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ALTERNATIVE SOURCES OF PROTEIN IN FEED FOR CULTURED FISH: A CASE STUDY ON ATLANTIC COD FRY (*GADUS MORHUA*)

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ABSTRACT

Commercial aquaculture is growing, thereby increasing the demand for aquaculture feeds. Traditionally, these feeds have been based on animal protein. However, due to cost and availability considerations, it is inevitable that more plant protein supplements will be utilised in the feeds in the future. Plant proteins are more cost effective than animal protein supplements. The increased acceptability and utilisation by the feed industry will be dependent on reliable nutritional research. Plant protein supplements are being used to some extent in all aquaculture feeds. Soybean meal is the most extensively evaluated and most commonly used. This project is divided into two parts: firstly, a review of the protein component in the feed for carnivorous and omnivorous fish, and secondly, a case study where the effects of substituting alternative sources of protein on growth and survival of cod fry (*Gadus murhua)* are investigated. Six isocaloric and isonitrogenus diets were tested, using protein of different origin. Superior fishmeal (LT) was used as reference diet and replaced partly or totally with special fishmeal (NSM), standard fishmeal, Corn Gluten Meal and HYPRO Soybean meal. Fish (initial weight 28 ± 0.5 g) were distributed among 18 circular 90 L fibreglass tanks, 50 cod in each tank. Each tank was randomly assigned diets in triplicate. The weight and length of the fish were measured every four weeks. Partial replacement of SUP with SPES; STANDARD; MGM and HYPRO or a full replacement with Standard did not result in a significant decrease in specific growth rate (SGR) in cod fry during the first four weeks of the trial but there was a tendency to reduced growth. The present observations suggest that it is possible to substitute high quality fishmeal with different protein in the feed for cod fry.

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1 INTRODUCTION

Aquaculture is the fastest growing sector of the world food trade, increasing by more than 10% per year and currently accounts for more than 30% of all fish consumed. While most farmed fish are omnivorous species, such as carp and tilapia, farming of carnivorous species, such as salmon, is a booming industry and the number of other farmed carnivorous species is growing rapidly (Lambert and Dutil 2001).

International trade in fish products has increased to a new record of US$55.2 billion. Net export trade from developing countries increased from US$10 billion in 1990 to US$18 billion in 2000, corresponding to a real growth of 45% (corrected for inflation) (FAO 2000).

Intensive farming of carnivorous fish may be detrimental to the environment. Detrimental effects include displacement of wild fish populations, harmful genetic interactions with wild fish, transfer of parasites and disease, discharge of untreated waste into coastal waters, use of chemicals and antibiotics, and the use of large amounts of wild fish as a feed (FDA 2001, Gorbach 2001).

Atlantic cod is the most important of all the marine resources in Iceland. In 2002 it represented 37% of the catch value and 38% of the total seafood export value (The Icelandic Ministry of Fisheries 2002). In order to maintain its market share, Iceland has been forced to investigate how cod farming could be a profitable industry in Iceland.

It is expected that increased production of farmed cod will lead to lower prices in the future, but as of today, prices of farmed cod are even higher than for wild cod products. Production costs are the factor that determines competitive advantage. The production cost of farming of cod is still high. Feed constitutes 60-70% of the production cost because ingredients are expensive. Culture farming of cod can be profitable using cheaper protein for feed.

Fish, like any other animal, requires nutrients to grow and stay alive, healthy, and active. Maximum diet performance involves two important aspects: reduction of feeding costs and decreased waste. The study of feeding behaviour in several fish species has revealed that the adjustment of feeding times to match natural rhythm improves nutritional efficiency, feeding frequency, food-conversion efficiency and can even influence the utilisation of certain nutrients (Gozdowska *et al.* 2003).

Protein is a basic component of fish diets, both in terms of quantity and quality, and protein is also the most expensive feed component. Fishmeal provides an adequate balance of amino acids, but increasing demand and price, as well as uncertain supply, make it necessary to find alternative protein sources. Other diet components (carbohydrates or fats) should be used to supply energy because they are usually less expensive. The economic importance of protein for farmers is related to its expense as a feed ingredient.

Traditionally, animal protein supplements were the foundation of any aquaculture feed formulation. However, given the limited world supplies and the increasing price of these animal protein supplements, nutritionists are considering alternative protein sources for fish feeds. Plant protein is generally cheaper than animal protein supplements. The increased use of plant protein supplements in aquaculture feeds will be dependent on reliable nutrition research (Purchase and Brown 2000).

Current aquaculture feed formulations are partly based on intuition and "unknown growth factors", rather than nutritional science. Animals do not require feed ingredients or formulas, but rather the nutrients, which are part of the chemical composition of these ingredients and feed formulas. Therefore, a feed formula is meaningless if we don't understand the nutritional principles involved in formulating the feed. In general, plant protein supplements are lower in some essential amino acids, energy, and minerals such as phosphorous as compared to animal protein supplements. These parameters need to be considered in feed formulations based on plant proteins (Sugiura *et al*. 2001).

The development of fresh water aquaculture began in Cuba in more than 1,200 dams, already built for irrigation purposes and those dams became production centres for imported species. Tilapia is one of the most important species in culture are.

In Cuba, one manufacturer produces balanced feeds. In 1992 the production capacity pf feeds was 2 metrics tonnes (MT)/hour. Their production has been dedicated mainly to the shrimp culture and in small quantities to tilapia and catfish.

The aquaculture development proposed by the Ministry of Fisheries started in 2001. It is characterised by high levels of production and a diversification of species, requiring increased industrial capacity and improvements in feed. To improve the diets it is necessary to know the nutritional requirements of the species, produce a pellet with optimal chemical and physical parameters at a minimum cost. Using alternative sources of protein, such as soybean meal, reduces the cost of feed in aquaculture (The Cuban Ministry of Fisheries 2001).

This project is divided into two parts: a literature study of the protein component in the feed for carnivorous and omnivorous fish and a case of study, which is a feeding trial on cod fish. The main objectives are:

1. To give an overview of the protein components in the feed for carnivorous and omnivorous fish.
2. To measure the effect of substituting alternative sources of protein on growth and survival on cod fry (*Gadus murhua).*

2 LITERATURE REVIEW

2.1 General overview of protein requirements in fish

The protein requirements, meaning the minimum amount needed to meet the requirements for amino acids and to achieve maximum growth, have now been measured in juvenile fish of many species (Table 1). They have been obtained mainly from dose-response curves in which graded amounts of high-quality protein were fed in partially defined diets. The response measured was weight gain. The values are expressed as a percentage of dry diet. Although the expression of protein as a proportion of dietary energy would have focused attention on protein as a substantial source of dietary energy, this approach was not possible for many of the studies because information on the digestible energy (DE) content of the diets was unavailable and values used for the energy composition of dietary components varied between authors. Many studies have been carried out to determine protein requirements for fish, with estimated protein requirements ranging from 30% to 55% according to fish species, size, dietary protein sources and environmental conditions (NRC 1993).

