



norden

Nordic Innovation Centre

March 2006

Feed for Atlantic cod

- Knowledge and practices for a successful cod farming industry
- Economical feed for cod: Cost of feed for cod may be reduced by 5-10%
- Competitive aquaculture of cod in the Nordic countries

Author: Rannveig Björnsdóttir, IFL, Iceland (project coordinator), et al.

Authors

Iceland

Rannveig Björnsdóttir, IFL / UnAk (Project coordinator)
Jon Árnason, Laxa Feedmill Ltd.
Helgi Thorarensen, Hólar Agricultural College
Soffía Vala Tryggvadóttir, IFL

Denmark

Bjarne Olsen, Dana Feed

The Faroe Islands

Durita í Grótinum, Kosin Seafood

Norway

Turid Mørkøre, Akvaforsk
Rolf Erik Olsen, Institute of Marine Research
Ørjan Karlsen, Institute of Marine Research

Sweden

Jana Pickova, SLU

Title: Feed for Atlantic cod		
Nordic Innovation Centre project number: 03029		
Author(s): Rannveig Björnsdóttir <i>et al.</i>		
Institution(s): Icelandic Fisheries Laboratories / University of Akureyri (IFL / Unak), Iceland <i>et al.</i>		
Abstract: Today the cost of feed is 50-60% of the total production of farmed cod. A 10% reduction in feed cost will therefore give a minimum of 5% reduction in production cost. The biggest single variable cost in cod farming, the high cost of feed, is the main obstacle in making cod farming economically feasible. The cost of feed can be reduced by minimizing protein in feed for cod and/or substituting cheaper plant ingredients for expensive marine fish meal and fish oil. To ensure access to available knowledge in the field of feed for cod a cooperation between the Nordic countries was considered very important. This cooperation was made possible by a grant from the Nordisk Innovation Centre. Overall, the R&D and the university partners carried out the feeding experiments and analysis of the feed, fillets and liver, and the partners from the industries provided experimental feed, processing of the farmed cod and expertise in marketing issues. The result of the project showed that it is possible to reduce the cost of feed for cod by 5-10%. The minimal protein requirements of small cod was found to be between 48-52%, which is lower than in traditional cod feed. Partial substitution of soy-meal and soy-bean oil was successful. The fish grew well and no health problems were observed in cod fed plant protein and plant oil. Analysis of fatty acids in the muscle and liver of cod fed a mixture of plant and fish oils showed that the fatty acids profile simulates the fatty acid profile in the feed. Sensory analysis revealed no difference in taste or texture between cod fed marine ingredients compared to partial substitution of plant sources. When provided with samples, Spanish buyers did not consider salted farmed cod to be a high quality product. The fillets of farmed cod are usually thicker and the texture is different, indicating that processing of farmed cod needs to be adapted to meet the demands of consumers.		
Topic/NiCe Focus Area: Aquaculture, farmed cod, cod feed, feeding trials, economical feed, feed efficiency, feed analysis, quality.		
ISSN: Not available	Language: English	Pages: 44
Key words: Aquaculture, farmed cod, cod feed, feeding trials, economical feed, feed efficiency, feed analysis, quality.		
Distributed by: Nordic Innovation Centre Stensberggata 25 NO-0170 Oslo Norway	Contact person: Rannveig Björnsdóttir Icelandic Fisheries Laboratories P.O. Box 224 IS-602 Akureyri Iceland Phone: +354-530 8600 E-mail: rannveig@rf.is	

Participants in the Feed for Atlantic cod project:



Rannveig Björnsdóttir, IFL/UnAk, Iceland
 Thorvaldur Thoroddsson, MSc student IFL/UnAk, Iceland
 Soffía Vala Tryggvadóttir, IFL, Iceland



Jón, Árnason, Laxá, Iceland



Helgi Thorarensen, HAC, Iceland
 Ingólfur Arnarson, HAC, Iceland



Thórhallur Jónasson, SVN, Iceland



Óttar Már Ingvason, Brim fiskeldi, Iceland



Jóhannes Gíslason, Primex, Iceland – not active



Turid Mørkøre, Akvaforsk, Norway
 1 PhD student and 2 MSc students



Rolf Erik Olsen, Institute of Marine Research, Norway
 Ørjan Karlsen, Institute of Marine Research, Norway

[Fjordmarin](#)

Gunnar Brandtzaeg, Fjord-Marin, Norway – not active



Jana Pickova, SLU, Sweden
 Lars Johnsson, SLU, Sweden



Christina Grenabo, COOP Sverige, Sweden



Bjarne Olsen, Dana Feed, Denmark
 Henrik Jarlbæk, Dana Feed, Denmark



Josianne Støttrup, DIFRES, Denmark



Hans Otto Sørensen, 999, Denmark



Durita i Gróttinum, Kosin Seafood, the Faroe Islands
 Johanna a Bergi, Kosin Seafood, the Faroe Islands
 John Mikkelsen, Kosin Seafood, the Faroe Islands



Ingolf Joensen, Fiskaaling A/S, Faeroe Islands
 Hannes Gislason, Fiskaaling A/S, Faeroe Islands
 Hørdur Wardum, Fiskaaling A/S, the Faroe Islands

Executive summary

Main objectives

The aim of this project was to bring the Nordic countries together in their research on feed for cod by creating a network of scientists and specialists from research institutes, universities and companies that are working in connection with cod farming. The main objective of the project was to optimize feed ingredients to achieve maximum growth, feed conversion, health and harvest quality of farmed cod for the minimum cost.

Objectives and expected achievements

1. To bring the Nordic countries together in their research on feed for cod:
 - By creating an extensive network of feed producers, producers of single feed components, future cod farmers as well as scientists from institutes and universities.
2. To reduce the cost of feed for cod:
 - By minimizing the protein content in the feed for cod
 - By partial substitution of plant protein and plant oil for more expensive marine nutritional sources.
3. To produce valuable, popular and healthy products from farmed cod:
 - High quality fresh cod for the fresh fish market
 - High quality processed cod for the salt fish market
 - Reasonably priced high quality, healthy product

The objectives have been met by:

The project partners were sixteen, from Iceland (6), Faroe Islands (2), Norway (3), Denmark (3) and Sweden (2) and included technicians and scientists from industries, universities and R&D institutes. The project consisted of partners from all the Nordic countries that are working today on developing a successful cod farming industry. Five project meetings were held, one in each country. At the project meetings the different results were discussed and evaluated and further experimental programs were planned. The Management Group of the project included 5 persons, three from the industry (Denmark, Iceland, Norway) and one from the R&D sector (Norway) in addition to the coordinator of the project (Iceland). An interactive homepage for the project was established. The partners have provided national funding for related projects and, thus, creating synergy with the funding from NICE. One of the valuable outcomes of the synergy work was that the partners were not repeating experimental work carried out in the other countries. Instead, each partner took on the responsibility of working on specific aspects of the problem and experiments were more focused. This increased the outcome of the project and raised the level of knowledge and practices for a successful cod farming industry.

Method/implementation

Feeding experiments, both on a small scale (100 g fish) and large scale (1000 g fish) were performed. Hólar, Laxá and IFL carried out two small scale trials, on small (100g) and medium (300g) sized cod in indoor tanks. Before the initiation of the trial the experimental feed was analysed for nutritional content and physiochemical testing was performed on the pellets. The third feeding experiment is in process at Hólar on approximately 500g fish. Akvaforsk and the Marine Research Institute in Norway carried out feeding trials on the bigger size groups of cod (in indoor tanks and sea pens). All the feeding experiments focused on finding less expensive feed ingredients compared to traditional cod feed today. The feed companies (Danafeed and Laxá) provided the experimental feed (dry feed) and planned the feed formulas in cooperation with the scientific partners. The market size fish from the Akvaforsk trial was partly analysed by Havforskninginstituttet in Norway and SLU in Sweden i.e for fatty acids, sterols and lipid classes. The fish processing company Kosin Seafood in Faroe Islands carried out a salt fish processing trials on market size cod from the Akvaforsk trial. SLU in Sweden and Akvaforsk in Norway performed a sensory preference test on market size cod from the Akvaforsk trial and a market study was carried out by COOP in Sweden

The feeding trials on the smaller size groups of cod (<100 g and 300-500 g):

- Different protein sources, both fish meal of different quality as well as plant proteins
- Optimizing the protein need for small cod (with high quality fish meal as a protein source)

The feeding trial on the large size group of cod (1000g):

- Partly substituting fish oil for plant oil, feeding the fish until it had at least doubled in weight.
A few weeks before the fish was slaughtered, the plant oil was removed and feed containing 100% marine oil was fed to the fish to successfully “wash out” the plant oil.

The following parameters were evaluated with respect to different feed formulas:

Growth of experimental fish:

- Specific growth rate
- Feed efficiency
- Health

Feed analysis

- Protein, fat, ash and water
- Fatty acid composition
- Amino acid composition

Product qualities post slaughtering:

- Weight and length
- Condition factor
- Liver index
- Liver discoloration

Analysis of cod fillets (fresh and after one year of frozen storage)

- Protein, fat and water
- Fatty acids
- Lipid classes
- Sterols (cholesterols among others)
- Texture analysis

Analysis of cod liver

- Fat and water
- Fatty acids
- Lipid classes
- Sterols

Sensory analysis on fillets (fresh and frozen)

- Consumer preference test

Processing trials of farmed cod for salt fish production

- Water content
- Consumers preference in Spain

Concrete results

Cost-effects of both the chemical composition and a raw material substitution in the experimental feed, depended on the raw material prices at any time and relative values of different currencies. The effects stated in this report refer to the situation as of the first half of December 2005.

1. Effect of protein raw material substitution:

The present project, as well as connected activities, has shown that Atlantic cod tolerates substantial substitution of protein raw materials.

It has been shown that it is possible to use up to 18% Hipro soy-bean meal in diets for cod without affecting growth. The effect of that finding gives a 6, 6% reduction of raw material cost compared to use of high quality fish meal as the only protein source in the feed.

2. Effect of oil substitution:

Trials run as part of this project have shown that a 40% substitution of fish oil by soy been oil had no effect on growth and feed utilization in cod. The substitution however affected the fatty acid composition in the fillet and the liver as it took after the fatty acid profile in the feed.

The substitution also affected the content of sterols in the liver but not in the fillet. The amount of cholesterol in cod liver fed 40% soy oil was decreased by 50%. Lipid fractions of cod after one year of frozen storage differed in the amount of free fatty acids, which increased with storage time. A sensory preference tests showed no significant difference in odor or taste between farmed cod fed soy oil or fish oil.

The fatty acid profile was not significantly different after one year of frozen storage in comparison to the fresh cod analysed at Akvaforsk.

The trials indicate that it is possible to design the fatty acid composition of the cods liver.

Given that the effect of feed lipid on liver composition is acceptable, at the present costs of raw material the effect of a 0% inclusion of soy-bean oil is equal to 1% reduction of the raw material cost.

3. Effect of reducing the concentration of crude protein in the feed:

A reduction of the content of crude protein represents approximately 1,2 percent units for each 10 grams reduction in the crude protein content of the diet.

The trials establishing the minimum protein requirements of cod of different size are ongoing and the final results will be available in 2006. The results for the cod with initial average weight of 60g indicate that the minimum need for crude protein was 480 – 520g per kg feed which is lower than is commonly used in commercial diets for cod today

4. Salt fish experiment with farmed cod:

Salt fish product from farmed cod did not have acceptable texture (soft, gaping). The color of the muscle has been found satisfactory, but not as good as of products from wild cod. The salt fish from farmed cod tasted fresher than from wild cod i.e. did not have as much “processing taste”. As for today the salt fish product from farmed cod can not be sold as high quality salted fish.

