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Protein requirements of Arctic charr

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Report summary

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Titill / Title	**Protein requirements of Arctic charr /** Próteinþörf bleikju		
Höfundar / Authors	Jón Árnason, Ólafur Ingi Sigurgeirsson, Jónína Jóhannsdóttir, Aðalheiður Ólafsdóttir and Joseph Ginindza		
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Ágrip á íslensku:	Fimm mismunandi fóðurgerðir með próteininnihaldi frá (29) 30 – 40% voru gefnar tveim stærðarhópum (100 gr. og 600 gr.) bæði í fersku og söltu vatni. Áhrif mismunandi fóðra voru metin út frá áhrifum þeirra á meltanleika, þyngdarþróun, dagvaxtar (SGR), fóðurnýtingar (FCR), efnasamsetningu flaka (í stærri fiskinum) og skynmat. Lokaþungi og dagvöxtur var lægstur hjá þeim fiskum sem fengu fóður með lægstu próteini, en engin áhrif fundust af próteini, umfram 37% í fóðri, á lokaþunga og SGR. Lágmarksþarfir fyrir prótein til vaxtar liggja því á milli 33% og 38% í fóðrinu. Ekki var um að ræða neinn verulegan mun á fóðursvörun milli stærðarhópa, jafnvel að áhrifin af lækkuðu próteini væru meiri hjá stærri fiskinum. Ekki var heldur hægt að sjá ein afgerandi áhrif af seltu á próteinþörfina. Próteininnihald í fóðri hafði ekki heldur nein afgerandi áhrif á flakasamsetningu eða skynmat á afurðum.		
Lykilorð á íslensku:	*Prótein, fóður, bleikja*		
Summary in English:	Four (five) different diets with protein varying from (29) 30 – 41% were fed ad libitum to two size groups of Arctic charr (100 gram and 600gram) in fresh- as well as seawater. The effect of the different diets was evaluated by digestibility, weight development, SGR, FCR, chemical composition of filet (in the bigger size groups) and sensory evaluation. The lowest final weights and SGR were found when fed the diets with lowest protein but here was no effect final weight and final weight between 38% and 41% protein in the diet, indicating that the minimum need for protein is between 33 and 38% protein in the diet. The same trend was shown in both size groups but the effect was more pronounced in the bigger fish than in the smaller fish. The results regarding size and growth were also the same in fresh- and seawater. The protein content in the diet did not have any marked effect on either chemical composition of filets or the sensory quality of the product.		
English keywords:	*Protein, Arctic charr*		

Contents

Introduction

There has been a considerable progress in the formulation of feeds for salmonids last decades. Consequently, the ratio of crude proteins (CP) and lipids (CL) in feed for large Atlantic salmon (*S. salar*) has been reduced from 55/8 in 1972 to 34/39 in 2004 (see www.Ewos.com). The feed conversion ratio (kg feed/kg fish growth) has also decreased as a result of increased energy content corresponding to increasing lipid levels in the diets. The objective is to spare the valuable proteins and improve the utilization of accessible CP for muscle growth while the energy from lipid primarily covers other energy usage. Results shows improved utilization of CP with decreased protein content when the lipid content is increased (Tabachek 1986; Arzel et al. 1995; Bendiksen et al. 2005). The minimum protein requirements are most probably reflected by the content of essential amino acids needed to fulfil muscle growth and other protein metabolism. The protein requirements of fish are size related since the basal metabolic rate, and hence the relative growth rate, is higher in smaller than bigger fish in similar environmental conditions. In larger fish the relatively less protein is needed for muscle growth and maintenance. In the effort to lower the feed cost, it is important to formulate the feed with maximum protein utilization and retention as an objective. Optimum nitrogen retention in the fish and less nitrogen excretion into the water will improve the quality of the rearing environment.

Although the salmonids are comparable in many terms, the nutritional requirements and feed utilization aren´t necessarily the same (e.g. Arzel et al 1992; Krogdahl et al. 2004; Azevedo et al. 2004a). The digestible energy requirement in relation to fish size is also different in salmonid species (Bailey, J. & Alanärä, A., 2006) and energy utilization changes with increased body size (e.g. Einen & Roem 1997; Azevedo et al.2004b; Bendiksen et al. 2003; Bendiksen et al. 2005). Hillestad & Johnsen (1994) tested different feeds containing 42, 39, 37 and 35% protein with increasing lipid content from 21,1–32,1% in salmon during the seawater growth phase. Fish fed the lowest protein content (35%) but highest energy content showed 27% faster growth compared with fish fed the highest protein content (42%). Furthermore, reduced feed conversion ratio was observed with increasing energy content. Similar results have been obtained for Atlantic halibut (Árnason et al. 2009a) and Atlantic cod (Árnason et al. 2009b).

