1 State of the art

Why do fish have such high requirements for protein? The protein requirement in terms of dietary concentration (% of diet) is high but the absolute requirement isn't. Protein (amino acids) is used as a major energy resource. Some economy can be made here if other dietary fuel is present in adequate amounts, e.g. increasing the lipid (fat) content of diet can help reduce dietary protein (amino acid) catabolism and requirement. This is referred to as protein-sparing effect of lipids (NRC. 1993).

Protein is required in the diet to provide indispensable amino acids and nitrogen for synthesis of non-indispensable amino acids. Protein in body tissues incorporates about 20 amino acids and among these, 10 amino acids must be supplied in the diet since fish cannot synthesis them. Amino acids are need for maintenance, growth, reproduction and repletion of tissues. A large proportion of the amino acid consumed by a fish is catabolised for energy and fish are well-adapted to using an excess protein this way (NRC. 1993).

Atlantic cod is a species with a high biological potential for aquaculture. Nowadays, as a result of the decline of natural stocks and increase in market value of cod (*Gadus morhua*), it is regarded as an alternative species with high potential for commercial farming. Protein is normally the single most expensive component of fish feeds (National Research Council, 1993) therefore; one of the priorities for development of a profitable commercial activity is formulation of cost-effective diets (Morais et al, 2001.) and satisfactory quality of the final product. Previous experiment with cod has pointed to a low protein sparing effect of lipid (Lie et al., 1988). Lipids serve as an important source of dietary energy for all fish, but perhaps to a greater extent for cold-water and marine fish, which have low ability to use carbohydrates for energy. The protein content of rainbow trout diets could be reduced from 48% to 35% without lose growth if the lipid concentration was increased from 15% to 20%. A similar result was found in yellowtail diets were protein concentration was reduced from 70% to 55% by increasing amount of fat. These studies support the recommendation that all diets should be formulated not only to meet the optimum ratio of energy to protein for that species, but also to contain an adequate amount of lipid (NRC. 1993).

The protein efficiency ratio, PER (live weight gain over protein intake), has been to estimate a protein-sparing effect of fat. Nevertheless the PER may be misleading in the case of cod, and the protein productive value, PPV (protein gain over protein intake), are more appropriate index. Because a lean fish such as cod with low fat fillet and a fatty liver (Lie et al. 1988, Morais, Sofia, J, et al. 2001.)

The protein requirements, meaning the minimum quantity needed to meet requirements for amino acids and to attain maximum growth, have now been measured in juveniles of many species (NRC, 1993). Although, our understanding of the feeding and nutrition of cod has been expanding steadily in the past decade, information on the nutrient requirement of this species is very spare (Lall, Santosh P. & Dominic Nanton, 2002).

In Iceland are aquaculture group around cod farming, The Icelandic Cod farming Project. In figure 1 are strategy in research and development in aquaculture described.

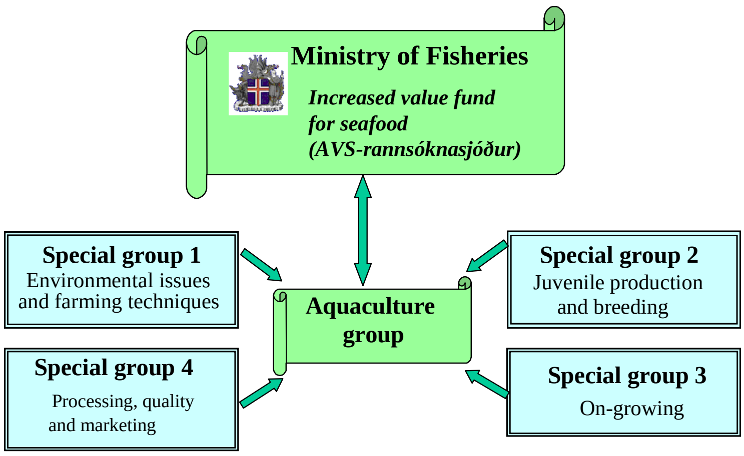

Figure 1. Strategy in research and development in aquaculture. (Gunnarsson, Valdimar Ingi. 2003)

The aquaculture group has a management team from the industry and the government. The objectives could be

- a liaison to the ministry of marine affairs
- leading for the work of special-topic groups to ensure further strategic work with the industry.
- Acquire more information concerning competitive competence
- To take possess/initiative in regular review of the progress of research and development.