Table 1: Estimated dietary protein requirement for maximal growth of some species of juvenile fish (as fed basis).

Species	**Protein source**	**Estimated protein requirement (%)**	**Reference**
Atlantic salmon	Casein and gelatin	45	Lall and Bishop (1977)
Channel catfish	Whole egg protein	32–36	Garling and Wilson (1976)
Chinook salmon	Casein, gelatin, and amino acids	40	Halver *et al*. (1958)
Coho salmon	Casein	40	Zeitoun *et al*. (1976)
Common carp	Casein	31–38	Ogino and Saito (1970); Takeuchi *et al*. (1979)
Estuary grouper	Tuna muscle meal	40–50	Teng *et al*. (1978)
Gilthead sea bream	Casein, fish protein concentrate, and amino acids	40	Sabaut and Luquet (1973)
Grass carp	Casein	41–43	Dabrowski (1977)
Japanese eel	Casein and amino acids	44.5	Nose and Arai (1972)
Largemouth bass	Casein and fish protein concentrate	40	Anderson *et al*. (1981)
Milkfish	Casein	40	Lim *et al*. (1979)
Plaice	Cod muscle	50	Cowey *et al*. (1972)
Puffer fish	Casein	50	Kanazawa *et al*. (1980)
Rainbow trout	Fishmeal, casein, gelatin, and amino acids	40	Satia (1974)
Red sea bream	Casein	55	Yone (1976)
Smallmouth bass	Casein and fish protein concentrate	45	Anderson *et al*. (1981)
Snakehead	Fishmeal	52	Wee and Tacon (1982)
Sockeye salmon	Casein, gelatin, and amino acids	45	Halver *et al*. (1964)
Striped bass	Fishmeal and soy proteinate	47	Millikin (1983)
Blue tilapia	Casein and egg albumin	34	Winfree and Stickney (1981)
Mossambique tilapia	White fishmeal	40	Jauncey (1982)
Nile tilapia	Casein	30	Wang *et al*. (1985)
Zillii's tilapia	Casein	35	Mazid *et al*. (1979)
Yellowtail	Sand eel and fishmeal	55	Takeda *et al*. (1975)

The protein requirements in fish diets are appreciably higher than those in the diets of terrestrial warm-blooded animals. The methods used to determine protein requirements, however, may overestimate requirements, in that excess dietary protein or amino acids, which cannot be stored, are catabolised preferentially over carbohydrates and fats and used for energy by some fishes (Wilson 1989). In addition, adequate consideration has not always been given to factors such as concentration of DE in the diet, amino acid composition of the dietary protein, and digestibility of the dietary protein (Wilson and Halver 1986, Wilson 1989). Understanding the nutritional constraints and limitations used in arriving at these reported protein requirements are important for their proper application.

2.1.1 Carnivorous fish

Fish may be broadly categorised as omnivorous, carnivorous or herbivorous (Jones 1982). Few species have exactly the same nutritional needs. Good nutrition is fundamental to the success and sustainability of the aquaculture industry in terms of economics, fish health and high quality product production with minimum environmental pollution. Few species of fish can be neatly categorised as strictly carnivorous (meat eaters) or herbivorous (plant eaters). Additionally, the type of foods fish consume in the wild may vary seasonally and change by size and age. This project focuses on the feed quality with emphasis on carnivorous and omnivorous fish.

Although carnivorous finfish species represented only about 11% of total finfish aquaculture production in 1994 (i.e. 1.5 million metric tonnes, wet basis) they consumed an estimated 804,000 MT of fishmeal and 276,000 MT of fish oil (i.e. 1 million metric tonnes of fishmeal and fish oil, dry basis) within commercial aquafeeds or about 70% and 80% of the total fishmeal and fish oil used in aquafeeds in 1994. Production of carnivorous, omnivorous and herbivorous finfish has increased at an average compound rate of 9.8% and 10.9% per year since 1984, respectively (Tacon 1994).

In general, carnivorous species have a limited ability to utilise starch and no capacity to digest non-starch polysaccharides. Plant materials, which are used as alternative sources of protein instead of fishmeal, will inevitably contain significant amounts of carbohydrates, predominantly in the form of non-starch polysaccharides and starch. Feeding plant materials to fish in aquaculture will be most efficient (less expensive) if the carbohydrate material is utilised.

2.1.1.1 Protein requirements according to fish size: fish fry

Fish must be given a diet containing graded levels of high quality protein and energy and adequate balances of essential fatty acids, vitamins and minerals over a prolonged period. These differences in apparent protein requirement are thought to be due to differences in culture techniques as well as diet composition and fish size.

Intensive tank rearing of channel catfish (*Ictalurus punctatus,* Rafinesque) fry can be successfully accomplished with commonly available “salmonid” starter diets. Ingredient substitution in starter diets is less important from the cost perspective but growth, survival and health of fish are major concerns. Several combinations of fishmeals and animal protein substitutes have been evaluated in diet formulations for hatchery rearing of catfish fry. A diet formulated on the basis of menhaden fishmeal (29.1%), herring fishmeal (25.1%) and animal by-product mixture (16.4%) gave numerically the highest growth rate after 13 weeks of feeding, although growth reductions in fish fed diets exclusively with menhaden meal (77.6%) as protein or substituted with 50% animal by-product protein were not significant. Instantaneous mortality rates were the highest between weeks 2 and 4 of rearing and increased almost proportionally with increased fishmeal protein substitution. The initial feeding can greatly affect physiological status of juvenile channel catfish (El-Saidy *et al*. 2000).

Gross protein requirements for young catfish appear to be less than those for salmonids. Initially feeding fry require that about 50% of the digestible components of the ration be protein, and the requirement decreases with size. African catfish (*Clarias gariepinus*) fry were fed a purified diet. Those fed three times a day consumed 1.27±0.27% of biomass per day. The fish fed twice a day exhibited better growth and food conversion. Despite the use of purified diets, performance indices for the group fed twice per day were good. The specific growth rate was 1.24±0.08% per day, the weight gain was 38.51±2.96% during 30 days and the food conversion ratio was 0.72±0.13. (Panagiotis *et al*. 2003).

A study was conducted to determine growth and feed utilisation by haddock fed diets containing graded levels of protein (35, 40, 45 and 50%). Haddock fingerlings with an average weight of 24 g were hand-fed one of the four isoenergetic (≈16.6 MJ digestible energy kg^{-1}) experimental diets to satiation, three times a day during a 9-week period. Increases in dietary protein improved weight gain, specific growth rate (SGR) and feed gain ratio. The highest weight gain (percentage/initial weight) was observed in fish fed 50% protein, although there was no significant difference between groups fed 45% and 50% protein. A similar effect was observed in SGR of fish fed 50% protein, which was the highest among the treatments. Although an increase in dietary protein resulted in a slight increase in feed intake, the lowest feed gain ratio was obtained in fish fed the diet with the highest protein. The broken-line regression of weight gain against protein level yielded an estimated protein requirement of 49.9%. (Kim *et al*. 2000).

