Project Report
31 - 06

Rannsóknastofnun fiskiðnaðarins

NOVEMBER 2006

Protein requirements of farmed cod

Rannveig Björnsdóttir, Rf
Helgi Thorarensen, Hólaskóli
Jón Árnason, Fóðurverksmiðjan Laxá
Soffía Vala Tryggvadóttir, Rf

AVS rannsóknasjóður í sjávarútvegi

Skýrsluágrip Rannsóknastofnunar fiskiðnaðarins

Icelandic Fisheries Laboratories Report Summary

Titill / Title	**Protein requirements of farmed cod**		
Höfundar / Authors	*Rannveig Björnsdóttir, Helgi Thorarensen, Jón Árnason, Soffía Vala Tryggvadóttir*		
Skýrsla Rf /IFL report	31 - 06	*Útgáfudagur / Date:*	November 2006
Verknr. / project no.	1585		
Styrktaraðilar / funding:	*AVS Research Fund, The Nordic Innovation Centre (NICe)*		

Ágrip á íslensku:

Þorskur er nýleg eldistegund og brýnt að unnt verði að þróa fljótt og vel hagkvæmt eldi á tegundinni. Þær þjóðir sem tekið hafa þátt í þróun þorskeldis eru mikið til að glíma við sömu vandamálin og viðfangsefnin því svipuð. Það var því mjög þarft framtak þegar sótt var um styrk til Norræna iðnþróunarsjóðsins með það að markmiði að stofna netverkefni sem sameinaði krafta þeirra þjóða sem hafa hagsmuna að gæta á þessu sviði.

Fóðurkostnaður er um 40-60% af heildarframleiðslukostnaði í þorskeldi og þótti því brýnt að einbeita sér að fóðurmálum. Með verkefninu var stefnt að því að allir legðust á eitt við að þróa hagkvæmara fóður fyrir þorsk. Við þróun ódýrara fóðurs þarf að huga að því að fóðrið innihaldi þá næringu sem fiskurinn þarf og gefi jafnframt hraðan vöxt, heilbrigðan fisk og hágæðaafurð.

Aðilar að verkefninu voru Rannsóknastofnun fiskiðnaðarins, Fóðurverksmiðjan Laxá hf, Hólaskóli, SR mjöl, Háskólinn á Akureyri, Brim fiskeldi og Primex. Norskir samstarfsaðilar voru Akvaforsk, Havforskningsinstituttet og Fjord Marin. Frá Færeyjum komu P/f Fiskaling og Kosin Seafood að verkefninu. Þátttakkendur frá Danmörku voru Danafeed, DIFRES og Tripple nine, og sænskir þátttakendur voru SLU og COOP-Sverige. Þarna tóku því saman höndum rannsóknastofnanir, háskólar og fyrirtæki sem vildu leggja sitt af mörkum til að stuðla að því að þorskeldi geti orðið arðbær atvinnuvegur.

Þar sem norræni styrkurinn var fyrst og fremst ætlaður til að standa straum af samskiptum þátttakenda þ.e. ferðum, fundum, samræmingu og stjórnun verkefnisins, var gert ráð fyrir að þáttakendur hver í sínu landi fjármögnuðu framkvæmd tilrauna með styrkumsóknum í innlenda sjóði eða með öðrum hætti. AVS rannsóknasjóður í sjávarútvegi styrkti rannsóknahluta Íslands, en markmið íslenska hluta verkefnisins var að finna kjörpróteininnihald fyrir tvo stærðarflokka af þorski þ.e. annars vegar 30-100g og hins vegar 300-500g þorsk. Þess var gætt í fóðurtilrauninni að orkuinnihald mismunandi tilraunafóðurs væri svipað. Prótein er dýrasta næringarefnið í fóðri fyrir fisk og þar af leiðandi mjög mikilvægt að lámarka innihald þess þannig að það fari fyrst og fremst til uppbyggingar á vöðum en ekki til orkunotkunar, þar sem ódýrari næringarefni svo sem fita geta komið að svipuðum notum.

Niðurstöður tilraunanna gáfu til kynna að próteinþörf stærri þorsksins (300-500g) sé minni en það sem venjulega er notað í verksmiðjuframleiddu fóðri í dag, en enginn munur kom fram á vaxtarhraða þorsks sem alinn var á fóðri sem innihélt frá 34-54% prótein. Eins og búast mátti við var próteinþörf minni fisksins (30-100 g) meiri en hjá þeim eldri en tilraunin sýndi að lágmarksþörf próteins í fóðri fyrir 30-100 g þorsk er á milli 44 and 56% og þarf að rannsaka betur hvar mörkin liggja . Mismunandi próteinhlutfall í fóðri hafði ekki áhrif á næringarefnainnihald fisksins sem neysluvöru.