Production of farmed cod is currently not very much but it is growing up. Most of the cod is on-growing of wild cod and it will be so next 2-3 years. There is offered 500 ton quota every year and the production next years will be around 1500 tons. Production with wild juveniles will be around 1000 tons after 2-3 years. This juveniles are caught west of Iceland by pelagic trawler and are first farmed on the land base and then in sea cages. Farming with reared juveniles started in 2002 and there was only few tons slaughtered in year 2003. The total quantity is estimated to be 1000 tons after 4-5 years. (Gunnarsson, Valdimar Ingi. 2003).

Production of cod juveniles are made by IceCod and the main objectives is selective breeding of cod.

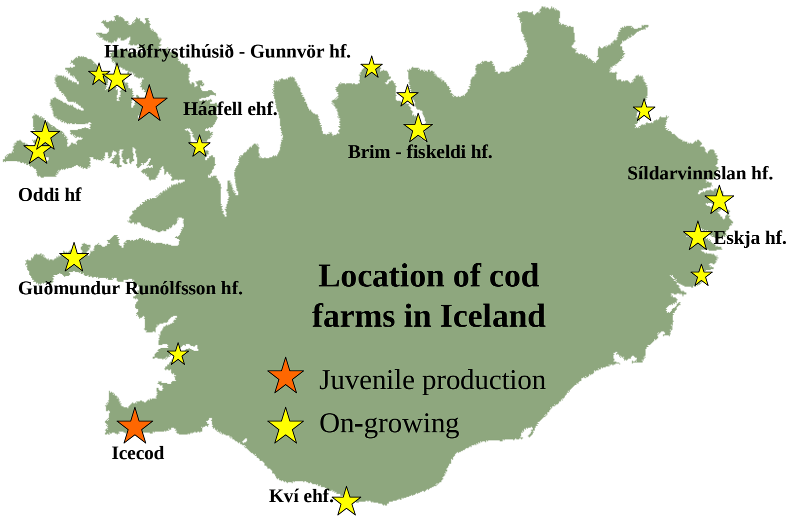

Figure 2. Location of cod farms in Iceland. (Gunnarsson, Valdimar Ingi. 2003)

In October 2002 was a cod conference to organize the strategy in research and development in cod farming in Iceland. Key researches of cod farming in Iceland are in:

- Feed and feeding
    - Effect of fat
    - Cheaper protein in cod feed
    - Feed for Atlantic cod
- Health studies
- Larval culture and selective breeding
- Slaughter and quality
- Growth and mortality
- Catch and on-growing (Gunnarsson, Valdimar Ingi. 2003).

Björn Björnsson, Marine Research Institute, has analyzed effects of fat content of feed in a nine month feeding trial with 2 kg cod at 7,2°C.

Table 1. Effect of fat content of feed in a nine month feeding trial with 2 kg cod at 7,2°C. (Björnsson, Björn. 2003)

Feed type	Fat capelin	Lean capelin	Shrimp
Fat content (%)	16,6	4,3	3,4
% of dry wt.	*50,2*	*21,1*	*13,9*
Dry wt. cont.(%)	33,1	20,4	24,5
SGR (% per day)	0,279	0,278	0,288
Feed conv. wet	2,28	4,16	4,3
Feed conv. dry	0.76	0.84	1,06
Liver index	17,3	9,3	9,2

The effect of fat content of feed in four month with 150g cod at 8-10°C has also been analyzed and the results are in table 2 here below. DANEX 1562 contains 15% fat and 62% protein, 50:50 contains 50% fat and 50% protein and DANEX 2446 contains 24% fat and 46% protein.

Table 2. Effect of fat content of feed in four month with 150g cod at 8-10°C. (Björnsson, Björn. 2003)

Feed type	DANEX 1562	50:50	DANEX 2446
W1	69,3	69,18	69,95
W2	230,77	216,13	196,87
SGR	1,002	0,949	0,862
Feed conversion	0,774	0,774	0,941
Liver index	11,5	13,5	13,8

SGR was most in DANEX 1562 and poorest in DANEX 2446, in that order. The liver index was reciprocally inverse as against SGR and feed conversion was the same in diet DANEX 1562 and 50.50 but little higher in DANEX 2446.