Recommendations for further work:

There is an interest in looking into the lipid metabolism and explore the effects of fatty acids and sterols, including cholesterol, on the nutritional quality as human food. Furthermore, the concept of “functional fish” is gaining a lot of interest, i.e. to examine if and how it is possible to control the functionality of fish as human food, through the feed used. There is also an interest of investigations towards the welfare of the fish and importance of oil (lipids) in the feed, which could affect the health of cod.

The preference tests show that a continuation of the sensory work is needed, in form of sensory panels to explore which differences are found between the materials from the marine and soy dietary origin.

At the final project meeting in Uppsala, Sweden in November 2005 the following project ideas for future work were listed.

New project ideas:

1. New feed resources for growth, health, welfare and quality of farmed cod.
2. Amino acid requirements (essential a.a.) for different size groups of cod.
3. Fatty acid requirements and tolerance of cod - define fatty acid requirements and tolerance for different size groups of cod - sources and ratios.
4. Tailored quality of cod through designed dietary composition and processing optimization - eating/consumer

Disseminations

Project meetings.

There were five project meetings during the project period.

- In Akureyri, Iceland Nov., 20-21, 2003 (meeting and workshop)
- In Tromsø, Norway Feb., 14, 2004
- In Horsens Denmark Nov., 25-26, 2004 (meeting and workshop)
- In Faroe Islands, May 2-4, 2005
- In Uppsala, Sweden Nov., 22, 2005

Presentations of the Feed for cod project.

- Bjarne Olsen (Danafeed). Presentation of the project at a meeting with the Norwegian fish farming industry in Denmark in January 2004.
- Rannveig Björnsdóttir (IFL). Presentation of the project at an open IFL-meeting in Isafjordur, West Iceland in April 2004.
- Rannveig Björnsdóttir (IFL). Presentation of the project and connected national projects for the Icelandic government (the board of the Fishing Industry) in Sept 2004.
- Rannveig Björnsdóttir (IFL). Presentation of the project and its results at a meeting with the Icelandic cod farming industry in Sept 2004.
- Turid Mörköre (Akvaforsk). Presentation of the Akvaforsk and Danafeed feeding trials at Sats paa torsk meeting in Bergen Feb 2005.
- Leaflets – presenting the project and its results. Distributed at the Sats paa torsk meeting in Bergen Feb 2005, at AquaNor in Trondheim, Norway in August 2005 and at the cod farming forum in Reykjavik Sept 2005.
- Turid Mörköre (Akvaforsk) and Thorvaldur Thoroddsson (IFL). Presentation of the project and results at the Sats paa torsk meeting in Bergen Feb 2005.
- Rannveig Björnsdóttir (IFL). Presentation of the project and its results at the annual meeting of the Aquaculture association of Iceland in Reykjavik May 2005.
- Turid Mörköre (Akvaforsk). A presentation of the project and results at AquaNor in Trondheim August 2005. After the conference there was a workshop.
- Rannveig Björnsdóttir (IFL). Presentation of the project in FishFarming International in August 2005.
- Rannveig Björnsdóttir (IFL). Presentation of the project and its results at an open IFL-meeting in Reykjavik in Sept 2005.
- Rannveig Björnsdóttir (IFL). Presentation of the project and results at the Cod farming forum in Reykjavik Sept 2005.
- Jana Pickova, SLU. Presentation of the project and results at the Sats paa torsk meeting in Tromsø in January 16-17th 2006. Jana Pickova has scientific publication in preparation.
- Turid Mörköre (Akvaforsk). Relevance of dietary oil source for contraction and quality of pre-rigor filleted Atlantic cod, *Gadus morhua*. Publication submitted to Aquaculture.

- Product quality of Atlantic cod fed diets supplemented with soybean oil. MSc thesis (Akvaforsk), which was delivered 16/6 6 2005.
- Results from the project have been presented in the NICE leaflet (#3, 2003) as well as various Norwegian and Icelandic media:
 - Norway: Havbruk, www.kyst.no, Nytt fra Akvaforsk, Halden, Arbeiderbladet.
 - Iceland: www.thorskeldi.is, Ægir, Morgunbladið (2004, 2005), Radio Saga

Table of contents

1. Introduction.....	2
2. The Icelandic part.....	5
Protein requirements of cod	5
2.1. <i>Materials and methods</i>	5
2.1.1. Fish and rearing conditions	5
2.1.2. Feed and feed formulation.....	6
2.1.3. Measurements and sampling of fish	7
2.1.4. Proximate analyses	8
2.1.5. pH measurement.....	9
2.1.6. Total amino acids	9
2.1.7. Water holding capacity (WHC).....	9
2.1.8. Statistics	9
2.2. <i>Results</i>	10
2.2.1. Smaller size group of cod.....	10
2.2.2. Larger size group of cod.....	12
2.3. <i>Discussion</i>	17
2.4. <i>References</i>	20
3. The Norwegian part I - Akvaforsk.....	22
Fatty acid profile, sterol composition and product quality in farmed Atlantic cod (<i>Gadus morhua</i> L) fed extruded diets with soybean oil.....	22
3.1. <i>Description of the study</i>	22
3.2. <i>Analyses</i>	23
3.3. <i>Important findings:</i>	23
4. The Norwegian part II – Institute of Marine Research.....	26
Effect of low quality protein sources on growth of Atlantic cod	26
4.1. <i>Introduction</i>	26
4.2. <i>Material and methods</i>	27
4.3. <i>Results</i>	28
4.4. <i>Discussion</i>	30
4.5. <i>References</i>	31

1. Introduction

Farming of Atlantic cod (*Gadus morhua*) is rapidly growing and is expected to become an important future industry for coastal communities around the North Atlantic. Cod farming in the Nordic countries is based on three different methods: (1) substandard wild cod from fisheries are fed in net cages for 6-8 months before harvesting (2) juveniles (5-10g) are caught and reared in land based tanks and then in net cages to market size (3) cod are reared from eggs in hatcheries and then juveniles are transferred to sea-pens for on-growing. However, large scale farming of cod will have to depend on control of the whole rearing process from fertilization of eggs to market size.

Feed represents around 60% of the total production cost in cod farming and the possibility of cod farming becoming a profitable industry are poor with the high cost of the feed presently used. Cod is a new aquaculture species and there is a gap of knowledge with respect to the nutritional requirements of this species (Lall and Nanton 2002). Any changes in the cost of feed will have a significant impact on the total cost of production and it is imperative to keep the cost of feed as low as possible while maintaining good growth rate besides producing healthy fish of high quality. Therefore, it is necessary to spend appreciable effort in developing a suitable feed for cod. Protein is the most expensive nutritional factor in the feed and currently most of the protein in fish feed comes from fish meal. Any diet for cod, as for other species of fish, must contain all necessary nutrients and energy to support maximum growth (Hertrampf and Piedad-Pascual 2000). Bendiksen (2005) demonstrated that the protein requirement of Atlantic salmon varies with fish size. The same has been shown for haddock among other species (NRC 1993, Hertrampf and Piedad-Pascual 2000).

Experiments with young cod have demonstrated that for maximum growth and protein retention, the feed should contain at least 50 – 60 % “crude” protein, 13-20% fat and less than 15% starch (Rosenlund *et al.* 2004). Hemre *et al.* (2003) conclude however that high starch content of feed should be avoided since this may limit growth and feed conversion, although formulations with up to 18% of high quality starch do not appear to limit digestibility in cod. According to Arnason (2004), older data on protein requirements in cod of different size are rather conflicting. Protein is the most expensive macro nutrient in the feed, and as protein requirements of various fish species have been shown to decrease with increasing size and age (Wilson 2002), it is of importance to know the need for protein in cod of different size.

The natural diet of wild cod consists mainly of protein and fat derived from small fish and crustaceans while carbohydrates do not appear to be equally important as an energy source for cod (Hemre *et al.* 1989), probably due to limited availability of carbohydrates in the marine environment. Capelin seems to be the most important feed source for large wild cod (Tacon 1993), a food selection that suggests that the protein requirements of cod are relatively high. Moreover, it can be expected that the high quality capelin meal and oil that are currently used in cod feed, are indeed a very suitable nutrition for cod. However, the fatty acid composition of cod liver does not exclusively reflect that of capelin (Sigurgísladóttir *et al.* 1993, Bragadóttir *et al.* 2002, Falch *et al.* 2006) indicating a diverse selection of food in the natural environment.

Fishmeal is an expensive protein raw-material and the market prices of fishmeal fluctuate widely depending on supply and demand. Therefore, it is of interest to look for alternative proteins in cod feed, e.g. from plant sources. The Atlantic salmon is also a carnivore and the nutritional needs of cod can be expected to be similar to those of the salmon. Extensive efforts in development of feed for salmon have resulted in increasing use of vegetable ingredients (Thorstensen *et al.* 2000). Long term growth study of Atlantic salmon (Refstie *et al.* 2001) showed that 18% replacement of low temperature dried fishmeal by commercial high protein soy meal was fully acceptable in grower diets for Atlantic salmon.

In the search for cheaper raw material of plant origin in fish feed, it is important to ensure that the substitution does not affect the growth rate or health of the fish as well as quality of the final product. The amino acid profile of plant protein is quite different from the amino acid profile of fish protein. Therefore, it is necessary to ensure that the amino acid profile of diets with plant protein provide adequate amounts of essential amino acids for the fish (Sugiura *et al.* 2001). It is likely that only partial substitution of plant protein and oil in feed for cod is feasible, since plant proteins usually does not contain the required amounts of some of the amino acids essential for fish. Furthermore, anti-nutritional components found in most grains, seeds and legumes may limit the use of plant protein in fish feed. One of these anti-nutritional factors is phytic acid that binds minerals, proteins and starch, thus reducing the intestinal absorption of these elements (Fallon and Enig 2000).

The future growth of the aquaculture industry depends on finding suitable sources for protein and oil in the feed. If the world aquaculture production continues to grow at the same rate as predicted, the aquaculture industry will need all the fish oil produced in the world within the next 10 years (Dersjant-Li 2002). No other fat source is as rich in omega-3 fatty acids as fish oil and

omega-3 fatty acids are very important in feed for cold water fish species. It is however necessary to distinguish between the consumers preference for omega-3 fatty acids in fish products and the actual needs of the species for these fatty acids. If fish feed contains the dietary requirement for essential fatty acids, soy oil can support fast growth and acceptable feed conversion (Storebakken *et al.* 2000). It's generally excepted that up to 50% of the fish oil can be substituted by plant oil without affecting the quality of farmed salmon. Similarly, studies with 2 kg cod do not suggest that inclusion of soybean oil affects the quality of the fish (Mørkøre 2006). Furthermore Mørkøre (2006) found that inclusion of soybean oil (40% of total lipids) resulted in faster energy depletion post-mortem, faster rigor contraction, faster reduction of the breaking strength and lower L*-values (lightness) compared to fish fed only fish oil.

The common aim of the project as a whole is to bring the Nordic countries together in their research on feed for cod by creating an extensive network of feed producers, producers of single feed components and cod farmers as well as scientists from institutes and universities. Cooperation between the Nordic countries is considered very important in order to benefit from the experience gained from farming of salmon and other fish species. A number of smaller projects have been run in parallel with this project within the participating countries. All of these projects have aimed on evolving economical feed for cod, and with the common goal to contribute to competitive aquaculture of cod in the Nordic countries.

