# Nutrition of Arctic Charr (*Salvelinus alpinus*) and Eurasian Perch (*Perca fluviatilis)* and Evaluation of Alternative Protein Sources

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Cover: Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*)
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## Abstract

The aim of this thesis was to estimate the dietary lysine requirement of Eurasian perch (*Perca fluviatilis*), to study the digestive enzyme activity in perch and Arctic charr (*Salvelinus alpinus*) and the utilization of meals of microbial and mussel meals.

Juvenile Eurasian perch were fed semi-purified diets containing graded levels of lysine (12.2-24.3 g $kg^{-1}$ dry matter (DM), (3.0-6.0 g/16 g nitrogen (N)) to estimate the lysine requirements. Fish fed diets containing 18.3-24.3 g lysine $kg^{-1}$ DM had higher final body weight, weight gain, protein gain and specific growth rate than fish fed diet containing 12.2-17.3 g lysine $kg^{-1}$ DM. The results indicate that the lowest lysine level required for optimal growth performance in Eurasian perch is 18.3 g lysine $kg^{-1}$ DM (4.5 g/16 g N).

The activity of digestive enzymes was studied in farmed Eurasian perch and Arctic charr. There were no differences in the activity of lipase, α-amylase and disaccharidases between slow and fast growing perch, or between perch of different age. The total lipase activity was higher than total carbohydrase activity in both species. Perch had a higher total activity of carbohydrases than charr, suggesting a greater capacity for digesting carbohydrates, especially starch.

In Artic charr, the apparent digestibility coefficients (ADC) of dry matter (DM), amino acids (AA) and energy was lower for intact *Saccharomyces cerevisiae* than for extracted *S. cerevisiae, Rhyzopus oryzae* and *Mytilus edulis*, whereas the ADC of crude protein (CP) did not differ between the ingredients. With the exception for arginine and histidine, differences were found in ADC of indispensable amino acids (IAA) between the test ingredients. In Eurasian perch, there were no significant differences in the ADC of DM, CP, IAA and energy between the test ingredients.

The results indicate that 40% of CP derived from fish meal in diets for Arctic charr can be substituted with blue mussel (*M. edulis*) meal or intact *S. cerevisiae* yeast without any negative effect on growth performance and nutrient utilization.



*Keywords:* Arctic charr, Eurasian perch, lysine requirement, digestive enzymes, alternative protein sources, microbes, mussel meal, essential amino acids, digestibility, growth.



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# Dedication

*To my wife Karin and my son Vidar*

# Contents

# List of Publications

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

I Langeland, M., Andersson, K., Lundh, T. & Lindberg, J. E. (2014). Dietary lysine requirements of juvenile Eurasian perch (*Perca fluviatilis*). *Journal of FisheriesSciences.com* 8(2), 161-175.

II Langeland, M., Lindberg, J. E. & Lundh, T. (2013). A comparison of digestive enzyme activity in Eurasian perch (*Perca fluviatilis*) and Arctic charr (*Salvelinus alpinus*). *Journal of Aquaculture Research & Development* 5(208). doi: 10.4172/2155-9546.1000208

III Langeland, M., Vidakovic A., Vielma, J., Lindberg, J. E, Kiessling, A. & Lundh, T. (2014). Digestibility of microbial and mussel protein meals in Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*). (*Submitted to Journal of Aquaculture Nutrition*).

IV Vidakovic, A., Langeland, M., Kiessling, A. & Lundh, T. (2014). Microbial and mussel protein meals as novel ingredients in feed to farmed Arctic charr (*Salvelinus alpinus*) (*manuscript*).

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# 1 Introduction

Aquaculture is currently the fastest growing animal food production sector in the world, and together with production from capture, is set to exceed beef, pork or poultry production in the next decade (FAO, 2012). Today, 50% of all fish eaten by humans originate from aquaculture production. FAO (2012) predicts that production of fish and crustaceans will have to increase by an additional 23 million tonnes by 2020 to maintain the current *per capita* consumption of aquatic foods. However, aquaculture is dependent on large inputs of wild fish for feed (Naylor *et al.*, 2000), as about half the 63 million tonnes of fish and crustaceans produced annually are feed-dependent (FAO, 2012). The use of fish meal and fish oil in the aquaculture industry is not a long-term sustainable production strategy and it has been argued for a long time that a reduction in the use of fish meal in feed for aquaculture is necessary (Hardy, 2010; Glencross *et al.*, 2007; Pickova & Mørkøre, 2007; Naylor *et al.*, 2000; Rumsey, 1993). Thus, alternative protein sources will be essential for the future growth and development of cultured fish and crustaceans.

To date, plant proteins have been the main protein sources used to replace fish meal in fish feed. However, inclusion of plant proteins may cause problems due to their content of anti-nutritional factors, which can impair fish health and performance. Moreover, their inclusion may reduce feed efficiency due to reduced digestibility and imbalanced amino acid profile and may impair feed intake due to low palatability (Oliva-Teles & Gonçalves, 2001). Single-cell proteins, derived from microbial organisms such as yeast, fungi, bacteria and some microalgae, have been identified as feed sources with high potential to replace fish meal in aquaculture (Nasseri *et al.*, 2011; New & Wijkström, 2002; Kiessling & Askbrandt, 1993; Rumsey *et al.*, 1991b). Furthermore, blue mussels (*Mytilus edulis*) may be another candidate with potential to replace fish meal protein in fish feed (Kiessling, 2009; Berge & Austreng, 1989).

When replacing fish meal with alternative protein sources, it is important that the properties of the new feed ingredient are known in detail. Therefore, it is necessary to have reliable data on both nutritional and anti-nutritional properties, in addition to the nutrient requirements of the fish species of interest. This will allow the formulation of fish feeds that support good performance and high welfare of the fish, while generating low costs for the producer and a low environmental impact.

Establishing the dietary amino acid requirements of farmed fish is of major importance when evaluating the nutritive value of alternative protein sources (Wilson, 2003). Lysine is the first-limiting indispensable amino acid in most protein sources used for commercial feed production (Hauler & Carter, 2001; Akiyama *et al.*, 1997). In addition, lysine is the amino acid found in the highest concentrations in the carcass of several fish species (NRC, 2011; Wilson & Cowey, 1985; Wilson & Poe, 1985).

Species-specific data on the presence and activity of digestive enzymes can be used in diet ingredient selection (Kolkovski, 2001) and can result in formulation of diets that match the digestive capacity of the fish (Furné *et al.*, 2005). Several enzymes are involved in the digestion of feed into sub-units appropriate for absorption in the gastrointestinal tract of fish, but there are variations in enzyme activity between species (Harpaz & Uni, 1999; Kuz'mina, 1996; Chakrabarti *et al.*, 1995). Studies on digestive enzymes in fish have helped to define limits for dietary inclusion of protein and carbohydrates (Furné *et al.*, 2005; Spannhof & Plantikow, 1983; Twining *et al.*, 1983).

This thesis examines the digestion of two types of microorganisms and blue mussels in Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*), the amino acid requirements of perch, and digestive enzyme activity in and between the two fish species. This knowledge will hopefully be useful in the future search for alternative protein sources in aquaculture.

# 2 Background

## 2.1 Aquaculture

Total production from capture fisheries and aquaculture in 2010 was about 148 million tonnes (Figure 1), with aquaculture contributing 60 million tonnes (excluding aquatic plants and non-food products). About 128 million tonnes of the total amount of fish produced were used as human food, in other words aquaculture provided about 50% of human fish consumption (FAO, 2012).

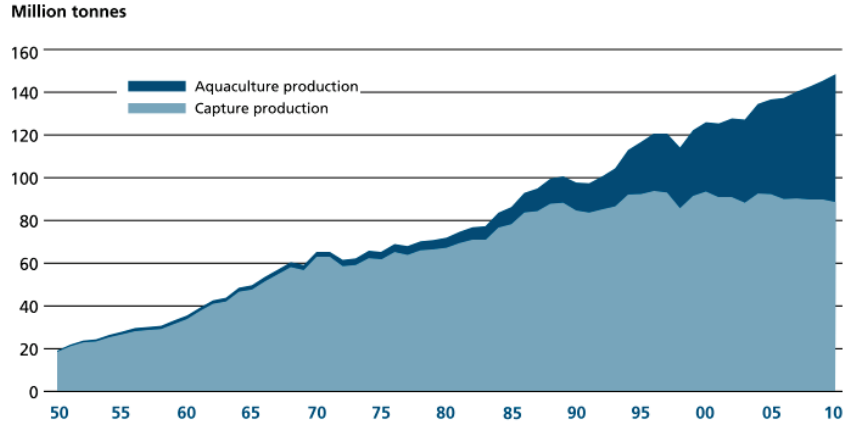


*Figure 1.* World capture fisheries and aquaculture production for the period 1950-2010 (source: FAO, 2012).

Since the 1950s, the worldwide aquaculture industry has increased by approximately 10% *per annum*, and has been the fastest growing food industry by far. In the next decade, total production from aquaculture together with capture is set to exceed the production from beef, pork or poultry. However,

aquaculture production has grown more slowly in the past decade than in the 1980s and 1990s.

Global aquaculture is dominated by Asia, accounting for 89% of production (by volume), with China being the main producer (36.7 million tonnes in 2010; FAO, 2012) (Figure 2). Production in Europe has increased from 567 000 tonnes in 1970 to 2.5 million tonnes in 2010, which is in share of world production a decline from 22.4% to 4.2%.

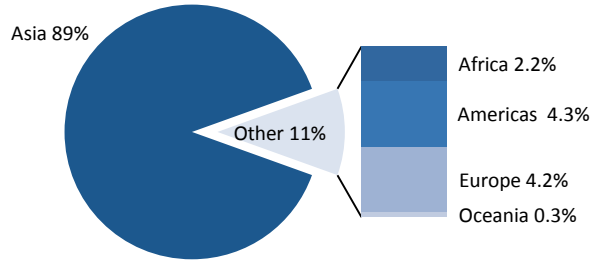


*Figure 2.* Global aquaculture by region (percentage of world production), excluding aquatic plants and non-food products (source: adapted from FAO, 2012).

World per capita fish consumption increased from an average of 9.9 kg in the 1960s to 12.6 kg in the 1980s, and reached 18.4 kg in 2009. However, the consumption differs considerably between and within countries and regions in terms of quantity consumed per capita, with a decrease in some countries in sub-Saharan Africa, for example. Moreover, the world population is growing and, to maintain at least the current per capita consumption of aquatic foods by 2020, the world will require at least another 23 million tonnes (FAO, 2012).

Aquaculture production in Europe has not followed the same expansion as in other parts of the world and is more or less stagnant or even slightly decreasing, apart from in Norway (Dalsgaard *et al.*, 2013; Paisley *et al.*, 2010). However, production in Sweden has increased, from average production of 6000 tonnes during the 1990s and 2000s to 12 447 tonnes in 2012 (Sweden Statistics, 2013) (Figure 3). Aquaculture production in Sweden is dominated by rainbow trout (*Oncorhynchus mykiss*), which comprises 84%, but in recent years the production of Arctic charr has increased (Sweden Statistics, 2013). Sweden has few offshore water sites appropriate for fish farming, but has great potential for increased production in open cages in hydroelectric dams and in land-based recirculating systems (Dalsgaard *et al.*, 2013; Paisley *et al.*, 2010; SOU, 2009:26).

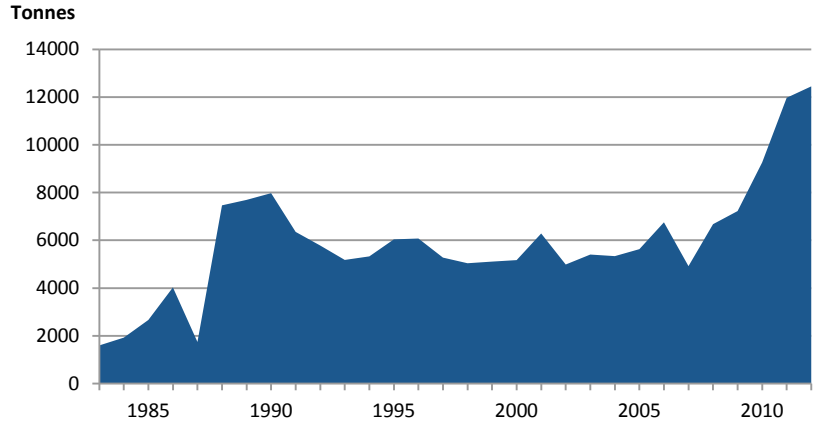


*Figure 3*. Swedish food fish production for the period 1983-2012.

Globally, 327 different fish species are farmed (FAO, 2012), but less than 10 different species are farmed in Sweden. Eurasian perch (*Perca fluviatilis*) is one species that has been identified as having potential for cultivation in Sweden (Strand, 2009; Kestemont & Dabrowski, 1996). There is a high market demand for perch in Europe and the catch of wild perch is not sufficient to meet this demand. One of the obstacles identified to profitable culture of perch is lack of information about its nutrient requirements.

Open-water farming of rainbow trout and Atlantic salmon (*Salmo salar*) typically results in low growth during colder seasons of the year, whereas Arctic charr can maintain growth in cold periods (Brännäs & Linnér, 2000). This trait, which could be exploited to increase annual fish production, creates potential for increased farming of charr, especially in the Nordic region, e.g. in Sweden. However, less is known about the nutritional requirements of charr than of other salmonids, and thus more research is needed. In addition, few nutritional studies including novel protein sources have been conducted with Arctic charr and Eurasian perch to date.

## 2.2 Protein sources

Consumption of global fish meal and fish oil in aquaculture is high and has increased over the past decade (FAO, 2012; Tacon *et al.*, 2011; Naylor *et al.*, 2009) (Figure 4). It has been reported that fish meal and fish oil contribute 75% of the protein and 35% of the energy in aquaculture feed (Tacon, 1999).

However, fish meal consumption in aquaculture has gradually decreased since 2006 and the inclusion of fish meal in diets for cultured fish has been reduced since 1995 (Tacon *et al.*, 2011; Tacon, 1999) (Figure 4).