This too experiments which are described above have not been written any article yet, that is purposed in June.

There is trial in Iceland which is on going and there are effects of different proteins on growth and health cod juveniles. The main protein materials in the diet are fishmeal, soybean meal and some type of maize-gluten meal and wheat-gluten meal. Results will be in April.

Øyvind Lie et al. (1988) did two feeding trials, one involved 40g cod fed for 26 days and the other 180g cod fed for 60 days. Feeds was mainly squid mantle, capelin oil and dextrin zed potato starch were made up to give from 75% to 27% of available protein energy, balanced with energy from fat from 11% to 61% energy, all with 12%-15% carbohydrate energy. It seems to be low protein-sparing effect of fat in cod. A diet containing 52% protein, 11% lipid and 17,5% carbohydrate was suitable for cod where 60%, 25% and 15% of available energy was supplied by protein, lipid and carbohydrate, respectively (Lie, Øyvind et al., 1988).

In an experimental trial with cod that Albektsen et al 2003 did, impaired feed conversion rate was found by feeding low quality fish meal NorSeaMink (NSM) compared to higher quality meal Norse-LT 94. The feed intake increased which is the reason for the reducing feed conversion. Reduced protein digestibility and reduced whole body preservation of protein and minerals, accompanied by increased fat content, was also found in cod fed with low quality fish meal. By using Norse-LT quality meal it may give a more cost-effective production of cod through higher growth, feed efficiency and whole body protein retention.

By exchanging Norse-LT fish meal with a combination of vegetable protein, corn gluten meal, wheat gluten and full-fat soybean meal, the feed conversion rate was slightly impaired. But the effect was to a smaller extent than by feeding NSM quality fish meal.

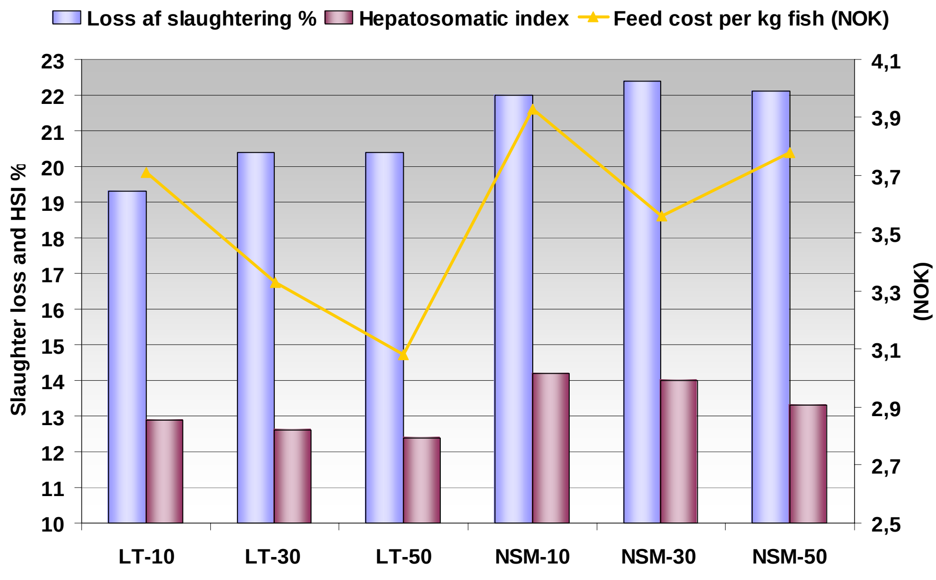

Figure 3. Feeding cost per kg cod. Loss at slaughtering % and the hepatosomatic index (HIS) of cod fed two fish meal qualities and the three dietary levels of vegetable protein 10, 30 and 50% of total feed protein (Albrektsen, Sissel. 2003).

Diets containing 50% of vegetable protein led to reduce liver size for both fish meal qualities. Muscle amino acid composition showed less than 10% variation irrespective of feed contents and diet composition.