The protein requirement for haddock fed isocaloric diets is reported to be 50% (as-fed basis). Preliminary results indicate that optimum growth and feed utilisation is achieved with diets containing high levels of dietary protein (55%) and relatively low levels of dietary lipid (10%). Unlike salmonids, the added cost of higher dietary lipid levels is not necessary as it only leads to increased liver growth (Tibbetts *et al*. 2001). In summary tests in feeding fry, fingerling, and yearling fish have shown that gross protein requirements are highest in initial feeding fry and that they decrease as fish size increases. To grow at the maximum rate, fry must have a diet in which nearly half of the digestible ingredients consist of balanced protein; at 6-8 weeks this requirement is decreased to about 40% of the diet for salmon and trout and to about 35% of the diet for yearling salmonids raised at standard environmental temperature. Given the high concentration of protein required in fish feeds, there is considerable dependency on fishmeal as a protein source and there are issues about substituting this, especially in diets for carnivorous fish.

2.1.1.2 Protein requirement according to fish size: juvenile fish

Growth and feeding have been investigated by several authors. Gross protein requirements have been determined for juveniles of several species of fish. One of which is the freshwater Percichthyid fish, Murray cod (*Maccullochella peelii peelii*, Mitchel). An experimental trial was carried out with juveniles of this species where the fish were fed isocaloric diets containing 40%, 45%, 50%, 55% or 60% protein. The final mean weight, increase in mean weight (%) and % SGR increased with increasing dietary protein up to 50%, with growth being better in Murray cod maintained on the last three diets than in those on 40% and 45% protein (Gunasekera *et al*. 2002).

A feeding trial of three dietary protein levels (30, 40 and 50%) and two dietary energy levels (19 and 21 MJ kg $^{-1}$ diet) using a factorial design with three replications was conducted to investigate the effect of dietary protein and energy levels for the growth of juvenile masu salmon (*Oncorhynchus masou,* Brevoort). Fish, average weight 21.9 g, were fed the experimental diets for 10 weeks. The weight gain and feed efficiency ratio of fish improved as dietary protein and energy levels increased ($P < 0.05$), whereas there was no significant difference between fish fed the diets containing 40 and 50% protein at the same dietary energy level. Weight gain of fish fed the high-energy diets was significantly higher than those fed the low-energy diets at 40 and 50% dietary protein levels. The results of this study indicate that a diet containing 40% protein and 21 MJ kg $^{-1}$ diet should be optimal for the growth and effective protein utilisation of juvenile masu salmon (Lee and Kim 2001).

A study conducted at Kentucky State University determined that juvenile palmetto bass could be fed diets containing as little as 15% fishmeal without adversely affecting growth or body composition as long as crude protein was maintained at 40% of the diet by using soybean meal and meat-and-bone meal (Webster *et al*. 1997).

A six week feeding trial was conducted to determine the dietary protein requirement of juvenile giant croaker, *Nibea japonica*. Semipurified diets were formulated with white fishmeal to contain graded levels of protein ranging from 30% to 55% of dry weight. Diets containing higher protein levels (45%−55%) produced significantly higher muscle ratio values than diets containing lower protein levels (30% 40%), whereas fish fed diets containing different protein levels had similar muscle ash, lipid, moisture and protein values. The optimal dietary protein requirement for juvenile giant croaker was determined to be 45% of dry diet in natural seawater based on weight gain, feed efficiency, protein efficiency ratio values and muscle ratio (HaeYoung *et al*. 2001).

A feeding trial was conducted with juvenile red drum to determine their quantitative dietary requirement for arginine. Experimental diets with 35 g crude protein/100 g from red drum muscle and crystalline amino acids were used. Based on least-squares regression of feed efficiency and protein estimated requirement data, the minimum requirement of red drum for arginine was estimated at 1.44 ± 0.15 and 1.48 ± 0.12 g/100 g diet (4.11 and 4.23 g/100 g dietary protein), respectively. The arginine requirements estimated from weight gain data were 1.75 (± 0.18) g/100 g diet or 5.0 g/100 g dietary protein. These values are similar to those reported for other carnivorous fish species (Daniel *et al.* 2000).

Protein requirements of euryhaline fish such as the rainbow trout (*Salmo gairdneri*) and the coho salmon (*Oncorhynchus kisutch*) reared in 20‰ is about the same as the requirements for juveniles in freshwater (Krogdahl *et al*. 2003). No data are available for the protein requirements of these species in full strength sea water (35‰). Salmon, trout and catfish can use more protein than required for maximum growth because of efficiency in eliminating nitrogenous wastes in the form of soluble ammonia compounds through the gill tissue.

Protein requirements, as a proportion of the diet, decrease as fish approach maturity. For example, 25% protein was adequate in the diet of channel catfish of 114 to 500 g, but 35% protein produced faster gains than did 25% protein in 14 to 100 g fish (Blanc and Margraf 2002). Somewhat similar results have been obtained for salmonids (Wilson and Halver 1986).

Little convincing evidence exists to show that protein requirements, expressed as a percentage of dry matter, are affected by water temperature. In general, all feeding and growth functions increase in parallel as water temperature rises, although growth rates may increase more rapidly because of an increased feed conversion efficiency coupled with higher food intake (Brett 1979).

2.1.2 Omnivorous fish

All species in this category feed on both algal and animal diets. The group can be subdivided into three groups according to the relative importance of feed of plant and animal origin.

1. Omnivores feeding predominantly on algae: animals make up 6-20% of their total diet.
2. Omnivores feeding largely on animals: algae forms 6-20% of the total diet of these “predators”.
3. Generalised omnivores: Most omnivorous predators fall into this group. They feed on both algae and animals in relatively equal amounts.

Carps are an important group of cultivated fish. Incorporation in the diet of soy protein crude up to 40% in the first feeding of carp larvae does not adversely affect survival or growth of carp larvae (Escaffre *et al*. 1997). However, Dabrowski and Kozak (1979) found that the growth of grass carp fry was depressed when the content of soybean meal in the diet was increased, despite the addition of deficient amino acids. The authors suggested that carp might be less sensitive to the appetite-suppressing factors in soybean products than other fish species.