2. The Icelandic part

Protein requirements of cod

This part of the project was conducted by the Icelandic Fisheries Laboratories, Hólar University College and Laxá Feedmill. *The objective was to identify the minimum protein requirements of cod of two different size groups.* The nutritional need of fish is highly dependant upon their age, and the growth rate of younger fish is proportionally faster than that of older fish. The protein requirements are therefore higher in younger fish compared with larger and older fish, and different feed formulations have to be developed for fish of different age groups.

2.1. Materials and methods

2.1.1. Fish and rearing conditions

Fish for all experiments were obtained from the Marine Research Institute in Staður and brought to the Holar University College facility in Sauðárkrókur where the experiments were conducted. In each experiment, the fish were distributed at random among 18 tanks with each feed formula fed in triplicate. The fish were habituated to the tanks for at least two weeks before the experiments commenced. Two size groups of fish were used in the studies, smaller fish (initial size 35g) and two groups of larger fish (initial size 356g). The fish were reared at 8-10 °C in 90 L tanks (smaller fish) and 800 L tanks (larger fish). The target salinity during the experiments was 33‰, however, the salinity fluctuated during one of the experiments on the larger fish, reaching occasionally levels as low as 20‰. The initial number of fish in each tank was 40 for the smaller fish and 30 for the larger fish. The smaller size group was reared for 151 days and the larger size group for 155 days.

The fish were individually weighed and their length measured at the beginning and at the end of each experiment. Fish from individual tanks were sampled at the end of the experiments, weighed (ungutted and gutted), and the length measured in addition to evaluation of the liver index and chemical analysis on muscle and liver samples.

In all experiments the fish were fed in excess. The smaller fish were fed by hand twice each day until uneaten feed pellets were clearly present in the tank. The larger fish were fed through automatic feeders as well as by hand to ensure adequate feed supply. The amount of feed offered

in each tank was recorded and the feed conversion rate (FCR) calculated as: total mass of feed presented / total gain in body mass.

The actual feed intake of the larger fish was measured by collecting uneaten feed particles with mesh traps in one tank of each triplicate of tanks for seven days in a row. The number of uneaten pellets was counted on a daily basis and the mass of uneaten feed estimated. The feed conversion for the larger fish was calculated based on the actual intake of feed and the gain in body mass (Árnadóttir 2006, results not included).

2.1.2. Feed and feed formulation

Six different types of isoenergetic feeds were formulated for each experiment. The feed for the smaller size group of fish was formulated to contain 40%, 44%, 52%, 58% and 60% protein, all from capelin meal. However, proximate analysis of the feed showed that the actual protein content was slightly higher (**Table 1**). Similarly, the feed for the larger fish was formulated to contain 36%, 39%, 43%, 48%, 52% and 57 % protein from capelin meal, whereas the actual protein content turned out to be somewhat higher for most formulations (**Table 2**).

Digestible energy was calculated from the nutritional content of the feed.

Total energy was determined as follows:

Protein; 23.7 MJ/kg

Fat; 39.5 MJ/kg

Carbohydrates; 17.2 MJ/kg

Digestible energy was determined as the following proportions of the total energy:

Protein; 85% or 20.1 MJ/kg

fat; 91% or 35.9 MJ/kg

carbohydrates; 72% or 12.4 MJ/kg

Table 1. Proximate analysis (dry weight basis) of the experimental diets for the smaller size group of cod.

Feed group	Protein % (± 0,4)	Lipid % ± (0,4)	Ash % (± 0,5)	Carbohydrates* (%) by difference	Digestible energy† (DE) MJ/kg
P40	47.7	16.0	10.7	25.7	18,5
P44	47.1	16.9	10.5	25.5	18,7

P48	51.9	13.1	11.2	23.9	18,1
P52	55.9	12.4	11.8	19.9	18,2
P56	59.0	10.5	12.1	18.3	17,9
P60	64.0	8.9	12.9	14.2	17,8

*Estimated from subtraction

† Calculated based on proximate analysis

Table 2. Proximate analysis (dry weight basis) of the experimental diet for the larger size group of cod.

Feed group	Protein (%)	Lipid (%)	Ash (%)	Carbohydrates* (%) by difference	Digestible energy (MJ/kg)†
P34	36.3	18.4	8.3	37.0	18.5
P38	38.6	17.5	8.4	35.5	18.5
P42	42.5	15.8	9.3	32.3	18.2
P46	47.7	14.2	10.1	28.0	18,2
P50	51.7	12.7	11.0	24.6	18.0
P54	57.0	10.5	11.6	20.8	17.8

* Estimated from subtraction

†Calculated based on proximate analysis.

2.1.3. Measurements and sampling of fish

All the fish were anaesthetized (3 mg TMS; Syndel International Inc., in 10L of water), weighed and measured at the beginning of the experiment and then every 1-2 months throughout the experiments. The mean specific growth rate of fish in different tanks was calculated as:

$$SGR = 100 \times \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1}$$

Where: W_1 = biomass in tank at the beginning of period
 W_2 = biomass in tank at the end of period

The condition factor of the fish was calculated as:

$$\text{Condition factor} = 100 \times \frac{\text{Body mass}}{\text{Lenght}^3}$$

At the beginning and at the end of the experiments, nine fish from each tank were sacrificed for chemical and physical analysis. The total mass and gutted mass of these fish was recorded as well as the liver mass. The liver index was calculated as:

$$\text{Liver index} = 100 \times \frac{\text{liver mass}}{\text{body mass}}$$

Proximate analysis (protein, water, fat and ash) was performed on nine fish from each tank pooled into three samples. The amino acid profile and total amino acid content of the muscle of the larger fish fed the lowest (P34) and highest (P57) amounts of protein in the feed was investigated. The water holding capacity of muscle in the larger fish was measured at the end of the experiment.

2.1.4. Proximate analyses

Protein (Ghb-e-AM-903). The sample is digested in sulphuric acid in the presence of copper as a catalyst. The sample is then placed in a distillation unit (2400 Kjeltac Auto Sampler System) and the acid solution is made alkaline by a sodium hydroxide solution. The ammonia is distilled into boric acid and the acid is simultaneously titrated with diluted H₂SO₄. The nitrogen content is multiplied by the factor 6.25 to get % crude protein.

Ref. ISO 5983-1979.

Water (Ghb-e-AM-904). The sample is heated in a heating oven at 103°C +/-2°C for four hours. Water corresponds to the weight loss.

Ref. ISO 6496 (1983).

Ash (Ghb-e-AM-905). The sample is ashed at 550°C, and the residue weighed.

Ref. ISO 5984-1978 (E).

Fat (Ghb-e-AM-901a). The sample was extracted with petroleum ether, boiling range 40-60°C (2050 Soxtec Avanti Automatic System).

Ref. AOCS Official Method Ba-3-38 with modifications according to Application note Tecator no AN 301.

2.1.5. pH measurement

Fish mince (5 g) is mixed with 5 ml of ionized water and the pH measured in Radiometer PHM within 15 minutes from mincing of the samples.

2.1.6. Total amino acids

The fish and feed samples are analysed for total amino acid content (Analycen AB, Sweden). The samples are first oxidized for 16 hours and then hydrolyzed with 6M HCl for 23 hours. During the process the samples go through pH adjustment and filtration, and finally amino acid analysis (cation exchange chromatography) with different pH buffers as eluents.

2.1.7. Water holding capacity (WHC)

Analysis of WHC is based on a method described by Børresen (1980) but was modified by reducing the speed from 1500 g's to 500 g's. Raw samples (n = 3) were coarsely minced for approximately 20 s at speed 4 (Braun Electronic, type 4262, Kronberg, Germany). Approximately 2 g of the mince is weighed with accuracy into a test tube of a known weight and centrifuged at 530g for 5 min with temperature maintained at 2-5 °C (SS-34 rotor; Sorvall RC-5B, Du Pont, Delaware, USA). Two parallels are run for each sample. After centrifugation, the total weight of each test tube and sample is recorded and used to calculate sample weight. WHC was calculated as percentage remaining water of initial water in sample:

$$\text{WHC}\% = 100 \cdot \frac{v_1 - \Delta_r}{100 - \Delta_r}$$

$v_1 = (\text{Weight before drying} - \text{Weight after drying}) / (\text{Weight before drying}) * 100\%$

$\Delta_r = (\text{Weight before centrifugation} - \text{Weight after centrifugation}) / \text{Weight before centrifugation} * 100\%$

2.1.8. Statistics

All statistical analyses were performed with the SYSTAT using the GLM option with nested design. The significance limits were set at $p < 0.05$

2.2. Results

2.2.1. Smaller size group of cod.

The initial average body-mass of the fish was 37g and, during the experiment, the fish more than tripled in size (**Figure 1**). No significant difference in the final body-mass of fish fed different protein levels was observed (**Figure 1**). However, the SGR of fish fed high protein diets was significantly higher than that of fish fed the lowest protein levels (40% and 44%) (**Figure 2**). The feed conversion rate of fish fed low protein diets was significantly higher ($p<0,002$) than in fish fed high protein diets (**Figure 3**). The HSI in the group fed the lowest protein and highest lipid levels was more than twice as high as in the other groups (**Figure 4**).

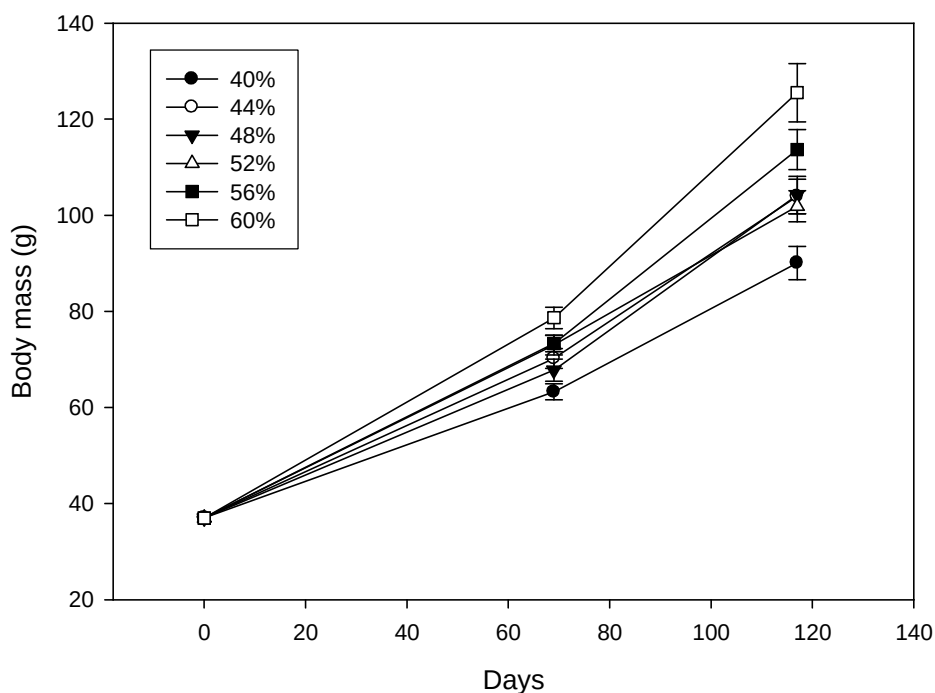


Figure 1. Growth of cod ($\pm$ SEM (smaller size group of cod) fed isocaloric diets with six different protein levels.