It seems to be a potential for application of low-cost vegetable protein in feed for cod, on the assumption that high quality fish meal is applied. Low quality fish meal may increase fatty liver and reduce slaughter weight in cod. This will not be cost-effective or beneficial in cod farming, unless the liver is utilized

Pågående och planerade projekt under 2003-2004 som handlar om torsk i Sverige. Förstudie om framtida torskodlingsförutsättningar, Sveriges lantbruksuniversitet, SLU, Institutionen för vattenbruk, Inst för livsmedelsvetenskap.

Ansökningar som är sända: Biogena aminer, Inst för livsmedelsvetenskap, SLU.

Muskelkvalitet i odlad och vild torsk med avseende på ffrämst struktur och textur samt vattenhållande förmåga; Inst för livsmedelsvetenskap, SLU.

Torskens lekplatser vid svenska västkusten; Havsfiskelaboratoriet, Lysekil, Fiskeriverket.

Torskens lekbeteende och reproduktion; Havsfiskelab, Fiskeriverket

Ägg och larvdistribution/abundans i Kattegat och östra Skagerrak; Havsfiskelab, Fiskeriverket.

Utveckla otolitkemisk metodik i syfte att avgöra enskilda individers beståndstillhörighet; Havsfiskelab, Fiskeriverket.

Åtgärdprogram för att få tillbaka ett kustfiskbestånd av torsk vid svenska västkusten; Havsfiskelab, Fiskeriverket.

Alternativa selektionsanordningar i torsktrålar i Östersjön; Havsfiskelab, Fiskeriverket.

Rist i bottentrålar (EU-projekt).

Överlevnad av torsk som selekteras ut under trålning; Havsfiskelab, Fiskeriverket.

Draggning efter tappade torskgarn i Östersjön; Havsfiskelab, Fiskeriverket.

Beräkning av omfattning av förluster av torskgarn i Östersjön samt den totala fiskeridödligheten orsakad av dessa (EU-projekt); Havsfiskelab, Fiskeriverket.

Nordisk torsknätverk deltagare, projekt 2003-2004, SLU.

Few experiments have been done in Canada on cod, but something has been done on haddock. Santosh P. Lall and Dominic Nanton (2002) reported that the protein requirements of most marine species ranges between 50 to 60% and the requirement for haddock has been estimated 49,9%. A diet containing high amount of protein (48-60%) and low of carbohydrate (10-14%) and lipid, less than 15%, with an adequate amount of n-3 long chain highly unsaturated fatty acids (HUFA) (1-1,5% eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for juveniles fish) as well as being fortified with vitamins and trace elements would be suitable for initial feed formulations of cod diets. Recent research on juvenile haddock (50-250g) suggests that high energy diets containing 15% to 24% lipid decrease the feed conversion ratio but growth rate was not improve. There is no real gain if the diets contain more than 15% lipid, but the growth rate decline when dietary lipid was reduced below 12% (Lall, Santosh P. & Dominic Nanton, 2002).

Jeong-Dae Kim and Santosh P. Lall did an experiment to determine the optimum level of protein required in the diet of juvenile haddock (6,9g). Haddock was fed by isoenergetic diets containing 45%, 50%, 55%, 60% and 65% protein. The experimental period was 6 weeks and in table 1 can see the growth and feed utilization of fish fed different levels of protein.

Table 3. Growth and feed utilization of fish fed different levels of protein for 6 weeks (Kim, Jeong-Dae & Santosh P. Lall. 2001).

Protein level (%DM)	Initial weight (g/fish)	Final weight (g/fish)	Weight gain (g/fish)	SGR (%)[#]	Feed intake (g DM/fish)	Feed/gain ratio[†]
45	6.9 ± 0.42^{ns}	23.0 ± 0.62^{ns}	16.1 ± 0.20^{ns}	2.88 ± 0.08^{ns}	12.27 ± 0.23^{a}	0.76 ± 0.01^{a}
50	6.9 ± 0.41	23.6 ± 0.75	16.7 ± 0.40	2.94 ± 0.08	11.80 ± 0.15^{a}	0.71 ± 0.01^{b}
55	6.9 ± 0.38	24.2 ± 1.22	17.3 ± 0.83	3.00 ± 0.02	11.78 ± 0.43^{a}	0.68 ± 0.01^{c}
60	6.9 ± 0.33	24.2 ± 0.35	17.3 ± 0.19	3.00 ± 0.09	10.85 ± 0.04^{b}	0.63 ± 0.01^{d}
65	6.9 ± 0.30	23.6 ± 0.70	16.8 ± 0.41	2.95 ± 0.04	10.44 ± 0.18^{b}	0.62 ± 0.01^{d}