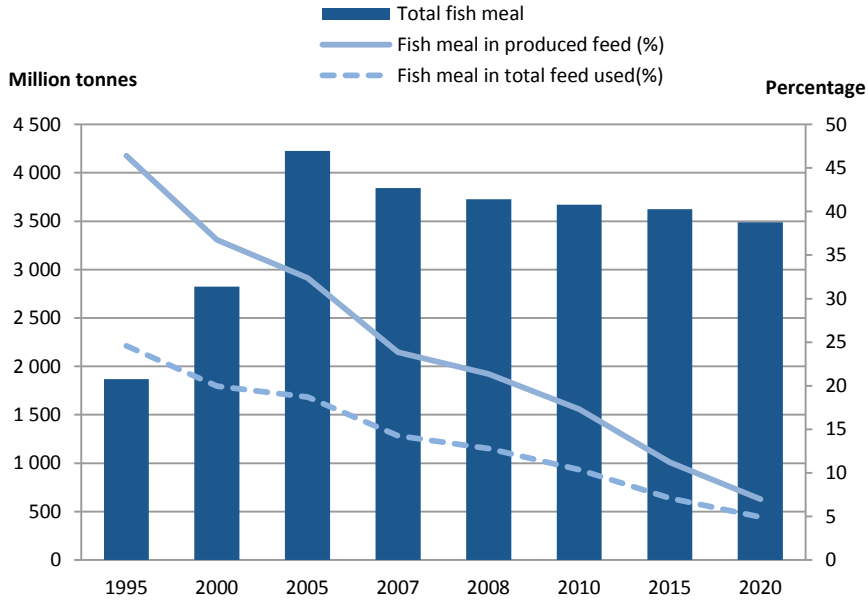


*Figure 4.* Actual and predicted total consumption of fish meal in aquaculture for the period 1995-2020, and in relation to inclusion of fish meal in total feed produced and used (source: adapted from Tacon *et al.*, 2011).

Nevertheless, the expansion of aquaculture production will demand good access to high-quality fish feed. The quantity of fish meal is already barely sustainable and it is believed that the predicted growth in demand worldwide for use in fish feed will result in the supply being exceeded (Gatlin *et al.*, 2007).

To achieve sustainable and successful production of fish, one major challenge for the expanding aquaculture industry is formulation of diets that result in high growth rate, good health and low environmental footprint. This is particularly important when unconventional and novel feed ingredients are introduced as replacements for fish meal in the diet. The protein content in fish meal is high and the amino acid profile of fish meal often fulfils the amino acid requirements of the farmed fish species. However, many of the alternative protein sources are often deficient in one or several amino acids and, in

addition to this, comprise a more complex structure than fish meal (Glencross *et al.*, 2007). Thus, some of these ingredients can have negative impact on the function of the gastrointestinal tract in fish (Krogdahl *et al.*, 2010; Naylor *et al.*, 2009; Krogdahl *et al.*, 2003), which can result in reduced digestibility of the feed and impaired growth.

Consequently, when searching for novel protein sources for aquaculture, chemical composition and digestibility have been pointed out as two key factors in ingredient valuation (Glencross *et al.*, 2007). The ingredients used in aquafeeds can be categorised according to their origin as: animal nutrient sources, plant nutrient sources and microbial nutrient sources.

### 2.2.1 Animal nutrient sources

The proteins originating from animals are divided into: aquatic animal proteins, including meals made from fish/shellfish, fish/shellfish by-products and zooplankton, fish/shellfish hydrolysates, silages and fermentation products, marine polychaeta meals; and terrestrial animal proteins, including meals made from meat, poultry and blood by-products and various invertebrate terrestrial products (Tacon *et al.*, 2011). The major aquatic animal protein used in aquaculture today is fish meal derived from wild harvested whole fish, with Peru as the largest producer and exporter. Recently, however, the focus has shifted to fish by-products as a total or partial substitute for fish meal (FAO, 2012; NRC, 2011).

Little attention to date has been directed to molluscs, such as blue mussels (*Mytilus edulis*), as a protein ingredient in fish feed. Blue mussels have a high protein content and an amino acid profile similar to that of fish meal (Jönsson & Elwinger, 2009; Berge & Austreng, 1989). In addition, blue mussels are filter feeders and thereby improve water quality, especially in eutrophic water bodies with an oversupply of nutrients. However, blue mussels can also absorb pollutants, a factor which needs to be taken into consideration when they are used as human food or animal feed source (Lindahl *et al.*, 2005). For example, bioaccumulation of algal toxins in the soft tissue of blue mussels has been observed in the Baltic Sea (Sipiä *et al.*, 2001). In addition, the bulk of shells have to be removed in order to obtain a suitable protein meal. Nevertheless, blue mussels are reported to have potential to act as both a dietary protein source (Kikuchi & Furuta, 2009; Berge & Austreng, 1989) and a feed attractant (Nagel *et al.*, 2013) in fish.

### 2.2.2 Plant nutrient sources

The major plant ingredients used in aquafeeds include cereals and cereal by-products (*e.g.* wheat, maize, rice, barley, sorghum, oats, rye, millet, wheat

gluten, dry distiller's grains with soluble, rice bran *etc*.), oilseed meals (soybean, sunflower, rapeseed and soybean protein concentrates *etc*.) and pulses and protein concentrate meals (*e.g.* peas, lupins) (NRC, 2011; Tacon *et al.*, 2011; Gatlin *et al.*, 2007). Ingredients of plant origin are traditionally used in aquafeeds as energy sources and as novel products developed through feed technology. Compared with fish meal, plant feedstuffs normally contain more indigestible organic matter, in the form of insoluble carbohydrate and fibre (Naylor *et al.*, 2009), as well as bioactive compounds that may positively or negatively affect the fish (Gatlin *et al.*, 2007).

### 2.2.3 Microbial nutrient sources

Microbe-derived feed ingredient sources for fish feed include some species of microalgae, yeasts, fungi, bacteria and/or combinations of these. The term 'single-cell proteins' is widely used to describe protein production from biomass originating from microbial sources. However, this term is not completely correct, since for technical and economic reasons the whole biomass is most often used in the feed and it also includes lipids, vitamins, pigments, minerals, trace elements etc. (Becker, 2007). At present, the low market availability and high price of microbe-derived feed ingredients limit their use in commercial aquafeeds. However, they are considered to be an innovative and promising protein source in aquaculture (FAO, 2012; Tacon *et al.*, 2011; Pfeffer, 1982).

Zygomycetes are microfungi that are rich in protein (40-50% depending on culture conditions) and have an amino acid profile similar to that of fish meal (Van Leeuwen *et al.*, 2012; Bankefors *et al.*, 2011; Edebo, 2008; Mydland *et al.*, 2007). Thus, it is suggested that zygomycetes *e.g. Rhizopus oryzae* have potential to replace fish meal protein in fish feed (Ferreira *et al.*, 2012; Edebo, 2008). In addition to protein, zygomycetes contain large but varying quantities of chitosan (glucosamine polymers) and chitin (N-glucosamine polymers). The variation in chitosan and chitin content (45-85%) is dependent on fungal strain and cultivation conditions (Edebo, 2008; Mydland *et al.*, 2007; Hjorth, 2005).

Yeasts are unicellular fungi that include both obligate aerobes and facultative anaerobes and have a long tradition of being used by humans for baking, brewing, distilling, wine making and food enhancing purposes (Tanguler & Erten, 2008; Mortimer, 2000). Among the yeasts, strains of *Saccharomyces cerevisiae* are the best known and also the most extensively studied (Graham & McCracken, 2006). The protein content in yeast normally varies between 45 and 65% and is comparable to that in fish meal, but yeast protein is often deficient in methionine (Nasseri *et al.*, 2011). In addition, the

production potential of yeasts are high, and it has been estimated that 45 kg of yeast can produce 250 tons of protein in 24 hours (Nasseri *et al.*, 2011).

To the best of our knowledge, none of these potential protein ingredients is being used as a protein source in commercial fish diets as yet, although *S. cerevisiae* strains are sometimes used in fish feed as immune-stimulants (Gatesoupe, 2007; Oliva-Teles & Gonçalves, 2001; Sakai, 1999). Thus more research is needed before they can be confidently used in aquaculture.

## 2.3 Digestion

Digestion is the process of solubilising, degrading and hydrolysing ingested nutrient polymers into smaller molecules and elements that can be transported across the intestinal wall to support physiological processes (NRC, 2011; Bakke *et al.*, 2010). The utilisation of nutrients depends on the presence and quantity of appropriate digestive enzymes, which are responsible for enzymatic hydrolysis of complex food polymers such as proteins, lipids and carbohydrates (Figure 5). The products from these processes are further digested at the epithelium of the intestinal tract by enzymes located at the brush border membrane of the absorptive cells. These processes produce molecules small enough for absorption in the fish, *e.g.* small peptides and amino acids, fatty acids and monosaccharides (Bakke *et al.*, 2010).

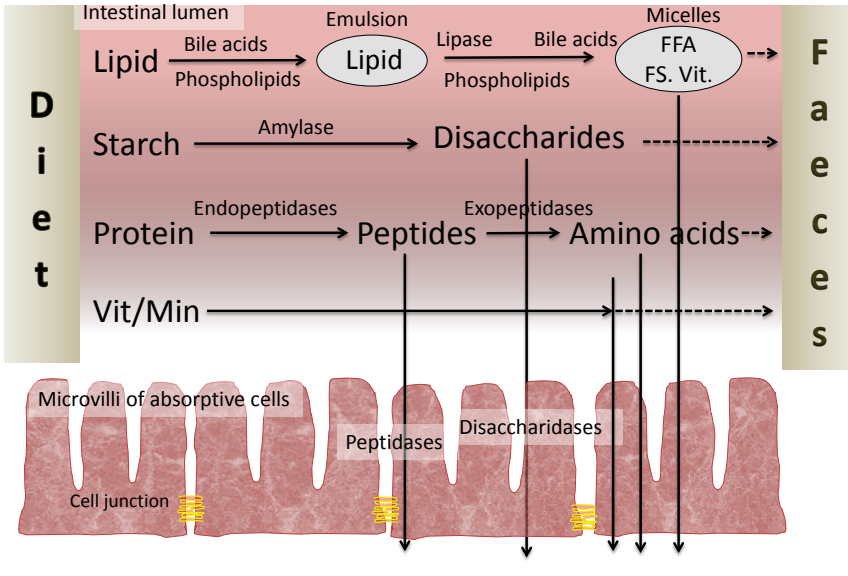


*Figure 5.* Main digestive processes along the digestive tract of fish. FFA = free fatty acids, FS .Vit.= fat soluble vitamins (source: adapted from Bakke *et al.*, 2010).

However, there are variations in enzyme activity between species (Harpaz & Uni, 1999; Kuz'mina, 1996; Chakrabarti *et al.*, 1995). Fish are commonly categorised according to their feeding habits into carnivorous, omnivorous and herbivorous, and it is generally believed that this is reflected in digestive enzyme activity (Falcón-Hidalgo *et al.*, 2011; Kuz'mina, 1996; Sabapathy & Teo, 1993; Kapoor *et al.*, 1976). However, this is not always the case. In some studies the relationships between feeding habits and proteolytic and α-amylase activity are unclear (Chan *et al.*, 2004; Fish, 1960), and it has been shown that carnivorous fish have α-amylase activity despite the lack of starch in their natural diet (Frøystad *et al.*, 2006; Krogdahl *et al.*, 2005).

In practice, species-specific data on the presence and activity of digestive enzymes can be used in the diet ingredient selection (Kolkovski, 2001) and can result in the formulation of diets that match the digestive capacity of the fish (Furné *et al.*, 2005). For example, studies on digestive enzymes in fish have helped to define limits for the dietary inclusion of protein and carbohydrates (Furné *et al.*, 2005; Spannhof & Plantikow, 1983; Twining *et al.*, 1983).

### 2.3.1 Protein digestion

The digestion of proteins to peptides and amino acids starts in the fish stomach, if present, with denaturation of the proteins by the low pH from HCl secretion, and hydrolysation of the peptide bonds by pepsins. In the intestine, proteins and peptides are diluted and dissolved in alkaline secretions and the pancreatic endopeptidases trypsin, chymotrypsin, elastases I and II, and the exopeptidases carboxypeptidase A and B. The short peptide chains remaining after the action of the pancreatic endopeptidases and exopeptidases are hydrolysed to amino acids by the aminopeptidases present at the brush border, or are taken up as small peptides.

### 2.3.2 Lipid digestion

Lipid digestion requires emulsifiers, mainly protein and phospholipids from the ingested feed, as well as endogenous bile acid and phospholipid excretion, to allow emulsification of the lipids released from the feed (NRC, 2011; Bakke *et al.*, 2010). Lipases with different substrate specificity are then responsible for further digestion of the lipids, resulting in free fatty acids, monoglycerides and, to a lesser extent, glycerols (Oxley *et al.*, 2007) incorporated into micelles. The further process of absorption of fatty acids in fish is not well understood, but is believed to occur as in mammals (Tocher, 2003). This involves diffusion or facilitated transport, aided by protein, of the fatty acids across the brush border membrane (NRC, 2011; Bakke *et al.*, 2010).

### 2.3.3 Carbohydrate digestion

It is generally accepted that fish do not have a definite requirement for dietary carbohydrates (NRC, 2011). Nevertheless, carbohydrates are included in fish feed in varying forms such as highly digestible mono-, di and oligosaccharides and insoluble and indigestible hemicellulose and cellulose (Krogdahl *et al.*, 2005). Inclusion of carbohydrates in the feed can save on the use of lipids and proteins as sources of energy (NRC, 2011). Another reason for carbohydrate inclusion in the feed is due to their binding properties, mainly of starches, pectins and hemicelluloses (Sørensen, 2012; Krogdahl *et al.*, 2005).

Polysaccharides *e.g.* starch and glycogen, are degraded by α-amylase to disaccharides, *e.g.* maltose and branched oligosaccharides, by hydrolysis of α-(1-4) glucoside linkages. Di- and oligosaccharides are further hydrolysed, mainly by disaccharidases in the brush border, into their constituent monosaccharides, which can be transported across the villi (NRC, 2011; Bakke *et al.*, 2010; Krogdahl *et al.*, 2005).

Chitinase is a polysaccharide-degrading enzyme present in some fish species. Chitin (β(1-4)linked N-acetyl glucosamine) is a common component of the cell walls of some microorganisms and of the exoskeleton of arthropods and the cuticle of annelids and molluscs (Bakke *et al.*, 2010; Krogdahl *et al.*, 2005). Chitinase activity in the digestive system has been reported for several fish species, but whether it originates from endogenous or exogenous production is unclear (Bakke *et al.*, 2010; Krogdahl *et al.*, 2005).

In addition, in several fish species, activity of cellulase and other polysaccharidases has been observed (Chakrabarti *et al.*, 1995). However, in this case too it is unclear whether these enzymes are of endogenous or microbial origin (Krogdahl *et al.*, 2005).

## 2.4 Amino acids

Proteins are built up from amino acids, which are organic compounds with an amino group at one end and a carboxyl group at the other (Figure 6). The amino acids are linked together by a covalent peptide bond between the α-carboxyl group of one amino acid and the α-amino group of another. Two or several amino acids can be linked together by successive peptide bonds to form di-, tri- and polypeptides. Proteins consist of several peptides arranged in varying sequences, cross-linked by disulphide bridges, hydrogen bonds and van der Waals forces. The side chain of the amino acids (R-group; Figure 6), can vary from a single hydrogen atom (glycine) to a methyl group (alanine) to a large heterocyclic group (tryptophan). The properties of an amino acid are

determined by the type of side chain, which affects the size, shape, electrical charge etc. (Berg *et al.*, 2002)

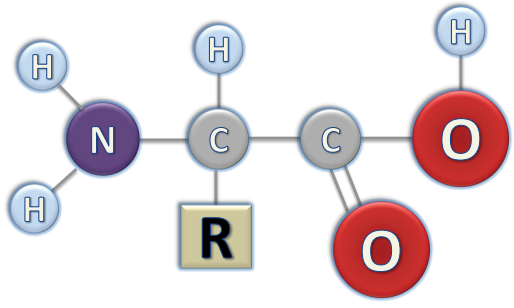


Figure 6. Chemical structure of an amino acid with the α-carboxyl end to the right and the α-amino end to the left. R indicates the side chain, which differs between the amino acids.