Silver perch (*Bidyanus bidyanus*, Mitchell) of 65 g average weight were reared at high density under controlled conditions on diets containing 24.8% and 40.6% protein. Diets were fed at 2% or 4% of the fish biomass per day. Both the protein concentrations and feeding levels influenced growth and proximate composition of the fish at the end of the 50-day growth trial. The results indicate that it is possible to obtain the same weight increment when feeding a 24.8% protein diet at a level of 4% body weight per day as compared with only 2% body weight per day of a 40.6% protein diet. This study indicates that the nutritional requirements for early grow-out are similar to those found with juvenile silver perch (Harpaz *et al*. 2001).

Carnivorous fish require high levels of fishmeal and fish oil. For example, farmed salmon are fed a diet that contains approximately 40% fishmeal and 25% fish oil (Tibbetts *et al*. 2001). In contrast, the diets of herbivorous and omnivorous fish, such as tilapia, carp, and silver perch, require much lower levels of fishmeal and fish oil, usually less than 10% and 1% respectively. Protein requirements of omnivorous fish like carnivorous fish decrease as fish approach maturity.

2.1.2.1 Tilapia

There are several commercial farms in Central America that are successfully growing tilapia to export fresh fillets to North America. Additional farms are coming online in the region. In Cuba the demand for tilapia has increased in the past few years and several farms are focusing on local markets. No matter where they are grown the feed is always the largest cost in the production budget for commercial tilapia farmers regionally (Meyer 2001).

Tilapias are very versatile and can be produced in diverse manners by ranching in large lakes, cage culture, pond culture both static and flow-through systems, pens or enclosures in irrigation canals, tanks and raceways, in both monoculture and polyculture, and more recently, in aquaponics systems. Tilapias are hardy and can withstand adverse water quality and low dissolved oxygen levels. However, this does not imply that they should be cultured under such conditions since poor water quality often retards feeding and growth and hence productivity. Tilapias are resistant to diseases, and when problems arise, they are readily treated. Tilapia production has been embraced by many for its ecologically sustainable characteristics and should be remembered when developing production systems (Fitzsimmons 2000).

Carnivorous fish require fishmeal or other animal proteins in their diets, which in general are more expensive than plant proteins. Nutritional studies which substitute plant proteins supplemented with specific amino acid supplements may lower costs, but still not to the level that can be achieved with tilapia diets. Considerable research has been conducted on complete diets and on fertilisation programmes for natural and man-made ponds/tanks. Development of supplemental diets directed to specifically provide limiting nutrients is a growing area of research (Popma and Michael 1999).

One of the great advantages of tilapia for aquaculture is that they feed on a low trophic level. The members of the genus *Oreochromis* are all omnivores, feeding on algae, aquatic plants, small invertebrates, detrital material and the associated bacterial films. The individual species may have preferences between these materials and are more or less efficient depending on species and life stage in grazing on these foods. They are all somewhat opportunistic and will utilise any and all of these feeds when they are available. This provides an advantage to farmers because the fish can be reared in extensive situations that depend upon the natural productivity of a water body or in intensive systems that can be operated with low cost feeds. (Arun and Amararatne 2003).

In extensive aquaculture, the fish will be able to grow by eating algae and detrital matter and the farmer can grow more fish in a given area because the fish are depending directly on the primary productivity of the body of water. Fish which feed on a higher trophic level, eating larger invertebrates or small fish, are secondary consumers and a system can only support a fraction of the biomass of secondary consumers compared to primary consumers (Lutz 2000).

In intensive systems, tilapia has the advantage that they can be fed a prepared feed that includes a high percentage of plant proteins. Tilapia was stocked in a pond using the optimum stocking rate of tilapia (2 fish/m2) (16 fingerling per each pen). Two metabolisable energy levels (300 and 3500 Kcal/kg diet) and two protein levels (30 and 3%) were used to prepare four protein/energy rations (cp/E ratio, mg/kcal/kg diet) (100, 110, 90 and 100). Improvement of tilapia body weight gain, specific growth rate and feed conversion ratio were achieved when dietary P/E increased while, decreased dietary P/E ratios reduced feed consumption and increased the condition factor. The results for the above parameters of tilapia performance increased significantly ($p << 0.05$) with increasing fishmeal protein at the expense of soybean meal protein in all experimental diets, and the highest values were obtained for diets containing 60:30 fishmeal protein: soybean protein (Hafez *et al*. 2000).

Protein requirements of tilapia are lower than in carnivorous fish. Dependency of tilapia upon fishmeal is not as great as that of carnivorous finfish and shrimp species. Tilapia is essentially an omnivorous fish. With this in mind, many studies have been made to replace fishmeal within compound aquafeeds for tilapia with alternative protein sources, including fishery and terrestrial animal by-product meals, oilseed meals and by-products, aquatic plants, single-cell proteins, and legumes and cereal by-products.

2.1.2.2 Protein requirements of tilapia according to size

Tilapia, as well as others species, exhibit their best growth rates when they are fed a balanced diet that provides a proper mix of protein, carbohydrates, lipids, vitamins, minerals and fibers. Shiau (1997), Santiago *et al*. (1985), El Sayed *et al*. (1991) and Chang *et al*. (1988), provide excellent reviews that examine the details of tilapia nutrition. Feed formulators will adjust protein sources to fit the desired pattern of amino acids through the growth cycle. Brood fish may require elevated protein and fat levels to increase reproductive efficiency (Table 2.).

Table 2: Typical protein requirements for tilapia (Santiago *et al*. 1985, Chang *et al*. 1988).

Fish size	**Percentage of protein**
First feeding fry	45 – 50%
0.02 - 2.0 g	40%
2.0 - 35 g	35%
35 - harvest	30 – 32%

The effects of dietary protein (25%, 30%, 35%, 40% and 45%) on growth, survival, feed conversion ratio (FCR), protein efficiency ratio (PER) and body composition were investigated for four sizes (0.51, 45, 96 and 264 g) of Nile tilapia, *Oreochromis niloticus* L. There was a progressive increase in growth with increasing dietary protein for all sizes. In fry (0.51 g), significantly higher growth, survival and feed conversion rates were recorded for fish fed 40-45% rather than 25-35% protein diets (Al Hafedh 1999).

Similar trends for growth and FCR were also noted in 45 g fish. For larger (96 and 264 g) tilapia, significant differences in growth and FCR were found only between fish fed 25% and 30-45% protein diets. FCR and PER decreased with increasing weight of fish, and both were found to be negatively correlated with dietary protein levels. Whole-body composition of the smallest fish was significantly influenced by dietary protein content. Percentage body protein of the fish fed 40-45% protein was higher than that of fish fed 25-35% protein diets, whereas lipid content decreased with increasing dietary protein level (Al Hafedh 1999).

