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Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I — Effects on growth and protein retention

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Abstract

A regression design with increasing levels of a plant protein mixture replacing fish meal in diets for Atlantic cod, was used to elucidate effects on growth, feed utilization (FCR), digestibility (ADC), and retention of nutrients (PER, PPV and HSI). The plant protein diets contained soybean meal, soy protein concentrate and wheat gluten meal, all of high quality, and were added crystalline methionine and lysine to achieve levels of indispensable amino acids for maximizing growth in other fish species. Initial fish weight was 1652±6 g (mean±SEM) and the experiment lasted for 28 weeks from December 2004. High growth (SGR 0.31–0.35) and feed utilization (FCR 1.06–1.26) were obtained up to 50% plant protein inclusion. Above this inclusion level, growth (SGR 0.30–0.14) and feed utilization (FCR 1.42–2.71) were reduced. None of the diets affected whole body, liver or muscle proximate compositions. Hepatosomatic index was not effected by diet up to 75% plant protein (HSI 12.7–14.3), but was significantly lower for the 100% plant protein diet group (HSI 10.1–11.4). Plasma and muscle free amino acid pools, sampled 5 h post-feeding, partly reflected diet amino acid composition. Reduced protein retention was found as dietary plant protein increased (PPV 0.45–0.16 and PER 2.11–0.73), however without any reductions in apparent digestibility coefficients. There was a decrease in vitamin B_{12} concentrations in the diets (120–10 μg kg^{-1}) as inclusion of plant protein increased, but no specific deficiency symptoms were detected in the fish. Furthermore, the sufficiency of dietary methionine supply in the highest replacement groups, can be questioned based on low levels in the muscle free amino acid pool. Overall conclusion is a great potential for using quite high inclusions of plant proteins in cod diets, provided that the plant ingredients are of high quality.

Keywords: Atlantic cod; Diets; Plant protein; Feed utilization; Growth; Tissue free amino acids

1. Introduction

Farming of Atlantic cod (*Gadus morhua* L.) has developed rapidly in recent years, stimulated by a steady decline in landings from wild stocks and a more predictable and safe production and supply of juveniles (Rosenlund and Skretting, 2006). Development of new diets and feeding regimes for cost-effective production and satisfactory quality of the final eatable product is crucial in order to establish a viable cod farming industry. To date, diets for cod have been based on high quality fish meal and oil. If cod production volumes are to be increased such diets will not be sustainable, neither cost

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nor feed-volume wise. New feed ingredients are therefore necessary to ensure a healthy production (FAO, 2002). Furthermore, as diets for cod in all life stages will have to contain high protein levels and relatively low fat levels (Rosenlund et al., 2004), more flexibility is required with respect to the use of protein ingredients.

There has been several trials where fish meal has been substituted with different plant proteins and at different inclusion levels in diets for cod (von der Decken and Lied, 1993; Albrektsen et al., 2006; Refstie et al., 2006a; Hansen et al., 2007). No effect on growth and feed intake was observed by Albrektsen et al. (2006) using a mixture of different qualities of soybean meal and corn gluten up to 68% of total protein. Similarly, Refstie et al. (2006a) found no effect on growth when various soybean qualities were used up to 24% of total protein. In all those studies increased feed intake and reduced digestibility of fat and amino acids, were reported. Hansen et al. (2006, 2007) found minor effects on growth, feed intake and macro-nutrient digestibility when including corn gluten in the diets (11–45% of protein in diet). No effects were seen when soybean meal (7–30% of protein in diet) was included, and a mixture of soy protein concentrate and wheat gluten (40 and 80% of protein in diet) showed even better growth than the fish meal control. This demonstrated a great potential of replacing fish meal with plant protein in diets for cod. However, to our knowledge, there have been no attempts to test the effect of a total replacement of fish meal with plant proteins in diets for cod.

Reduction in growth and feed efficiency at high levels of plant protein inclusions in diets for salmonids has been explained by imbalanced dietary amino acid concentration, reduced mineral content, increased fibre, reduced palatability and presence of anti-nutrients. Soybean is especially low in methionine and wheat gluten is low in lysine. Imbalanced amino acid concentration is associated with lower protein synthesis or increased degradation, or a simultaneous change in both components of protein turnover. The plasma amino acid pool reflects the content of amino acids in the diet, and can be a tool to determine the adequacy of dietary amino acids and to reflect nitrogen balance (Mente et al., 2003). Protein catabolism is shown to increase when the dietary indispensable/dispensable amino acid ratio decrease (Gómez-Requeni et al., 2003; Vilhelmsson et al., 2004). Espe et al. (2007) have shown that close to 100% fish meal replacement is possible in diets for Atlantic salmon (*Salmo salar* L.) with no negative effect on growth, if the amino acid profile in the feed is well balanced and if feed intake is comparable to a high fish meal control diet.

Fibres and anti-nutrients, e.g. protease inhibitors, are associated with reduced digestibility in fish (Krogdahl et al., 1994; Francis et al., 2001). Changes in intestinal morphology were however not observed in the above referred studies on cod, even when finding reductions in digestibility of macro-nutrients (Førde-Skjærvik et al., 2006; Hansen et al. 2006; Refstie et al., 2006b).

Alanine aminotransferase (ASAT) and aspartate aminotransferase (ALAT) are two enzymes, mostly active in liver and kidney, which are quantitatively important in transamination of amino acids. Vitamin B_6, in the form pyridoxal-5-phosphate, functions as a co-factor catalyzing the action of ASAT and ALAT. Deficiency of vitamin B_6 can lead to reduced activity of these enzymes in liver (Albrektsen et al., 1993). Vitamin B_{12} (cobalamine) is involved as a coenzyme in methionine synthetase which converts homocysteine to methionine (Hilton, 1989). Since vitamin B_{12} is absent from plant protein resources, but present in fish meal, a replacement of fish meal with plant ingredients might lead to too low levels if not added in sufficient amounts. Vitamin B_{12} deficiency may result in megaloblastic anemia, observed as lowered haemoglobin levels (Waagbø, 1999). Changing the protein ingredients to high plant protein inclusions, might therefore result in insufficient availability of this co-factor, if protein metabolism in cod changes in a similar manner as reported for rainbow trout (*Oncorhynchus mykiss*) (Martin et al., 2003).

The aim of the present study was to elucidate how Atlantic cod could utilize high dietary levels of plant proteins. Fish were fed diets where a mixture of soybean meal, soy protein concentrate and wheat gluten, replaced fish meal in a regression design up to 100% of the dietary protein. Measurements included growth, feed conversion factor, digestibility of macro-nutrients, efficiency of protein and lipid depositions, organ proximate compositions, plasma and muscle free amino acid profiles, transaminase activities in liver, and concentrations of vitamin B_6 and B_{12} in liver and muscle.