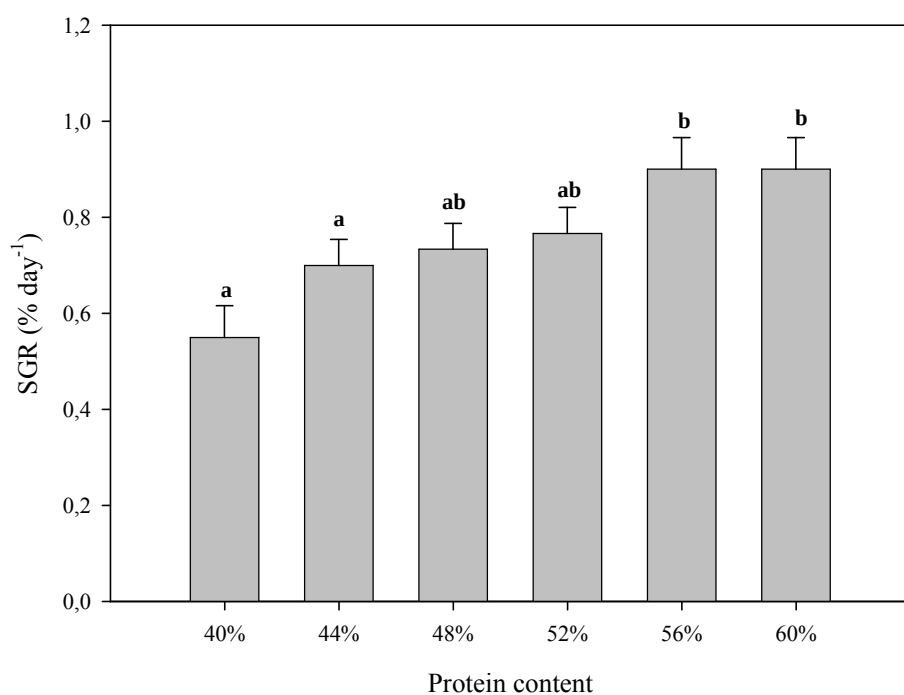


Figure 2. Specific growth rate ($\pm$ SEM) of cod from the smaller size group, fed six isocaloric feed formulations containing different protein levels. Bars identified with different labels are significantly different.

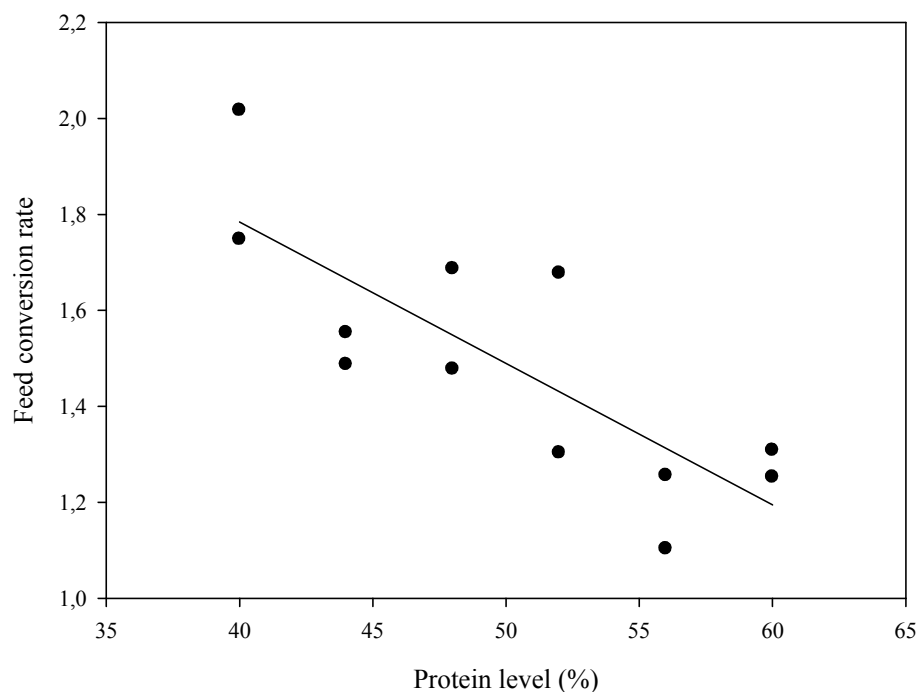


Figure 3. Feed conversion rate of the smaller size group of cod fed different protein levels ($p < 0.002$; R^2 : 0.647).

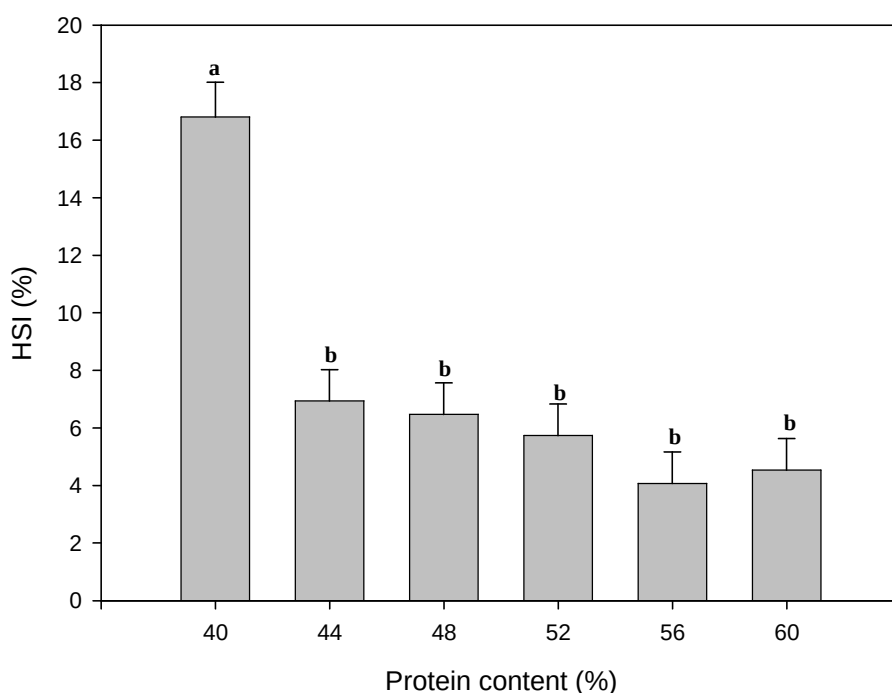


Figure 4. The hepatosomatic index of the smaller size group of cod fed different protein levels.

2.2.2. Larger size group of cod

Two experiments were performed with the larger fish. Although the first experiment was terminated because of an outbreak of *Vibrio* bacteria, similar results were obtained. In the second experiment, the fish more than doubled in size over a period of 160 days (Figure 5). No significant difference in the final body mass (Figure 5) or the growth rate (Figure 6. S) was observed in groups fed diets containing different protein content. Furthermore, no significant difference in the condition factor of the groups was observed (Figure 7).

The liver index increased during the experiment (Table 3). The HSI was significantly ($p < 0.0001$) higher in fish fed lower amounts of protein and a significant ($p < 0.002$) difference in the HSI values of different groups was observed when body mass was used as a covariate (Figure 8). The HSI was higher in groups fed diets containing lower protein and higher lipid levels and gutted weight of the fish increased with higher protein and lower lipid content of the feed (Table 3). The proximate analysis did not reveal any significant difference in the chemical composition or the physical properties of the fillets from fish fed different diets (Table 4).

The amino acid composition of the lowest (34%) and highest (54%) protein diets and the whole body tissue of fish fed the two different diets for 155 day are shown in table 5. The amino acid composition of three other species is also presented in the table for comparison (Wilson 1989). Although the difference in protein content in the experimental feed is as high as 20%, the amino acid composition in muscle does not seem to be affected.

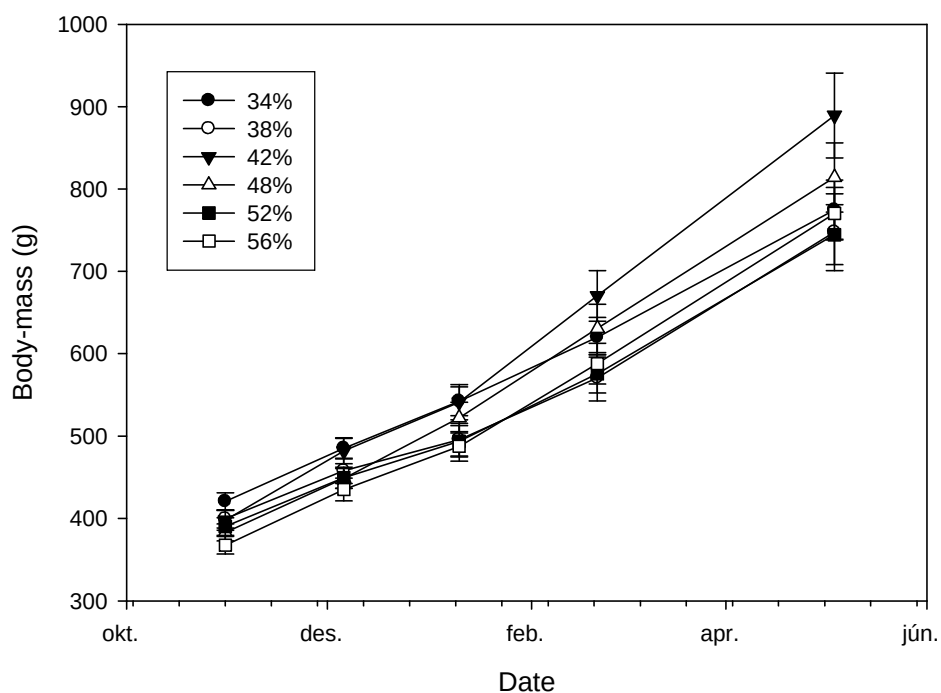


Figure 5. Growth of the larger size group of cod ($\pm$ SEM) fed isocaloric diets containing various protein levels.

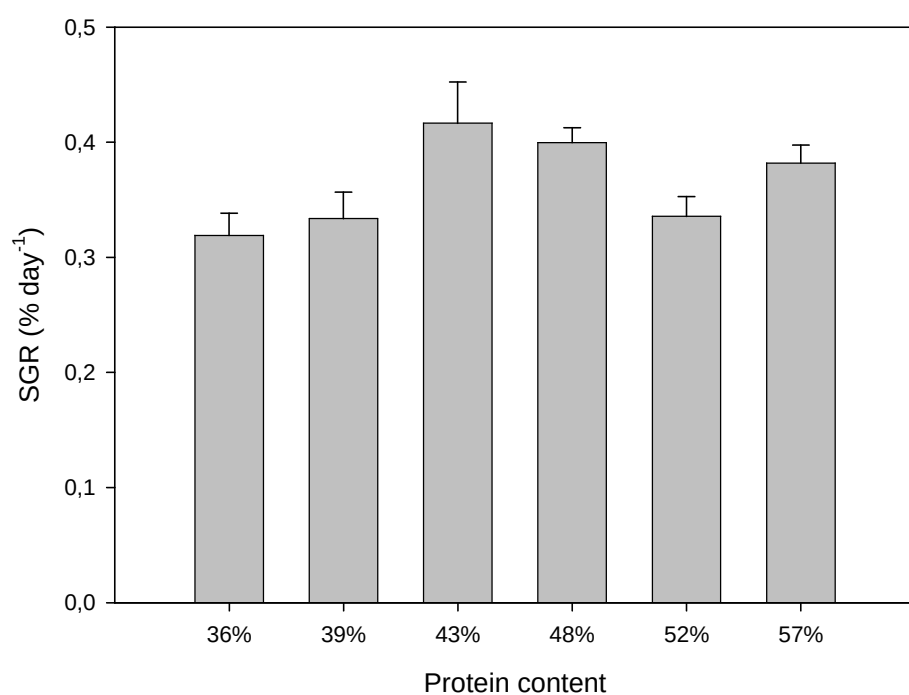


Figure 6. Specific growth rate ($\pm$ SEM) of larger size group of cod fed isocaloric diets with different protein levels.