In 45 g fish, both protein and lipid contents were higher in fish fed 25% and 30% protein diets than in those fed 35-45% protein diets. In larger tilapia, no significant influence of dietary protein levels on body protein content was found. The percentage of lipids decreased with increasing dietary protein levels, and no definite trend in ash content was found. The results of these studies indicate that *O. niloticus* fry (0.51 g) should be reared on a practical diet containing 40% protein, and larger tilapia (96-264 g) on a diet containing 30% protein (Al Hafedh 1999).

The nutritional requirements are slightly different for different species of tilapia and more importantly vary with life stage. Fry and fingerlings require a diet higher in protein, lipids, vitamins and minerals and lower in carbohydrates as they are developing muscle, internal organs and bone with rapid growth. Sub-adult fish need more calories from fat and carbohydrates for basal metabolism and a smaller percentage of protein for growth. Of course the absolute amount the fish is eating will still be increasing as the fish is much larger. Adult fish need even less protein; however the amino acids that make up that protein need to be available in certain ratios.

2.1.2.3 Replacement of fishmeal with soybean meal in diet for tilapia

According to recent estimates, world production of cultured tilapia *(Oreochromis* sp.*)* is in excess of 1 million metric tonnes. Tilapias are cultured in a great variety of aquatic environments and with many different management protocols. The management of modern commercial tilapia production systems is an aquatic analog to North American feedlots used for beef production. The fish are held in cages and raceways at stocking densities that can exceed 100 fish/m3. The fish are fed to satiation several times each day using specially formulated feeds. When large enough, the fish are harvested. Modern manufactured fish feeds are not well assimilated by tilapia. Typically only a small fraction (< 30%) of the total content of N and P in the diet is incorporated into the fish's biomass (= growth). The remaining amounts of each macronutrient are never ingested (feed not consumed), excreted into the pond water, lost as part of faecal material, or used for maintenance (Deyab *et al*. 2003).

Egyptian researchers compared a commercial tilapia diet containing 20% fishmeal and 30% soybean meal to diets with all of the protein coming from soybean meal with graded levels of L-lysine supplementation formulated for Nile tilapia fingerlings. After feeding for ten weeks, the diet containing 55% soybean meal and 0.5% L-lysine was significantly (P>0.05) better than the commercial tilapia diet with respect to final weight, weight gain, feed conversion, protein efficiency ratio and feed intake. The diet had the highest digestibility coefficients for protein, fat and energy. These researchers suggested that a diet with 55% soybean meal supplemented with 0.05% L-lysine can totally replace fishmeal in a diet for Nile tilapia fingerlings without adverse effects on fish performance (El-Saidy and Gaber 2002).

Two feeding trials were conducted at the Central Luzon State University Freshwater Aquaculture Centre in the Philippines to determine the viability of using yeast and composted rice straw as alternative protein sources in tilapia diets. In the first phase, the experimental diets were prepared using a meat grinder to make pellets and fed to tilapia in ponds. In the second phase, the diets were fed to tilapia in cages in a common pond. In both experiments, the fish fed the diet incorporating the composted straw demonstrated the highest growth rate. Based on the results of these trials it was concluded that these low-cost supplemental feeds would increase the yield from ponds and the composted rice straw would be a better protein source for the replacement of fishmeal compared to the variety of yeast used in the diet (Kevin *et al*. 1999).

Considerable work has been done to evaluate the nutritive value of soybean meal as a substitute for fishmeal. Soy protein is considered the best plant protein source for meeting the essential amino acid requirements of tilapia and other fish species commercially grown. It is highly digestible by fish and the digestion coefficients are comparable or higher than for fishmeal protein.

2.2 Effects of using alternative protein raw materials to feed carnivorous fish

The protein source affects growth and body composition of fish. Fishmeal is an important source of protein in the diets of carnivorous fish species because of its high protein quality and palatability. However, fishmeal is quite expensive and can substantially increase feed costs. Total or partial substitution of fishmeal with less expensive plant and animal protein feedstuffs may help to reduce feed costs, although these sources may be lower in digestibility, palatability and essential amino acids (Robinson and Li 1996).

Fish processing creates a large amount of waste of high nutrient content which, if not properly processed for use in human or animal nutrition, is likely to be deposited in the environment creating pollution problems. By-products from rainbow trout processing for smoking, consisting of heads, bones, tails and intestines, were used as ingredients for gilthead bream diets. The growth and body composition data from this preliminary experiment indicated that trout waste could be used successfully as an ingredient of sea bream diets (Kotzamanis *et al*. 2001).

Feed intake maintained at 3% of biomass/day for the three diets at the difference with other species (red drum, rainbow trout, and striped bass) with up to 75% replacement of FM protein with soybean protein. A replacement of fishmeal with soybean meal for red drum juveniles (Davis *et al*. 2002) reduced the palatability of the feed. Provided that 20% poultry by-products meal was present, fishmeal was reduced to 5% of the diet and replacing it with solvent extracted soybean meal. A similar approach was reported on trout (Cho *et al*. 2001.) and a combination of soy concentrate and crude protein soya (P80) would overturn the problem of succulence of the diet in absence of regular fishmeal. On a 34% CP diet with protein supplied by soybean meal and fishmeal, diets containing only soybean meal protein were poorly consumed by red drum (Turano *et al*. 2002) which lost weight (Cuzón *et al*. 2002).

In an experiment with Atlantic halibut using three isonitrogenous and isocaloric diets containing 0%, 18% and 36% toasted full-fat soybean were prepared through replacement of fishmeal, wheat and fish oil. The researchers concluded that up to 36% toasted full-fat soybean meal may be replacement in the diets of Atlantic halibut without negative effects on growth, feed efficiency or intestinal morphology (Grisdale-Helland *et al*. 2002).

Four experimental diets were fed to triplicate groups of red drum, rainbow trout, and striped bass, testing the effects of the protein source and the use of an attractant. The diets contained 61% FM or 37% FM and 28% soy protein concentrate (SPC) with or without a coating of squid meal. In the SPC diets, 44% of the nitrogen was supplied from the SPC. There were no significant effects of dietary treatment on specific growth rates and mean body weight, nor were there any effects of protein source on digestibility or protein or energy retention in whole fish (Berge *et al*. 1999).

Atlantic salmon (*Salmo salar* L.) (280 g, initial weight) were fed for 60 days on diets in which fishmeal was substituted with graded levels of extracted soybean meal (SBM) comprising 0%, 10%, 15%, 20%, 25% or 35% of the total protein. The effects on feed intake, growth, feed conversion, apparent digestibility and utilisation of macronutrients and energy, pathohistological response of the distal intestine (DI), activities of digestive enzymes in the mid and distal intestinal mucosa, and faecal trypsin and plasma insulin concentrations were studied. A negative, dose-dependent effect of SBM was observed on nearly all performance parameters with the notable exception of feed intake. The results suggest that caution should be exercised in the use of even low levels of extracted SBM in salmon feeds (Krogdahl *et al*. 2003).