2. Material and methods

2.1. Feed and experimental design

Atlantic cod were fed five different extruded diets, produced as 9 mm pellets by Skretting Aquaculture Research Centre (Stavanger, Norway). The experimental design was a regression design with increasing levels of a plant protein mixture replacing 0–100% of the fish meal (LT; low temperature quality) in the diet. The plant ingredients chosen were soybean meal (14%), soy protein concentrate (36%) and wheat gluten (50%). These plant sources were chosen due to

high protein content required to reach a target protein level of 52% of total diet. Furthermore, concentrated plant proteins hold low levels of anti-nutrients, and soy protein concentrate and wheat gluten have also shown good results at high inclusion in previous trials with cod (Hansen et al., 2007). Soybean and wheat gluten are complementary in lysine level (soybean; lys 6.2%, wheat gluten; lys 1.7%). A practical replacement approach was applied implying that only limiting indispensable amino acid were added according to requirement levels determined in other fish species (NRC, 1993). Thus, the diet with the highest level of plant proteins (100% replacement) was added DL-methionine and L-lysine. The control diet was based on fish meal as the main protein source, additionally the wheat contributed with a small proportion of protein. Fish oil was added as main lipid source (target level of 18%), but in order to adjust for the differences in the levels of the long $n-3$ fatty acids (EPA and DHA) in response to decreasing levels of fish meal, increasing levels of South American fish oil was added in diets with plant proteins, resulting in similar levels of these fatty acids in all diets. The same quantities of vitamin and mineral premixes were added to all diets, and to the 75% and 100% replacement diets, mono-sodium-phosphate (MSP) was added to meet NRC-recommendations for phosphorous. Yttrium oxide was added to all diets as a digestibility marker. Diet abbreviations used were FM (100% fish meal), 25PP (25% plant protein), 50PP (50% plant protein), 75PP (75% plant protein) and 100PP (100% plant protein). The composition of the experimental diets is given in Table 1.

2.2. Fish and experimental conditions

The experiment was carried out at Austevoll Aquaculture Research Station, Institute of Marine Research (Austevoll, Norway). At experimental setup 950 cod (*G. morhua* L.) (1652 ± 6 g, mean ± SEM, $n=10$ cages) were randomly distributed into 10 sea cages of $5\times5\times5$ m. The trial lasted for 28 weeks, starting in December 2004. All cages were exposed to 24 h light (400 W metal halide, submerged) and fed every morning for 1.5 h using automatic feeders, feeding controlled and topped by hand feeding at the end of the feeding session. Mortalities were registered daily, and dead fish were removed and weighed. Mean water temperature was 8 °C from December to February, 5 °C from February to April and 9 °C from April to June.

2.3. Sampling

All fish were weighed (g) and fork length (mm) measured individually at start, after 9 weeks (period 1), after 20 weeks (period 2) and at the end of the experiment (28 weeks; period 3). Five fish from the start population and from each cage at all following samplings were anaesthetized and killed with a blow to the head, before blood samples were drawn from the caudal vessels using heparinized medical syringes. The blood samples were centrifuged (2000 ×*g* at 4 °C for 10 min) within 10 min of sampling. The resulting plasma was pooled to one sample per cage, frozen on dry ice, and stored at −80 °C until analysed. At final sampling blood was withdrawn exactly 5 h post-feeding to enable comparison of plasma and organ amino acid concentrations.

At final sampling five fish were dissected, and carcass and liver were weighed. Four liver samples (2–5 g), taken from the same area of liver on each fish, were immediately frozen on dry ice, and stored in at −80 °C until analyses of aspargine aminotransferase (ASAT) and alanine aminotransferase (ALAT) activity, and vitamin B_6 and B_{12} concentrations. The remaining liver was homogenised with a kitchen machine and stored at −20 °C until analysis of fat, protein ($N\times6.25$), dry matter and glycogen concentrations, using one pooled sample (5 fish) per cage. The total left-hand side of the muscle fillet was dissected from the same five fish and two samples (5 g) were immediately frozen on dry ice for analyses of free amino acids, vitamin B_6 and B_{12} concentrations. The remaining of the left-hand fillets were then pooled, homogenised with a kitchen machine and frozen on dry ice for later analyses of proximate composition. In addition to the five fish collected for organs, faeces was collected from five fish by stripping (Hemre et al., 2003) before the fish were pooled and homogenised for analyses of whole body proximate composition. A similar pooled sample for proximate composition of whole fish was also obtained from 10 fish from the start population.

2.4. Analytical methods

Feeds were analysed for proximate composition by means of conventional methods. Dry matter was determined gravimetrically after drying at 104 °C for 24 h. Total nitrogen was determined with a nitrogen element analyser (LECO, FP-428; system 601-700-500, Perkin Elmer Coop., Norwalk, CT, USA), and crude protein calculated as $N\times6.25$. Fat was determined gravimetrically after acid hydrolysis and extraction with di-ethyl ether and ash gravimetrically after combustion at 540 °C for 16 h. Starch was analysed using an enzymatic method as described by Hemre et al. (1989). Amino acids were hydrolysed by 6 M hydrochloric acid, derivatised with phenylisothiocyanate (PICT), and analysed in a Waters HPLC amino acid analyser system using L-norleucine as the internal standard (Cohen and Strydom, 1988). Vitamin B_6 was determined according to a modified AOAC Method (1980; 43: 768–769). Vitamin B_{12} was determined microbiologically using *Lactobacillus delrueckii susp. lactis* (ATCC4797).

Pooled homogenised samples of whole fish, muscle and liver were analysed for moisture (gravimetric determination after drying at 104 °C for 24 h), protein (as described for feed), fat (gravimetrically after ethyl acetate extraction according to Lie (1991)) and glycogen according to Hemre et al. (1989). In addition, muscle and plasma were analysed for free amino acids with post-column Ninhydrin detection on a Biochrom 20 plus (Biochrom Ltd, Cambridge, England). Vitamin B_6 and B_{12} were analysed in muscle and liver as described for feed. Liver was also analysed for ASAT and ALAT activity on MAXMAT™ PL (MAXMAT S.A., Montpellier, France) using DIALAB kit (Vienna, Austria).