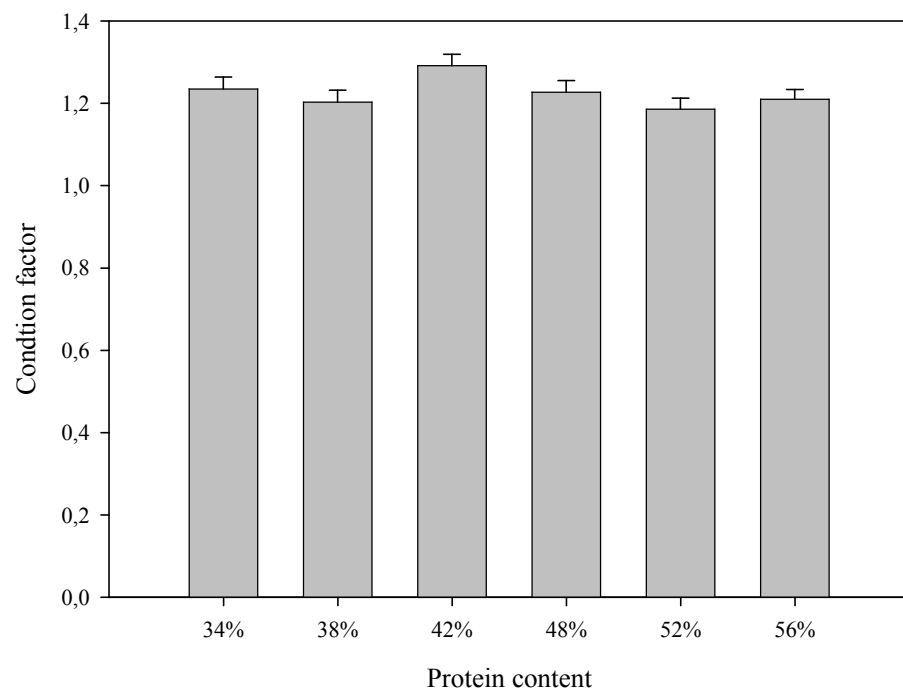


Figure 7. Final condition factor of larger size group of cod fed different protein levels.

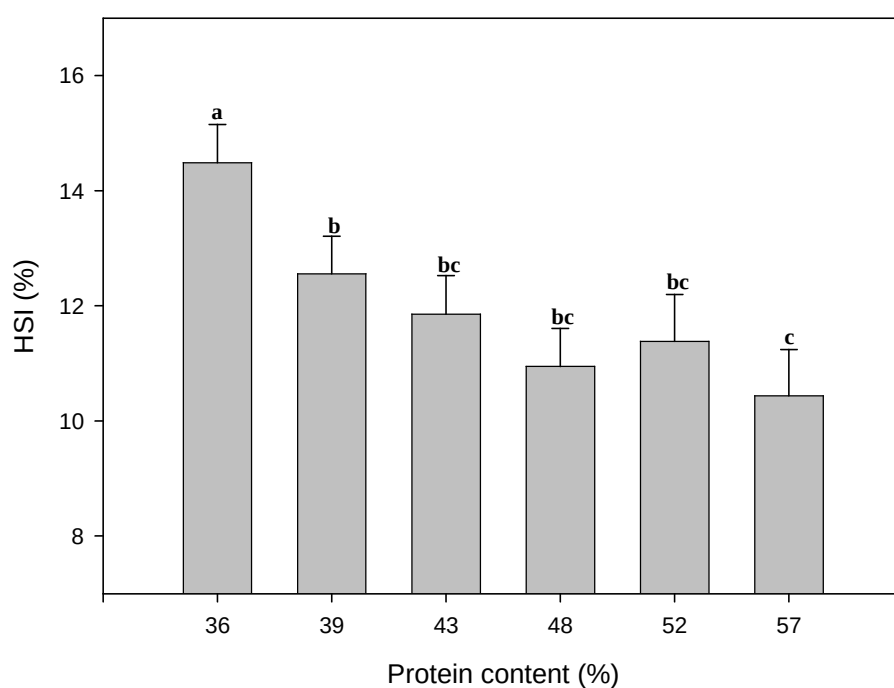


Figure 8. The size adjusted HSI of larger size group of cod fed different protein levels.

Table 3. Liver index % ($\pm$ Stdev) and gutted mass as percentage of total mass of the larger size group of cod at the end of the 155 day growth trial where cod was fed different protein levels (n=15 for each feed group).

	Protein content (%) in feed						
	Initial value	36	39	43	48	52	57
Liver index % (gutted fish)	10.0 $\pm$ 0.5	17.3 $\pm$ 4.5	16.1 $\pm$ 7.2	16.2 $\pm$ 4.0	14.1 $\pm$ 6.3	13.2 $\pm$ 4.1	14.3 $\pm$ 8.2
Gutted mass of total mass (%)	84.8	76.8	81.1	81.6	79.4	80.3	81.6

Table 4. Proximate analysis, pH and water holding capacity of samples from fillets of larger size group of cod at the end of the growth period

Protein content of diet	Protein (%±SD)	Lipids (%±SD)	Water (%±SD)	Ash (%±SD)	pH (mean±SD)	Water holding capacity (%±SD)
Intital value	18.5±0.9	0.3±0.0	80.4±0.5	1.3±0.0		
36 %	20,2 ±0,4	0,2 ±0,1	79,0 ±0,5	1,3 ±0,1	6,32 ±0,08	76,5 ±3,9
39 %	20,2 ±0,3	0,1 ±0,0	79,2 ±0,2	1,2 ±0,1	6,30 ±0,09	74,5 ±3,3
43 %	19,7 ±0,5	0, 2±0,1	79,6 ±0,5	1,2 ±0,0	6,26 ±0,03	70,9 ±3,8
48 %	19,6 ±0,2	0,2 ±0,0	79,6 ±0,0	1,2 ±0,0	6,33 ±0,16	74,3 ±3,1
52 %	19,8 ±0,1	0,2 ±0,0	79,4 ±0,1	1,2 ±0,0	6,34 ±0,18	74,0 ±2,1
57 %	20,0 ±0,4	0,2 ±0,1	79,3 ±0,4	1,2 ±0,0	6,30 ±0,08	74,4 ±3,3
Average	20,0 ±0,1	0,2 ±0,1	79,3 ±0,4	1,2 ±0,0	6,30 ±0,08	74,4 ±3,3

*The mean values for each group are based on three samples, each pooled from five fish.

Table 5. Lipid and water content (%) of liver in the larger size group of cod at the end of the growth period.

Feed Protein level (%)	Liver Lipid %	Liver Water %	Feed Lipid % (± 0.4) (<i>Table 2</i>)
36	67.4 ± 0.5	24.3 ± 0.4	16.0
39	68.7 ± 1.7	23.7 ± 1.0	16.9
43	66.2 ± 4.5	26.0 ± 3.2	13.1
48	68.4 ± 1.3	24.6 ± 1.1	12.4
52	67.9 ± 0.4	24.1 ± 0.6	10.5
57	67.5 ± 1.3	25.5 ± 1.3	8.9

Table 6. Amino acid composition* of feed and muscle samples of the larger size group of cod fed the lowest (34%) and highest (54%) protein content. Included in the table is the amino acid composition of three other farmed fish species for comparison.

Feed samples			Muscle samples			Amino Acid composition of certain fish species for comparison (Wilson 1989)		
			Initial (day 0)	At the end of growth trial				
	Feed P34	Feed P54	Muscle	Muscle P34	Muscle P54	Rainbow trout	Atlantic salmon	Channel catfish
Cystine	1.4	1.2	1.4	1.3	1.4	0.8	1.0	0.9
Methonine	2.7	2.9	3.4	3.5	3.4	2.9	1.8	2.9
Aspartic acid	9.7	10.0	10.6	10.7	10.6	9.9	9.9	9.7
Threonine	4.4	4.5	4.5	4.6	4.6	4.8	5.0	4.4
Serine	4.5	4.5	4.4	4.6	4.6	4.7	4.6	4.9
Glutamic acid	18.0	16.3	15.5	15.3	15.3	14.2	14.3	14.4
Proline	5.3	4.4	3.2	3.2	3.3	4.9	4.6	6.0
Glycine	6.0	6.2	4.9	4.7	4.8	0.8	1.0	0.9
Alanine	5.9	6.2	6.2	6.3	6.3	6.6	6.5	6.3
Valine	5.5	5.6	5.5	5.5	5.6	5.1	5.1	5.2
Isoleucine**	4.7	4.8	5.1	5.1	5.0			
Leucine	8.0	8.2	8.4	8.4	8.4	7.6	7.7	7.4
Tyrosine	3.3	3.4	3.9	3.9	3.9	3.4	3.5	3.3
Phenylalanine	4.3	4.3	4.3	4.3	4.2	4.4	4.4	4.1
Histidine	2.6	2.6	3.1	3.1	3.1	3.0	3.0	2.2
Ornithine**	0.2	0.2	0.0	0.0	0.0			
Lysine	7.3	7.8	9.5	9.4	9.3	8.5	9.3	8.5
Arginine	6.2	6.2	6.3	6.4	6.3	6.4	6.6	6.7
Hydroxyproline	< 0.1	0.5	< 0.1	< 0.1	< 0.1			

*The values are expressed as g/100 g amino acids

** References did not present these amino acid values for rainbow trout, salmon and catfish

2.3. Discussion

The results of these experiments suggest that the protein requirements of the larger size group of cod are lower than commonly used in commercial diets for cod. No difference in the growth rate of fish fed semi isoenergetic (Calculated DE varying from 17,8-18,5 MJ/kg) diets containing 34-54% protein was observed (**Figure 5**). These results are in accordance with findings in other studies, showing no difference in the growth rate of similar sized cod fed diets containing 40-60% protein (Helland pers. com. 2006) and they suggest that 34% protein in the diet is enough to maintain maximum growth for cod. However, it should be noted that the higher liver index of the fish fed lower protein diets may mask some of the effects of dietary protein on protein accretion

in the fish. The lipid content of the liver in the larger size group of cod neither reflects the protein nor the lipid content of the feed (**Table 5**), but individual differences in the lipid content of the liver have to be taken into account, as these were considerable.

As expected, the smaller fish appear to be more affected by dietary protein levels than the larger fish. The growth rate of the smaller fish was significantly higher in groups fed higher protein levels (**Figure 2**), although the final size of the fish was not significantly different from other groups (**Figure 1**). The results of this study suggest that the minimum protein levels that are required to maintain maximum growth in 35-70g cod are between 44% and 56% (**Figure 2**). The protein requirements of cod seem to be similar (slightly lower) to the protein requirements of Atlantic salmon of similar size, i.e. around 49% for 85 gram fish and 42% for 750 gram fish (Bendiksen 2005). The results also indicate that smaller cod may be able to compensate for low protein levels in the diet by increasing feed intake, which will of course result in poorer feed conversion (**Figure 3**).

The growth rate of the fastest growing groups of fish used in this experiment, was comparable to what is predicted by the growth model of Björnsson and Steinarsson (2002) for Icelandic cod, while of some of the groups grew at a slower rate. Protein requirements of cod could depend on the growth rate and therefore possible that the protein content of the feed may have a greater effect on growth of cod than these results indicate. However, the apparently low protein requirements of the larger fish are supported by results of other studies (Helland pers. com. 2006).