2.3 Favourable effects on the growth of fish using alternative raw materials

The high quality of fishmeal proteins makes substitution difficult. However, partial substitutions are being made (Li *et al*. 2002, Li and Selle 2002, Tabe *et al*. 2002, Chen *et al*. 2002). Tacon (1987) recommended a crude protein level varying from 35% to 42% for omnivorous fish without affecting growth.

For optimal growth fish require not only a minimum level of protein, but also that the essential amino acids are balanced to meet the requirements of each single one. This can easily be done by using fishmeal as the main protein source. However, the amount of fishmeal on a world wide basis is not high enough to cover the need for the constantly growing aquaculture industry. Protein requirements vary between species (Finke *et al*. 1987), but are generally high (>30%) even for most omnivorous species (Tacon 1987) where all the protein (98%) can come from a mixture of plant protein - in commercial diets mainly from soybean. Using this almost “all-veggie” feed still results in satisfactory growth and feed utilisation.

For the more strictly carnivorous species, such as Atlantic cod and Atlantic salmon, there is still a challenge to optimise dietary proteins focussing on alternatives to fishmeal, and only special qualities of plant proteins, or low levels of these (no more than 7%), can be used without any negative effects on production results (Albriktsen *et al*. 2003). In intensive aquaculture in Norway and the European Union, most species are strictly carnivorous, with protein requirements from 38% and higher, and giving the best production results when using high quality fishmeals such as LT-meal with minor additions of other protein sources. In addition to well-balanced dietary amino acids, the protein digestibility and absorption rates of each amino acid are of utmost importance. To increase protein retention from the diet, the quality and mixture of different proteins and the inclusion of partly pre-digested proteins have shown good results.

2.4 Differences in the ability of carnivorous and omnivorous fish to use alternative protein raw materials

Soybean meal (SBM) has been used as a major protein source in diets for aquatic animals, especially for the replacement of fishmeal (FM), which is costlier, less available, and less stable in supply and price than soybean. A significant amount of research has been conducted on the replacement of FM with SBM as a protein source in feeds for tilapia *Oreochromis* spp (El-Saidy and Gaber 2002, Hafez *et al*. 2000, Fontaínhas-Fernandes *et al*. 1999, El-Sayed 1998, Kevin *et al*. 1999).

In general, omnivores have higher rates of intestinal sugar transport and lower rates of amino acid transport than carnivores, if each is studied while eating their respective natural diets. It was unclear whether these differences involve a genetic contribution, when omnivores are switched from a high-protein to a high-carbohydrate diet. A group of researchers studied eight fish species with differing natural diets and fed them the same manufactured diet. They reversibly increase sugar transport and suppress amino acid transport following Michaelis-Menten kinetics (Michaelis and Menten 1913, Wong 1975, Borghans *et al*. 1996). Values of the apparent Michaelis-Menten constant increased with values of the maximal transport rate, probably as a result of unstirred layer effects (Buddington *et al*. 2002):

1. The ratio of proline to glucose uptake decreased in the sequence: carnivores greater than omnivores. The intestine's uptake capacity for the non- essential nutrient glucose was much higher in herbivores than in carnivores, correlated with species differences in carbohydrate content of the natural diet. Proline uptake varied much less among species, since species with different natural diets still have similar protein requirements.
2. Since all species were studied while eating the same diet, these species differences in uptake are not phenotypic but genetic adaptations to the different natural diets.
3. In two fish species, which normally switch from carnivore towards omnivore as they mature, we observe a "hard-wired" developmental change in intestinal uptake. Larger animals had lower proline uptake relative to glucose uptake than did smaller animals, even though both were being maintained on the same diet in the laboratory.
4. Carnivorous fish tend to allocate absorptive tissue to pyloric caeca or a thick mucosa, while omnivorous fish tend towards a long thin intestine.

Results suggest that protein catabolism increases in SPC-rich diets, possibly because of rapid assimilation and utilisation of the methionine supplement. The importance of amino acid supplements and the beneficial effects of protecting these, either by coating them in protein or incorporating them in a protein-lipid emulsion, was investigated (Day and González 2003). Growth data provided some indication that the utilisation of SPC may be improved by incorporating methionine and lysine supplement in a protein-lipid emulsion prior to diet preparation.

2.5 The necessity of substituting fishmeal in diets

Unlike farmed herbivorous and omnivorous fish, such as carp, catfish and tilapia, which consume a plant-based diet, farming carnivores requires a diet containing large amounts of fishmeal and fish oil. Farming carnivorous fish will result in a net loss of fish if fish is used as feed for them. Even with improvements in feed and breeding, three pounds or more of wild fish are still required to produce one pound of farmed salmon or other carnivorous fish (Tacon 1994). Aquaculture production is to maintain its current expansion (i.e. within developing countries) and continue to play an important role in the food security of developing countries as a provider of an *affordable* source of high quality animal protein.

Production systems continue in developing countries to be targeted towards the production of lower-value herbivorous/omnivorous finfish for domestic markets, rather than toward to the culture of higher-value fish. Omnivorous/herbivorous finfish and crustacean species feed lower on the aquatic food chain and are therefore less demanding and more efficient in terms of nutrient resource use. In this respect it is also high time to change farming systems entirely based on the production of non-carnivorous animal species (Dey *et al*. 2002).

Raw material and farm operating costs, in addition to an often static even decreasing market value for many farmed species, necessitates that the farmer reduce production costs so as to maintain profitability. Food and feeding (including fertilisation) usually represent the largest single operating cost item. Therefore attention must be focused on further reducing feed costs per unit of production through the development and use of improved feeds and on-farm fertiliser/feed and water management techniques (Delgado *et al*. 1999).

Finally, the dietary value and importance of aquaculture products in human nutrition as a much needed source of “affordable” animal protein should not be overlooked; fish being one of the cheapest sources of animal protein in rural and coastal communities. For example, at present freshwater aquaculture (mainly cyprinids and tilapia) offers one of the cheapest sources of high quality animal protein within the major rural inland communities of Asia, including China, India, Indonesia, and the Philippines (Delgado *et al*. 2002).

Because an appropriate food supply is crucial to the rearing of fish, we have focused on the diet composition and feeding selectivity in early life of cod. Therefore, the aims of the case study were to measure partial substitution of different kinds of fishmeal, corn gluten meal and soybean meal, in diets of juvenile cod through the examination of survival, growth, and feed performance.

3 CASE STUDY

The effect of substituting alternative sources of protein on growth and survival on cod fry (Gadus murhua) are studied in this section.

3.1 Material and methods

3.1.1 Source of fish

Cod fry from Þorlákshöfn (the Marine Research Institute) was transported to Sauðárkrókur branch of Hólar Agricultural College and the feeding experiment was run there (at Sauðárkrókur, Hólar Agricultural College). 0-year-old cod were maintained on a commercial high quality fishmeal diet for two weeks before the start of the study in early December.