Table 1
Feed ingredients and analysed feed composition (given as g 100 g^{-1} except for B_6 given as mg kg^{-1}, B_{12} as µg kg^{-1} and gross energy given as MJ kg^{-1})

Diet	FM	25PP	50PP	75PP	100PP
Feed ingredients					
Fish meal [a]	69.4	54.4	36.2	18.2	0
Wheat gluten [b]	0	8.6	17.8	27.6	36.9
Soy protein concentrate [c]	0	6.1	12.8	19.7	26.4
Soya bean meal [d]	0	2.3	4.8	7.4	10.0
Wheat	19.0	16.2	15.0	11.7	8.2
Fish oil, Nordic	11.2	11.4	11.6	11.8	12.3
Fish oil, South-America	0	0.5	1.3	2.0	2.6
DL-methionine [e]	0	0	0	0	0.028
L-lysine [f]	0	0	0	0	0.573
Mono-sodium-phosphate [g]	0	0	0	1.1	2.7
Constants [h]	0.485	0.485	0.485	0.485	0.485

Diet	FM	25PP	50PP	75PP	100PP	Statistics		
Analysed feed composition						Linear regression line	R^2	P
Crude protein	50.7	52.2	53.3	53.8	53.2		0.71	ns
Crude fat	18.1	16.7	16.7	16.9	16.6		0.50	ns
Starch	11.2	10.2	10.1	8.2	6.8	$Y=1.47-0.04x$	0.93	<0.01
Dry matter	91.8	91.6	93.2	92.4	92.2		0.16	ns
Ash	10.8	9.3	7.4	5.9	4.9	$Y=10.68-0.06x$	0.997	<0.001
Remaining [i]	1.0	3.3	5.7	7.6	10.7	$Y=0.9+0.1x$	0.996	<0.0001
Gross energy (MJ kg^{-1}) [j]	21.1	20.8	21.0	20.9	20.4		0.54	ns
Vitamin B_6 (mg kg^{-1})	13.4	12.7	13.2	12.0	12.3		0.59	ns
Vitamin B_{12} (mg kg^{-1})	120	80	60	40	10	$Y=114-1x$	0.98	<0.001

Linear regression equation (where Y is the response and x the level of plant protein in diet), R^2 and P (significant when $P<0.05$) are also given.
[a] Norse LT, Vedde Herring Oil Factory, Egersund, Norway.
[b] Gluvital 21000, Cerestar Scandinavia, Charlottenlund, Denmark.
[c] Soycomil FG, ADM Europort BV, Koog aan de Zaan, The Netherlands.
[d] HP340, Hamlet Protein, Horsens, Denmark.
[e] Degussa, Hanau, Germany.
[f] Ajinomoto Eurolysine, Paris, France.
[g] Trouw Nutrition, Boxmeer, The Netherlands.
[h] Constant ingredients; 3.31 g vitamin and mineral premix (proprietary composition, Skretting ARC, Stavanger, Norway), 1 g yttrium premix (100 mg Y_2O_3 kg^{-1} diet), 0.42 g Betafin (Danisco Animal Nutrition, Marlborough, UK), 0.12 g Lutavit C (BASF, Ludwigshafen, Germany).
[i] Remaining=100 %−% protein−% fat−% starch−% ash−% water.
[j] Estimated using the following caloric values: starch 17 MJ kg^{-1}, fat 39 MJ kg^{-1} and protein 24 MJ kg^{-1}.

Plasma was analysed for concentrations of glucose, total protein, cholesterol and triacylglycerols (TG) on MAXMAT™ PL. (MAXMAT S.A., Montpellier, France) using DIALAB kit (Vienna, Austria).

Yttrium concentration was determined in feeds and faeces using ICP-MS after complete digestion of homogenised and freeze-dried samples in nitric acid after cooking in a microwave oven for 1 h.

2.5. Calculations

$$\text{Hepatosomatic index(HSI)} = (\text{g liver weight/g live body weight}) \times 100$$

$$\text{Specific growth rate(SGR)} = ((\ln W_2 - \ln W_1)/D) \times 100$$

where W_1 and W_2 are the initial and final body weight in grams, D is the number of experimental days.

$$\text{Gonadosomatic index(GSI)} = (\text{g gonad weight/g live body weight}) \times 100$$

$$\text{Condition factor(CF)} = (\text{live weight, g}) \times 100/(\text{length, cm})^3$$

$$\text{Protein efficiency ratio(PER)} = (\text{g live weight gain/g protein intake})$$

$$\text{Protein productive value(PPV)} = (\text{g protein retention/g protein intake})$$

$$\text{Apparent digestibility coefficient(ADC)} = 100 - 100 \times (\text{nutrient in faeces/yttrium oxide in faeces}) * (\text{yttrium oxide in feed/nutrient in feed})$$

$$\text{Feed conversion ration(FCR)} = (\text{g dry feed given/g live weight gain}).$$

2.6. Statistics

Dunnetts' test was used to test if the test diets (25PP–100PP) were different from the FM control diet. Large variation in growth between two independent parallel cages exceeded the variation between diet groups and might mask possible diet effects, and one way ANOVA could not be applied. A simple linear regression model was therefore chosen as statistical approach, with responses as dependent variable and plant protein inclusion level as independent (predictor) variable. Pearson Correlation was used to evaluate correlation between amino acid concentrations in feed and muscle and plasma free amino acids. Significance level was set to $P<0.05$. All statistical analyses were performed using Statistica™ 7.0 software program (Statsoft, Tulsa, OK, USA, 2005). Initial data are given as mean±SEM (n is individual fish and is specified in tables), and final data are given as range (min–max) with $n=2$ cages.

3. Results

3.1. Diets

Feed ingredients and the analysed feed composition are given in Table 1. Protein, fat and moisture levels were close to the target levels of 52%, 18% and 8%, respectively, in all diets. Dietary starch ($R^2=0.93$, $P<0.01$) and ash ($R^2=0.997$, $P<0.001$) level decreased, while the remaining (mostly fibre) increased with inclusions of dietary plant protein ($R^2=0.996$, $P<0.0001$). The levels of vitamin B_6 were the same in all diets, while the concentration of vitamin B_{12} was 90% lower in the 100PP diet than in the FM diet, and decreased in a linear manner due to increases in plant proteins ($R^2=0.98$, $P<0.001$).

Protein ingredients with different amino acid pattern were mixed and DL-methionine and L-lysine were added to support assumed minimum requirement levels of all indispensable amino acids in the diets. However, the amino acid patterns (g amino acid per g protein) were not similar, giving significant variations between diets (Table 2). Of the indispensable amino acids (IAA) phenylalanine increased and valine, methionine, lysine, arginine and threonine decreased with increasing inclusion of plant protein. Of the dispensable amino acids (DAA) glutamic acid, serine, proline and tyrosine increased and aspartic acid, glycine, taurine and alanine decreased with increasing inclusion of plant protein (Table 2). As a consequence of this, the ratio IAA/DAA decreased in a linear manner ($R^2=0.92$, $P<0.01$) from 2.12 in the FM diet to 1.75 in the 100PP diet. Tryptophan, glutamine and cysteine could not be analysed by the method used, and are therefore not part of the evaluation.

3.2. Growth, feed and protein utilization

Low mortality (<4%) and maturation (<2%) were registered in all groups throughout all feeding periods, with no diet dependent variations. All diet groups had approximately the same initial weight of 1652±6 g (mean±SEM, $n=10$ cages), with no significant differences in start weight between cages. In the first period (Dec–Feb) poor appetite and thus low feed intake, was observed in one cage given the 50PP diet resulting in lower growth compared with the replicate cage (Fig. 1). Although feed intake improved in the second (Feb–April) and third period (April–June) growth in this cage was always inferior to that in the replicate cage (Fig. 1). The deviation in growth compared with the general growth pattern observed in all other cages, suggests that the underperforming cage fed the 50PP diet should be treated as an outlier when treating data related to dietary influence on growth.