Lykilorð á íslensku: *Fiskeldi, eldisþorskur, fóður, þorskfóður, próteinþörf, vaxtarhraði*

Summary in English:

There is a considerable and growing interest in cod farming in many countries around the N-Atlantic. Similar problems have been encountered in farming of the Atlantic cod in all countries and a trans-national research co-operation is preferred to speed up the development of this industry for the benefit of everyone. One such collaborative network was established through funding from the Nordic Innovation Centre and through national funding sources.

The cost of feed is 40-60% of the total cost of production in cod farming and any reduction of feed cost will therefore significantly reduce the production cost. The aim of the Nordic cooperation was to bring together the interested parties with the aim to develop a more profitable feed for cod.

The participants from Iceland were the Icelandic Fisheries Laboratories (IFL), Laxá Feed Mill Ltd, Holar Agricultural College (HAC), SR mjöl Ltd, The University of Akureyri (UnAk), Brim aquaculture and Primex. The Norwegian partners were: Akvaforsk (Institute of Aquaculture Research), Havforskningsinstituttet (Institute of Marine Research) and Fjord Marine Seafood. The partners from the Faroe Islands were P/f Fiskaling A/S and Kosin Seafood. Danafeed A/S, DIFRES and Tripple Nine participated from Denmark and the Swedish partners were Swedish University of Agricultural Sciences (SLU) and Coop Sverige. The aim of this cooperation was to join research institutes, universities and industries in an attempt to contribute to a more profitable cod farming industry.

The funding from NIC´s was mainly intended for synergy, i.e. travel, meetings and administration expenses of the project. The participants were therefore expected to seek additional funding of their respective research from funds within the individual Nordic countries or from other sources.

The AVS research fund in Iceland supported the research part of the Icelandic participants. The aim of the Icelandic part was to optimize the protein content in feed for two groups of cod of different sizes, i.e. 30-100g and 300-500g, respectively. Protein is the most expensive macronutrient in feed for fish and therefore it is very important to minimize the protein content without compromising the growth and well being of the fish.

The results indicate that the protein requirements of the larger size group of cod is less than previously believed. In fact, they require less protein than is presently used in commercial feed for cod. As expected the need for protein was higher in the smaller size group of cod (30-100 g), but the minimum need for protein in feed for 30-100 g cod was shown to be between 44 - 56%. The protein content of the feed did not appear to affect the quality and nutritional value of the fish.

English keywords: *Aquaculture, cod farming, fish feed, cod feed, protein requirements, growth rate*

Table of contents

1. Introduction

Farming of Atlantic cod (*Gadus morhua*) is growing and is expected to become an important industry for costal communities around the North Atlantic in the future. 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 until they reach market size, (3) cod are reared from eggs in hatcheries and 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 for farmed fish must support a high growth rate and result in healthy fish of high quality. However, information on the nutritional requirements of cod has been lacking. Feed represents around 60% of the total production cost in cod farming and any changes in the cost of feed will have a significant impact on the total cost of production. It is imperative for the future growth of cod farming to reduce the cost of feed while maintaining good growth rate of the fish. Therefore, it is necessary to spend appreciable effort into 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. Therefore, it is important to limit the protein content of the diet. The protein requirements of fish vary with size (Bendiksen 2005; NRC 1993; Hertrampf and Piedad-Pascual 2000) and, different feed formulations may therefore be necessary for fish of different sizes. 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). High starch content of feed may limit growth and feed conversion in cod (Hemre *et al.* 2003), although formulations with up to 18% of high quality starch do not appear to limit digestibility in cod. However, results from other studies on protein requirement for cod of different size are rather conflicting (Arnason 2004).

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 a limited availability of carbohydrates in the marine environment. Capelin seems to be the most important feed source for large wild cod (Tacon

1993), which 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.

Protein is the most expensive nutritional factor in the feed and currently most of the protein in fish feed comes from fishmeal. Fishmeal is an expensive protein source for feed. Moreover, the market prices of fishmeal tends to fluctuate, depending on the variable catches of pelagic species used for making the fishmeal. The quality of fishmeal can affect the growth of the fish and feeding cod with a low quality fishmeal can lead to an increased lipid deposition in the liver and compromise the slaughter quality of the cod (Albrektsen *et al.* in press).

The aim of this project was to define the minimum need for protein in the feed for maximum growth of cod of different sizes (50 -100 grams and 300 – 500 grams). This was done in dose/response trials with graded amounts of crude protein in the feed and with high quality capelin meal as the protein source in iso-energetic diets.