The different protein levels in feed did not affect the nutritional composition of the muscle at the end of the feeding trial. Consequently, no trend was seen that feed containing higher protein level resulted in higher protein content of flesh. The proximate analysis of the fish flesh therefore suggest that different protein levels in feed for cod do not affect the nutritional composition of the fish as food (**Table 2** and **Table 4**).

Water holding capacity is on the average $74.4\% \pm 3.3$. These are lower values than observed in a study by Tryggvadóttir *et al.* (2004), where the water holding capacity of farmed cod post rigor was on the average $80\% \pm 2.0$. For comparison, the water holding capacity of wild cod is usually around 87% (Tryggvadóttir *et al.* 2004). Tryggvadóttir *et al.* (2004) furthermore conclude that low water holding capacity of muscle of farmed cod could therefore explain the finding by sensory evaluations that farmed cod is found dryer and tougher compared to wild cod. The water

holding capacity of fish muscle was found to be similar in groups fed different protein levels (**Table 4**), with the exception of the fish fed 43% protein diet where the water holding values registered were considerably lower than in other groups ($70.9\% \pm 3.8$). Furthermore, the protein content of the experimental diets did not affect the amino acid composition of the flesh (**Table 6**).

To be able to reduce the protein content, the protein has to be replaced with one or more of the other macro-ingredients in the feed. To avoid a liver of excessive size, there are limitations to how much lipid there can be in the feed for cod. There is still uncertainty how much starch or carbohydrates the cod can tolerate before growth is affected. High inclusion of carbohydrates as replacement for protein and lipid will affect the energy content of the feed and thereby increase the feed conversion ratio (FCR). However, studies on replacement of fish meal with plant protein indicate however that cod tolerates relatively high amounts of fiber in the diet. Recent research in Norway has shown that cod can tolerate high ash content (up to 22%) in the diet without adverse effect on growth (Toppe *et al.* 2005, Toppe *et al.* 2006). More research is therefore needed on the tolerance for lipid, carbohydrates and ash in feed for cod, sparing the protein.

The cost of feed is between 40% and 60% of the total production cost in cod farming and protein is the most expensive part of the feed. Therefore, lowering the protein levels in feed will significantly reduce the production cost. The results of this and other studies suggest that the minimum protein levels for maximum growth of larger cod are even lower than 34%. However, cod appear to be able to compensate for lower protein levels in the feed by increasing feed intake (**Figure 3**). Therefore, the economical benefit from reducing the protein levels must be weighed against the possibility of having to increase the feeding rate and feed conversion efficiency. Further and more detailed studies are required to determine the optimum protein levels in feed for cod.

2.4. References

- AOCS Official Method Ba-3-38 with modifications according to Application note Tecator no AN 301.
- Árnadóttir, G.S. 2006. Fóðurþörf, vöxtur og efnaskipti þorsks í eldiskerfum. B.Sc ritgerð í fiskeldi og umhverfisfræði við Auðlindadeild Háskólans á Akureyri (in Icelandic).
- Arnason, J. 2004. Fóður og fóðurgerð fyrir Þorsk. In: Hafrannsóknastofnun. Fjölrit nr. 111 Björn Björnsson og Valdimar Ingi Gunnarsson (ritstj.) s. 121 – 126.
- Bendiksen, E. Å. 2005: Poster presented at Aquaculture Europe in Trondheim, August 2005.
- Björnsson, B and Steinarsson, A. 2002. The food-unlimited growth rate of Atlantic cod (*Gadus morhua*). Can. J.Fish.Aquat.Sci. 59:494-502.
- Bragadóttir, M., Pálmadóttir, H and Kristbergsson, K. Seasonal Changes in Chemical. 2002. Composition and Quality Parameters in Capelin (*Mallotus villosus*). Journal of Aquatic food Product Technology, Vol 1(3/4)
- Børresen, T. 1980. Nyutviklete metoder for bestemmelse av vannbindingsevne, saltvannbindingsevne og koketap I fiskemuskel. Rapport no 663. 1-7-2. 36 pp. Fiskeriteknologisk Forskningsinstitutt, Trondheim, Norway.
- Dersjant-Li, Y. 2002. The use of soy proteinin aquafeeds. Proceedings from Memórias del Sexto Simposium Internacional de Nutrición Acuicola, Sept 3-6, Cancun Quintana Roo, Mexico.
- Fallon, S and Enig, M.G. 2000. Newest Research on why you should avoid soy. Nexus Magazine, Volume 7(3).
- FAO 2002. The state of world fisheries and aquacultuer 2002. The Food and Agricultural Organization of the United Nations. Rome, Italy.
- Falch, E., Rustad, T., Jonsdottir, R. Shaw, N.B., Dumay, J., Berge, J.P., Arason, S., Kerry, J P., Sandbakk, M. and Aursand, M. 2006. Geographical and seasonal differences in lipid composition and relative weight of by-products from Gadidae species. Journal of Food Composition and Analysis, 19, 727–736.
- Helland S. 2006: Personal communication.
- Hemre, G-I., Karlsen, Ø., Mangor-Jensen, A. and Rosenlund, G.2003. Digestibility of dry matter, protein starch and lipid by cod, *Gadus morhua*: comparison of sampling methods. Aquaculture, 225, 225-232
- Hemre, G-I., Lie,Ø., Lied, E. and Lambertsen, G. 1989. Starch as an energy source in feed for cod (*Gadus morhua*): Digestibility and Retention. Aquaculture, 80, 261-270.
- Hertrampf, J.W. and Piedad-Pascual, F. 2000. Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic. Dordrecht, The Netherlands.
- Lall, S.P. and Nanton, D. 2002. Nutrition of Atlantic Cod. Bulletin of the Aquaculture Association of Canada 102(1), 23-26.

- Mørkøre, T. 2006. Relevance of dietary oil source for contraction and quality of pre-rigor filleted Atlantic cod, *Gadus morhua*. *Aquaculture* 251, 56-65.
- NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC.
- Refstie, S., Storebakken, T., Baeverfjord, G. and Roem A. 2001. Long-term protein and lipid growth of Atlantic salmon (*Salmo salar*) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid level. *Aquaculture* 193, 91-106.
- Rosenlund, G., Karlsen, Q, Tveit, K., Mangor-Jensen, A and Hemre, G.I. 2004. Effect of feed composition and feeding frequency on growth, feed utilisation and nutrient retention in juvenile Atlantic cod, *Gadus morhua* L.
- Sigurgísladóttir, S. and Pálmadóttir, H. 1993. Fatty Acid Composition of Thirty-Five Icelandic Fish Species., *Journal of American Oil Chemists' Society*, 70: 1081-1087.
- Storebakken, T., Refstie, S. and Ruyter, B. 2000. Soy products as fat and protein sources in fish feeds for intensive aquaculture, *In: Drackley, J.K. (Ed.), Soy in Animal Nutrition*. Federation of Animal Science Societies, pp. 127-170.
- Sugiura, S.H., Gabaudan, J., Dong, F.M. and Hardy, R.W. 2001. Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout fed soybean meal-based diets. *Aquaculture Research*, 32(7):583-592.
- Tacon, A.G.J. 1993. Feed ingredients for warm water fish. Fishmeal and other processed feedstuffs. FAO (The Food and Agriculture Organization of the United Nations). *Fish.Circ.*, No 856. Rome, Italy.
- Toppe, J., Aksnes, A., and Albriksen, S. 2006: Growth and tissue mineralization in Atlantic cod (*Gadus morhua*) fed diets with different levels of salmon bones. Presentation at the XII International Symposium Fish Nutrition & Feeding. Biarritz, France
- Toppe, J., Aksnes, A., Hope, B. and Albriksen, S. 2005. Inclusion of fish bone and crab by-products in diets for Atlantic cod, *Gadus morhua*. *Aquaculture*, *In press*.
- Torstensen, B.E., Lie, Ø. and Froyland, L. 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.) - effects of capelin oil, palm oil and oleic acid -enriched sunflower oil as dietary lipid sources. *Lipids* 6, 653-664.
- Tryggvadóttir, S.V., Thorkelsdóttir, A., Jónsson, Á. and Arnarson, G.Ö. 2004. Framtíðarþorskur: Gæðamat á eldisþorski (Future cod: quality evaluation of farmed cod). IFL report 10-04 (In Icelandic).
- Wilson, R.P. 1989. Amino Acids and Proteins. *In: Fish Nutrition* (ed. Halver, J.E.). Academic Press Inc. San Diego, California.
- Wilson, R.P. 2002. Amino acids and proteins. *In: Fish nutrition* (eds. Halve, J.E. and Hardy, R.W.), pp. 144-179. Academic Press San Diego.

3. The Norwegian part I - Akvaforsk

Fatty acid profile, sterol composition and product quality in farmed Atlantic cod (*Gadus morhua* L) fed extruded diets with soybean oil.

Results from this extensive study are reported in detail in previous sub-reports. In the following selected results are given.

3.1. Description of the study

The fish used were farmed Atlantic cod (*Gadus morhua* L.) with an initial body weight of 1.84 ± 0.01 kg. From August 26 until October 21, the cod were fed extruded dry feed (produced by DanaFeed) where the added oil was either marine fish oil (FO diet) or soybean oil (SO diet). Each diet was fed to duplicate net pens at AKVAFORSK research station, Averøy, Norway. During the period October – December, all fish were fed the FO diet. The crude fat content was 15.5% on average, while the crude protein content was 56%. The fish were fed to satiation once a day. No supplementary illumination was used. This study was carried out in connection with three other projects: A) A sub-study within a project on cost efficiency in cod culturing with emphasis on dietary composition financed by the Norwegian Research Council (NRC), B) a project on optimising seafood packaging financed primarily by the Norwegian industry, and C) a project focusing on optimising analyses of composition of farmed Atlantic cod using computer tomography (CT) financed by NRC.

Scientific partners involved and responsibility:

AKVAFORSK, Norway (Institute of Aquaculture Research). Responsibility for the study. Running the feeding trial, quality analyses of fresh and frozen samples. Supervision of two master students and one PhD student

Swedish University of Agriculture Science, SLU (Sweden). Responsible for designing sensory analyses of frozen baked samples and carrying out sterol analyses

Havforskningsinstituttet (Institute of Marine Research, Norway). Responsible for carrying out plasma analyses.

Institutt for husdyrfag og akvakulturvitenskaper (IHA, UMB, Norway). Performing X-ray analyses and supervision of one master student

MATFORSK (Norway). Responsible for MAP and vacuum packaging, and analyses of microbiology, GC-MS and sensory analyses of raw muscle

Industry involved and responsibility

DanaFeed (Denmark). Responsible for producing the experimental feeds

Coop (Sweden). Involved in sensory analyses of the frozen baked samples (consumer panel)

Kòsin Seafood (Faroe Islands). Responsible for processing the cod and for evaluating the farmed cod salt fish production (also evaluated by an external expert panel).

Students: Three master students and one PhD-student at University of Life Sciences, Norway.

3.2. Analyses

Every second week during the whole experimental period:

- Fresh fillets: Fat content, fatty acid profile in muscle and organs (liver, spleen, kidney, heart, gonads)
- Fresh and frozen/thawed fillets: dry matter, pH, texture, colour, water holding capacity in fresh and frozen fillets.