Omitting the one outlier fed 50PP, specific growth rate (SGR) over the total period of 28 weeks, decreased slightly with increased inclusion of up to 75% plant protein in diet ($Y=0.338-0.001x$, $R^2=0.81$, $P<0.01$) (Table 3). A further increase in plant protein inclusion up to 100% significantly reduced SGR from around 0.3 to 0.15, thus the control group (FM) increased its average weight with 1540 g, while the 100PP group only increased their average weight with 533 g. The growth rate in all diet groups was also lower (SGR from 0.08 to 0.29) in the cold period (second period; Feb–April) compared to the first period (Dec–Feb) where SGR values from 0.17 to 0.39 were obtained, and the last period (April–June) where SGR values ranged between 0.12 and 0.40, depending on dietary treatment (Table 3).

The sea cages used were not equipped for waste feed collection; therefore, feed intake was carefully monitored visually based on appetite, resulting in quite reliable feed intake estimates except for three cages; the poor appetite in the 100PP group (two cages) and in one cage fed the 50PP diet, made it difficult to control feeding in these cages.

FCR (estimated from feed offered) ranged between 1.10 and 2.78 in the first feeding period (Des–Feb), 1.05–2.93 in the second feeding period (Feb–April) and 1.02–2.77 in the third feeding period (April–June). This resulted in an increased FCR with increased dietary plant protein in all periods, and when evaluating the total trial ($R^2=0.72$, $P<0.01$) (Table 3). These effects were also seen when the 100PP treatment was excluded from the regression model ($R^2=0.73$, $P<0.01$).

PER ranged between 0.73 and 2.11, and PPV ranged between 0.16 and 0.45, and both PER and PPV decreased with increased inclusion of plant protein (PER; $R^2=0.72$, $P<0.001$, PPV; $R^2=0.82$, $P<0.01$) (Table 3).

3.3. Digestibility

The apparent digestibility coefficients (ADC) of dry matter, protein and fat ranged between 64 and 79%, 85 and 92% and 91 and 97%, respectively, and were not significantly affected by diet ($P>0.05$) (Table 4). However, a noticeable drop in ADC_{dm} and $ADC_{protein}$ was found in the 100PP diet group. ADC_{starch} showed small but significant reduction as the level of plant protein increased ($R^2=0.58$, $P<0.01$), and with a major decrease in starch digestibility when plant protein level increased from 75PP to 100PP. Excluding the 100PP diet from the regression led to a non-significant effect on ADC_{starch} ($P=0.304$), implying that the reduced digestibility of starch was related to the 100PP diet only.

Table 2
Amino acid concentration in feed (mg g protein^{-1})

	FM	25PP	50PP	75PP	100PP	Statistics		
						Regression line	R^2	P
Indispensable amino acids (IAA)								
Valine	51	52	49	47	47	$Y=51.23-0.04x$	0.78	<0.05
Leucine	80	82	80	81	83		0.49	ns
Isoleucine	44	46	47	46	45		0.07	ns
Phenylalanine	42	46	47	51	53	$Y=42.3+0.1x$	0.99	<0.001
Methionine	29	28	22	19	16	$Y=29.4-0.1x$	0.97	<0.01
Lysine	80	72	59	49	46	$Y=79.5-0.4x$	0.96	<0.01
Arginine	60	58	55	53	50	$Y=60.7-0.1x$	0.99	<0.001
Histidine	24	22	21	20	21		0.75	ns
Threonine	46	43	39	36	33	$Y=45.5-0.1x$	0.99	<0.0001
Dispensable amino acids (DAA)								
Aspartic acid	96	93	84	78	73	$Y=96.8-0.2x$	0.98	<0.001
Proline	44	57	71	86	98	$Y=43.7+0.5x$	0.999	<0.0001
Glutamic acid	148	185	222	261	294	$Y=148.2+1.5x$	0.999	<0.00001
Serine	44	45	47	50	51	$Y=43.8+0.1x$	0.95	<0.01
Glycine	64	59	51	45	39	$Y=64.1-0.3x$	0.997	<0.0001
Taurine	8	6	4	2	nd	$Y=7.8-0.1x$	0.999	<0.00001
Alanine	65	59	50	43	35	$Y=65.2-0.3x$	0.998	<0.0001
Tyrosine	33	35	35	36	37	$Y=33.18+0.04x$	0.93	<0.01
IAA/DAA	2.12	1.98	1.84	1.75	1.75	$Y=2.081-0.004x$	0.92	<0.01

Linear regression equation (where Y is the response and x the level of plant protein in diet), R^2 and P (significant when $P<0.05$) are also given.
nd: not detected, ns: not significant.
Tryptophan, glutamine and cysteine were not analysed.

3.4. Fish composition and liver size

HSI ranged from 12.7 to 14.3 for diet groups FM, 25PP, 50PP and 75PP at final sampling, and was not influenced by increases in dietary plant protein. For the 100PP group final HSI ranged between 10.1 and 11.4 (Table 3), and was significantly different from the FM control group. There were no diet dependent variations in fat, protein, glycogen or dry matter levels in the whole body, muscle or liver, except for significantly lower whole body dry matter content in the 100PP group compared to the FM group (Table 5).

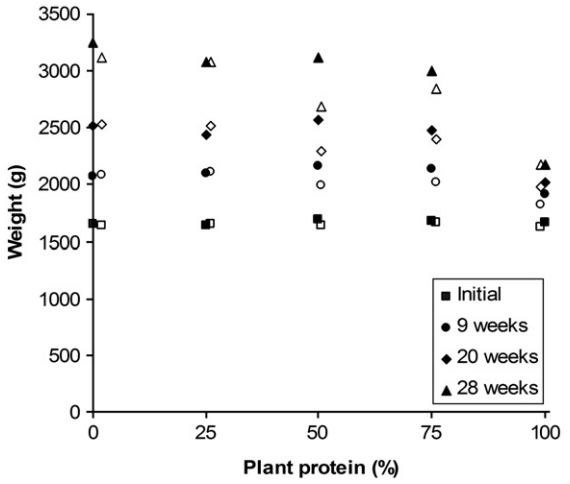

Fig. 1. Weight (g) of cod given diets with an increasing level of plant protein (0–100%) in duplicate cages from the start of the trial and after 9, 20 and 28 weeks.