2. Materials and methods

2.1. Fish and rearing conditions

Fish for all experiments were obtained from the Marine Research Institute in Staður, Grindavík and brought to the Holar University College facility in Sauðárkrókur where the experiments were conducted. In each experiment, the fish were distributed randomly into 18 tanks with each feed formulation 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, both at the beginning and at the end of each experiment as well. 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 the experiments, the fish were fed in excess. The smaller fish were fed manually twice each day until uneaten feed pellets were clearly visible in the tank. The larger fish were fed through automatic feeders as well as manually 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.

2.2. Feed and feed formulation

Six different types of iso-energetic feeds were formulated for each experiment. The feed for the smaller size group of fish contained 40%, 44%, 52%, 58% and 60% protein, all from capelin meal (Table 1). However, proximate analysis of the feed showed that the actual protein content was slightly higher (Table 2). Similarly, the feed for the larger fish was formulated to contain 36%, 39%, 43%, 48%, 52% and 57% protein from capelin meal (Table 3), whereas the actual protein content turned out to be somewhat higher for most formulations (Table 4).

The total energy of the feed was estimated from the nutrient content: Protein, 23.7 MJ/kg, lipids 39.5 MJ/Kg and carbohydrates 17.2 MJ/Kg. The digestible energy was calculated based on the estimates of total energy: protein 20.1 MJ/Kg (85% og total energy), lipids 39.5 MJ/Kg (91% of total energy) and carbohydrates 12.4 MJ/Kg (72% of total energy).

The formulation of the feed for the smaller cod is shown in Table 1 and the actual values following proximate analysis of the feed in Table 2. Similarly, the formulation of the feed for the larger size group of cod is shown in Table 3 and the actual values following proximate analysis in Table 4.

Table 1. Feed formulation (wet weight basis) model used in the experimental diets for the smaller cod.

Feed group	Dry matter %	Crude protein %	Crude fat %	Starch %	Ash %	*DE MJ/kg
P40	89.8	40.0	18.0	18.0	8.9	17.3
P44	89.7	44.0	17.3	18.0	9.0	17.3
P48	89.7	48.0	15.2	16.2	9.3	17.0
P52	89.7	52.0	13.7	13.9	9.6	17.0
P56	89.7	56.0	12.3	11.5	9.9	17.0
P60	89.7	60.0	10.8	9.3	10.2	17.0

*Estimated from formulation

Table 2. 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 3. Feed formulation model used in the experimental diet for (wet weight basis) the larger size group of cod.

Feed group	Dry matter (%)	Protein (%)	Lipids (%)	Starch (%)	Total energy* (MJ/kg)	Digestible energy* (MJ/kg)
P34	91.7	34.0	20.2	23.7	21.0	17
P38	92.6	38.0	19.2	21.5	20.9	17
P42	93.0	42.0	17.9	19.0	20.8	17
P46	90.2	46.0	16.7	16.5	20.7	17
P50	91.8	50.0	15.4	14.0	20.7	17
P54	91.4	54.0	14.2	11.5	20.6	17

*Estimated from formulation

Table 4. Proximate analysis (dry weight basis) of the experimental diet for the larger size group of cod (as fed basis).

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.

2.3. Measurements and sampling of fish

At the beginning of all experiments, fish were anaesthetized (3 mg TMS; Syndel International Inc., in 10L of water), weighed and measured, 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 slaughtered 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. 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 Kjeltec 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 an 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.

2.5. 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.

2.6. 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.

2.7. Water holding capacity (WHC)

Analysis of WHC is based on a method described by Børresen (1980), 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 the percentage of remaining water of the initial water in the sample:

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

v1 = % water in sample before centrifugation = (Weight before drying-Weight after drying) / (Weight before drying) x 100%

Δr = (Weight before centrifugation - Weight after centrifugation) / Weight before centrifugation × 100%

2.8. Statistics

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

3. Results

3.1. 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).