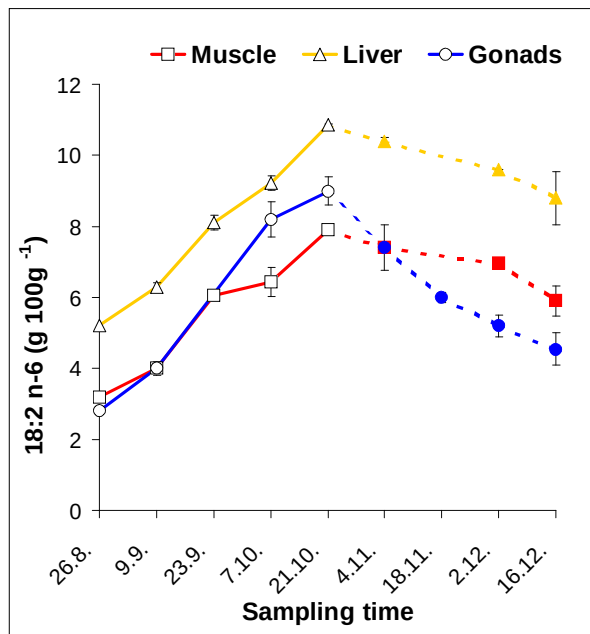
Additional analyses after eight weeks of feeding (October 21)

- rigor development
- post-mortem metabolism
- early quality changes (0 – 72hours after slaughter)
- Sterol composition
- microbiological, sensory and rheological stability during storage of fillets packed in modified atmosphere or vacuum
- suitability for salting
- sensory evaluation of baked fillets (after six and 12 months of frozen storage, consumer panel).
- Conformation and composition using X-ray CT

3.3. Important findings:

- Fatty acid profile was significantly altered in cod fed soybean oil where especially 18:2n-6 increased linearly during the feeding period. Incorporation of the characteristic “soy-fatty acids” was faster than recovery of the marine fatty acid profile during the “wash out” period. The rate of incorporation and dilution varied significantly between tissues
- Feeding cod soybean oil had no general negative effect on product quality of the following products: fresh fillets, fillets packed in vacuum, fillets packed in MAP, frozen/thawed fillets, baked fillets evaluated six and 12 months after frozen storage, or salted fish or fillets.

- Cod fed soybean oil had significantly faster rigor contraction and energy depletion *post mortem*. Early quality development differed between dietary treatments.
- Phytosterols were only detected in the soy feed and the main phytosterol was sitosterol followed by campesterol and stigmasterol. The liver analyses indicated a significant difference in cholesterol content between treatments. The soy oil fed cod had lower levels of cholesterol in the liver lipid. The other sterols were only found in trace amounts. Phytosterol oxidation products were not detected.
- Glucose level in plasma was higher in the FO group and triacylglycerol levels in plasma were higher in the FO group after eight weeks of feeding.
- Gonadal development was delayed in cod previously fed soybean oil
- Suitability of farmed cod for value added products seems to be good, also for cod fed soybean oil. However, our results clearly showed that value added methods used for captured cod are not directly transferable to farmed cod since farmed and captured cod differ in so many ways (body shape, muscle structure, muscle composition etc.).
- *Pre-rigor* filleted cod had higher liquid loss during storage when packed in MAP compared with *post-rigor* cod, which opens for further optimisation regarding time and methods for packaging (i.e. it is possible that the cod were packed too early after *pre-rigor* filleting). These results are especially interesting, as farmed cod has been regarded as a prima product at the fresh fish market in for example EU. No consistent dietary effects were found on shelf life of early packed cod fillets.
- Aquaculture opens unique possibilities to tailor the product composition. From the obtained results we suggest that further studies are conducted to evaluate further the possibility to control the content and composition of cod fillets in order to tailor the nutritional quality of cod as human food. For example the cholesterol level and fatty acid profile are interesting to look further into. Optimisations of processing and packaging methods are other issues that must be addressed in order to achieve long- term economical sustainability in cod culturing.



Development in 18:2n-6 and n-3/n-6 ratio in the lipid fraction extracted from muscle, liver and gonads of cod fed diets supplemented with soybean oil (8 weeks), followed by a wash out period (8 weeks) where the cod were fed a 100% fish oil based diet

4. The Norwegian part II – Institute of Marine Research

Effect of low quality protein sources on growth of Atlantic cod

Ørjan Karlsen & Rolf Erik Olsen, Institute of Marine Research, Norway.

4.1. Introduction

Interest in Atlantic cod (*Gadus morhua* L.) farming has increased during the last few years, and in parallel with increases in total production of carnivorous species in aquaculture the demand feed in the aquaculture industry has increased, simultaneously as traditional marine resources are exploited to the highest possible level. The market price for cod demands that the production cost being kept low, and one of the most important costs in cod farming is feed prices. High quality fishmeal (LT quality) is the most common protein source in feed for cod, and intensive fish farming is currently dependent upon the use of fishmeal as the sole or major source of dietary protein and lipid in formulated compound diets. There are alternatives to LT fish meal, as lower prices NS fishmeal, and vegetable sources.

As protein quality of fish meal will depend both on the freshness of the raw material used and the processing conditions in the manufacturing of the fish meal, the quality of fishmeal varies. NorSeaMink quality (NS) fishmeal differentiates from the Low Temperature (LT) fishmeal in that drying temperature is higher, and the quality is slightly lower in the NS which allows an upper limit of 90 mg Total Volatile Nitrogen (TVN) compared to the upper limit of 50 mg/100 g in the LT quality. The variability in quality is higher in NS meal. Gross chemical content are reasonable similar in both meals, with a protein content of about 70-71%, fat (Soxhlet) about 11-12 and ash about 14 %. Cadaverin content is higher in NS meal (1.8 g/kg compared to 1 g/kg in LT meal). In spoiled raw material, the concentration of cadaverine, histamine and putrescine increases during storage. Cadaverin has been shown to be a good indicator for raw material freshness (Aksnes and Brekken, 1988; Pike, 1993; Clancy et al., 1995) and this diamine is used commercially as a criterion to evaluate raw material freshness for fishmeal.

Fishmeal may vary considerably in quality and growth may be affected due to this variation. Pike et al. (1990) found an average of 15% increase in growth, while feed conversion ratios were reduced by 10% with the use of high quality fish meal compared with fish meal of fair average quality. A similar effect of fishmeal quality was observed in turbot (Danielssen et al., 1989). For wolf fish the effect of fish meal quality was shown to be more important (Moksness et al., 1995)

as specific growth rates were reported to be 0.3 and 0.5 %, and feed efficiencies were 0.45 and 0.83 for ‘medium’ quality and ‘good’ quality fish meals, respectively. Fish meal produced from stale raw material resulted in a 28% reduction in specific growth rate of salmon compared with fish that were fed fish meal produced from fresh raw material (Pike, 1993). Further, McCallum and Higgs (1989) and Pike et al. (1990) showed that increased processing temperature reduced both protein digestibility and growth performance of salmon.

The aim of the present work was to determine the impact of replacing LT fishmeal with NS fishmeal, and replacing LT fishmeal with Soya protein in diets for Atlantic cod.

4.2. Material and methods

Farmed Atlantic cod were fed 5 different diets in triplicate tanks for 13 weeks. The 1.5 m diameter and 1 m water depth round tanks were stocked with 60 fish each with a mean initial weight of 135 g. The tanks were equipped with a waste feed collection system, and the fish were fed a pre-weighed ration (in slight excess of satiation) once a day 7 days a week from 0900-1100, where after the feed not eaten were collected, dried at 65°C overnight and weighed. The fish originated from intensively reared cod at Institute of Marine Research Austevoll, and were kept on a continuous light regime prior to and during the experimental period. Mean temperature was 8.74 °C the first period, 8.78 the second period and 8.76 °C during the whole period.

The cod were measured individually at start (11. March 05), after 7 weeks (28. April 05), and finally after 13 weeks (9. June 05). At the final sampling samples were taken from intestines (fixated in McDowell's fluid for later histological analysis), and samples from liver and muscle were frozen in liquid nitrogen for later chemical analysis. These analyses are not completed and will, therefore not be presented here. The diets used are summarised in table 1.

Table 1: The replacement of LT protein in the diets.

Protein source	Diet				
	LT	NS25	NS50	NS75	SO
LT protein	100	75	50	25	75
NS protein	0	25	50	75	0
Soya	0	0	0	0	25

Due to an accident one of the LT tanks were lost in week 4 of the experiment, and consequently not included in any of the analysis.

Differences in size and condition between dietary groups were analysed using nested ANOVA, where tanks are nested under diet. Specific growth rate (SGR) and food conversion ratio (FCR) were compared using Kruskal-Wallis one-way ANOVA.

4.3. Results

The mortality was low and not related to feed type.

There was no difference in length or weight (Fig. 1) between tanks at start of the experiment (nested ANOVA, $p > 0.05$). During the first 7 weeks the fish grew to a mean of all tanks 182 g, and at the end the fish were 265 g. There was no significant difference in weight or length between diets after 7 or 13 weeks (nested ANOVA, $p > 0.05$). The condition factor was 1.10 at start, and increased to 1.11 and 1.18 after 7 and 13 weeks, respectively. There was no significant difference in condition factor between dietary groups at any of the measurements (nested ANOVA, $P > 0.05$).

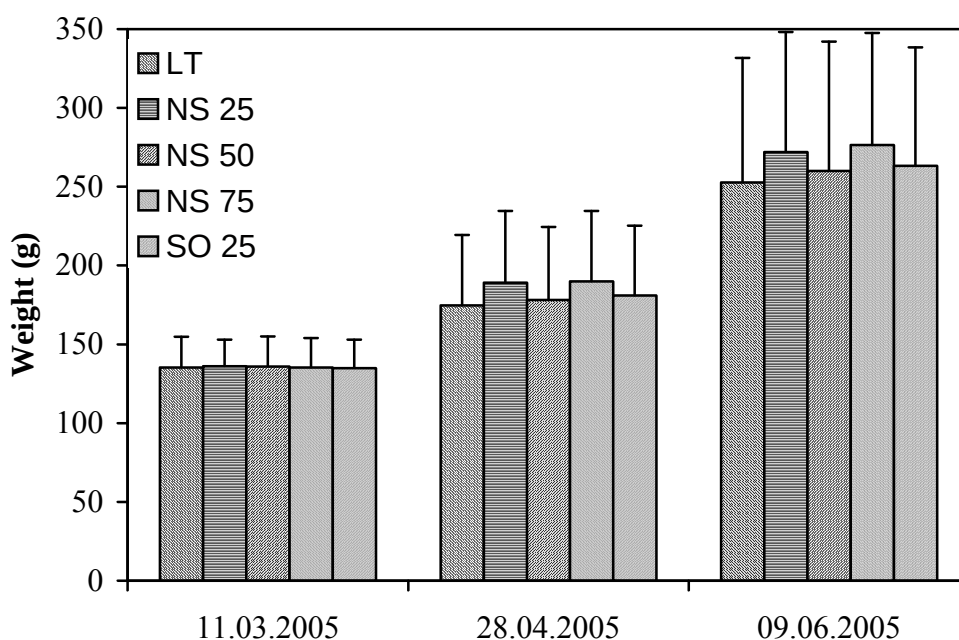


Figure 1. Weight as mean of the diets at start, after 7 weeks and after 13 weeks. Bars represents SD. Diet codes as in table 1.

The specific growth rate (SGR; %/day) was about 0.6 %/day the first period measured as the mean of all treatments, and 0.9 %/day the second period (Fig. 2). The SGR was unaffected by treatment in all periods (KW $P > 0.05$).