3.5. B-vitamin concentrations, ASAT and ALAT activities in liver

Concentrations of vitamins B_6 and B_{12} in muscle and liver, and transaminase (ASAT and ALAT) activities in liver are given in Table 5. The levels of vitamins B_6 and B_{12} in muscle were not influenced by diet. In liver, all experimental diets had lower concentration of vitamin B_6 compared to the FM control diet (Dunnetts $P<0.05$). ALAT activity in liver tended to be lower in all groups given plant protein (238–409 U g^{-1}) compared to the FM diet group (415–467 U g^{-1}).

3.6. Free amino acid concentrations in plasma

As methionine is the major limiting IAA in soybean, and soybean constituted a major part of the plant protein mixture, the level decreased by 45% when replacing 100% of the fish meal with plant protein (100PP diet). This was clearly reflected in plasma methionine concentrations 5 h post-feeding where the level decreased from 16.5 μmol 100 ml^{-1} (mean) in fish fed FM to 9.4 μmol 100 ml^{-1} (mean) in fish fed 100PP diet ($R^2=0.86$, $P<0.001$) (Table 6). The second limiting PP ingredient lysine (in wheat gluten) was also reduced by 40% as the plant protein inclusion increased, but no linearity was

Table 3
Specific growth rate (SGR) and feed conversion ratio (FCR) for period 1 (Dec.–Feb.), 2 (Feb.–April), 3 (April–June) and for the whole trial

Diet	FM	25PP	50PP	75PP	100PP	Linear regression line[a]	R^2	P
Period 1								
SGR	0.35–0.37	0.38–0.39	0.31–0.39	0.31–0.38	0.17–0.22	$Y=0.40-0.002x$	0.52	<0.05
FCR	1.10–1.14	1.22–1.26	1.29–1.55	1.45–1.59	2.69–2.78	$Y=0.94-0.01x$	0.74	<0.01
Period 2								
SGR	0.29–0.29	0.23–0.26	0.21–0.26	0.22–0.26	0.08–0.13	$Y=0.30-0.002x$	0.69	<0.01
FCR	1.05–1.10	1.19–1.26	1.28–1.80	1.41–1.55	2.69–2.93	$Y=0.92+0.01x$	0.72	<0.01
Period 3								
SGR	0.32–0.40	0.32–0.36	0.25–0.30	0.26–0.30	0.12–0.15	$Y=0.38-0.002x$	0.77	<0.01
FCR	1.02–1.07	1.15–1.24	1.20–1.43	1.38–1.50	2.34–2.77	$Y=0.89+0.01x$	0.73	<0.01
Whole trial								
SGR	0.33–0.35	0.32–0.32	0.25–0.31	0.27–0.30	0.14–0.15	$Y=0.36-0.002x$	0.73	<0.01
FCR	1.06–1.10	1.19–1.25	1.26–1.59	1.42–1.55	2.68–2.71	$Y=0.88+0.01x$	0.72	<0.05
PPV	0.36–0.45	0.36–0.41	0.26–0.36	0.27–0.29	0.16–0.19	$Y=0.43-0.002x$	0.84	<0.001
PER	2.08–2.11	1.78–1.86	1.31–1.66	1.39–1.55	0.73–0.75	$Y=2.12-0.01x$	0.89	<0.0001
Final sampling								
CF[b]	1.4–1.5	1.4–1.5	1.3–1.5	1.3–1.4	1.2–1.2	$Y=1.487-0.003x$	0.67	<0.01
HSI[b]	12.7–14.1	13.3–13.6	13.1–14.3	13.2–13.5	10.1–11.4		0.32	ns

Protein efficiency ratio (PER) and protein productive value (PPV) are also given for the whole trial. Condition factor (CF), hepatosomatic index (HSI) and weight (g) are only given for the final sampling. All data are given as range (min–max, $n=2$ cages). Linear regression lines (where Y is the response and x are the level of plant protein in diet), R^2 and P (significant when $P<0.05$) are also given.
ns: not significant.
[a] One replicate of 50PP treated as an outlier and are excluded from the statistics.
[b] Initial mean±SEM; CF: 1.4±0.0 ($n=10$), HSI: 11.7±0.3 ($n=10$).

found in plasma lysine concentration 5 h post-feeding. Further, plasma valine, leucine and isoleucine concentrations increased with increasing level of plant protein in diet (Table 6). Of the dispensable amino acids (DAA) serine, glutamine and glutamic acid increased, and aspartic acid and taurine decreased with increasing dietary plant protein levels. Several of the diet dependent variations in plasma amino acid concentration correlated significantly ($P<0.05$) with the concentrations of the respective amino acids in the feeds (Glu; $r=0.79$, His; $r=-0.72$, Thr; $r=-0.74$, Pro; $r=0.80$, Met; $r=0.89$, Ser; $r=0.70$). The plasma IAA/DAA ratio decreased ($R^2=0.62$, $P<0.05$) with increasing level of plant protein in diet. Plasma ammonia increased from 19.2 μmol 100 ml^{-1} in the fish given the FM diet to 36.9 μmol 100 ml^{-1} in fish given the 100PP diet ($Y=16.7+0.2x$, $R^2=0.73$, $P<0.01$).

3.7. Free amino acid concentrations in muscle

Of the muscle free IAA valine and leucine increased, while lysine and arginine decreased with increased inclusion of plant proteins in diets (Table 7). These muscle free amino acid concentrations were significantly ($P<0.05$) correlated with dietary amino acid concentrations (Val; $r=-0.67$, Leu; $r=0.70$, Lys: $r=0.99$, Arg; $r=0.91$). For methionine no significant linear decrease could be established due to concentrations below detectable levels for the 75PP and 100PP diets, but a clear decrease was registered (from 0.022 mg g^{-1} in FM to not detected in 100PP). Of the DAAs alanine and tyrosine increased following increased dietary PP, and tyrosine correlated with the tyrosine level in the feeds ($r=0.82$, $P<0.05$). The muscle IAA/DAA ratio decreased ($R^2=0.87$, $P<0.0001$) with increasing level of plant protein in diet.

Table 4
Apparent digestibility coefficients (ADC) for protein, fat and starch in cod fed diets with increasing levels of plant proteins in the diet for 28 weeks

Nutrient	FM	25PP	50PP	75PP	100PP	Statistics[a]	
						R^2	P
Dry matter	74–75	78–79	73–77	76–77	64–70	0.33	ns
Crude protein	85–88	88–89	88–90	92	88–90	0.42	ns
Crude fat	95–96	96–97	93–96	94–95	91–96	0.23	ns
Starch[b]	91	92–93	87	88–92	80–82	0.59	<0.05

Given as range (min–max, $n=2$ cages), only one value is given when min and max are the same. Linear regression coefficient (R^2) and P (significant when $P<0.05$) are also given.
ns: not significant.
[a] One replicate of 50PP treated as an outlier and are excluded from the statistics.
[b] $Y=92.8-0.1x$.