[*] Values (means ± SE of three replicate groups) in the same column sharing a common superscript were not significantly different ($P > 0.05$) from each other.
[#] Specific growth rate = (ln(final wt.) − ln(initial wt.))/days × 100.
[†] Feed intake, DM/wet wt. gain.

Highest SGR was in the group with 55% and 60% protein and lowest in group 45%, but there was no statistical differences were found (P=0,05). All fish had more than a three-fold increase in body weight.

Fish fed 60% and 65% protein measured with lower feed intake and feed gain ratio than the others group.

Nitrogen (N) intake increased with an increase in the feed, which resulted better N gain in of the fish whole body. There was no significantly different N gain by fish fed 45% protein and 50% protein.

Diet 55% and 60% had the same nitrogen gain (0,43 g), that was significantly ($P<0,05$) higher than fish fed the diet containing 45% protein. The broken-line regression of N gain against dietary protein level yielded an estimated protein requirement of 53,8% on dry matter basis.

Gross energy intake of fish varied from 283 to 229 kJ/fish for fish fed 45% and 65%, in that order. The highest energy gain was obtained with fish fed 50% or 119 kJ, resulting in the highest energy retention efficiency of 44,1%.

Table 4. Lipid contain of the feed and liver index of haddock juveniles (Kim, Jeong-Dae & Santosh P. Lall. 2001).

Protein (%)	Lipid (g/100g)	HSI (%)
45	18,07	10,71
50	16,60	10,34
55	13,78	8,99
60	11,42	8,66
65	8,69	7,32

The hepatosomatic index of the initial fish was 6,82% and in table 4 may see how the HSI has change over the experimental period. HSI significantly ($P<0,05$) decreased from 10,7% to 7,3% as the dietary protein contain increased from 45% to 65%.

Another study was conducted to determine growth and feed utilization by haddock fed diets containing various levels of protein (35, 40, 45 and 50%). An average weight of the juveniles was 24 g and was hand-fed one of the isoenergetic experimental diets to satiation. The juvenile was fed three times a day for 9 weeks. Proportionate weight gain of fish fed the experimental diets at the end varied from 119% to 175%, which is a linearly increase with an increase in dietary protein level. Both SGR and weight gain (percentage/initial weight) were highest in diet containing

50%, but it is not significantly different (P>0,05) from that of fish fed 45% protein. Feed intake was the lowest in fish fed 35% and highest in fish fed 50% protein (table 3).

Table 5. Growth and feed utilization of fish fed different levels of protein for 9 weeks (Kim, Jeong-Dae, S. P. Lall & J. E. Milley. 2001)

Protein level (% DM)	Initial weight (g fish^{-1})	Final weight (g fish^{-1})	Weight gain (%/initial weight)	SGR[2] (%/d)	Feed intake (g DM fish^{-1})	Feed : gain ratio[3]
35	24.8 ± 0.42^{NS}	54.1 ± 0.62^{c}	119.4 ± 9.70^{c}	1.24 ± 0.08^{c}	27.5 ± 0.45^{b}	0.94 ± 0.03^{a}
40	23.8 ± 0.41	56.9 ± 0.75^{b,c}	138.7 ± 6.66^{b,c}	1.38 ± 0.04^{b,c}	28.4 ± 0.54^{a,b}	0.86 ± 0.04^{a}
45	23.7 ± 0.38	61.1 ± 1.22^{a,b}	158.0 ± 4.64^{a,b}	1.50 ± 0.02^{a,b}	28.2 ± 0.34^{a,b}	0.76 ± 0.01^{b}
50	23.9 ± 0.33	65.8 ± 0.35^{a}	175.4 ± 0.83^{a}	1.61 ± 0.01^{a}	29.4 ± 0.35^{a}	0.70 ± 0.01^{b}

[1]Values (mean ± SE of three replicate groups) in the same column sharing a common superscript letter are not significantly different ($P > 0.05$) from each other.
[2]Specific growth rate = [ln(final weight) − ln(initial weight)]/duration × 100.
[3]Feed intake, dry matter/wet weight gain.