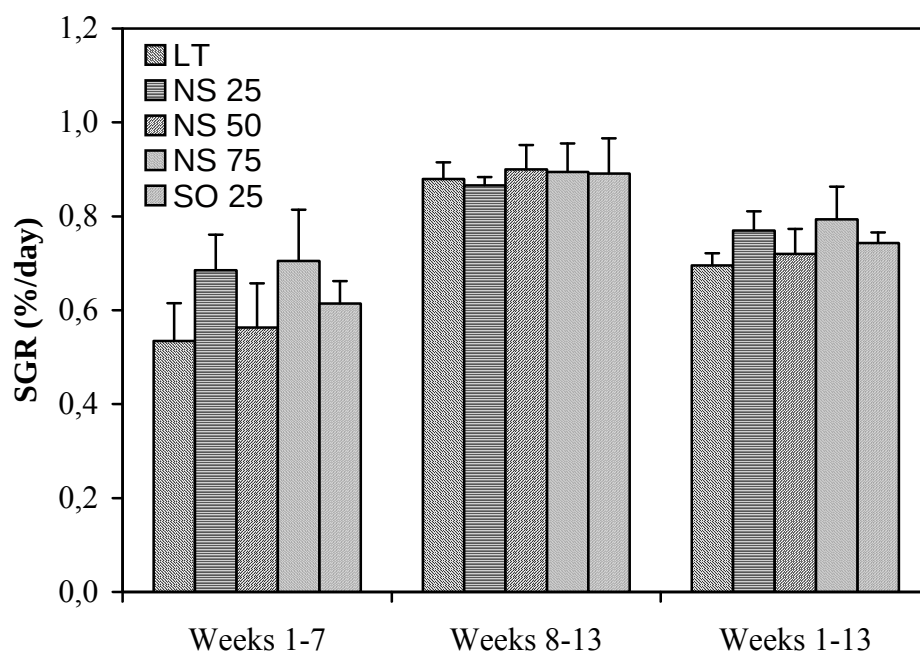


Figure 2: Mean SGR for the diets for the experimental periods. Bars represents SD. Diet codes as in table 1.

The energy stores at the end of the experiment (13 weeks) was not related to dietary treatment, (nested ANOVA, $p > 0.05$), with a mean of about 12 % (Fig. 3).

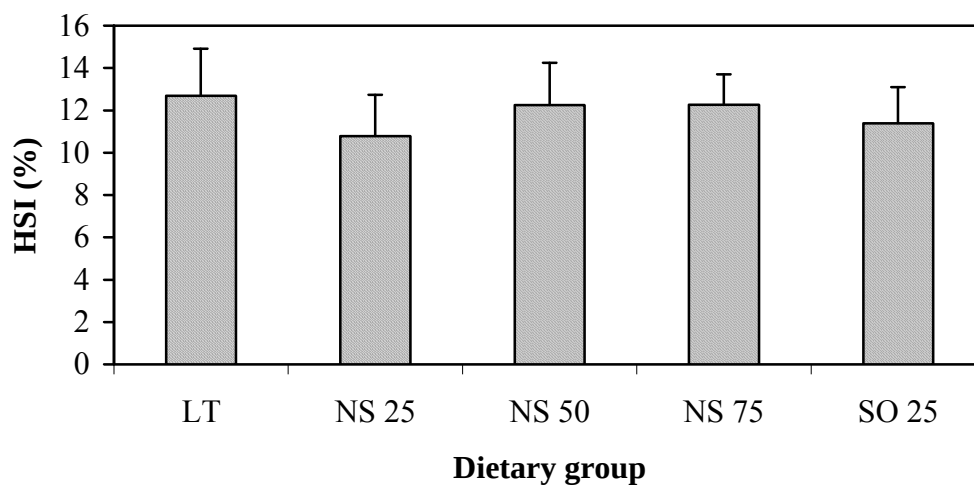


Figure 3: HSI (%) for the mean of the dietary groups at end of the experiments. Bars represents SD. Diet codes as in table 1.

Food conversion (gram food eaten/gram weigh gain) ranged between 0.84 and 0.90 (Fig. 4), and was not significant different (KW $p > 0.05$) between dietary groups

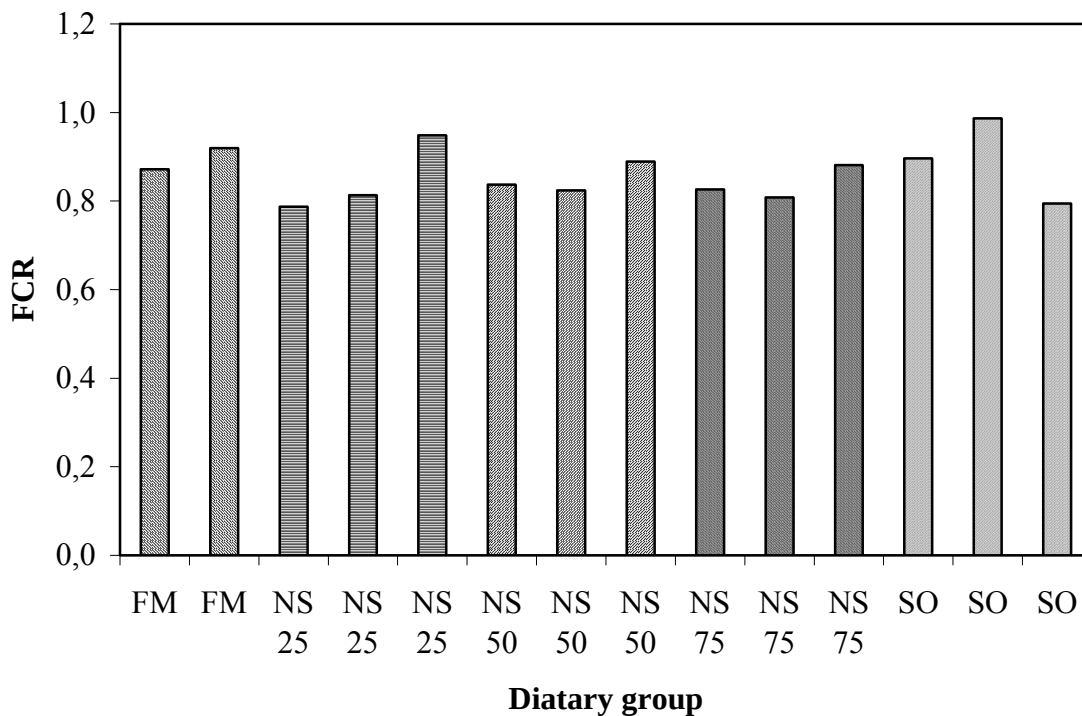


Figure 4. Feed conversion ratio for the individual tanks during the whole experimental period. Diet codes as in table 1.

4.4. Discussion

The replacement of high quality LT fish meal with more variable NS fish meal had no significant effect on mortality, growth, liver indexes or feed conversion. Previous investigations have shown that the use of low quality fish protein in diets for different fish species resulted in inferior growth compared to high quality fish meal (op. cit.). The lack of any significant effect on growth in our study may be due to two factors; either that the NS meal used in the diets was of relatively high quality, or that cod are able to utilise low quality NS meal better than expected.

Anderson et al. (1993) reported that the true availability of amino acids to Atlantic salmon could vary from 84.9 to 94.3% in trials with four different commercial fish meals, and Romero et al. (1994) found that true protein digestibility, measured in rainbow trout, could vary from 84.5 to 97.0% in 27 samples of fish meal. Clancy et al. (1995) did not find any or only small effects of either raw material freshness or processing temperature on protein digestibility. Cod may compensate for less than optimal dietary composition by increased feed intake and FCR

(Rosenlund et al., 2004). No increased FCR was not observed in our study, and may therefore indicate that the NS meal used in the diets were of relatively high quality.

Replacement of LT fishmeal with Soya protein did not affect any of the analysed factors. This is in accordance with previous studies in cod (Hansen et al., 2005a,b).

Our preliminary conclusion is therefore that it is possible to replace some of the high quality fishmeal with lower quality fishmeal, or Soya protein without compromising growth. Final conclusions await chemical analysis of blood, liver and muscle, and histological examination of the gut.

4.5. References

- Aksnes, A. & Mundheim, H. (1997). The impact of raw material freshness and processing temperature for fish meal on growth, feed efficiency and chemical composition of Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* 149: 87-106.
- Aksnes, A. and Brækken, B. (1988). Tissue degradation, amino acid liberation and bacterial decomposition of bulk stored capelin. *J. Sci. Food Agric.* 45: 53-60.
- Anderson, J.S., Lall, S.P., Anderson, D.M. & McNiven, M.A. (1993). Evaluation of protein quality in fish meals by chemical and biological assays. *Aquaculture* 115: 305-325.
- Clancy, S., Beams, R., Higgs, D., Dosanjh, B., Haard, N. & Toy, B. (1995). Influence of spoilage and processing temperature on the quality of marine fish protein sources for salmonids. *Aquaculture Nutrition* 1: 169-177.
- Danielssen, D.S., Gulbrandsen, K.E. & Hjertnes, T. (1989). Fish meal quality in dry feed for turbot (*Scophthalmus maximus* L.). *EAS Spec. Publ. No. 10*, pp. 83-84.
- Hansen, A.-C., Rosenlund, G., Karlsen, Ø., Olsvik, P. & Hemre, G.-I. (2006a). The inclusion of plant protein in cod diets, its effects on macronutrient digestibility, gut and liver histology and heat shock protein transcription. *Aquaculture Research*, 37: 773-784.
- Hansen, A.-C., Karlsen, Ø., Rosenlund, G., Rimbach, M. & Hemre, G.-I. (2006b). Dietary plant-protein utilisation and its effects on metabolism in Atlantic cod, *Gadus morhua*. *Aquaculture Nutrition*, in press.
- McCallum, I. and Higgs, D., 1989. An assessment of processing effects on the nutritive value of marine protein sources for juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* 77: 181-200.

- Moksness, E., Rosenlund, G. and Lie, O.. 1995. Effect of fish meal quality on growth of juvenile wolffish, *Anarhichas lupus*. Aquaculture Research 26: 109-115.
- Pike, I.H. (1993). Freshness of fish for fish meal - effect on growth of salmon. In: S.J. Kaushik and P. Luquet, (Editors). Fish Nutrition in Practice. INRA, Paris, pp. 843-846.
- Pike, I.H., Andorsdottir, G. & Mundheim, H. (1990). The role of fish meal in diets for salmonids. IAFMM, Techn. Bull. No. 24, March, 35 pp.
- Romero, J.J., Castro, E., Diaz, A.M., Revoco, M. & Zaldivar, J. (1994). Evaluation of methods to certify the 'premium' quality of Chilean fish meals. Aquaculture 124: 351-358.
- Rosenlund, G., Karlsen, Ø., Tveit, K., Mangor-Jensen, A & Hemre, G.-I. (2004). Effect of feed composition and feeding frequency on growth, feed utilisation and nutrient retention in juvenile cod, *Gadus morhua*. Aquaculture Nutrition, 10.



norden

Nordic Innovation Centre

Nordic Innovation Centre

The Nordic Innovation Centre initiates and finances activities that enhance innovation collaboration and develop and maintain a smoothly functioning market in the Nordic region.

The Centre works primarily with small and medium-sized companies (SMEs) in the Nordic countries. Other important partners are those most closely involved with innovation and market surveillance, such as industrial organisations and interest groups, research institutions and public authorities.

The Nordic Innovation Centre is an institution under the Nordic Council of Ministers. Its secretariat is in Oslo.

For more information: www.nordicinnovation.net