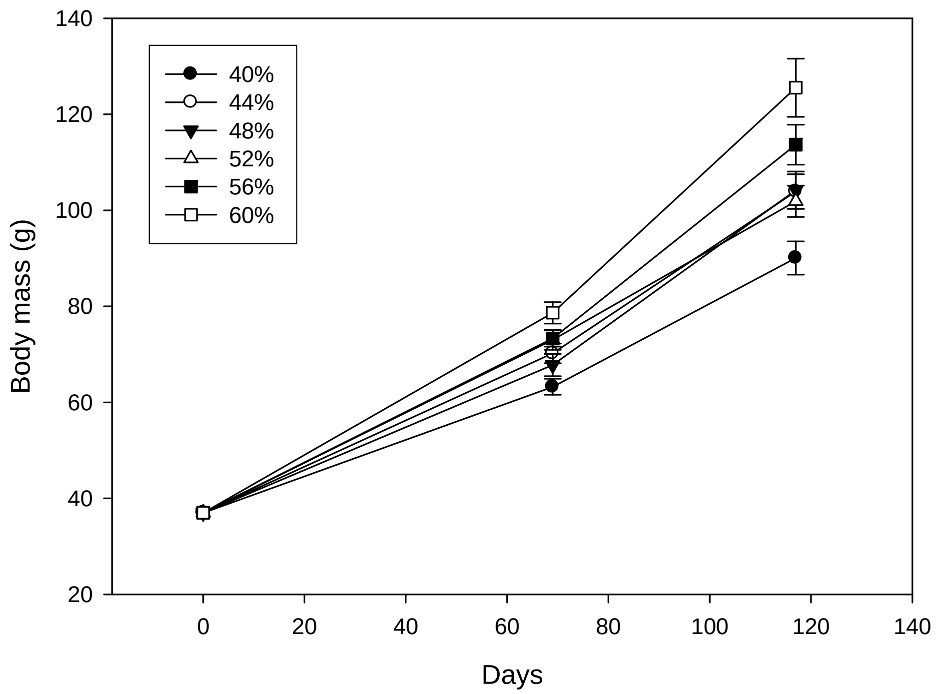

Figure 1. Growth of cod (±SEM (smaller size group of cod) fed isocalorific diets with six different protein levels.

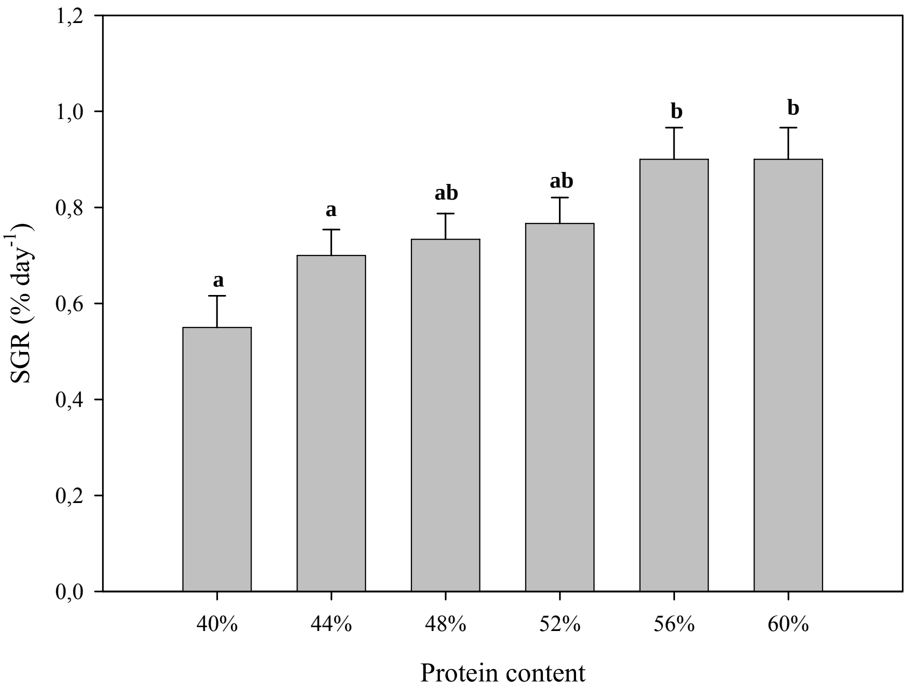

Figure 2. Specific growth rate (±SEM) of cod from the smaller size group, fed six isocaloric feed formulations containing different protein levels. Bars identified with different labels are significantly different.