The broken-line regression of weight gain against protein level yielded an estimated protein requirement of 49,9% and a higher amount of dietary lipid (>16,7%) caused increase in the HIS of juvenile fish.

If this study's are compared, a lower SGR was found, it seemed to be due to a difference in body weight. The feed gain ratio values for diet 45% and 50% protein contain are comparable with those of the previous study, even though the great difference in body weight and feed intake.

The effect of protein/lipid ratios in extruded diets for Atlantic cod has been proved by Morais et al. The aim of this research was testing four extruded diets differing in the levels of protein (48% or 58%) and lipid (12% and 16%). Diet 58/12 was used as the control diet, against which they compared the effect of cheaper choice, such as diets 48/12 and 48/16. To examine a potential protein economizing effect by dietary lipid was diet 48/16 formulated. Diet 58/16 was included in the study to verify whether a higher performance can be achieved with a more nutrient dense diet, though protein sparing dietary lipid. A second objective was to verify whether the producers could profit from the lipid-rich livers characteristic of farmed cod, exploiting it as a source of cod liver oil for human health purposes.

All fish fed the experimental diets for 16 week and was conducted with juvenile cod (233g, average total weight) using three replicates per treatment. The effect of the diets was estimated by growth (SGR and GF3), feeding performance (FCE, PER), condition, hepatosomatic index and biochemical composition.

The tested diets introduce good performance results, with a protein sparing effect by lipid and a more efficient use of protein in diets content being clear at 12 weeks but not so apparent after 16 weeks of culture. The hepatosomatic index and lipid content in the liver was higher in the diets with high lipid level. The fatty acids profile of farmed cod liver oil showed promising properties, even though having a slightly higher content of saturated fatty acids and linoleic acid, compared to natural cod liver oil. (Morais, Sofia, J, et al. 2001.).

The statistical analyses conducted for weight, length and condition factor of cod submitted to difference experimental diets did not detect significant differences between treatments (P> 0,05), at any of the sampling dates. The size dispersion within each tank was high and, as a consequence, the ANOVA analysis was less sensitive to the dietary effect. The highest mean weight and length results after 12 weeks of culture were achieved in diet 58/16 while the lowest mean growth value were attained in the replicates fed diet 48/12. On the other hand, after 16 weeks of culture, the high specific growth rate (SGR) observed in fish submitted to treatments 58/12 and 48/12 resulted in a very similar weight and length in cod fed the different experimental diets. The condition factor was comparable between treatments.

Table 6. Growth and performance parameters of cod at the start of the experimental period and after 12 and 16 weeks of culture (Morais, Sofia, J et al. 2001)