For biosynthesis of protein in living cells, 20 primary amino acids are needed (NRC, 2011). All fish are unable to synthesise 10 particular amino acids and, hence they need them in the diet, as do most other animals (Ketola, 1982). These 10 amino acids, referred to as indispensable or essential, are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. In addition, cysteine is synthesised from methionine and tyrosine is synthesised from phenylalanine. Thus, these two amino acids are regularly referred to as conditionally indispensable (NRC, 2011; Ketola, 1982).

### 2.4.1 Metabolism

Protein synthesis is fundamental to all living organisms and occurs together with protein catabolism (degradation) as continuous processes. Knowledge of these processes is vital in understanding how to quantify intake, digestion, circulation, synthesis and hydrolysis where protein/amino acids are in a state of change (Dabrowski & Guderley, 2002) (Figure 7).

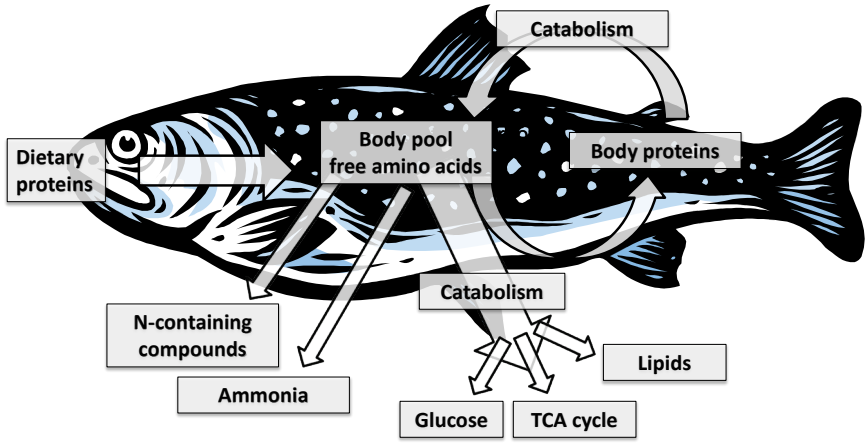


*Figure 7.* Main pathways of protein and amino acid metabolism, N = nitrogen, TCA = tricarboxylic acid.

The high concentrations of di- and tripeptides present in the intestinal lumen, resulting from the hydrolysis of dietary proteins, are absorbed either directly or after hydrolysis to amino acids (Bakke *et al.*, 2010; Verri *et al.*, 2010). Once absorbed by the fish, the main organ for amino acid catabolism is the liver which explains the presence of high concentrations of free amino acids (Ballantyne, 2001). In the liver the primary catabolism of amino acids takes place in the tricarboxylic acid (TCA) cycle, to generate energy in the form of adenosine triphosphate. In the TCA-cycle, the mechanism for degradation of amino acids is trans-deamination, in which the amino group is transferred to α-ketoglutarate to form glutamate. Other metabolic pathways for amino acid catabolism in the liver are: gluconeogenesis, with glucogenic amino acids being the main source of carbon to form glucose, and lipogenesis, with amino acids being the preferred carbon source for lipid synthesis. Ammonia is the end product of amino acid catabolism in fish (Ballantyne, 2001).

### 2.4.2 Amino acid requirements

Like other animals, fish do not have an exact protein requirement but have a requirement for a well-balanced composition of indispensable and dispensable amino acids (Wilson, 2002). Meeting the amino acid requirements of fish can be a challenge and demands reliable information on their requirements of indispensable and dispensable amino acids and on the chemical composition of feed ingredients (Kaushik & Seiliez, 2010).

However, the quantitative requirements for each of the 10 indispensable amino acids are known only for a limited number of fish species (NRC, 2011;

Kaushik & Seiliez, 2010; Wilson, 2002). The indispensable amino acid requirements differ greatly between species when expressed as a proportion of the diet, but the differences decrease when they are expressed as a proportion of dietary protein (Wilson, 2002; Cowey, 1994). One important reason for this variation is differences in the methods used to estimate the requirements (Cowey, 1995), *e.g*. mode of expression of the data (relative to dietary dry matter (DM), crude protein or digestible energy level *etc*.), type and composition of the experimental feed, rate of growth, the criteria used in estimation of requirements and the statistical model used in the data analyses. The most common approach used is dose-response experiments to obtain curves in which weight gain or nitrogen (N) utilisation is used as the response parameter. Other approaches include quantifying the indispensable amino acid requirement by direct or indirect measurements of amino acid oxidation, metabolic responses (Cowey, 1995), single amino acid deletion or reduction (Fournier *et al.*, 2002; Green & Hardy, 2002) or diet dilution techniques (Liebert & Benkendorff, 2007).

### 2.4.3 Lysine requirement

Lysine is the amino acid found in the highest concentrations in the carcass of several fish species (NRC, 2011; Wilson & Cowey, 1985) and it is the first-limiting indispensable amino acid in most protein sources used for commercial feed production (NRC, 2011; Hauler & Carter, 2001). In addition, due to its highly reactive Ɛ-amino group, lysine is sensitive to heat damage and to non-enzymatic glycolysis reactions, resulting in the production of Maillard reaction products (Moughan & Rutherfurd, 1996). Lysine deficiency has been shown to reduce growth and feed efficiency and to cause health issues such as dorsal and caudal fin erosion in rainbow trout and common carp (*Cyrpinus carpio*) (NRC, 2011; Ketola, 1982). The lysine requirements for some fish species are given in Table 1. In general, the lysine requirement for fish appears to range from 4-6 g/16 g N.

### 2.4.4 Quantification of lysine requirement and the ideal protein concept

The most commonly used method for quantification of the dietary lysine requirement in fish is the conventional growth response assay. A basal diet, lacking lysine but meeting all other known nutrient requirements, is formulated and supplemented with graded levels of lysine. This basal diet is generally synthetic or semi-synthetic, containing N from amino acids or a mixture of amino acids, casein and gelatine (NRC, 2011).

However, in some experiments high inclusion of crystalline amino acids has resulted in impaired growth compared with diets containing intact protein (Walton *et al.*, 1982; Robinson *et al.*, 1981), and decreased palatability, resulting in reduced feed intake (Brown *et al.*, 1993).

Table 1. *Lysine requirement (g/16 g N) for some fish species*

| Fish species | Requirement | Based on | Reference |
|---|---|---|---|
| African catfish | 5.7 | Growth studies | Fagbenro *et al.* (1998) |
| Atlantic salmon | 4.0 | Growth studies | Anderson *et al.* (1993) |
| | 3.2-3.6 | Growth studies | Berge *et al.* (1998) |
| Indian carp | 6.2 | Growth studies | Ravi and Devaraj (1991) |
| Channel catfish | 5.1 | Growth studies | Wilson *et al.* (1977) |
| | 5.0 | Growth studies | Robinson *et al.* (1980) |
| Chinook salmon | 5.0 | Growth studies | Halver *et al.* (1958) |
| *Clarais* hybrid | 4.8 | Growth studies | Unprasert (1994) |
| Common carp | 5.3 | Protein accretion | Ogino (1980) |
| European sea bass | 4.8 | Growth studies | Tibaldi and Lanari (1991) |
| Hybrid striped bass | 4.0 | Growth studies | Griffin *et al.* (1992) |
| Milkfish | 4.0 | Growth studies | Borlongan and Benitez (1990) |
| Nile tilapia | 5.1 | Growth studies | Santiago and Lovell (1988) |
| Rainbow trout | 3.7 | Growth studies | Kim *et al.* (1992b) |
| | 4.2 | Growth studies | Walton *et al.* (1984) |
| | 4.2 | Growth studies | Pfeffer *et al.* (1992) |
| | 5.3 | Protein accretion | Ogino (1980) |
| | 6.1 | Growth studies | Ketola (1983) |
| Red drum | 5.7 | A/E ratios | Moon and Gatlin (1991) |
| Red sea bream | 4.4 | Growth studies | Forster and Ogata (1998) |
| Turbot | 5.0 | A/E ratios | Kaushik (1998) |
| White sturgeon | 5.4 | Protein accretion | Ng and Hung (1995) |

When the requirement of dietary lysine is known, it can be used to predict the requirements of other indispensable amino acids when combined with the 'ideal protein concept'. This has been defined as the amino acid profile that exactly meets the requirements with no excess or deficit. The ideal protein concept proposes that the optimum dietary indispensable amino acid requirements be considered as a proportion of total indispensable amino acids, usually expressed as per cent of lysine (Wang & Fuller, 1989). The ideal protein concept has been applied for estimation of the amino acid requirements for several fish species, based on the amino acid profile of whole-body tissue

of the given species (Furuya *et al.*, 2004; Rollin *et al.*, 2003; Green & Hardy, 2002). Arai (1981) represented the concept using the A/E ratio as:

$$\frac{Indispensable\ amino\ acid\ content \times 1000}{Total\ indispensable\ amino\ acid\ content\ including\ cysteine\ and\ tyrosine}$$

and showed this to be highly correlated (r = 0.96) with the indispensable amino acid requirement of channel catfish.

## 2.5 Digestibility in fish

Modern feed formulations are normally based on the digestible nutrient and energy values, whereas gross nutrient and energy values were commonly used in the past. Thus, the digestibility of feed ingredients has been identified as one of the key factors in evaluation of prospective novel feed ingredients to be included in aquaculture (Glencross *et al.*, 2007). Digestibility has been defined as: the amount or proportion of nutrients, or categories of nutrients such as crude protein, that disappears from a meal as it passes through the digestive system and is excreted in faeces (NRC, 2011).

In practice, digestibility is primarily a measure of disappearance of nutrients from the ingested feed. Diet design, feeding strategy, faeces collection method and calculation method are all parameters affecting the apparent digestibility coefficient (Glencross *et al.*, 2007).

### 2.5.1 Digestibility determination

Determining the digestibility of a diet or feed ingredient in animals requires the collection of faecal material and this is more challenging with aquatic animals than with terrestrial animals. Both direct and indirect methods can be used for collecting faeces. In the direct method, all feed consumed by the organism needs to be measured and complete collection of faeces is required. This method is seldom used for fish due to problems with separating faecal material excreted by the fish from uneaten feed particles (NRC, 2011).

The method more commonly used in fish is the indirect method, in which a non-digestible marker such as titanium, chromic or yttrium oxide is added to the feed. The marker passes through the digestive tract at the same rate as food and is unaffected by the digestive processes. A representative sample of faeces is collected and it is assumed that the collected sample is representative of faeces excreted over a period of time. However, with the indirect method, faeces can be collected in an active or passive way. The active collection of faeces involves techniques such as manual stripping, dissection or vacuum

removal. In the passive faeces collection method, faeces naturally excreted by the fish are collected (NRC, 2011). The active method can result in underestimation of the apparent digestibility, while the passive method can result in its overestimation (Storebakken *et al.*, 1998; Hajen *et al.*, 1993; Cho *et al.*, 1982).

# 3 Aims of the thesis

The overall aim of this thesis was to estimate the amino acid requirements of Eurasian perch (*Perca fluviatilis*) and to study digestive enzyme activity and the utilisation of alternative protein sources in perch and Arctic charr (*Salvelinus alpinus*).

Specific objectives Papers I-IV were to:

- Assess the dietary lysine requirement of juvenile Eurasian perch
- Estimate the requirement of other indispensable amino acids (except tryptophan) in Eurasian perch using the ideal protein concept
- Compare digestive enzyme activity in slow-growing and fast-growing groups of Eurasian perch of different ages
- Quantify the digestive enzyme activity along the gastrointestinal tract of Eurasian perch and Arctic charr
- Compare the digestive enzyme activity in Eurasian perch and Arctic charr
- Assess the digestibility of dietary components, amino acids and energy in novel protein sources of fungal origin and mussel meal fed to Eurasian perch and Arctic charr
- Evaluate the growth performance and nutrient utilisation in Arctic charr fed diets in which protein of fungal origin and from mussel meal replaced with fish meal.

The hypotheses tested in Papers I-IV were that: *I)* The dietary amino acid requirements of Eurasian perch are different from those of yellow perch (*Perca*

*flavescens*); II) fast-growing perch have higher digestive enzyme activity than slow-growing perch and there are differences in digestive enzyme activity along the gastrointestinal tract in and between Eurasian perch and Arctic charr; III) extracted baker's yeast and mussel meal have higher digestibility than intact baker's yeast and zygomycete meal in Eurasian perch and Arctic charr; and I*V*) growth performance in Arctic charr is not negatively affected by partial replacement of fish meal with protein of fungal origin and mussel meal.

# 4 Materials and methods

## 4.1 Experimental design

The first experiment with Eurasian perch was organised as a randomised block design, with three replicates for each of the eight inclusion levels of lysine (Paper I). In the second experiment, with five groups of perch and two groups of Arctic charr, the activity of a total of eight digestive enzymes was measured in nine different parts of the digestive tract and chyme (Paper II). The digestibility experiments with perch and charr were organised as a randomised block design, with four replicates for each experimental diet (Paper III). The final experiment, with charr, was organised as a fully randomised design, with three replicates for each experimental diet (Paper IV). In all experiments, fish were mildly anaesthetised (tricaine methane sulphonate, MS222; Western Chemical Inc., Ferdale, WA, USA) before handling, and had an adaption period in the experimental unit prior to the start of the experiment.

## 4.2 Fish and housing

All perch used in the experiments were obtained from a commercial fish hatchery (Östgös AB, Söderköping, Sweden) and brought to the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. These perch were first generation offspring from wild eggs. The charr used in the experiments originated from the Swedish breeding programme (Nilsson *et al.*, 2010), and were obtained from Kälarne Research Station (KRS), Bräcke, Sweden.

The experiments presented in Paper I, part of Paper II (regarding perch) and Paper III were carried out at SLU, Uppsala. The experiments presented in the other part of Paper II (regarding charr) and Paper IV were carried out at KRS, Bräcke. The experiments were approved in advance by the local ethics

committee and were performed in compliance with laws and regulations on procedures and experiments in live animals in Sweden, which are overseen by the Swedish Board of Agriculture.