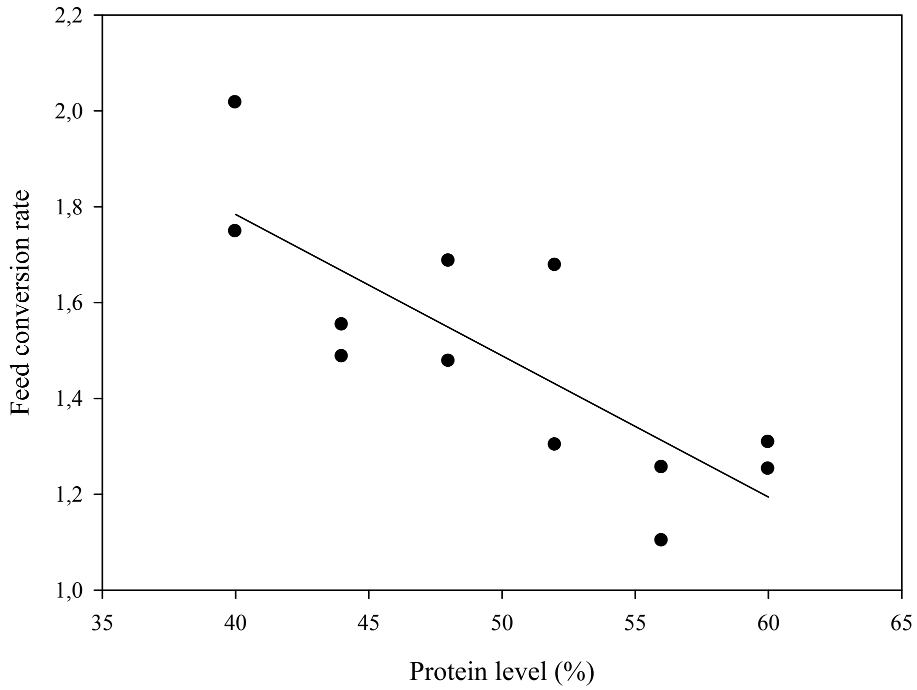

Figure 3. Feed conversion rate of the smaller size group of cod fed different protein levels (p<0.002; R^2: 0.647).

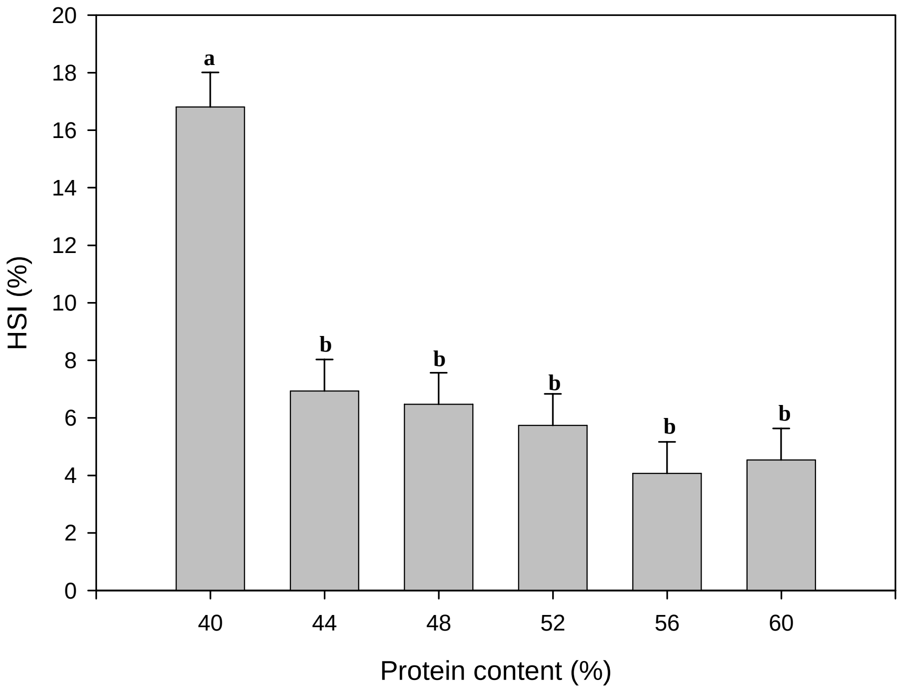

Figure 4. The hepatosomatic index of ther smaller size group of cod fed different protein levels.

3.2. 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 a repeated experiment. In the repeated experiment, the fish more than doubled in size over a period of 160 days (Figure 5). There was no significant difference in the final body mass (Figure 5) or the growth rate (Figure 6) of the fish fed diets containing different protein levels. The feed intake was higher in fish fed low protein diet compared with fish fed high protein diet (Figure 7). No significant difference in the condition factor of different groups was observed (Figure 8).

The liver index increased during the experiment (Table 5). The HSI was significantly ($p<0.0001$) higher in fish fed low amounts of protein and more lipids when body mass was used as a covariate. 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 5) 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 6).

The amino acid composition of the lowest (36%) and highest (57%) protein diets and the whole body tissue of fish fed the two different diets for 155 day are shown in Table 8. 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.

The proportional lipid content was similar in all groups (Table 7). The average proportion of fat in liver of all groups was 67.7 % ± 0.9%.