	58/12	58/16	48/12	48/16
0 weeks				
Initial mean weight (g)	233 ± 69.0	233 ± 69.0	233 ± 69.0	233 ± 69.0
Initial mean length (cm)	26.6 ± 2.4	26.6 ± 2.4	26.6 ± 2.4	26.6 ± 2.4
Condition factor	1.21 ± 0.42	1.21 ± 0.42	1.21 ± 0.42	1.21 ± 0.42
HSI (%)	9.3 ± 1.8	9.3 ± 1.8	9.3 ± 1.8	9.3 ± 1.8
12 weeks				
Mean weight (g)	435 ± 111.5	445 ± 120.1	430 ± 106.0	436 ± 118.3
Mean length (cm)	32.8 ± 2.7	32.9 ± 2.7	32.4 ± 2.4	32.4 ± 2.5
Condition factor	1.22 ± 0.15	1.23 ± 0.13	1.24 ± 0.14	1.26 ± 0.12
HSI (%)	8.5 ± 1.3	10.0 ± 1.5	8.5 ± 0.3	11.2 ± 1.0
Weight gain (g)	202 (68)	211 (73)	197 (82)	203 (80)
SGR (% per day)	0.74 (0.61)	0.77 (0.64)	0.73 (0.76)	0.75 (0.72)
GF3	2.08	2.16	2.04	2.09
Feed consumption (g)	273.4 (106.4)	273.4 (106.4)	273.4 (106.4)	273.4 (106.4)
FCE	0.74 (0.64)	0.77 (0.69)	0.72 (0.77)	0.74 (0.75)
PER	1.25 (1.08)	1.32 (1.17)	1.36 (1.45)	1.44 (1.45)
16 weeks				
Mean weight (g)	529 ± 1256	524 ± 129.5	517 ± 135.4	520 ± 152.0
Mean length (cm)	34.6 ± 2.6	34.7 ± 2.7	34.3 ± 2.6	34.5 ± 3.00
Condition factor	1.26 ± 0.14	1.23 ± 0.14	1.25 ± 0.13	1.24 ± 0.12
HSI (%)	6.2 ± 1.7^{a}	7.3 ± 1.5^{ab}	6.6 ± 2.0^{a}	8.4 ± 2.0^{b}
Weight gain (g)	296 (94)	291 (80)	284 (87)	287 (84)
SGR (% per day)	0.73 (0.70)	0.72 (0.59)	0.71 (0.66)	0.72 (0.63)
GF3	1.96	1.93	1.90	1.91
Feed consumption (g)	400.8 (127.4)	400.8 (127.4)	400.8 (127.4)	400.8 (127.4)
FCE	0.74 (0.74)	0.73 (0.63)	0.71 (0.68)	0.72 (0.66)
PER	1.25 (1.25)	1.24 (1.07)	1.33 (1.29)	1.38 (1.27)

Values in the same row with different superscript letters are significantly different ($P < 0.05$).
For mean weight, condition and HSI, values are presented as mean ± S.D. and values in parenthesis were calculated relative to the previous 4 weeks.
HSI—hepatosomatic index, SGR—specific growth rate, GF3—growth factor, FCE—food conversion efficiency, PER—protein efficiency ratio.
For mean weight, length and condition factor, $n = 150$. For HIS at 0 weeks, $n = 6$; at 12 weeks, $n = 3$ and at 16 weeks, $n = 12$.

As for the hepatosomatic index (HIS), the statistical analysis detected significant differences between treatments (P = 0,002), at the end of the experimental, with diet 48/16 inducing a significantly higher HSI than diets 58/12 and 48/12.

If other parameters are analyzed, 12 weeks after the onset of the growth trial, the SGR, growth factor (GF3) and feed conversion efficiency (FCE showed a more efficient use of diet 58/16, followed by diets 58/12 and 48/16 and diet 48/12 was responsible for the poorest results. After 12 weeks of culture was the PER for diet 48/16 the most efficient use of the dietary protein, followed by diets 48/12, 58/16 and finally 58/12. At the end of the experiment did this different reduce.

The muscle composition of farmed cod appears to be very homogenous, with regard to lipid, protein, moisture and ash. The muscle total lipid did decrease from the beginning to the end of the experiment period. At the same time did the moisture and total protein composition increase, but there was no changes occurred in the ash content. There were statistical differences found for total protein, moisture and ash, with the biggest differences being observed between diet 48/12 and 58/16 and 48/16.

The sparing effect of dietary lipid were evident after 12 weeks of culture, with the highest growth rate and FCE being found in cod fed a diet with high levels of both protein and lipid (58/16). The protein sparing effect is suggested by the similar FCE and growth results achieved with diets 58/12 and 48/16. Unsurprisingly, the worst results were obtained with diet 48/12. The most efficient use of dietary protein was performed in diets with lower protein content. Within these, the protein efficiency ratio and net protein utilization points to some degree of dietary protein sparing by lipid, since higher values were achieved with diet 48/16. This allowed the energetic requirements to be met by the dietary lipid, with more protein being used for growth. However, after 16 weeks the protein sparing effect was no longer obvious, given the faster growth of cod fed diets 58/12 and 48/12 in the last 4 weeks of breeding.

The authors suggest that the 48/16 diet appeared to be the best compromise between growths, feed utilization and cost (Morais, Sofia, J et al. 2001).

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