3.1.2 Diet preparation

The experimental diets were prepared at Laxa Feedmill Ltd and proximate composition analysis was carried out on three subsamples (each in triplicate). Based on table values of content in the ingredients, six isocaloric and iso-nitrogenus diets of different raw materials were produced as extruded dry feed. The raw materials tested were: high quality fishmeal - superior (LT), high quality fishmeal - special (NSM), fishmeal–standard, maize gluten meal (MGM) and HYPRO soya bean meal. Table 3 shows the protein content and the relative price of protein in the protein raw material used. Table 4 shows the combination of the raw material in order to obtain six diets for the experiment trial. In addition, the amino acid and fatty acid content of each of the diets were calculated from table values (Table 5). In the diet formulation it was ensured that the essential fatty acid and amino acid content was above the limiting levels for carnivorous fish species.

Table 3: Protein content and relative price of different raw materials from Laxa Feedmill Ltd.

	Protein content %	Relative price of protein
Superior fishmeal (LT)	70	100
Special fishmeal (NSM)	70	93
Standard fishmeal	70	90
Corn Gluten meal	60	65
HYPRO Soybean meal	47	62

Table 4: Percentages of raw material in the trial.

NR	Diet	SUP %	SPES %	STAND %	MGM %	HYPRO %
1	SUP	78	0	0	0	0
2	SUP/SPES	39	39	0	0	0
3	SUP/STANDARD	39	0	40	0	0
4	STANDARD	0	0	80	0	0
5	SUP/MGM	66	0	0	15	0
6	SUP/HYPRO	70	0	0	0	15

Table 5: Diet calculated chemical parameters of the experimental diet.

Nr.	Diet	DE Fish	CP	CF	S	CA	CF	NFE	P	Ly	Me	Cy	DM
1	SUP	16.2	580.0	89.8	124.0	98.6	4.4	139.0	14.9	60	16.6	6.3	908.8
2	SUP/SPES	16.2	580.0	89.8	124.0	98.6	4.4	139.0	14.9	60	16.6	6.3	908.8
3	SUP/STANDARD	16.4	580.0	90.4	120.0	107.6	4.2	134.3	15.0	52.3	17.0	5.8	910.6
4	STANDARD	16.4	580.0	91.0	116.0	116.4	4.1	116.7	15.0	43.6	17.1	5.2	912.2
5	SUP/MGM	16.4	580.0	80.1	132.9	87.7	5.6	156.0	13.6	52.8	16.6	7.2	905.2
6	SUP/HYPRO	16.0	580.0	95.6	87.8	97.2	9.2	128.8	14.0	58.4	14.0	6.5	15.7

DE fish: Digestible energy (MJ); CP: Crude protein (g/kg); CF: Crude Fat (g/kg); S: Starch (g/kg); CA: Crude Ash (g/kg); CF: Crude fibre (g/kg); NFE: Non feeding energy (g/kg); P: Phosphorus (g/kg); Ly: Lysine (g/kg); Me: Metionine (g/kg); Cy: Cysteine (g/kg); DM: Dry matter (g/kg).

3.1.3 Husbandry

Fish (initial overall average weight 29 g; SE±0.5 g) were distributed among 18 circular fibreglass tanks (50 cod each tank) of 40 L-90 L capacity. Tanks are within a recirculation system with a biofilter and a waste trap solid (flow rate of 2 l min^{-1}), the water within each tank was aerated. Temperature was 12±1.5 C, pH 7.2, and mean levels of ammonia and nitrite below 0.1 ppm. A 24 hour light cycle is maintained in the facility. Each tank was randomly assigned one of six diets (Table 4), and fish were then acclimatised for 2 weeks before being weighed and length measured individually. Weight and length of fish were measured every four weeks throughout the feeding experiment.

The experiment lasted for 16 weeks. However, this project covers only the first four weeks of the trial. At the end of the experiment, fishes' individual weights will be determined. Fish are fed to apparent satiation once a day.

At the start of the experiment, six fish were taken for proximate analysis in the beginning for each dietary treatment. Freshly killed fish was stored until proximate analysis. In the subsequent sampling the quantity of fish that will be sacrificed will take into account optimal density of the fish in the tanks at any time.

3.1.4 Growth parameters

The following formula was used to assess growth.

Specific growth rate (%SGR) = $(\ln w_2 - \ln w_1)\, 100 \div (t_2 - t_1)$,

where w_2 and w_1 are mean weight, based on the total biomass in each tank, in grams at times t_2 and t_1 respectively.

3.1.5 Statistical analysis

All data are subjected to one-way analysis of variance followed by Duncan's multiple range tests for comparison of the means among different treatments. All analyses will be conducted using a software package used at the Icelandic Fisheries Laboratories.

3.2 Results

Survival of cod fry for each of the treatments is shown in Table 6. The highest survival was in the treatments with dietary regimes SUP/STANDARD and SUP/MGM each with 93.2%. The lowest survival was in the treatment with the dietary regime STANDARD. Survival in relation to the dietary regime was not statistically significant.

Table 6: Survival of cod fish maintained under different dietary regimes during the first four weeks of the experimental trial.

Nr.	Diet	Survival %
1	**SUP**	90.9 ± 0.2
2	**SUP/SPES**	89.4 ± 1.3
3	**SUP/STANDARD**	93.2 ± 3.5
4	**STANDARD**	85.6 ± 5.1
5	**SUP/MGM**	93.2 ± 3.5
6	**SUP/HYPRO**	91.6 ± 1.1

Growth parameter indicators of cod fry for each of the treatments are given in Table 7. Final mean weight, increase in mean weight as a percentage of initial weight and SGR increased with increased high quality fishmeal. There are no significant differences in any of the parameters calculated. Final mean weight was highest in cod maintained under the dietary regime SUP with only superior fishmeal, with 43.0 g, cod maintained on treatment STANDARD had the lowest final weight with 39.3 g. The other treatments, partial replacement of SUP with SPES; STANDARD; MGM and HYPRO, were intermediate. Increases in mean weight and SGR show the same tendency as seen in Table 7.

Table 7: Growth parameters (±SE) of cod fish maintained under different dietary regimes during the first four weeks of the experimental trial.