Table 5
Proximate composition (given as g 100 g^{-1} wet weight) and vitamin B_6 (mg kg^{-1}) and B_{12} (μg kg^{-1}) in whole body (a), muscle (b) and liver (c) from cod given increased level of plant protein for 28 weeks

Diet	FM	25PP	50PP	75PP	100PP	Statistics[a]	
						R^2	P
a) Whole body							
Crude fat	7.7–8.3	9.1–9.3	6.0–8.3	8.2–8.6	4.6–7.2	0.27	ns
Crude protein	18	18	17–18	17–18	18	0.11	ns
Dry matter	29	30	28	29	25–27*	0.37	ns
b) Muscle							
Crude fat	0.4–0.5	0.5	0.4–0.6	0.4–0.5	0.5–0.6	0.16	ns
Crude protein	21	21	22	21–22	20–21	0.30	ns
Glycogen	0.3–0.4	0.2–0.3	0.5	0.3–0.4	0.2	0.05	ns
Dry matter	22	22–23	22	22	20–22	0.35	ns
Vitamin B_6	2.8–3.1	2.9–3.2	2.9	3.0–3.1	2.7–3.0	0.03	ns
Vitamin B_{12}	3.0	4.0	2.0	2.0	2.0–4.0	0.03	ns
c) Liver							
Crude fat	65.2–69.6	67.3–69.0	64.9–68.6	66.1–70.6	65.9–72.9	0.06	ns
Crude protein	3–4	3	3–4	3	3–4	0.05	ns
Glycogen[b]	2.5–3.9	3.3–3.4	2.6–3.0	2.7–3.3	1.7–1.8	0.47	<0.05
Dry matter	73–77	75–76	73–75	74–77	74–75	0.003	ns
Vitamin B_6	0.9	0.6–0.7*	0.7*	0.6–0.7*	0.7*	0.32	ns
Vitamin B_{12}[c]	17–18	18–22	8–18	11–12	12	0.48	<0.05
ASAT (U g^{-1})[d]	357–361	209–333	216–325	204–219	218–260	0.50	<0.05
ALAT (U g^{-1})	415–467	245–362	263–409	272–318	238–310	0.37	ns

Values given as range (min–max, $n=2$ cages), only one value given when min and max are the same. Linear regression coefficient (R^2) and P (significant when $P<0.05$) are also given.
ns: not significant.
*Different from FM control diet using Dunnetts' test.
[a] One replicate of 50PP treated as an outlier and are excluded from the statistics.
[b] $Y=3.47-0.01x$.
[c] $Y=18.4-0.1x$.
[d] $Y=333.3-1.2x$.

3.8. Plasma nutrients

Plasma concentrations of protein and TG did not vary with diet at sampling in February, whereas in April and June a decrease was found in plasma protein and TG concentrations with increasing level of dietary plant protein (Table 8). These effects were also seen when the 100PP group was excluded from the regression. Plasma glucose concentration was affected by diet at the sampling in February, decreasing as dietary plant protein increased ($R^2=0.61$, $P<0.01$), but this effect was not seen at following samplings. Plasma cholesterol level decreased with increased dietary plant protein at all samplings, also when the 100PP group was excluded from the diet (Table 8).

4. Discussion

The high growth registered in all groups, except the 100% PP treatment, shows a great potential for plant protein usage in cod diets, and exceeds earlier recommendations (Refstie et al., 2006a). The difference in growth obtained between cages fed the 50PP diet was due mainly to an initial delay in feed intake. Thus excluding this outlier, our conclusion is that up to 50% plant protein can be recommended in practical cod diets without any adverse effects on growth or feed utilization. The slightly reduced growth up to 75% PP coincided with reduced PER and PPV, and agrees with earlier studies evaluating plant proteins in cod diets (Refstie et al., 2006a) and diets for several other species (Refstie et al., 2000; Martin et al., 2003; Opstvedt et al., 2003). However, our experiment was the first to evaluate diets holding up to 100% plant protein. In comparison, the recent study by Refstie et al. (2006a) included 24% as plant protein only. The increased FCR, associated with increased plant protein up to 75% inclusion was also observed by Refstie et al. (2006a) and can be explained as a compensatory intake to meet demands for protein to maintain maximum growth in line with findings for Atlantic salmon (Espe et al., 2007). Also, the plant protein mixture in our study

Table 6
Concentration of free amino acids in plasma (mmol 100 ml^{-1}) from cod fed diets with increasing levels of plant protein (0–100%) for 28 weeks

	FM	25PP	50PP	75PP	100PP	Statistics[a]		
						Regression line	R^2	P
Indispensable amino acids (IAA)								
Valine	24.0–30.0	27.0–27.6	32.6–43.6	35.6–40.6	36.5–44.8	$Y=26.8+0.2x$	0.62	<0.05
Leucine	22.1–27.4	23.6–25.2	30.7–43.3	34.6–40.0	34.1–43.3	$Y=24.4+0.2x$	0.59	<0.05
Isoleucine	11.4–14.8	11.8–13.5	15.7–21.6	17.0–20.0	16.7–21.6	$Y=12.9+0.1x$	0.52	<0.05
Phenylalanine	13.2–14.9	14.3–15.3	16.7–21.1	18.8–22.6	13.6–19.0		0.23	ns
Methionine	15.7–17.3	14.0–14.3	13.1–15.5	10.1–11.3	9.2–9.7	$Y=16.5-0.1x$	0.86	<0.001
Lysine	31.2–31.5	22.1–24.2	28.8–32.1	15.1–17.4	26.5–26.2		0.19	ns
Arginine	9.7–9.8	7.7–8.2	11.6–14.3	9.7–10.8	9.6–13.9		0.19	ns
Histidine	3.6–3.8	4.4–7.0	5.6–7.1	7.1–7.4	4.4–9.1		0.38	ns
Threonine	11.5–12.5	11.0–12.6	14.4–15.9	13.1–13.4	14.3–16.8	$Y=11.71+0.03x$	0.50	<0.05
Dispensable amino acids (DAA)								
Cysteine	1.9–2.7	2.1–2.6	2.0–3.0	1.6–2.5	1.7–2.0		0.17	ns
Aspartic acid	1.5–1.6	1.0–1.2	0.0–1.2	0.8–0.9	0.7–0.9	$Y=1.42-0.01x$	0.75	<0.01
Proline	nd	nd	24.9–28.4	29.5–35.9	46.9–49.7		0.03	ns
Glutamic acid	3.5–4.8	3.4–4.0	4.3–6.3	5.5–5.9	5.3–7.2	$Y=3.66+0.02x$	0.62	<0.05
Glutamine	12.5–13.2	15.1–18.5	31.7–32.1	29.5–40.1	23.0–78.7	$Y=10.4+0.4x$	0.51	<0.05
Serine	4.0–4.8	5.4–6.1	6.5–6.8	5.3–5.9	7.5–7.7	$Y=4.65+0.03x$	0.67	<0.01
Glycine	5.0–6.3	6.2–6.8	7.3–8.3	5.7–6.1	5.2–5.3		0.09	ns
Taurine	25.3–36.4	17.8–30.8	8.2–18.3	8.2–10.4	2.8–11.2	$Y=29.4-0.3x$	0.74	<0.01
Alanine	25.6–30.9	26.7–42.0	37.2–42.8	28.7–31.7	35.7–39.1		0.15	ns
Tyrosine	11.3–12.0	12.9–16.2	21.2–23.3	22.6–26.8	14.5–21.0		0.39	ns
IAA/DAA	1.45–1.54	1.17–1.46	1.01–1.46	1.13	0.95–1.08	$Y=1.45-0.004x$	0.62	<0.05