The fish were stored in 1000-L flow-through tanks prior to the start of experiments at SLU and fed commercial diets formulated to match the nutrient requirements of each species to apparent satiation twice daily. Feed waste and faeces were removed daily. The average water temperature was 20°C for perch and 9°C for charr, the dissolved oxygen concentration ranged from 6.4 to 8.7 mg $L^{-1}$ (HQ 40d, Hach Lange GmbH, Berlin, Germany), the pH fluctuated between 7.5 and 8.0, and ammonium-N and nitrate-N were below the detection limit (Sera Aqua-test Box, Heinsberg, Germany). The photoperiod was set to 12 h light and 12 h darkness. The tanks were provided with artificial plants to provide shelter for the fish.

## 4.3 Test ingredients

The ingredients tested in Papers III and IV consisted of intact baker's yeast (*Saccharomyces cerevisiae*), extracted baker's yeast (*S. cerevisiae*), zygomycetes (*Rhizopus oryzae*) and blue mussel (*Mytilus edulis*). The intact *S. cerevisiae* (Jästbolaget®, Stockholm, Sweden) was cultured on molasses, ammonia, phosphorus, magnesium and vitamins, and dried on a fluidised bed. The extracted *S. cerevisiae* (Alltech Serbia AD, Senta, Serbia) was produced by autolysis. The liquid and solid fractions (cell content and cell walls) were separated by centrifugation, and the liquid slurry was then dried. The *R. oryzae* was cultivated on spent sulphite liquor, a side-stream from a magnesium sulphite paper pulp mill. The spent sulphite liquor was transferred into an air-lift fermenter, supplemented with ammonium and phosphate, and inoculated with *R. oryzae*. The culture was sparged with air and run at 35°C in fed batch mode. Fungal biomass was harvested on a sieve, washed with water and dried with hot air (Cewatech AB, Gothenburg & Nordic Paper Seffle AB, Säffle, Sweden. The *M. edulis* (Royal Frysk Muscheln GmbH, Emmelsbüll-Hornsbül, Germany) was dried (55°C for 24 h) in our laboratory. Both *R. oryzae* and *M. edulis* were milled to pass through a 1-mm screen prior to inclusion in the feed. The proximate composition, energy and amino acid content of the test ingredient diets are given in Table 2.

Table 2. *Proximate composition (g kg$^{-1}$ DM), energy content (MJ kg$^{-1}$ DM) and amino acid content (g/16 g N) of fish meal and the test ingredients; intact Saccharomyces cerevisiae, extracted S. cerevisiae, Rhizopus oryzae and Mytilus edulis meals*

| | Ingredient | | | | |
|---|---|---|---|---|---|
| | Fish meal[1] | Intact *S. cerevisiae* | Extracted *S. cerevisiae* | *R. oryzae* | *M. edulis* |
| Dry matter | 920 | 935 | 938 | 947 | 868 |
| Crude protein | 711 | 466 | 779 | 479 | 657 |
| Sum of amino acids | - | 428 | 498 | 220 | 472 |
| Crude lipid | 83 | 10 | 2 | 94 | 69 |
| Neutral detergent giber | - | 0 | 0 | 104 | 14 |
| Ash | 155 | 63 | 153 | 121 | 89 |
| Gross energy | - | 19.9 | 18.1 | 19.7 | 22.8 |
| **Indispensable amino acids** | | | | | |
| Arginine | 5.6 | 4.8 | 2.2 | 1.8 | 5.6 |
| Histidine | 2.4 | 2.2 | 1.3 | 1.5 | 1.6 |
| Isoleucine | 4.7 | 4.9 | 3.4 | 2.9 | 3.3 |
| Leucine | 7.6 | 6.9 | 4.7 | 3.8 | 5.1 |
| Lysine | 7.8 | 7.4 | 5.0 | 3.8 | 5.9 |
| Methionine[2] | 3.0 | 2.1 | 1.7 | 1.7 | 2.9 |
| Phenylalanine | 4.1 | 4.1 | 2.7 | 2.1 | 2.8 |
| Threonine | 4.3 | 4.9 | 2.6 | 2.0 | 3.3 |
| Valine | 5.4 | 6.0 | 4.2 | 3.5 | 3.8 |
| Total | 44.9 | 43.4 | 27.8 | 23.0 | 34.3 |
| **Dispensable amino acids** | | | | | |
| Alanine | - | 5.2 | 4.9 | 3.2 | 3.9 |
| Aspartic acid | - | 9.7 | 6.9 | 3.3 | 7.6 |
| Cysteine[3,4] | - | 2.1 | 1.5 | 1.9 | 2.2 |
| Glutamic acid | - | 14.3 | 9.8 | 5.1 | 9.6 |
| Glycine | - | 4.7 | 3.4 | 2.9 | 4.7 |
| Ornithine | - | 0.2 | 0.0 | 1.6 | 0.0 |
| Proline | - | 3.3 | 4.3 | 1.6 | 2.8 |
| Serine | - | 3.9 | 2.7 | 1.4 | 3.2 |
| Tyrosine[3] | - | 4.9 | 2.5 | 2.0 | 3.3 |
| Total | - | 48.5 | 36.0 | 23.0 | 37.4 |

[1] Commercial fish meal, anchovy (NRC, 2011).
[2] Amount present after oxidation of methionine to methionine sulphone.
[3] Amount present after oxidation of cysteine and cystine to cysteic acid.
[4] Conditionally indispensable (NRC, 2011).

## 4.4 Experimental diets

In Paper I, eight semi-purified, iso-nitrogenous and iso-energetic diets containing graded levels of lysine (final concentration in diet varying between 12.2 and 24.3 g $kg^{-1}$ DM, corresponding to 3.0-6.0 g/16 g N) were produced. Fish meal, casein and gelatine were used as protein sources, and were supplemented with mixtures of dispensable amino acids and indispensable amino acids, excluding lysine. Approximately 40% of the total nitrogen content in the diets was in the form of protein-bound amino acids.

In Paper II, the perch and charr were fed commercial iso-nitrogenous fish feeds, formulated to match the nutrient requirements of each species. The nutrient composition was therefore not identical with respect to lipid and nitrogen-free extractives.

In Paper III, the diets consisted of one reference diet and four experimental diets containing 70% reference diet and 30% test ingredient (Cho & Slinger, 1979). The test ingredient in these four diets was intact *S. cerevisiae*, extracted *S. cerevisiae*, *R. oryzae* and *M. edulis* meal, respectively.

In Paper IV, the experimental diets consisted of one reference diet and four experimental diets, and were formulated to be iso-nitrogenous and iso-energetic. The reference diet contained fish meal as the main protein source, while in the test diets 40% of the fish meal crude protein was replaced with crude protein from intact *S. cerevisiae*, extracted *S. cerevisiae*, *R. oryzae* and *M. edulis*.

## 4.5 Experimental conditions

In Paper I, 240 perch (41.7 ± 8.1 g; mean ± s.d.) of two different ages (young and old) and two different sizes (small and large) were randomly placed in groups of ten in 24 experimental units (consisting of 200-L glass aquariums divided in two by a sponge filter) organised into three floors. Fish within each floor were randomly assigned to one of the eight treatments. The experimental aquariums were connected to an indoor recirculating system with mechanical and biological filtration and UV-treatment. During the first week of the experimental period, the fish were fed the experimental diets by hand to apparent visual satiation, to allow them to adapt to the diets and to estimate the apparent visual satiation. Excess rations (100% of satiation=full ration) of each diet were constantly fed by automatic feeders for 11 hours a day, 6 days a week.

In Paper II, perch fingerlings (mean weight 6.0 g) and charr (mean weight 30.5 g) were fed by automatic feeders to apparent satiation twice daily, corresponding to approximately 1.7% and 2.0% of biomass in the tank for

perch and charr, respectively. Perch and charr were held in 1000-L flow-through tanks.

In Paper III, in which each experiment was divided into two periods per species, 200 charr (106.6 ± 4.9 g and 113.8 ± 7.9 g in period 1 and 2, respectively), and 300 perch (51.8 ± 5.3 g and 45.1 ± 6.0 in period 1 and 2, respectively) were used. The experimental unit comprised ten 90-L PVC tanks equipped with waste feed and faeces collectors (Cho *et al.*, 1982). The PVC tanks were connected to an indoor recirculating system with mechanical and biological filtration and UV-treatment. The fish were randomly allocated to the experimental tanks (10 fish per tank for charr and 15 fish per tank for perch) and the experimental diets were randomly assigned to the tanks. Charr were fed by hand twice daily until provided feed was rejected. Perch were fed 1.5% of their tank biomass twice daily, which was in excess. The different feeding procedures were due to the stress observed in perch during human activity above the tanks.

In Paper IV, 750 charr (48.1 ± 0.6 g) were marked with a passive integrated transponder tag and randomly allocated to groups of 50 in fifteen 700-L tanks. The fish were fed the experimental diets by automatic feeder at a restricted ration of 1% of the tank biomass, with the rate adjusted weekly to an expected increase in tank biomass.

## 4.6 Sample collection

In Papers I and IV, all fish were individually weighed at the end of the experiment. In Paper I, a blood sample was collected from the caudal vein and the fish were then dissected and liver, intraperitoneal fat and final parts of viscera removed and weighed. In Paper IV, faeces from the most distal part of the intestine were collected for digestibility assessment and stored at -25°C until analysis.

For the measurements of carbohydrase and lipase activity in Paper II, fish were starved for 24 hours prior to sampling, killed, the liver, stomach, pancreas, pyloric caeca, mid-intestine and distal intestine were dissected, and the stomach, mid-intestine and distal intestine were opened longitudinally and rinsed in ice-cold saline. The samples were frozen in liquid N and stored at -80°C until analysis. For measurements of trypsin and chymotrypsin activity, the pyloric caeca and chyme from mid-intestine and distal intestine were sampled from non-starved fish and immediately frozen in liquid N and stored at -80°C.

In Paper III, feed waste was collected and stored at -25°C and the tanks were drained of 50% of the water to ensure that the cleaning procedure was

accurate. A 50-ml tube was attached to the bottom of the faeces collector column and covered with ice. The tube was removed just before the next feeding started, the contents were centrifuged at 5000×g for 10 minutes and the supernatant was rejected. The pellet obtained was stored at -25°C until analysis. Total collection time in the experiment with charr was two weeks per period and in the experiment with perch three weeks per period.

## 4.7 Determination of enzyme activity (Paper II)

Preliminary tests were performed for each enzymatic assay during which various dilutions of the homogenates were tested, as well as different pH and temperatures, to determine which parameters resulted in the greatest initial rectilinear change in absorbance in a certain time. The enzyme activity of α-amylase and disaccharidases was determined with a microplate reader (Multiscan FC; Thermo Fisher Scientific Inc., IL, USA) and that of lipase, trypsin and chymotrypsin with a spectrophotometer (UV-1800, Shimadzu Europa GmbH, Duisburg, Germany).

Enzyme activity was expressed as specific activity in U $mg^{-1}$ protein or U $mg^{-1}$ sample (U = μmol substrate hydrolysed $min^{-1}$). Protein was analysed using a Micro BCA Protein Assay kit, no. 23235 (Thermo Fisher Scientific Inc., IL, USA).

### 4.7.1 Preparation of homogenate

For carbohydrase and lipase activity assays and protein determination, tissue samples were homogenised (1:20, ice-cold malic acid buffer, pH 5.4) in an electrical homogeniser (Ultra turrax tube dispenser, IKA Werke GMBH & Co.KG, Staufen, Germany), centrifuged for 10 minutes at 15,800×g and the supernatant stored at -80°C for later analysis. The protease inhibitor phenylmethylsulphonyl fluoride (PMSF; 0.5 mM; Sigma no. P7626, Sigma-Aldrich Sweden AB, Stockholm, Sweden) was added before the homogenate was analysed for lipase and α-amylase activity and protein content.

For trypsin and chymotrypsin activity assays, the pyloric caeca with chyme were homogenised (1:20, ice-cold buffer; 0.2 M Tris HCl, 0.05 M $CaCl_2$, pH 8.0, containing enterokinase solution (25.0 μg enterokinase $mL^{-1}$) in an electric homogeniser (Ultra turrax tube dispenser, IKA Werke GMBH & Co. KG). The pyloric caeca samples were incubated for about 16 hours at 4°C to allow enterokinase to convert trypsinogen to trypsin, thereby activating chymotrypsinogen. The samples were then centrifuged for 10 minutes at 15,800×g. The supernatant was immediately analysed for trypsin and chymotrypsin activity. Chyme samples from mid-intestine and distal intestine

were suspended and homogenised as described for pyloric caeca above, but without the addition of enterokinase solution to the buffer since trypsin was present and therefore converting trypsinogen to trypsin. The samples were centrifuged for 10 minutes at 15,800×g, and the supernatant stored at -80°C for later analysis.

### 4.7.2 Disaccharidases

The activity of disaccharidases (maltase, isomaltase, sucrase and trehalase) was determined according to the methods described by Dahlqvist (1968), using maltose (Sigma no. M5885), isomaltose (Sigma no. I7253), sucrose (Sigma no. 84097) and trehalose (Sigma no. T9531), respectively as substrates. Incubation with 10 µL homogenate and 10 µL substrate buffer was performed at 37°C for 60 minutes and then stopped by adding 300 µL Tris-glucose oxidase reagent. This was followed by another 60 minutes of incubation at 37°C for the colour to develop. Absorbance was recorded at 450 nm.

### 4.7.3 Alpha-amylase

Alpha-amylase activity was determined with a commercial kit (Ceralpha Method, AOAC Official Method 2002.01., Megazyme International Ireland Ltd., Wicklow, Ireland) using benzylidene end-blocked p-nitrophenyl α-D-maltoheptaoside as substrate. Test tubes, containing 100 µL homogenate and 20 µL amylase high-range reagent solution were incubated for 20 minutes at room temperature. The reaction was stopped by adding 300 µL stopping solution (alkaline solution) and absorbance was recorded at 405 nm.

### 4.7.4 Lipase

Lipase activity was determined according to Albro *et al.* (1985) using p-nitrophenyl myristate (p-NPM; Sigma no. 70124) as substrate. In brief, the reaction mixture was prepared in a final volume of 1.0 mL and contained 100 mM Tris HCl (pH 8.0) 10 mM deoxycholate, 6 mM DL-dithiothreitol and 0.5 mM p-NPM. Ethylene glycol monomethyl ether was used to dissolve p-NPM at 50°C. The reaction was started by adding 100 µL homogenate and was monitored at 400 nm for 2 minutes at 30°C. The background (control without homogenate) change was subtracted from all values.

