY

Pokud posuzujeme rybu jako potravinu, soustředíme se především na n-3 LC-PUFA, zvláště na EPA a DHA mastné kyseliny. Kromě toho je zřejmé, že také proteiny a peptidy v rybách mají nejen vysokou nutriční hodnotu, ale také dopad na lidské zdraví. Navíc může být ryba považována za dobrý zdroj některých minerálů, vitamínů a mikroživin. Obecně je třeba zdůraznit, že z pohledu lidské výživy a souvisejících zdravotních dopadů je nemožné se soustředit na jednu skupinu živin odděleně od ostatních. Celkovým cílem této dizertační práce bylo zdůraznit různé faktory, které ovlivňují výživovou jakost ryb a zaměřit se na nutriční hodnotu některých komerčně významných říčních druhů ryb z České republiky. Dílčím cílem bylo otestovat různé udržitelné alternativy k nahrazení rybího oleje (FO) a rybího masa (FM) v krmivu masožravých ryb. Podle našich výsledků se ve vybraných sedmi druzích sladkovodních ryb z České republiky lišilo složení mastných kyselin s ohledem na jejich přirozené stanoviště a rozdíly v krmivu a jeho dostupnosti. Zároveň jsme ve všech analyzovaných druzích pozorovali velice příznivé složení MK s dobrým podílem n-3 PUFA, zahrnující EPA a DHA, což odráží složení přirozené stravy. V důsledku toho byly nízké hodnoty IA a IT a tedy blízké hodnotám při takzvané Eskimo dietě, která souvisí s velmi nízkým výskytem srdečních onemocnění. Na základě našich výsledků můžeme usuzovat, že vybrané druhy mají standardní obsah proteinů, minimum cukrů a relativně nízký obsah tuku, který se může do určité míry lišit v různých lokalitách, což většinou souvisí s dostupností a složením krmiva.

Kombinací drastického nárůstu potřeb pro akvakulturní krmivo a úbytku zdrojů FM a FO získává více pozornosti využití alternativních zdrojů krmiva. Na základě našich výsledků je možné v krmivu sivena severního (*Salvelinus alpinus*) nahradit FO kvasničným olejem produkovaným z lignocelulózy. Mezi kontrolním krmivem a krmivem s kvasničným olejem nebyly pozorovány významné rozdíly při testování zvýšení hmotnosti a délky ryb, poměru konverze krmiva, specifické růstové rychlosti, indexu kondice a hepatosomatického indexu. Podle výsledků další studie, kdy bylo komerční krmivo pstruha duhového nahrazeno hmyzem částečně (25 % pelet nahrazeno živým cvrčkem domácím, 25 % nahrazeno potemníkem brazilským, kombinace 12,5 % hrubé energie z každé skupiny) nebo úplně (50 % každého druhu), nevykazuje toto krmivo žádné negativní účinky na růst, přežití, poměr konverze krmiva a využití hrubé energie. Přídavek hmyzu do krmiva byl spojen s nižším obsahem nutričně cenných n-3 MK (EPA a DHA). V naší studii je ukázáno, že úplné nahrazení krmiva směsí hmyzu je vhodnou živou stravou pro lososovité ryby, protože způsobuje lepší růstový výkon ve srovnání s komerčním krmivem podobné energetické hodnoty. Při vyšším podílu hmyzu v rybím krmivu vykazovalo rybí moučka pro konečné konzumenty nižší atraktivitu a přijatelnost. Ve stravě okouna bylo při nahrazení 25 % rybího masa potravou ze směsi cvrčka domácího (*Acheta domesticus*) a potemníka brazilského (*Zophobas morio*) (s úpravou aminokyselinového složení) jen v malé míře ovlivněno přežití, růst, konverze krmiva se zvláštním důrazem na lipidové změny a složení MK v rybích filetách. Nicméně nižší nárůst ryb ve skupině krmené hmyzem poukazoval na nižší nutriční hodnotu a stravitelnost krmiva spojené s chutí krmiva. Za zmínku také stojí, že díky bohatému obsahu kyseliny 18:2 n-6 v obou druzích hmyzu byl ve skupině ryb krmené hmyzem naměřen vyšší obsah této mastné kyseliny, ale tato hodnota je z výživového hlediska nedůležitá. K rozšíření této pilotní práce je potřeba dalšího hodnocení různých typů zmíněného hmyzího krmiva se stupňujícím se přídavkem hmyzí složky nebo využití i jiných druhů hmyzu ke krmení masožravých ryb.

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LIST OF PUBLICATIONS

Peer-reviewed journals with IF

Blomqvist, J., Pickova, J., **Khalili Tilami**, S., Sampels, S., Mikkelsen, N., Brandenburg, J., Sandgren, M., Passoth, W., 2018. Oleaginous yeast as a component in fish feed. Scientific Reports 8: 15945. (IF 2017 = 4.122)

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Khalili Tilami, S., Sampels, S., 2018. Nutritional value of fish: lipids, proteins, vitamins, and minerals. Reviews in Fisheries Science and Aquaculture 26: 243–253. (IF 2017 = 4.75)

Khalili Tilami, S., Sampels, S., Zajíc, T., Krejsa, J., Másílko, J., Mráz, J., 2018. The nutritional value of several commercially important river fish species from the Czech Republic. PeerJ 6:e5729. (IF 2017 = 2.118)

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Manuscripts

Khalili Tilami, S., Červený, D., Lepič, P., Kozák, P., Burkina, V., Sakalli, S., Noguchi, S., Mráz, J., Sampels, S., 2019. Insect meal as a partial replacement for fish meal in a formulated diet for perch (*Perca fluviatilis*). (manuscript)

Khalili Tilami, S., Sampels, S., 2019. Determination of the quality changes of minced carp separate during frozen storage. (manuscript)

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Abstracts and conference proceedings

Khalili Tilami, S., Sampels, S., 2016. Determination of the quality changes of minced carp separate during frozen storage. In: Book of abstracts "The 14th Euro Fed Lipid Congress", 18–21 September 2016, Ghent, Belgium, pp. 75. (lecture)

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Khalili Tilami, S., Sampels, S., 2016. Storage stability of carp separate. FABA-International Symposium on Fisheries and Aquatic Sciences. 3–5 November 2016, Antalya, Turkey. (lecture)	2016
Khalili Tilami, S., Sampels, S., Rodina, M., 2016. Fatty acid composition in fat of Siberian sturgeon eggs, subjected to different separation methods. FABA-International Symposium on Fisheries and Aquatic Sciences. 3–5 November 2016, Antalya, Turkey. (poster presentation)	2016

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English training to the bachelor and master students, discipline Fishery at USB FFPW in range of 90 teaching hours	2015
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Leading of the International Summer school project entitled Measurement of fish oxidation products in fish muscle (27th June–22th July 2016)	2016
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