Rasmussen, R.S. and Ostenfeld, T.H (2000) compared growth, quality traits and feed utilization in rainbow trout and brook trout (*S. fontinalis*), using commercial salmon feed (CP=49%; CL = 23%). The results indicated that the protein was more efficiently utilized in the brook trout. A comparative study carried out by Azevedo et al. (2003) indicated that feed utilization was up to 20% better in rainbow trout compared to Atlantic salmon. A number of studies carried out on rainbow trout fry indicate that the optimal protein content in feed is around 40% CP (Takeuchi et al. 1978; Kim et al. 1991) which is considerably lower than is commonly used in formulated feeds for Arctic charr fries and fingerlings in Iceland. Research on the nutritional requirements of Arctic charr is scarce compared with research on salmon and rainbow trout. The results of Jobling and Wandsvik (1983) indicate that the protein requirement (CP) of Arctic charr (18g initial weight) is 36-44% and Tabachek (1986) found that formulated feed containing 54% CP and 20% CL gave best growth of Arctic charr fingerlings (initial weight 4.6g). The results of Tabachek (1986), however, indicate that using a diet containing 44% CP and 20% CL represented the cheapest feed and led only to minimal reduction of growth compared to higher protein content.

The results of Skúlason et al. (1993) indicate a good protein efficiency of Arctic charr while the quality of the fish meal used did not significantly affect the growth rate of the fish. The protein levels tested in that study were between 42-45% CP. Guðmundsson & Pétursdóttir (1998) studied the digestibility and the growth rate in three different initial weight classes of Arctic charr (60, 360 and 980g) and two water temperatures (7 and 11°C). Altogether, nine types of formulated feeds containing 32, 41 and 52% CP and 7.5 14.5 and 21% CL were tested. The results indicate that increased protein content had insignificant effect on growth rate and less effect was observed on bigger fish. The high growth rate (SGR) observed for 60g fish fed a diet containing 32% CP was of particular interest. Only insignificant difference in growth was observed for this size group fed varying protein levels at 11°C. Therefore more study on the effect of 32-41% protein level in feed for A. charr is needed.

In a recent Canadian study, different protein/lipid ratio of 25,6-60,8% CP and 24.7-10.8% CL in feed did not significantly affect the growth rate of small trout (initial weight 2,56g) (Cameron et al. 2002). An exception was the feed containing the lowest protein content and highest lipid content (CP= 25,6%; CL = 24,7) which resulted in the lowest growth rate, highest feed conversion ratio (FCR) and protein utilization rate (PER). The content of essential AA in the feed was not specified in this research and the growth rate was rather poor.

Digestibility of the feed is of key importance for nutrient uptake. Protein digestibility measures give some information on accessible protein for muscle growth and protein metabolism in the fish. Studies of the digestibility are therefore important when studying feed utilization. In the seawater growth phase of Atlantic salmon the protein content of the feed is reduced progressively as the fish grows bigger, despite the increased growth rate following the seawater transfer. This reflects the good feed utilization of the salmon. Arctic charr can be grown in saline water and it is of interest to study whether the same applies for Arctic charr, taking the size and production time of both species into account.

Previous findings of the research group clearly indicate that protein requirements of Arctic charr are size dependent (Figure 1) (Sigurgeirsson et al 2009). Formulated feeds containing 35-52% CP were tested on different fish size groups. No effects on growth rate of fish above 100 g fed on diet containing 35-52% protein were found in this study. Hence, there is a reason to believe that minimum protein requirement for maximum growth rate of Arctic charr above fingerling size is not higher than 35% in the diet. The aim of the present study is to determine the minimal protein requirements for Arctic charr of 100g initial weight to harvesting size, with the intention of lowering the production cost and increase the efficiency of Arctic charr farming.