### 4.7.5 Trypsin and chymotrypsin

Trypsin and chymotrypsin activity was determined according to Rick (1974) and Worthington (1982), respectively. For trypsin, benzoyl-arginine-p-nitroanilide (BAPNA; Sigma no. B4875) was used as substrate and the reaction was followed at 390 nm for 2 minutes at 25°C. For chymotrypsin, N-benzoyl-

L-tyrosine ethyl ester (BTEE; Sigma no. B6125) was used as substrate and the reaction was monitored at 256 nm for 2 minutes at 25°C

## 4.8 Chemical analysis

Feed and faeces samples and carcasses for nutritional analyses were freeze-dried and milled to pass through a 1-mm screen before analysis. DM was determined by drying samples at 103°C for 16 h in a well-ventilated oven, and ash after ignition at 550°C for at least 3 h until the ash had a white colour. For both analyses, samples were cooled in a desiccator before weighing. Total nitrogen was determined by the Kjeldahl method using a 2020 digestor and a 2400 Kjeltec Analyser unit (FOSS Analytical A/S, Hilleröd, Denmark). Crude protein content was calculated as N×6.25 (Nordic Committee on Feed Analysis, 1976). Crude lipid content was determined according to the Official Journal of the European Communities (1984), using a 1047 Hydrolysing Unit and a Soxtec System HT 1043 Extraction Unit (FOSS Analytical A/S, Hilleröd, Denmark). In Paper III, neutral detergent fibre was determined according to Chai and Udén (1998), with a 100% neutral detergent solution and the use of amylase and sulphite (for reduction of starch and protein). Gross energy was determined with an isoperobol bomb calorimeter (Parr 6300, Parr Instrument Company, Moline, IL, USA).

In Paper I, the amino acid content of feeds and fish was analysed by high-performance liquid chromatography at a certified laboratory (Eurofins Food & Agro Testing Sweden AB, Linköping, Sweden), as described by Llames and Fontaine (1994). In brief, the samples were oxidised with performic acid for 16 h prior to hydrolysis for 23 h with 6N HCl. Individual amino acids were separated on an ion-exchange chromatograph (Biochrom 30 amino acid analyser, Biochrom Ltd., Cambridge, England) and the peaks were identified, integrated and quantified with EZChrom Elite (Biochrom Ltd., Cambridge, England).

In Papers III and IV, the amino acid content in the feed was analysed using the Waters AccQ•Tag™. The samples were hydrolysed in 15 mL 6M HCl containing 1% phenol in a microwave oven (Synthos 3000, Anton Paar Nordic, AB Sweden). For analysis of methionine and cysteine, 50 mg of sample were added to 2 mL cold, freshly prepared formic acid:perhydrol (9:1) and incubated overnight at 4°C. Thereafter, 2 mL freshly prepared sodium bisulphite (0.17 g sodium bisulphite $mL^{-1}$ water) solution was added to each tube and the contents mixed for 15 min. The samples were then hydrolysed as described above. In Papers III and IV, titanium oxide ($TiO_2$) was analysed according to Short *et al.* (1996).

## 4.9 Calculations

In Papers I, III and IV, weight gain (WG) and specific growth rate (SGR) were used as indicators of performance, while in Papers III and IV feed conversion ratio (FCR) was used as the performance indicator. Protein gain (PG) was used as an indicator of protein utilisation in Paper I and nutrient retention (NR) was used as an indicator of nutrient utilisation in Paper IV. The WG, SGR, FCR, PG and NR were calculated according to the following equations:

$$\text{WG (\%)} = (\text{FW} - \text{IW}) \times \frac{100}{IW}$$

$$\text{SGR (\% day}^{-1}) = (\log FW - \log IW) \times \frac{100}{days}$$

$$\text{FCR (g g}^{-1}) = \frac{FI}{WG}$$

$$\text{PG (\%)} = \left(\frac{FW \times CPF}{100} - \frac{IW \times CPI}{100}\right) \times \frac{100}{IW \times \frac{CPI}{100}}$$

$$\text{NR (\%)} = \frac{Nutrient\ retained\ in\ the\ body \times 100}{Nutrient\ ingested}$$

where FW is the final body weight (g), IW is the initial weight (g), CPF is the final crude protein content in the body (%) and CPI is the initial crude protein content in the body (%). In Papers I and IV, the relative weight of liver, viscera and intraperitoneal fat was expressed as hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitoneal fat (IPF), respectively, calculated according to the following equations:

$$\text{HSI (\%)} = \frac{W_{Liv} \times 100}{LW}$$

$$\text{VSI (\%)} = \frac{W_{Vis} \times 100}{LW}$$

$$\text{IPF (\%)} = \frac{W_{IntrF} \times 100}{LW}$$

where LW is live weight of fish (g), $W_{Liv}$ is weight of liver (g), $W_{Vis}$ is weight of viscera (g) and $W_{IntrF}$ is weight of intraperitoneal fat (g).

In Papers III and IV, the apparent digestibility coefficient (ADC) for the nutrient and energy content of the experimental diets was calculated according to the following equation (Cho *et al.*, 1982):

$$\mathrm{ADC_{diet}} = 1 - \left(\frac{F}{S} \times \frac{D_i}{F_i}\right)$$

where F = % nutrient (or kJ $g^{-1}$ gross energy) in faeces, D = % nutrient (or kJ $g^{-1}$ gross energy) in the diet, $D_i$ = % digestion indicator of the diet and $F_i$ = % digestion indicator of faeces.

In Paper III, apparent digestibility coefficient for the test ingredients was calculated based on the following equation (Bureau *et al.*, 1999):

$$\mathrm{ADC_{test\ ingr.}} = ADC_{test\ diet} + \left[\left(ADC_{test\ diet} - ADC_{ref\ diet}\right) \times \left(\frac{0.7 \times D_{ref}}{0.3 \times D_{test\ ingr.}}\right)\right]$$

where $D_{ref}$ = % nutrient (or kJ $g^{-1}$ gross energy) in the reference diet (as is) and $D_{test\ ingr.}$ = % nutrient (or kJ $g^{-1}$ gross energy) in the test ingredient (as is).

## 4.10 Statistical analysis

The statistical analysis was conducted using SAS programme version 9.3 (SAS Institute, Inc, Cary, NC, USA). The level of significance in all papers was set at $P<0.05$.

In Paper I, the effect of fish size on carcass composition was analysed using the GLM procedure, whilst the effects of diet on growth, survival, carcass composition, body indices and haematocrit value were evaluated using the Mixed procedure, followed by Tukey's Multiple Comparison test. The model included the fixed factor of treatment (lysine level) and the random factor of aquarium.

The estimated most adequate dietary lysine levels for weight gain and protein gain are presented in this thesis, but not in Paper I, by using the broken-line model as a function of lysine level (R software version 2.12.1). The treatment with a lysine level of 17.3 g $kg^{-1}$ DM was excluded from the calculations, as the response deviated from that observed the treatments containing 15.3 and 18.3 g lysine $kg^{-1}$ DM and as the coefficient of determination ($R^2$) for the model was markedly improved when this treatment was excluded from the analysis.

In Paper II, three different comparisons were made: The first of these compared the effects of age and growth rate on carbohydrase and lipase activity in perch, for which the statistical model GLM included the fixed effects of groups (young and small, young and large, old and small, old and large) and tissues (liver, stomach, pancreas, pyloric caeca, mid-intestine, distal intestine). In the second comparison, the digestive enzyme activity was analysed separately for perch and charr, with tissues as fixed effect. In the third comparison, the effect of species on enzyme activity in perch and charr was analysed, for which the statistical model included the fixed effects of species

and tissues. Two-way interactions between fixed factors (groups/species and tissues) were tested and excluded from the models if found to be non-significant ($P>0.05$).

To compare the overall activity of individual carbohydrase and lipase in the gastrointestinal tract in Paper II, enzyme activity in all tissues was summarised (referred to as 'total activity'). In addition, the total carbohydrase activity (sum of total sucrase, maltase, isomaltase, trehalase and α-amylase activity) was compared with the total lipase activity within and between species. The PDIFF option was used to calculate P values when testing differences between LSMs.

In Paper III, the effect of diet (reference diet, and diets with intact *S. cerevisiae*, extracted *S. cerevisiae*, *R. oryzae* and *M. edulis*) on apparent digestibility within fish species (Arctic charr and Eurasian perch) was evaluated using the Mixed procedure, followed by Tukey's Multiple Comparison test. The model included the fixed factor of treatment and the random factor of period (period 1 and 2).

In Paper IV, effect of test diets on growth performance (start and final weights, weight gain, specific growth rate, and relative organ weights (hepatosomatic index and viscerosomatic index) were evaluated using the hierarchical model mixed procedure, including the fixed factor of test diets and the random factor of tank within test diet. Effect of test diet on feed conversion ratio, apparent digestibility, nutrient- and energy retention was evaluated using the statistical model GLM, including the fixed factor of test diet. To adjust for multiple comparisons Tukey's Multiple Comparison test were used for all comparisons. Tank was the experimental unit and significance level was set to $P<0.05$.

# 5 Summary of main results

## 5.1 Dietary lysine requirements in perch (Paper I)

Fish fed diets containing 20.1 g lysine $kg^{-1}$ DM had significantly higher final weight than fish fed diets containing 12.2-17.3 g lysine $kg^{-1}$ DM. However, when broken down into treatment levels, weight gain only resulted in significant differences between the lowest and third lowest inclusion levels of lysine (12.2 and 15.3 g $kg^{-1}$ DM, respectively) and the highest inclusion level (24.3 g $kg^{-1}$ DM). The highest inclusion level of lysine resulted in significantly higher protein gain than diets containing ≤17.3 g lysine $kg^{-1}$ DM.

The hepatosomatic index was lower in fish fed diets containing 18.3, 20.1 and 24.3 g lysine $kg^{-1}$ DM compared with fish fed diets containing 12.2 and 17.3 g lysine $kg^{-1}$ DM. The viscerosomatic index followed the same pattern as the hepatosomatic index. Intraperitoneal fat, haematocrit value and body composition were not affected by dietary lysine level in this experiment.

However, comparisons between treatments with inclusion levels 12.2-17.3 and 18.3-24.3 g lysine $kg^{-1}$ DM resulted in significant differences in final body weight (51.6 vs. 69.7 g), weight gain (44.9 vs. 65.4%), protein gain (27.5 vs. 47.9%), specific growth rate (0.44 vs. 0.60% $day^{-1}$), hepatosomatic index (2.8 vs. 2.3) and viscerosomatic index (11.1 vs. 10.4 %).

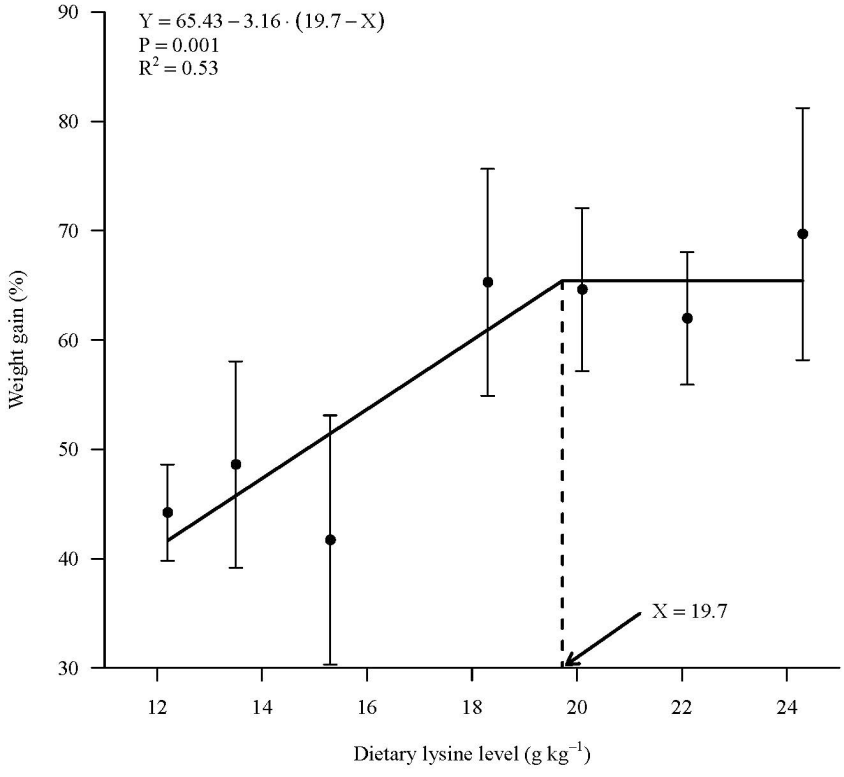


*Figure 8.* Linear broken-line model for the relationship between weight gain and dietary lysine level in Eurasian perch.

Weight gain and protein gain were used as parameters in estimation of the most adequate dietary lysine requirements of juvenile Eurasian perch. The results suggested that at least 19.7 g lysine $kg^{-1}$ DM (4.9 g/16 g N) are needed for optimal weight gain (r=0.73; Figure 8) and 20.3 g lysine $kg^{-1}$ DM (5.0 g/16 g N) for optimal protein gain (r=0.74; Figure 9).

In Paper I, the indispensable amino acid requirements of juvenile Eurasian perch were estimated using the ideal protein concept (Table 3). The average indispensable amino acids composition of whole-body tissues of perch fed a commercial diet was used for this estimation. The results showed that the indispensable amino acid content in the whole body of Eurasian perch is very similar to that of yellow perch, but differs from the profile of rainbow trout and Arctic charr.

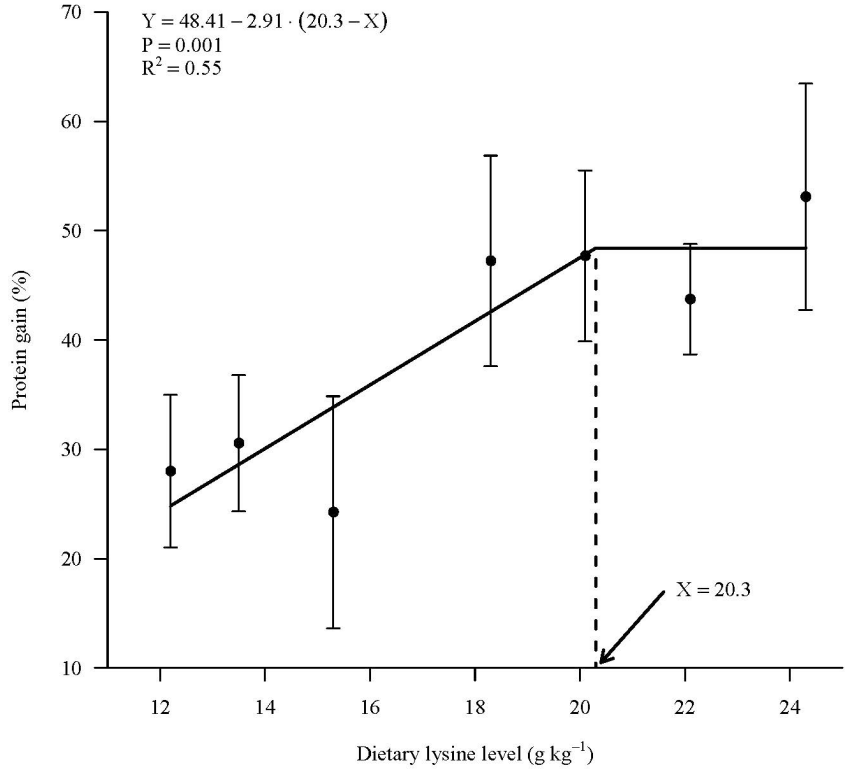


*Figure 9.* Linear broken-line model for the relationship between crude protein gain and dietary lysine level in Eurasian perch.