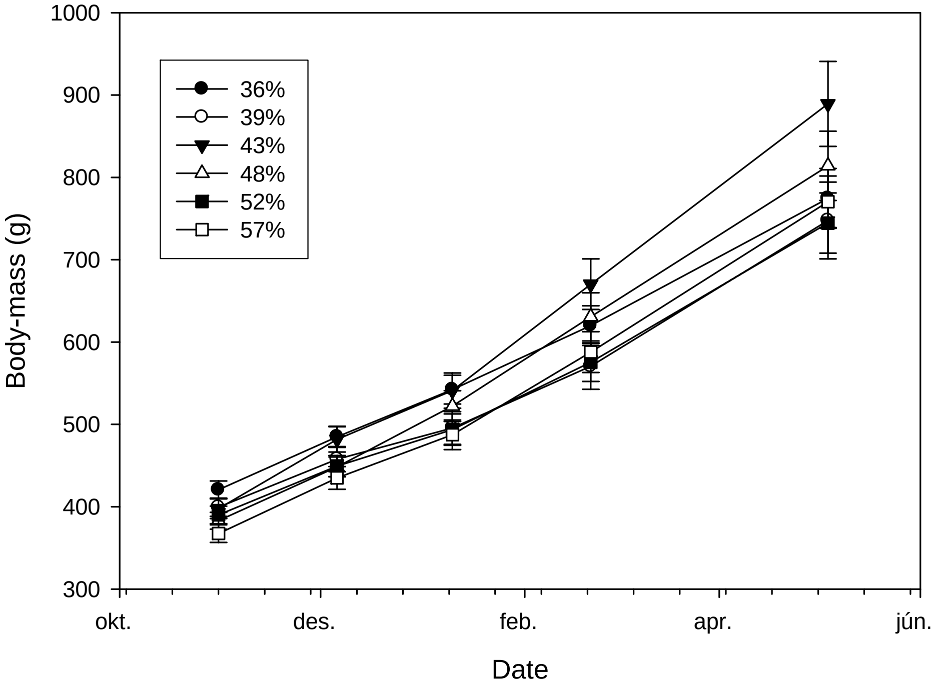

Figure 5. Growth of the larger size group of cod (±SEM) fed iso-caloric diets containing various protein levels.

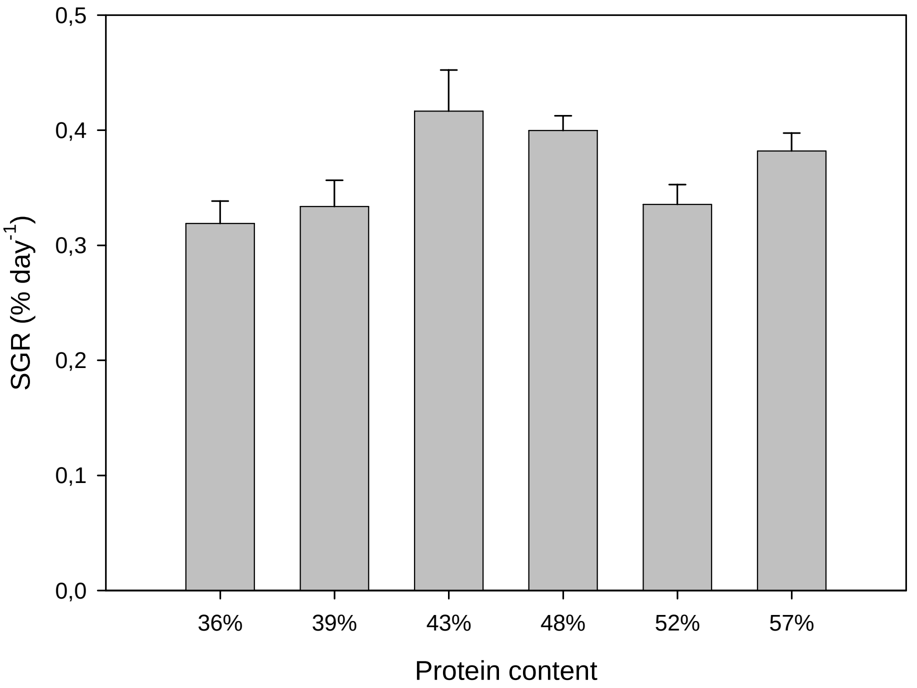

Figure 6. Specific growth rate (±SEM) of larger size group of cod fed iso-caloric diets with different protein levels.