Nr.	Diet	Initial mean weight (g)	Final mean weight (g)	Increase mean weight % of initial	SGR (% day $^{-1}$)
1	**SUP**	28.0 ± 0.5 [a]	43.0 ± 2.3 [a]	53.6 ± 8.5	1.3 ± 0.2
2	**SUP/SPES**	29.0 ± 1.5 [a]	42.7 ± 1.4 [a]	47.3 ± 2.2	1.2 ± 0.1
3	**SUP/STANDARD**	28.0 ± 0.5 [a]	40.4 ± 1.1 [a]	44.1 ± 1.0	1.1 ± 0.0
4	**STANDARD**	28.7 ± 0.2 [a]	39.3 ± 2.0 [a]	37.0 ± 8.1	1.0 ± 0.1
5	**SUP/MGM**	28.3 ± 0.2 [a]	41.1 ± 0.2 [a]	45.2 ± 0.1	1.1 ± 0.0
6	**SUP/HYPRO**	28.7 ± 0.2 [a]	41.2 ± 0.1 [a]	43.6 ± 2.2	1.1 ± 0.0

Averages are based on mean values for all three replicates. Parameter values with the same superscript are not significantly different ($P > 0.05$).

3.3 Discussion

Cod fish is a carnivorous species, which is considered to have a high culture potential in aquaculture (Ingram 2000).

Carnivorous species generally require high dietary protein content, and cod is no exception to this rule, as the protein requirement for optimal growth of cod fry is at least around 50%, by dry weight of the diet (Gunasekera *et al*. 2002). The protein sparing capabilities tend to differ amongst species, both to the extent to which protein is spared, and the energy source utilised for the sparing. For example, in salmonids lipid acts as the energy source (Hardy 2000), whilst in anguillid eels carbohydrates are the alternative energy source (Hidalgo *et al*. 1993, García-Gallego *et al.* 1995). On the other hand, in Asian seabass (*Lates calcarifer,* Bloch) (Catacutan and Coloso 1997) and hybrid tilapia (Shiau 1997) lipid is known to be spared by carbohydrates as an energy source. However, the findings on protein-sparing capabilities in some warm water fish species, such as tilapia, are not uniform (El-Sayed and Teshima 1991, De Silva *et al*. 1991, Hanley 1991, Shiau 1997).

In the present study Superior fishmeal (LT) SUP was partly or fully replaced by other protein raw materials. The raw materials tested as replacements for the Superior fishmeal were Special quality fishmeal (NSM) SPES, Standard fishmeal STANDARD, Maize Gluten Meal MGM and HYPRO Soya bean meal HYPRO. Partial replacement of SUP with SPES; STANDARD; MGM and HYPRO or a full replacement with Standard did not result in a significant decrease in growth rate in cod fry, although a trend was evident to this effect. Similar effects have been reported by Albriktsen *et al.* (2003). They found a significant effect of fishmeal quality on growth (LT versus NSM) in a trial using different inclusion levels of plant protein raw materials (Full-fat Soya meal (2.0, 8.0, and 14.0%) and Maize-gluten meal (4.0, 16.0, 28.0 %). At higher levels of plant protein there was a tendency to a negative effect on growth. Some of the actual raw materials (such as soya bean meal) that have been used to replace fishmeal in diets for fish, can contain Trypsin inhibitor activity (TIA) that can limit utilisation of protein in diet (Perez-Maldonado *et al*. 1999).

In this project, there is a tendency of reduction in survival, growth and SGR when plant protein raw material or lower quality fishmeal is used as a protein source, but the effects are not significant. Similar results have been reported in the experiment mentioned before (Albriktsen *et al*. 2003) where significant effects of replacements were observed. It is presumably due to the fact that the results from the present experiment only represent four weeks and the fish might need more time to grow for a longer period to find statically significant differences.

In experimental trials it is very important to reduce time and costs. It could be profitable to sample digestive enzymes of fish in order to find out about its kinetic behaviour. Trypsin is the most important digestive enzyme in cod (Lemieux *et al*. 1999) as well as in other aquatic species (Escaffre *et al*. 1997, Krogdahl *et al*. 2003, Li and Selle 2002). Change in trypsin activity reflects protein quality, in particular if one can expect TIA activity, of feed, which in turn will affect the growth. Change in Trypsin activity is fast: in 24 hours the effect in enzymatic activity will take place. This activity has also has been related to growth in several fish species (Wong 1975) Therefore trypsin activity can be used as a tool to measure the effects of feed quality on performance. This method can accurately predict results of future experiments and suggests new lines of work and new ways of thinking. There is a continual need to refine biological assays to increase the efficiency of experiments (Bergmeyer *et al.* 1974).

In the present study with cod fry, the SGR (% day^{-1}) showed a tendency to increase with increasing quality of the fishmeal. Such trends are apparent indicators of protein-sparing effect of protein quality (van der Meer *et al*. 1997). It is, therefore, possible that in the present study the dietary protein levels used were such that they were sufficient to satisfy the basic requirement for protein accretion in all the diets even though there were differences in the quality of the protein component. On the other hand at lower protein levels (near to minimum needs of digestible protein in the diet), a reduction in high quality fishmeal, with a concurrent increase in dietary regime of plant protein raw material could compromise growth, SGR and or feed utilisation. The present observations suggest that it is possible to substitute high quality fishmeal with different protein raw material in the feed for cod fry.

The present study shows that cod fry could be successfully reared on artificial diets, and that high quality fishmeal was the best food in early life. It is known that most carnivorous fish species require high protein diets during their earliest stages of development (Huet 1972). A slight tendency was found to an increased final mean weight, increased mean weight as a percentage of initial weight and SGR with high quality fishmeal compared to other diets, supporting the notion that protein quality affects feed utilisation, which is in line with other studies on Atlantic salmon (Aksnes 1995, Hemre *et al*. 1995, Anderson *et al*. 1997). However, it is possible that small cod do not use soybean meal and corn gluten meal as well as adult fish. Other experiments confirmed that high quality fishmeal for cod fish fry is the best initial food (Kim *et al*. 2000). Later on when fish grow up to fingerling and grower stages and their protein requirements get lower as a result of age or size they can tolerate replacements of fishmeal to a greater extent. This is supported by other authors that have conducted several studies on carnivorous fish (Albriktsen *et al*. 2003, Kim *et al*. 2000, Lee and Kim 2001, Gunasekera *et al*. 2002, Davis *et al*. 2002, Cho *et al*. 2001, Grisdale-Helland *et al*. 2002). The results from the present study show that feed with a large proportion of animal matter of marine origin and relatively small part of plant matter would be suitable feed for growing cod.

3.4 Conclusions

Cod fry can be successfully reared on artificial diets but high quality fishmeal appears to be the best food for early life stage.

Partial replacement of SUP with SPES; STANDARD; MGM and HYPRO or a full replacement with Standard did not result in a significant decrease in growth rates (SGR) in cod fry.

The present observations suggest that it is possible to substitute high quality fishmeal with different protein in feed for cod fry.

3.5 Recommendations

Regarding future experimental work the results of this trial should be reconfirmed and greater substitution tested.

It could be profitable to sample digestive enzymes of fish in order to find out more about its kinetic behaviour and use that to interpret the effects of feed quality on growth.

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