## 5.2 Digestive enzyme activity in perch and charr (Paper II)

### 5.2.1 Enzyme activity in the gastrointestinal tract of perch

Growth rate and age had no effect on the activity of carbohydrases and lipase in different regions of the gastrointestinal tract in perch. However, there were significant differences between the different regions of the gastrointestinal tract. The highest disaccharidase activity was found in the liver (sucrase), pyloric caeca (maltase), mid-intestine (isomaltase) and pancreas (trehalase). For both α-amylase and lipase, activity was highest in the pancreas, while trypsin and chymotrypsin activity was highest in the pyloric caeca.

Total lipase activity was significantly higher than total carbohydrase activity (142 and 6.2 U $mg^{-1}$ protein, respectively). Within the carbohydrases, maltase had the highest total activity (4.8 U $mg^{-1}$ protein).

Total trypsin activity was markedly lower than total chymotrypsin activity (0.27 and 110 U $mg^{-1}$ sample, respectively).

### 5.2.2 Enzyme activity in the gastrointestinal tract of charr

Differences in carbohydrase, lipase and proteolytic activity were found in different regions of the gastrointestinal tract in charr. The highest sucrase activity was found in the liver, the highest maltase and trehalase activity in the pyloric caeca and mid-intestine, and the highest isomaltase activity in the pyloric caeca. Alpha-amylase was found to have the highest activity in the pancreas, while the highest activity of lipase was found in the pyloric caeca and mid-intestine. The highest activity of trypsin and chymotrypsin was found in the mid-intestine.

In charr, total lipase activity was significantly higher than total carbohydrase activity (111 U $mg^{-1}$ protein and 2.4 U $mg^{-1}$ protein, respectively). Maltase was found to be the carbohydrase with the highest total activity. Total trypsin activity was significantly lower than total chymotrypsin activity (0.24 and 193 U $mg^{-1}$ sample, respectively).

### 5.2.3 Comparison of enzyme activity in the gastrointestinal tract of perch and charr

The activity of sucrase and maltase was significantly higher in the liver (only sucrase), the pyloric caeca and the mid-intestine of perch than of charr. Isomaltase activity was higher in the distal intestine of perch than of charr, while trehalase and lipase activity was higher in the pancreas, but lower in the pyloric caeca and mid-intestine, of perch than of charr.

There were significant interactions between species and tissues in terms of trypsin and chymotrypsin activity. In charr, the activity was highest in the mid-intestine, while in perch the activity was highest in the pyloric caeca. There were no significant differences between the two species in terms of trypsin and chymotrypsin activity in the distal intestine. The lipase, trypsin and chymotrypsin activity was higher in the mid-intestine than in the distal intestine in charr, whereas no such differences were observed in perch.

Perch had higher total carbohydrase activity than charr, with higher activity of maltase, sucrase and α-amylase. Total lipase and total trypsin activity did not differ between species, whilst total chymotrypsin activity was significantly higher in charr than in perch.

## 5.3 Digestibility in perch and charr (Papers III and IV)

In charr, inclusion of extracted *S. cerevisiae, R. oryzae* and *M. edulis* in the diet had a positive effect on the apparent digestibility coefficient for DM in the diet, compared with the reference diet and the intact *S. cerevisiae* diet (Paper III). The extracted *S. cerevisiae* had a higher apparent digestibility coefficient for crude protein than the diet containing intact *S. cerevisiae.* An effect of dietary treatment was observed on the apparent digestibility coefficient for indispensable amino acids, in general with higher values for extracted *S. cerevisiae* and *M. edulis* diets. The apparent digestibility coefficient for gross energy in the diets was lower for intact *S. cerevisiae* than for extracted *S. cerevisiae, R. oryzae* and *M. edulis*.

The apparent digestibility coefficient of test ingredients in Paper III showed significant differences for charr, but not for perch. Intact *S. cerevisiae* had lower apparent digestibility coefficients for DM, sum of amino acids and gross energy than the other test ingredients. No effect of dietary treatment was observed on apparent digestibility coefficient for crude protein. However, an effect was observed for isoleucine, lysine, methionine, phenylalanine, threonine and valine, with higher values for extracted *S. cerevisiae* and *M. edulis* and lower values for intact *S. cerevisiae*.

For the experiment with perch (Paper III), an effect of dietary treatment was observed on the apparent digestibility coefficient of isoleucine, leucine, threonine and valine for the experimental diets, generally with higher values for the extracted *S. cerevisiae* diet than the *R. oryzae* diet. No significant differences were found for the apparent digestibility coefficient of indispensable amino acids.

In Paper IV, the *M. edulis* diet had a higher apparent digestibility coefficient for DM than the reference, intact *S. cerevisiae* and *R. oryzae* diets. The apparent digestibility coefficient for crude protein was highest in the reference, extracted *S. cerevisiae* and *M. edulis* diets. No effect of dietary treatment of gross energy was observed.

## 5.4 Performance in charr (Papers III and IV)

A significantly higher weight gain was observed for fish fed diets containing intact *S. cerevisiae* and *M. edulis* than for fish fed extracted *S. cerevisiae* and *R. oryzae* diets (Paper III).

In Paper IV, replacement of 40% of the fish meal with extracted *S. cerevisiae,* intact *S. cerevisiae, R. oryzae* and *M. edulis* had no effect on the final weight compared with fish fed the reference diet. However, there were

significant differences in weight gain between fish fed the reference diet (180.8%) and fish fed diets containing extracted *S. cerevisiae* (157.7 %), and *R. oryzae (*152.7%). Feeding charr with the *R. oryzae* diet also resulted in lower weight gain, compared with the intact *S. cerevisiae* and *M. edulis* diets.

Specific growth rate was significant higher for the reference diet than for the extracted *S. cerevisiae* and *R. oryzae* diets. Consequently, fish fed the reference diet had a significantly better feed conversion ratio than fish fed the extracted *S. cerevisiae* and *R. oryzae* diets. Replacement of fish meal with intact *S. cerevisiae* and *M. edulis* did not result in significant differences in specific growth rate or feed conversion ratio compared with the other diets. Body indices, hepatosomatic index and viscerosomatic index were not affected by dietary treatment.

However, inclusion of *R. oryzae* and extracted *S. cerevisiae* in the diets resulted in lower N retention compared with the reference diet, whilst no significant differences in lipid and energy retention were observed between the dietary treatments.

# 6 General discussion

## 6.1 Chemical composition of baker's yeast, zygomycete and blue mussel meals

Chemical analysis of the test ingredients used in Papers III and IV showed large variations in the crude protein content (466-779 g $kg^{-1}$ DM). Moreover, the indispensable amino acid profile of the test ingredients differed from that of fish meal (Figure 10). Intact *S. cerevisiae* had an indispensable amino acid profile that was most similar to fish meal, expressed as g/16 g N, but also the lowest crude protein content. It has been shown that the chemical composition of yeasts and zygomycetes can vary depending on strain, growth medium and growing conditions (Lennartsson *et al.*, 2011; Halasz & Lasztity, 1991). This has to be considered when evaluating these types of protein sources.

The indispensable amino acid profile of *R. oryzae* differed most from that of fish meal, and had only about 50% of the lysine content in fish meal, whilst the other test ingredients had about 99, 79, and 67% of the lysine content in fish meal (intact *S. cerevisiae*, *M. edulis* and extracted *S. cerevisiae*, respectively). Intact and extracted *S. cerevisiae* and *R. oryzae* were all deficient in the sulphur-containing amino acid methionine, which is common in microbial proteins (Kuhad *et al.*, 1997; Murray & Marchant, 1986). Murray and Marchant (1986) reported an increased growth rate in rainbow trout fed microbial biomass supplemented with L-methionine.

Furthermore, microorganisms are known to have high nucleic acid content, resulting in elevated plasma uric acid levels when ingested by terrestrial animals, causing toxicological effects and disturbances in metabolism (Rumsey *et al.*, 1992; Rumsey *et al.*, 1991b). However, salmonid fish appear to be capable of metabolising high levels of nucleic acid by their very active liver uricase (Kiessling, 2009; Rumsey *et al.*, 1992; Rumsey *et al.*, 1991b; Kinsella *et al.*, 1985).

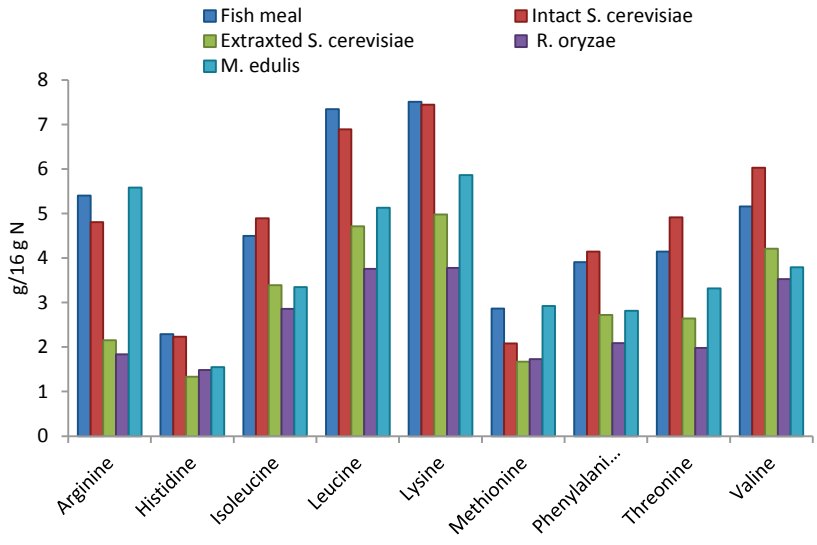


*Figure 10.* Amino acid composition (g/16 g N) of fish meal, intact and extruded *Saccharomyces cerevisiae*, *Rhizopus oryzae* and *Mytilus edulis*.

When novel protein sources are to be included in fish diets, it may be a challenge to meet the nutrient requirements of the fish. Ingredients with a lower crude protein content than in fish meal has to be included in high amounts to meet the targeted digestible protein content in the diet. This may compromise the supply of other nutrients required. In addition, for novel protein sources to be successfully included in fish feed, the physical properties of the ingredient must allow extrusion.

## 6.2 Dietary essential amino acid requirements in perch

In Paper I, perch fed 18.3 g lysine $kg^{-1}$ DM, corresponding to 4.5 g/16 g N, or more had a higher weight and protein gain than fish fed diets containing less lysine. The response in weight and protein gain to increasing lysine content in the diet was not proportional at the lowest levels of inclusion. In addition, there were large variations in growth response between replicates, which may be related to poor feed intake of individual fish within replicates. The perch used in all experiments in this thesis were undomesticated and apparently sensitive to stress, which may reduce growth gain due to the increased stress-related energy consumption and lower feed intake (Strand *et al.*, 2007; Jentoft *et al.*, 2005). The estimation of dietary lysine requirement resulted in 19.7 g $kg^{-1}$ DM

(4.9 g/16 g N) for optimal weight gain and 20.3 g $kg^{-1}$ DM (5.0 g/16 g N) for optimal protein gain.

The lowest dietary lysine level resulting in improved weight gain reported in Paper I was comparable with values reported for other species; 20.0 and 22.0 g $kg^{-1}$ DM (5.0 and 4.0 g/16 g N, respectively) for Atlantic salmon (Espe *et al.*, 2007; Anderson *et al.*, 1993), 21.0 g $kg^{-1}$ DM (4.5 g/16 g N) for Asian sea bass (*Lates calcarifer*, Murillo-Gurrea *et al.*, 2001), 20.0 g $kg^{-1}$ DM (4.0 g/16 g N) for milkfish (*Chanos chanos*, Borlongan & Benitez, 1990) and 14.3 g $kg^{-1}$ DM (5.1 g/16 g N) for Nile tilapia (*Oreochromis niloticus*, Santiago & Lovell, 1988). However, the results found in Paper I are lower than the values reported for silver perch (*Bidyanus bidyanus*; 23.2 g $kg^{-1}$ DM (6.0 g/16 g N)) by Yang *et al.* (2011), but higher than the dietary lysine requirement reported for hybrid striped bass (*Morone chrysops* x *M. saxatilis*; 14.0 g $kg^{-1}$ DM (4.0 g/16 g N)) by Griffin *et al.* (1992).

Several authors have reported that protein utilisation is a more sensitive parameter of dietary lysine deficiency than weight gain (Grisdale-Helland *et al.*, 2011; Encarnacao *et al.*, 2004; Rodehutscord *et al.*, 1997; Gahl *et al.*, 1991). Body weight gain includes proteins as well as lipids, ash and water. This will affect the precision of the estimates if the retention of these compounds differs between treatments. A higher response to lysine intake has been found for protein and lysine gain than for weight gain in Atlantic salmon (Grisdale-Helland *et al.*, 2011), but not in rainbow trout (Kim *et al.*, 1992b), black sea bream (*Sparus macrocephalus*; Zhou *et al.*, 2010) or grass carp (*Ctenopharyngodon idella*; Wang *et al.*, 2005).

In a dose response trial, with growth as response parameter, it is of great importance that the fish reach their maximum growth rate (Cowey, 1995). The specific growth rate of the perch in Paper I varied between 0.41 and 0.63% $day^{-1}$, which can be considered an acceptable growth rate. Few studies have been published regarding the specific growth rate of Eurasian perch. Fiogbe and Kestemont (2003), Kestemont *et al.* (2001) and Melard *et al.* (1996) reported specific growth rates of 0.5% $day^{-1}$. Peres and Oliva-Teles (2005) reported depressed growth in turbot (*Scophthalmus maximus*) when high levels of dietary protein (>19%) were replaced with crystalline amino acids. In contrast, Cho *et al.* (1992), Kim *et al.* (1992a) and Kim *et al.* (1992b) obtained acceptable growth rate with diets containing high levels of crystalline amino acids. However, it has been shown in several fish species that crystalline amino acids are more rapidly absorbed and/or absorbed earlier in the alimentary canal than protein-bound amino acids (Zarate *et al.*, 1999; Zarate & Lovell, 1997; Cowey & Walton, 1988; Kaushik & Dabrowski, 1983; Yamada *et al.*, 1981a). This might lead to a slight metabolic dyssynchrony compared with amino acids

derived from protein digestion and a greater proportion of the crystalline amino acids being catabolised, probably resulting in a temporarily higher concentration of amino acids in tissues or plasma (Zarate *et al.*, 1999; Cowey & Walton, 1988). To avoid this metabolic dyssynchrony in our study, the feed was provided at very short time intervals. Yamada *et al.* (1981b) reported that more frequent feeding intervals increased weight gain and metabolic utilisation of crystalline amino acids in common carp. In contrast, Zarate *et al.* (1999) did not notice any interaction between protein-bound lysine and crystalline lysine fed at different feeding frequencies to channel catfish (*Ictalarus punctatus*).