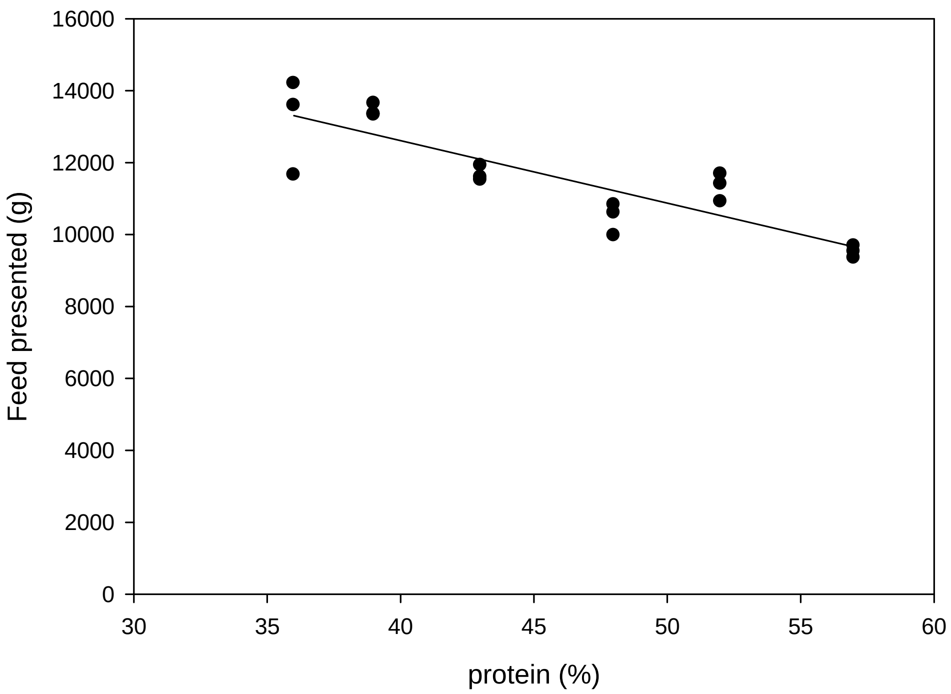

Figure 7. Feed presented to the larger size group of fish.

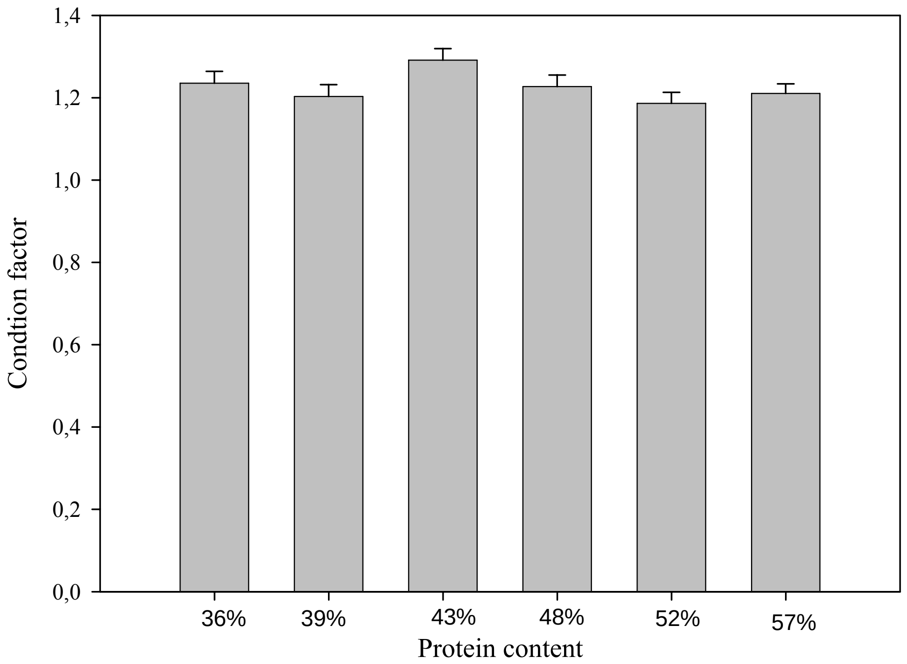

Figure 8. Final condition factor of larger size group of cod fed different protein levels.