Concentrations given as range (min–max, $n=2$ cages), and linear regression equation (where Y is the response and x the level of plant protein in diet), R^2 and P (significant when $P<0.05$) are also given.
nd: not detected, ns: not significant.
Tryptophan not analysed.
[a] One replicate of 50PP treated as an outlier and are excluded from the statistics.

differed from that used by Albrektsen et al. (2006) which included full-fat soybean meal and corn gluten meal. The reduced appetite registered in the 100PP group, indicates that this inclusion level exceeded the level where the fish accepted the feed, possible as a result of reduced palatability. However, many other factors varied in this diet, e.g. vitamin B_{12}, amino acid pattern and mineral level. For the 100PP group, all plasma nutrients were lower, reflecting fish close to a food-deprivation status (Hemre et al., 1993; Krogdahl et al., 1999).

As no attempts were made to standardize amino acid patterns in the diets beyond supplementation of limiting amino acids (lysine and methionine) the amino acid pattern varied between the diets used in this trial. Amino acid requirements are not established for cod, but the diets were formulated to meet requirement levels for IAA described for rainbow trout in NRC (1993). Efficient protein synthesis requires sufficient availability of all IAA (Dabrowski and Guderly, 2002). Unbalanced amino acid concentrations in a diet will result in increased protein degradation (Langar et al., 1993; von der Decken and Lied, 1993), and thereby increased protein turnover (Martin et al., 2003). A 48% increase in plasma ammonia from the FM diet to the 100PP diet, was observed in the present trial. Ammonia is an end product from amino acid catabolism contributing 60 to 90% of the nitrogen excreted (Cowey and Walton, 1988). Excretion of ammonia is found to be low when protein synthesis is high (Lied and Braaten, 1984). Therefore increased plasma ammonia concentration can be an indicator of reduced protein synthesis, expressed as lower growth and protein retention. Amino acids as such can not be stored in tissues (Geiger, 1947) and excess free amino acids will be readily oxidized. In our study the IAA/DAA ratio decreased with increased inclusion of plant proteins. As protein catabolism increases when the dietary IAA/DAA ratio decreases (Gómez-Requeni et al., 2003; Vilhelmsson et al., 2004) the decrease in IAA/DAA ratio with increasing dietary plant protein in our study, suggests a corresponding increase in protein catabolism. Espe et al. (2007) showed that 95% replacement of fish meal with plant protein was possible in diets for salmon provided that the amino acid profile

Table 7
Concentration of free amino acids in muscle (μg g^{-1}) from cod fed diets with increasing levels of plant protein (0–100%) for 28 weeks

	FM	25PP	50PP	75PP	100PP	Statistics [a]		
						Regression line	R^2	P
Indispensable amino acids (IAA)								
Valine	12–18	6–21	25–29	29–31	31–33	$Y=16.2+0.2x$	0.89	<0.001
Leucine	0–16	4–5	23–30	29–30	28–33	$Y=7.8+0.3x$	0.62	<0.05
Isoleucine	nd	nd	16	15	nd		–	–
Phenylalanine	nd	8–14	15–18	20–25	7–17		0.03	ns
Methionine	14–30	10–13	4–9	nd	nd		0.55	ns
Lysine	522–581	466–490	362–366	224–233	148–214	$Y=559-4x$	0.96	<0.00001
Arginine	138–187	112–145	92–104	80–89	32–46	$Y=162-1x$	0.89	<0.001
Histidine	113–135	155–247	166–278	217–242	60–126		0.01	ns
Threonine	43–55	53–71	76–99	75–84	44–63		0.05	ns
Dispensable amino acids (DAA)								
Cysteine	51–78	43–49	35–49	20–28	17–31	$Y=60-0.4x$	0.76	<0.01
Aspartic acid	27–30	28–30	27–29	28–30	25–28		0.11	ns
Proline	108–152	131–246	575–730	504–603	367–518	$Y=17.5+4x$	0.48	<0.05
Glutamic acid	81–104	99–106	86–103	84–112	77–89		0.09	ns
Glutamine	39–43	36–38	76–102	45–68	29–115		0.22	ns
Serine	39–60	51–102	69–79	59–93	64–77		0.11	ns
Glycine	173–265	234–247	177–216	195–211	207–254		0.01	ns
Taurine [b]	>1000	>1000	>1000	>1000	>1000			–
Alanine	366–372	384–423	413–420	423–459	353–480	$Y=371+1x$	0.85	<0.01
Tyrosine	6–8	16	23–25	31–40	19–31	$Y=10.1+0.2x$	0.61	<0.05
IAA/DAA	0.69–0.85	0.64–0.67	0.40–0.42	0.32–0.35	0.29–0.34	$Y=0.74-0.005x$	0.87	<0.001

Concentrations given as range (min–max, $n=2$ cages), one value given when min and max are the same. Linear regression equation (where Y is the response and x the level of plant protein in diet), R^2 and P (significant when $P<0.05$) are also given.
nd: not detected, ns: not significant.
Tryptophan not analysed.
[a] One replicate of 50PP treated as an outlier and are excluded from the statistics.
[b] Above upper detection limit and not included in the IAA/DAA ratio.

mimicked that in fish meal and the fish accepted the feed. This indicates that differences in amino acid profiles may explain the reduction in growth in the 100PP group compared with the FM control group.

Methionine was the IAA with lowest concentration in the 100PP diet, and this was highly reflected in plasma and muscle free methionine concentrations, as also found in other species when diet level of methionine is below requirement levels (NRC, 1993). Cysteine in diets were not analysed, but plasma showed stable cysteine values, while muscle cysteine decreased. These results confirm that sulphur containing compounds were limiting, especially in the 100PP diet group. Also, a decrease in muscle free lysine of 67% was seen when comparing the fish meal group and the 100PP group, correlating well with diet levels. Thus, methionine and lysine seem to be the limiting amino acids in cod diets based on soybean and wheat gluten. Carter et al. (2000) proposed that the lowest relative concentration of an IAA in the muscle free pool should be considered in relation to its potential to limit the efficiency of protein synthesis and retention.