The body composition of perch fed diets with inclusion of different levels of lysine (Paper I) did not differ between treatments. The indispensable amino acid profile of Eurasian perch is very similar to that of yellow perch, but differs from that of rainbow trout and Arctic charr (Table 3).

Table 3. *Indispensable amino acid content (g $kg^{-1}$ DM) in whole body of Eurasian perch, yellow perch, rainbow trout and Arctic charr, and estimated indispensable amino acid requirements of Eurasian perch using the ideal protein concept*

| Amino acid | Eurasian perch[1] | Yellow perch[2] | Rainbow trout[3] | Arctic charr[4] | Indispensable amino acid requirements of Eurasian perch[1] |
|---|---|---|---|---|---|
| Arginine | 42 | 41 | 64 | 63 | 15.7 |
| Cysteine[5] | 7 | 4 | - | - | 2.6 |
| Histidine | 18 | 18 | 29 | 25 | 6.5 |
| Isoleucine | 28 | 29 | 43 | 31 | 10.6 |
| Leucine | 47 | 47 | 76 | 70 | 17.5 |
| Lysine | 54 | 53 | 85 | 89 | 20.3 |
| Methionine | 20 | 18 | 29 | 28 | 7.3 |
| Phenylalanine | 26 | 29 | 44 | 48 | 9.6 |
| Threonine | 29 | 27 | 48 | 50 | 10.6 |
| Tryptophan | - | - | - | - | - |
| Tyrosine[5] | 19 | 20 | 9 | - | 7.2 |
| Valine | 31 | 33 | 51 | 41 | 11.6 |

[1] Data from Paper I.
[2] Data from Ramseyer and Garling (1994).
[3] Data from Arai (1981).
[4] Data from Gurure *et al.* (2007).
[5] Conditionally indispensable (NRC, 2011).

## 6.3 Effect of digestive enzyme activity on age and growth rate in perch

No effect of age or growth rate on enzyme activity in Eurasian perch was observed (Paper II). In contrast, Kuz'mina (1996) noted a decrease in sucrase and α-amylase activity in adult Eurasian perch compared with fry. These differing results might be due to less variation in age between the groups compared in Paper II. In the wild, Eurasian perch changes its feeding habits as it grows, from initially eating predominantly plankton as fry to gradually eating more benthos as it continues to grow in size and finally becoming piscivorous (Allen, 1935). Furthermore, Blier *et al.* (2002) found no difference in digestive enzyme activity between slow-growing and fast-growing Coho salmon (*O. kisutch*), and Kuz'mina (1996) found no difference in pike (*Esox lucius*). In contrast, higher proteolytic activity has been found in fast-growing bream (*Abramis abrama*), roach (*Rutilus rutilus*) (Kuz'mina, 1996) and Arctic charr (Ditlecadet *et al.*, 2009).

## 6.4 Differences in digestive capacity between charr and perch

The α-amylase activity in both Eurasian perch and Arctic charr was found to be highest in diffuse pancreatic tissues (Paper II), as also reported by Fish (1960). For omnivorous/herbivorous species such as the Mozambique tilapia (*Oreochromis mossambicus*), goldfish (*Carassius auratus*) and river garfish (*Hyporhampus regularis ardelio*), the amylase activity shows a more widespread presence in the gut (Day *et al.*, 2011; Fish, 1960; Sarbahi, 1951). The total α-amylase activity in the different tissues was higher in perch than in charr (0.13 vs. 0.03 U $mg^{-1}$ protein, respectively), indicating a species-specific effect resulting in a higher capacity to digest starch in perch. Previous studies report higher α-amylase activity in herbivorous and omnivorous species than in carnivorous species (Falcón-Hidalgo *et al.*, 2011; Hidalgo *et al.*, 1999; Kuz'mina, 1996; Al-Hussaini, 1949). The disaccharidase with the highest activity in both perch and charr was maltase, followed by isomaltase, trehalase and sucrase, with higher activity for all except trehalase in perch. High maltase activity has previously been reported for hybrid tilapia (*O. niloticus* × *O. aureus*), hybrid striped bass (*Morone saxatilis* × *M. chrysops*), rainbow trout and Atlantic salmon (Krogdahl *et al.*, 2005; Krogdahl *et al.*, 2004; Harpaz & Uni, 1999). In the present study, sucrase, maltase and isomaltase activity was higher in Eurasian perch than in Arctic charr. The higher activity of both α-amylase and disaccharidases in Eurasian perch indicates that its capacity to digest carbohydrate-rich feed is higher than that of Arctic charr.

The highest lipase activity was found in the pyloric caeca and mid-intestine of the charr, which also had the highest total lipase activity (Paper II). For perch, the highest lipase activity was found in the diffuse pancreatic tissue. Carnivorous fish normally consume a fat-rich diet (Chakrabarti *et al.*, 1995), which explains the much higher activity of lipase than of carbohydrases in both perch and charr.

In charr, the activity of trypsin was highest in the second half of the small intestine and first part of the large intestine (Paper II), confirming earlier results for Atlantic salmon and rainbow trout reported by Torrissen (1984). In contrast to this, perch had the highest trypsin activity in the pyloric caeca. In Atlantic salmon, trypsin and chymotrypsin are stored in and secreted from the pancreas (Einarsson & Davies, 1996), but enter the gut through the pyloric caeca (Einarsson *et al.*, 1996). We suspect that the same mechanisms are present in charr and perch, so the high proteolytic activity found in the pyloric caeca of perch is unexpected. Furthermore, the proteolytic activity declined in the distal intestine in charr, but was unchanged in perch, confirming the results reported by Fish (1960). Consequently, the results found in Paper II indicate that the hydrolysis of proteins and peptides, to a higher extent, take place in the pyloric caeca in perch compared with salmonids.

Total chymotrypsin activity was higher in charr than in perch, and higher than the trypsin activity in both species. This is in agreement with findings in several other species: Atlantic salmon (Einarsson & Davies, 1996; Einarsson *et al.*, 1996), silver carp (*Hypophthalmichthys molitrix*) and common carp (Jonas *et al.*, 1983), *Limia vittata* and *Gambusia punctata* (Falcón-Hidalgo *et al.*, 2011) and spotted sorubim (*Pseudoplatystoma corruscans*). In contrast, Cuvier-Peres and Kestemont (2001), Blier *et al.* (2002), Jonas *et al.* (1983) and Rungruangsak-Torrissen *et al.* (2006) found the opposite relationship between trypsin and chymotrypsin activity in Eurasian perch larvae, Coho salmon, wels catfish (*Silurus glanis*) and Atlantic salmon, respectively.

In Paper II, no distinction was made between endogenous and exogenous digestive enzymes. For example, Askarian *et al.* (2012) found amylase-, lipase- and protease-producing bacteria in Atlantic cod. Thus, the extent to which the gut microbiota contribute to digestion of nutrients in fish is unclear, partly due to methodological difficulties, as the substrates concerned are utilised differently *in vitro* and *in vivo* (Ray *et al.*, 2012).

## 6.5 Apparent digestibility of baker´s yeast, zygomycetes and blue mussel in charr and perch

The findings in Paper III demonstrated that extracted *S. cerevisiae* and *M. edulis* have a higher digestibility for charr than intact *S. cerevisiae* (Table 4). No such differences were observed for perch, indicating a species-related difference for the digestibility of intact *S. cerevisiae* and *R. oryzae* between Arctic charr and Eurasian perch. Furthermore, the results showed that the digestibility of intact *S. cerevisiae* for charr may be improved by removing cell walls, given the increased digestibility of extracted *S. cerevisiae*.

In general, the digestibility of *M. edulis* in Papers III and IV agrees with results for rainbow trout (Berge & Austreng, 1989), based on faecal samples collected by stripping. However, in the study by Berge and Austreng (1989) the digestibility of *M. edulis* decreased with increasing inclusion level, which may be due to the inclusion of mussel shells that could have affected the digestibility of the feed.

In Paper III, the higher apparent digestibility coefficients observed for DM, gross energy and sum of amino acids in extracted compared with intact *S. cerevisiae* for charr could be an effect of the absence of cell walls, eliminated through the extraction process. Similar results have been reported for lake trout (*Salvelinus namaycush*) and rainbow trout fed brewer's yeast after cell wall disruption (Rumsey *et al.*, 1991a; Rumsey *et al.*, 1990). The cell walls of yeast consist of 85-90% polysaccharides, which are a mixture of mannans and glucans with a small amount of chitin (Klis *et al.*, 2006; Nguyen *et al.*, 1998).

The apparent digestibility of the intact *S. cerevisiae* presented in Paper III was similar to values reported by Oliva-Teles and Gonçalves (2001), based on faeces collection from a settling column. In contrast, lower digestibility of intact *S. cerevisiae* has been reported in several other studies (Øverland *et al.*, 2013; Cheng *et al.*, 2004; Rumsey *et al.*, 1991a), based on faeces collection from a metabolic chamber, settling column and stripping, respectively. It has been reported that apparent digestibility coefficients are affected by the faeces collection method (Storebakken *et al.*, 1998; Hajen *et al.*, 1993). Faeces collection based on settling of particles has been shown to overestimate apparent digestibility coefficients compared with the stripping and dissection methods (Hajen *et al.*, 1993). This might also explain the higher apparent digestibility values for the experimental diets in Paper III compared with Paper IV.

Furthermore, in this thesis the experimental diets (Papers III and IV) were extruded, which may have increased the digestibility of yeast by disrupting the cell walls. In contrast, the experimental diets in studies by Rumsey *et al.*

(1991a), Oliva-Teles and Gonçalves (2001), Cheng *et al.* (2004) and Øverland *et al.* (2013) were cold-pelleted.

The higher digestibility of DM and gross energy of intact *S. cerevisiae* found in perch may be due to a different digestive enzyme profile, *e.g.* higher glycosidase activity, than in charr.

In general, the apparent digestibility coefficients of *R. oryzae* for charr and perch were high in Paper III, but low for charr in Paper IV. Mydland *et al.* (2007) reported decreasing apparent digestibility, based on stripping, with increased inclusion of *R. oryzae* in rainbow trout. However, in Paper III and in the study by Mydland *et al.* (2007), the digestibility of the sum of amino acids was higher than the digestibility of crude protein.

Table 4. *Comparison of performance and digestibility in charr fed the experimental diets in Paper III and IV*

| | Reference | Intact *S. cerevisiae* | Extracted *S. cerevisiae* | *R. oryzae* | *M. edulis* | s.e. | P-value |
|---|---|---|---|---|---|---|---|
| **Weight gain (%)** | | | | | | | |
| Paper III | 11.6$^{ab}$ | 17.7$^{a}$ | 3.6$^{b}$ | 3.6$^{b}$ | 13.8$^{a}$ | 2.11 | <0.001 |
| Paper IV | 181.80$^{a}$ | 169.73$^{ab}$ | 157.77$^{bc}$ | 152.71$^{c}$ | 167.22$^{ab}$ | 3.89 | <.0001 |
| **Specific growth rate (% day$^{-1}$)** | | | | | | | |
| Paper III | 0.64$^{ab}$ | 0.97$^{a}$ | 0.20$^{bc}$ | 0.21$^{b}$ | 0.76$^{ac}$ | 0.11 | <0.001 |
| Paper IV | 1.08$^{a}$ | 1.04$^{ab}$ | 0.98$^{b}$ | 0.97$^{b}$ | 1.02$^{ab}$ | 0.03 | 0.002 |
| **Feed conversion ratio (%)** | | | | | | | |
| Paper III | 1.56 | 0.77 | 2.87 | 1.89 | 0.75 | 0.53 | 0.077 |
| Paper IV | 0.89$^{a}$ | 0.95$^{ab}$ | 0.98$^{ab}$ | 1.01$^{b}$ | 0.93$^{ab}$ | 0.03 | 0.064 |
| **ADC dry matter (%)** | | | | | | | |
| Paper III | 80.7$^{a}$ | 78.0$^{a}$ | 87.2$^{b}$ | 86.3$^{b}$ | 85.7$^{b}$ | 1.12 | <0.001 |
| Paper IV | 70.4$^{a}$ | 70.6$^{a}$ | 71.9$^{ac}$ | 62.2$^{b}$ | 73.7$^{c}$ | 0.51 | <.0001 |
| **ADC crude protein (%)** | | | | | | | |
| Paper III | 88.1$^{ab}$ | 87.5$^{a}$ | 92.8$^{b}$ | 89.9$^{ab}$ | 91.7$^{ab}$ | 1.01 | 0.018 |
| Paper IV | 87.0$^{a}$ | 83.8$^{b}$ | 89.5$^{a}$ | 80.4$^{c}$ | 88.2$^{a}$ | 0.56 | <.0001 |
| **ADC gross energy (%)** | | | | | | | |
| Paper III | 84.3$^{ab}$ | 80.8$^{a}$ | 89.4$^{c}$ | 88.3$^{bc}$ | 88.1$^{bc}$ | 1.09 | <0.001 |
| Paper IV | 78.8 | 85.3 | 78.7 | 71.5 | 87.1 | 4.41 | 0.178 |

Values within rows with different superscripts are significantly different (P<0.05).
Data are least square means. s.e. = pooled standard error.

## 6.6 Growth performance and nutrient utilisation of baker´s yeast, zygomycetes and blue mussel in Arctic charr

To evaluate the potential of intact and extracted *S. cerevisiae, R. oryzae* and *M. edulis* as protein sources in diets to charr, growth performance and nutrient utilisation were studied (Paper IV). In the pure digestibility study (Paper III), diets were formulated to evaluate the apparent digestibility of the test ingredient by mixing a reference diet and one test ingredient in the ratio 7:3, according to the procedure of Cho and Slinger (1979). As a result, the nutrient and energy composition of the experimental diets differed, depending on the composition of the test ingredient. The diets used in Paper IV were iso-nitrogenous and iso-energetic and, in addition, the experiment lasted for 99 days, in which the fish were able to increase their weight by 153-182%. However, in both Paper III and Paper IV the performance of charr fed intact *S. cerevisiae* was higher than for fish fed extracted *S. cerevisiae* and *R. oryzae*, indicating lower nutrient utilisation of the latter ingredients for charr (see Table 4 for comparison).

The results from Paper IV suggest that intact *S. cerevisiae* and *M. edulis* can be used as a partial replacement for fish meal to up to 40% on a crude protein basis in diets for charr, without a reduction in growth performance. This is in line with values reported for rainbow trout fed grain distiller's dried yeast (Hauptman *et al.*, 2014), but in contrast to a study by Øverland *et al.* (2013) with Atlantic salmon fed intact *S. cerevisiae.* In Paper IV, reduced growth performance was observed for fish fed diets containing extracted *S. cerevisiae* and *R. oryzae*, compared with the reference diet. In contrast, Mydland *et al.* (2007) reported that inclusion of 10 and 20% *R. oryzae* in diets for rainbow trout did not affect specific growth rate and feed conversion ratio. The reduced growth performance in fish fed diets containing extracted *S. cerevisiae* and *R. oryzae* may also be related to the imbalanced amino acid profile in the two test ingredients.