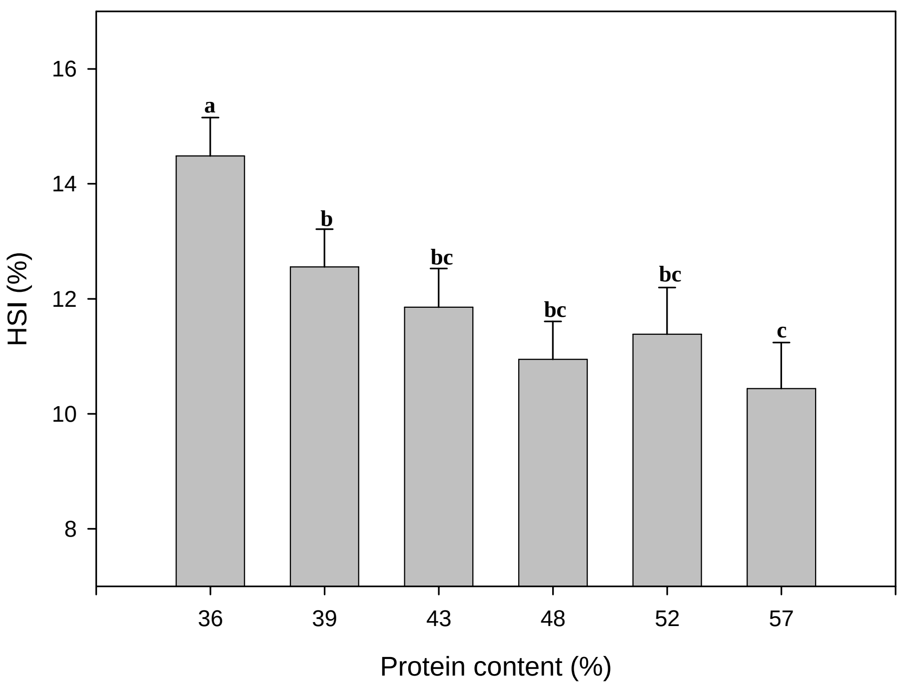

Figure 9. The size adjusted HSI of the larger size group of cod fed different protein levels. Mean values identified with different letters are significantly different.

Table 5. Liver index % (±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±0.5	17.3 ±4.5	16.1 ±7.2	16.2 ±4.0	14.1 ±6.3	13.2 ±4.1	14.3 ±8.2
Gutted mass of total mass (%)	84.8	76.8	81.1	81.6	79.4	80.3	81.6

Table 6. 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 7. 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) (*Table 4*)
36	67.4 ± 0,5	24.3 ± 0.4	18,4
39	68.7 ± 1.7	23.7 ± 1.0	17,5
43	66.2 ± 4.5	26.0 ± 3.2	15,8
48	68.4 ± 1.3	24.6 ± 1.1	14,2
52	67.9 ± 0.4	24.1 ± 0.6	12,7
57	67.5 ± 1.3	25.5 ± 1.3	10,5

Table 8. Amino acid composition* of feed and muscle samples of the larger size group of cod fed the lowest (36%) and highest (57%) 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 P36	Feed P57	Muscle	Muscle P36	Muscle P57	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
Ornitine**	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

4. 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 36-57% 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 indicate that 36% 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.

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 indicate 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 appears to be similar (slightly lower) to the protein requirements of Atlantic salmon of similar size, i.e. around 49% for fish 85 g and 42% for fish 750 g (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). Similarly, the larger fish fed low protein diets appear to compensate by increasing food intake (Figure 7).

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, although some of the groups grew at a slower rate. Protein requirements of cod could depend on the growth rate and therefore it is 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. Thus, the protein content of the feed did not appear to affect the muscle protein content or the nutritional value of the flesh.

Water holding capacity was on average 74.4% ± 3.3 (Table 6). These are lower values than observed in a study by Tryggvadottir *et al*. (2004), where the water holding capacity of farmed cod post rigor was 80 % ± 2.0. In 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 considered 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 6), 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 % ±3.8). Furthermore, the protein content of the experimental diets did not affect the amino acid composition of the flesh (Table 8).

To be able to reduce the protein content of the feed , the protein has to be replaced with one or more of the other macro-ingredients. To avoid a liver of excessive size, there are limitations to how much lipid can be in the feed for cod. There is still uncertainty about 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. Resent research indicates 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 appears to be able to compensate for lower protein levels in the feed by increasing feed intake (Figure 3 and Figure 7). 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.

5. References

Albrektsen, S., Mundheim, H and Aksnes, A. (2006) Growth, feed efficiency, digestibility and nutrient distribution in Atlantic cod (*Gadur morhua*) fed two different fish meal qualities at three dietary levels of vegetalbe protein sources. Aquaculture (In press)

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

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.

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.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC.

Rosenlund, G., Karlsen, Q, Tveit, K., Mangor-Jensen, A and Hemre, G.I. 2004. Effect of feed composition and feeding frequency on growth, feed utilixation 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.

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 Albriktsen, 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 Albriktsen, S. 2005. Inclusion of fish bone and crab by-products in diets for Atlantic cod, *Gadus morhua*. Aquaculture, *In press*.

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.