In salmonids, increases found in whole body fat content with the use of dietary plant proteins, were explained by unbalances in amino acid concentrations (Bjerkeng et al., 1997; Kaushik et al., 2004). Furthermore, it is suggested that unbalanced amino acid composition influences energy metabolism. Vilhelmsson et al. (2004) found an up-regulation of several proteins involved in energy metabolism in rainbow trout liver when fed 100% plant protein and concluded that plant protein increases the energy demands of fish. However, in the present trial no increases in fat deposits were registered, finding similar proximate compositions of whole body, liver and muscle in all diet groups, and that all values were in the same range as in earlier cod trials (Rosenlund et al., 2004). Previous studies showed that dietary macro-nutrients balance, as well as feed energy density, are of utmost importance to control cod liver growth (the only fat storing organ in this species), implying that diets can be designed to steer lipid deposition, and thereby relative liver sizes (HSI) (Rosenlund et al., 2004; Karlsen et al., 2006; Hansen et al., 2007). HSI values in our trial were

Table 8
Plasma nutrients values in cod fed diets with increasing levels of plant protein in December (initial sampling), in February (9 weeks), in April (20 weeks) and in June (28 weeks), given as range (min–max, $n=2$ cages), one value given when min and max are the same

Diet	FM	25PP	50PP	75PP	100PP	Statistics [a]		
						Regression line	R^2	P
Protein (g l^{-1})								
Initial	44							
February	38	44–45	39–42	41–43	33–38		0.10	ns
April	46–47	43–45	41–42	33–37*	31–37*	$Y=46.8-0.1x$	0.89	<0.001
June	48–49	45–49	43–45	42–45	36*	$Y=49.6-0.1x$	0.80	<0.01
Glucose (mmol l^{-1})								
Initial	8.0							
February	6.3–7.5	5.6–10.3	4.9–6.0	4.0–4.3	2.4–3.5	$Y=7.90-0.05x$	0.61	<0.01
April	3.4–4.9	4.7–5.6	2.6–5.2	3.3–4.3	2.5–3.3		0.32	ns
June	5.4–6.9	7.0–8.4	4.3–4.7	5.7–6.2	3.0–5.8		0.27	ns
TG (mmol l^{-1}) [b]								
Initial	5.0							
February	4.4–4.8	6.0–6.1	4.6–5.3	4.7–5.2	3.1–3.9		0.29	ns
April	5.7–6.4	5.5–5.7	5.3–5.5	3.6–4.6*	2.4–3.6*	$Y=6.29-0.03x$	0.83	<0.001
June	4.5–5.2	4.6–4.9	4.3–4.4	4.0–4.1	2.3–3.1*	$Y=5.11-0.02x$	0.75	0.01
Cholesterol (mmol l^{-1})								
Initial	7.3							
February	8.9	8.9–9.4	6.6–7.3*	6.0–6.7*	4.4–4.9*	$Y=9.6-0.05x$	0.90	<0.0001
April	9.1–10.9	9.7–10.9	7.0–7.6*	5.4–5.9*	4.4–4.8*	$Y=10.7-0.1x$	0.87	<0.001
June	10.4–11.4	9.3–10.1	7.1–7.3*	6.3*	4.7–4.8*	$Y=10.9-0.1x$	0.97	<0.0001

Linear regression equation (where Y is the response and x the level of plant protein in diet), R^2 and P (significant when $P<0.05$) are also given.
*Different from FM control diet using Dunnetts' test.
[a] One replicate of 50PP treated as an outlier and are excluded from the statistics.
[b] Triacylglycerols.

about 1% higher than predicted based on the macronutrient composition (Hansen et al., 2007) possibly related to the larger size of fish used in the present study. Furthermore, several compounds for which no exact requirements are known for cod were reduced with increasing levels of plant protein in the diets. They included levels of minerals naturally occurring in fish meal, the presence of specific growth stimulating peptides also naturally found in LT-fish meal (Aksnes et al., 2006), and vitamins (e.g. vitamin B_{12}).

Vitamin B_6 is an important co-factor for the enzymes ASAT and ALAT involved in transamination of alanine and aspartic acid. Compared to earlier findings (Hansen et al., 2007) vitamin B_6 concentrations in the present study were in the same range in muscle, but lower in liver, probably due to lower concentrations in the present diets. Decreasing liver ASAT activity and a tendency to lower liver ALAT activity in fish given diets containing plant proteins compared to the FM control diet, indicate reduction in substrates available for transamination as a consequence of reduced dietary levels of these amino acids. Furthermore, the stable vitamin B_6 concentrations in liver and muscle in all diet groups, suggest that the balance between several nutrients, and not dietary vitamin B_6, was insufficient. Vitamin B_{12} requirement is established to 0.02 mg kg^{-1} for salmonids but not for cod (Woodward, 1994). The 100PP diet (0.01 mg kg^{-1}) might therefore have been below requirement pointing at a need to establish B_{12} requirement data for development of sustainable cod diets based on high levels of plant proteins. Also, decreasing dietary vitamin B_{12} levels due to increasing levels of plant sources can easily be counteracted by increased supplementation in the premixes. Typical vitamin B_{12} deficiency signs such as low haemoglobin levels (Waagbø, 1999) were, however, not seen even in the 100PP group in the present study.

The plant protein diets contained increasing amounts of fibre (calculated; Table 1) and anti-nutrients known to reduce digestibility of nutrients (Francis et al., 2001; Krogdahl et al., 2005). However, a small reduction in digestibility was only observed for starch in this trial in contrast to earlier trials finding reduced digestibility also for protein and fat (Førde-Skjærvik et al., 2006; Hansen et al., 2006). On the other hand, we registered a reduction in plasma cholesterol which may be attributed to increased dietary fibre (Goto et al., 2001; Kaushik et al., 2004).

All diet groups had lower growth in the cold period (Feb–April), and more pronounced in groups given plant proteins; in the 100PP group SGR dropped 47% from period 1 (Dec–Feb) to period 2 (Feb–April). This may suggest that cod are more sensitive to nutrient imbalances caused by plant proteins at low water temperatures.

5. Conclusion

The growth was hardly affected up to 50% plant protein inclusion, but with 75% and 100% plant proteins SGR was lowered with 16% and 57%, respectively, compared to the fish meal based diet. Although the FCR and protein utilization (PER and PPV) are approximate values based on feed given and not feed intake (with waste feed collectors), the PER and PPV decreased and FCR increased with increased with increasing plant protein level. The reduced growth and retention values can partly be explained by unbalanced amino acid concentrations in diet, resulting in increased protein turnover. Especially lysine and methionine were low in the diets with 100% plant protein content (46 and 16 mg g protein^{-1} corresponding to 24.5 and 8.5 g kg diet^{-1}, respectively). Furthermore, reduced dietary minerals, increased fibre and anti-nutrient levels, and substantially lowered levels of vitamin B_{12} add to this explanation. Dietary vitamin B_6 levels seemed, however, to be sufficient.

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