Hepatosomatic index and viscerosomatic index (Paper IV) were not affected by dietary treatments, confirming the results reported by Hauptman *et al.* (2014) and Oliva-Teles and Gonçalves (2001) for rainbow trout and sea bass (*Dicentrarchus labrax*).

The growth performance reported in Papers III and IV is in agreement (Table 4). However, fish fed diets containing intact *S. cerevisiae* and *M. edulis* in Paper III had a higher growth rate than fish fed diets containing extracted *S. cerevisiae* and *R. oryzae*, an effect which was not seen in Paper IV. This discrepancy in growth performance between the two studies may also be attributed to different feeding regimes. In Paper III fish were fed *ad libitum*, whereas fish in Paper IV were restrictively fed.

# 7 Conclusions

Based on the results from studies performed under the experimental conditions specified in this thesis, it can be concluded that:

- The lowest dietary lysine content for optimal growth performance in juvenile Eurasian perch appears to be 18.3 g $kg^{-1}$ DM, which corresponds to 4.5 g/16 g N. However, estimations with weight gain and protein gain as response parameters indicated a slightly higher dietary requirement; 19.7 3 g $kg^{-1}$ DM (4.9 g/16 g N) for optimal weight gain and 20.3 g lysine $kg^{-1}$ DM (5.0 g/16 g N) for optimal protein gain. Until further studies are conducted on dietary requirements for the other indispensable amino acids in Eurasian perch, it is recommended that the indispensable amino acid profile in the whole body be used to predict the dietary requirements for the other indispensable amino acids.
- Growth rate and age have no effect on disaccharidases, α-amylase and lipase activity in juvenile Eurasian perch, which indicates that other factors are affecting the high variability in growth rate in Eurasian perch. For example, differences in proteolytic enzymes and metabolic capacity may be responsible.
- Arctic charr have lower total carbohydrase activity than Eurasian perch, which indicates lower capacity to digest carbohydrates. The total lipase and protease activity is higher than total carbohydrase activity in both species, which confirms previously reported feeding habits of carnivorous species. However, since there are differences in digestive enzyme activity between these species, feed formulation should be different for Arctic charr and Eurasian perch.
- There were large variation in the crude protein content and amino acid profile between intact *S. cerevisiae*, extracted *S. cerevisiae*, *R. oryzae* and *M. edulis*. The amino acid profile of intact *S. cerevisiae* and *M. edulis* is most similar to that of fish meal. Thus, it is of utmost importance to have

access to a detailed analysis of the chemical composition of alternative feed ingredients to fish meal to be able to formulate nutrient balanced diets.

- Apparent digestibility of intact *S. cerevisiae*, extracted *S. cerevisiae*, *R. oryzae* and *M. edulis* does not differ in Eurasian perch, whilst in Arctic charr the digestibility of *R. oryzae* and, in particular, intact *S. cerevisiae* is lower. For Arctic charr, the lower digestibility may be related to the cell walls present in both *R. oryzae* and intact *S. cerevisiae*. This indicates that there are species-related differences in digestibility, and that focus should be put on how to increase the digestibility of the cell walls of intact *S. cerevisiae*.
- Inclusion of intact *S. cerevisiae* and *M. edulis* to up to 40% on a crude protein basis is possible without decreased growth performance and protein retention in Arctic charr. In contrast, inclusion of *R. oryzae* and extracted *S. cerevisiae* on a 40% crude protein basis suppresses growth and N retention. This illustrates important differences in nutritional properties between the studied alternative feed ingredients that warrants further research to unravel the reason for these effects.

# 8 Future perspectives

On reviewing the data in this thesis, some additional issues in need of further investigation can be identified. For the indispensable amino acid requirement in Eurasian perch, further studies should be performed to confirm the dietary lysine requirements determined in this study.

The wide variation in growth rate of Eurasian perch is a potential problem for farmers because it could be challenging to market different fish sizes. Enzyme activity was assessed for Eurasian perch to determine whether this was causing difference in growth rate and was found not to be the case. Metabolic capacity, gender and seasonal variations could be factors influencing growth rate and thus ought to be explored further.

It is difficult to perform experiments with Eurasian perch, because they are sensitive to human interaction and tank rearing conditions. In addition, Eurasian perch occasionally reject experimental diets, especially with different texture and taste, which makes it difficult to perform nutrition studies. For future experiments, more emphasis should be placed on optimal housing conditions, such as type of tank, enrichment, group size, feeding system and other factors, and breeding programmes to improve Eurasian perch farming.

Intact *S. cerevisiae* showed potential as a novel protein source in fish feed and more attention should be directed to increased digestibility, *e.g.* disruption of cell walls. In addition, synthetic amino acids and/or preferably combinations with other protein sources could be used to formulate diets with an optimal amino acid profile for Arctic charr. One possibility could be to study the blood concentrations of free amino acids to better understand the utilisation of amino acids from *S. cerevisiae*, as well as other microorganisms.

Preliminary studies indicated a change in the gut microbiota of Arctic charr fed microorganisms. Experiments in this thesis did not investigate these changes, but further studies are needed on the synergistic effects on the gut

microbiota in Arctic charr fed diets containing *S. cerevisiae* and other microorganisms.

At the end, the produced fish are about to be consumed by humans. Thus, the sensory quality of the fish is a very important factor. It has been reported that the inclusion of novel feed ingredients in diets occasionally affects taste and texture of the flesh (Bjerkeng *et al.*, 1997). In addition, it was recently demonstrated that the amino acid composition of the feed may improve fish fillet firmness (Larsson *et al.*, 2014). In this thesis, the effect of dietary inclusion of microbial and mussel meals on the sensory quality of the fish, were not studied. However, it is an important research area if these products are going to be included to a greater extent in future fish feeds.

This thesis provides new information on the nutrition of Eurasian perch and Arctic charr, but more research is needed.

# 9 Svensk sammanfattning

## 9.1 Bakgrund

Akvakultur, odling av organismer i vatten, har under de senaste 60 åren haft en kraftig tillväxt och världsproduktionen har ökat från en miljon ton per år under 50-talet till 64 miljoner ton under år 2011. Humankonsumtionen av fisk och andra vattenlevande organismer uppgår till 130 miljoner ton per år varav cirka hälften härrör från akvakultursektorn (FAO, 2012). I Sverige har produktionen av matfisk ökat från en genomsnittlig produktion på 5000 ton under 1990- och 2000- talet, till 12 500 ton år 2012. Den största delen (84 %) utgörs av regnbågslax (*Oncorhynchus mykiss*), men produktionen av röding (*Salvelinus alpinus*) ökade med 64% mellan åren 2011 och 2012 (Sweden Statistics, 2013). Enligt FAO (2012) måste världsproduktionen av odlad fisk och skaldjur öka med ytterligare 23 miljoner ton till år 2020 för att kunna försörja den växande världsbefolkningen med protein.

Användandet av fiskmjöl och fiskolja i akvakulturindustrin är högt och den totala konsumtionen har ökat under de senaste decennierna (FAO, 2012), men har minskat i relation till den totala mängden producerad fisk (Tacon *et al.*, 2011). Om akvakultursektorn ska fortsätta växa måste fiskmjölsanvändningen minska och ersättas av alternativa och mer uthålliga proteinfodermedel (Glencross *et al.*, 2007).

Hittills har fiskmjöl främst ersatts av växtprotein, vilket inte är problemfritt. Vid utfodring med soja och andra växtbaserade fodermedel kan det uppstå problem som är relaterade till osmältbara kolhydrater och antinutrionella substanser. Detta kan leda till negativa fysiologisk effekter och ofta ett sämre foderutnyttjande.

Mikrobiellt protein (t.ex. från mikroalger, jäst, svampar och bakterier) har pekats ut som en lovande proteinfoderkälla som alternativ till vegetabiliskt protein (Tacon *et al.*, 2011; Pfeffer, 1982). Mikroorganismer är de effektivaste och snabbast växande proteinbildande organismerna vi känner, och kan odlas

på restprodukter från pappers-, jordbruks- och livsmedelsindustrin som består av för människor osmältbara kolhydrater såsom cellulosa och pentoser. Mikroorganismer är därför ett attraktivt alternativ som proteinfodermedel, där man dessutom kan återanvända restprodukter som växtsubstrat för att producera ett hållbart fodermedel med ett högt näringsvärd. Till skillnad från människor har fiskar inte problem med de höga halter av urinsyra som bildas vid nerbrytning av de i mikrober rikligt förekommande nukleinsyrorna (Rumsey *et al.*, 1992).

Bagerijäst (*Saccharomyces cerevisiae*) är en encellig svamp med ett högt proteininnehåll, vanligen mellan 45-56%, men har ofta ett bristfälligt innehåll av aminosyran metionin. Kopplingssvampar (zygomyceter) är ett släkte av cirka 1060 svamparter, i vilken *Rhizopus oryzae* ingår. *R. oryzae* har ett proteininnehåll på 40-50%. Mikroorganismer, som jäst och zygomyceter, består till 20% av cellväggar. Dessa är mestadels uppbyggda av polysackarider, som kan vara svåra att bryta ned för fiskar. Blåmusslor (*Mytilus edulis*) har också ett högt proteininnehåll med en aminosyraprofil lik den i fiskmjöl. Musslor tar upp näringsämnen från vattnet, vilket gör att de är intressanta som biologiska reningsverk i övergödda vattendrag. De kan också absorbera toxiner från den omgivande miljön, vilket måste tas med i beräknandet när de ska användas i mat eller foder. Dessutom måste skalet avlägsnas för att få ett rent proteinmjöl lämpligt till fiskar.

Abborre (*Perca fluviatilis*) är en ny art inom fiskodling. Den har förutspåtts ha en framtid som kommersiellt odlad art men kunskap saknas om dess näringsbehov. För både abborre och röding har hittills få studier gjorts där näringsutnyttjandet av alternativa proteinfodermedel har studerats.

I denna avhandling var syftet att:

- Skatta behovet av essentiella aminosyror hos abborre
- Jämföra aktiviteten av digestionsenzymer i långsam- och snabbväxande abborre av två olika åldrar samt i röding
- Utvärdera näringsutnyttjandet av intakt jäst, extraherad jäst, zygomycet och blåmussla hos abborre och röding.

## 9.2 Utförda försök

Totalt har fyra studier genomförts. I det första försöket (artikel I) undersöktes behovet av aminosyran lysin hos abborre genom utfodring med åtta olika dieter med samma näringsinnehåll förutom lysin som ingick i gradvis ökande mängd. Fiskarnas viktökning, proteinansättning och kroppssammansättning registrerades. Genom helkroppsanalyser av aminosyrasammansättningen hos abborre kunde behovet av de andra essentiella aminosyrorna uppskattas.

I det andra försöket (artikel II) mättes åtta olika digestionsenzymer på nio olika ställen i långsam- och snabbväxande abborrar av två olika åldrar samt i röding. Syftet var att undersöka om fiskens förmåga att bryta ned fodret inverkar på tillväxthastighet och om den skiljer mellan abborre och röding. Uppmätta enzymaktiviteter indikerar vilken förmåga fisken har att tillgodogöra sig olika mängd och typ av framförallt kolhydrater, men även fett och protein. Denna kunskap kan vara till hjälp när man ska optimera fodrets sammansättning.

I de två sista försöken (artiklar III och IV) studerades smältbarheten av intakt jäst, extraherad jäst, zygomycet och blåmussla hos abborre och röding. Fodermedlens smältbarhet värderades genom uppsamling av fekalier i specialdesignade fisktråg. Även foderkonsumtion och tillväxt hos röding mättes, även om försöket pågick under för kort tid för att dra säkra slutsatser. För att studera tillväxten genomfördes även ett längre produktionsförsök med röding, där tillväxt, foderomvandlingsförmåga, proteinansättning mättes och fodrets smältbarhet skattades (artikel IV).

## 9.3 Resultat och slutsatser

Resultaten från artikel I visade att abborrens lysinbehov är snarlikt det som skattats för andra odlade fiskararter, men det finns dock skillnader mellan arter. Helkroppsanalysen visade att abborrens essentiella aminosyrabehov är snarlikt behovet hos den nordamerikanska släktingen ”Yellow perch” (*Perca flavescens*). Denna information kan vara till nytta vid sammansättningen av artspecifika foder till abborre, samt vid val av foderingredienser.

Vid jämförelsen av digestionsenzymer återfanns inget samband mellan enzymaktivitet och tillväxthastighet eller ålder hos abborre. Däremot fanns det skillnader mellan abborre och röding. Båda arterna hade högre aktivitet av de fettnedbrytande enzymerna (lipaser) än av de kolhydratnedbrytande (karbohydrater), vilket är vanligt hos karnivora fiskarter. Däremot hade abborre högre aktivitet av de kolhydratnedbrytande enzymerna än röding, vilket indikerar att abborre har högre kapacitet att smälta kolhydrater, framförallt stärkelse. Vi fann också att det fanns skillnader mellan arterna var i digestionskanalen respektive enzym hade högst aktivitet.

Smältbarhetstudien visade att hos röding var smältbarheten högst för extrakterad jäst och blåmusslor, följt av zygomycet och intakt jäst. Hos abborre fanns inga tydliga skillnader i smältbarhet mellan de testade fodermedlen, vilket tyder på artspecifika skillnader, möjligen kopplat till skillnaderna i aktivitet i digestionsenzymerna. Den lägre smältbarheten av intakt jäst hos röding är troligen beroende av förekomsten av cellväggar, då den extraherade

jästen saknade cellväggar och hade hög smältbarhet. I vår studie var smältbarheten av hel jäst högre än vad som tidigare rapporterats för andra arter, vilket troligen beror på att fodret i vårt försök var extruderat. I denna process utsätts fodermassan för högt tryck och temperatur, vilket kan ha gjort cellväggarna mer nedbrytningsbara. Tillväxten och foderutnyttjandet visade dock att röding växte sämre och hade ett lägre näringsutnyttjande av de dieter som innehöll extraherad jäst och zygomycet. Orsaken kan vara en sämre aminosyraprofil i dessa fodermedel.

Vidare visade vi i dessa studier att fiskmjöl kan till 40% bytas ut mot hel jäst och musselmjöl utan att tillväxt eller näringsutnyttjande försämras, och att ett högre utnyttjande av hel jäst kan uppnås om smältbarheten ökas genom upplösning/borttagning av cellväggarna.

Denna avhandling har bidragit med ökad kunskap om nutrition hos abborre och röding och om näringsutnyttjandet av alternativa proteinfodermedel. Avhandlingen visar också att mer forsking behövs inom detta område för att vi ska kunna utnyttja den potential som finns i alternativa proteinfodermedel till odlad fisk.

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