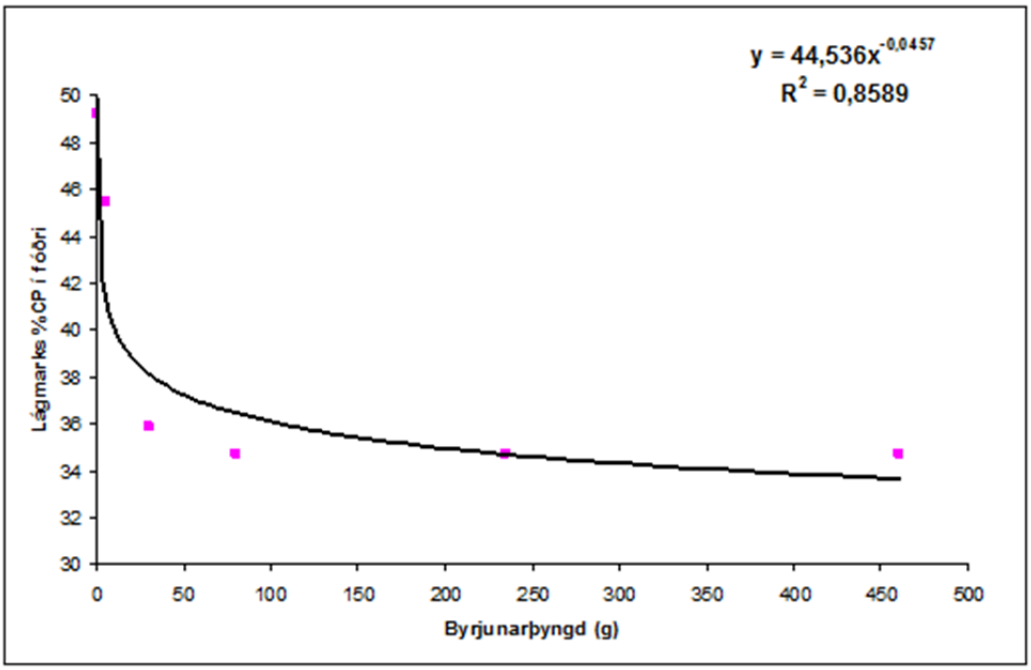

Figure 1: The relation between initial fish weight and minimum protein requirement in in diets on growth of Arctic charr (Sigurgeirsson et al 2009).

Growth trials

Materials and methods

Experimental system

The experiments were conducted in a partial re-circulation system at Verið in Sauðakrókur (trial 1a & b and trial 2b) and in Holalax fish farm (trial 2a).

In Trials 1a and 1b the mean temperature during the trial period was 10,0 °C (fig.2) and average Oxygen saturation was 102% in the effluent water (fig. 4). Trial 1a was run in fresh water but the salinity in Trial 1b (Salt water) was on average 26, 6 ppt. (fig. 3). In Trials 2a and 2b the mean temperature was 7,0±2 °C and average oxygen saturation was 102% in the effluent water. Trial 2a was carried out in fresh water but the salinity in Trial 2b (Salt water) was on average 26,6 ppt.

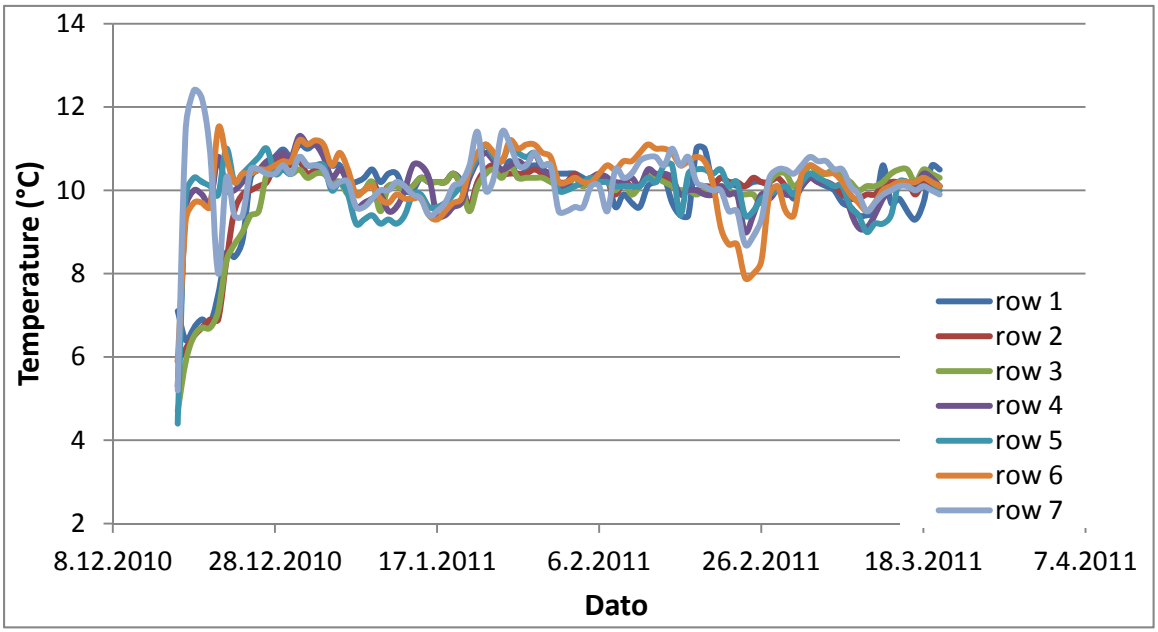

Fig. 2 Temperature in the experimental tank system, during the trial period in trial 1a & trial 1b (initial fish size 100g). Row 1-3 in FW (mean T= 9,9°C), row 4-7 in SW (mean T= 10,1°C).

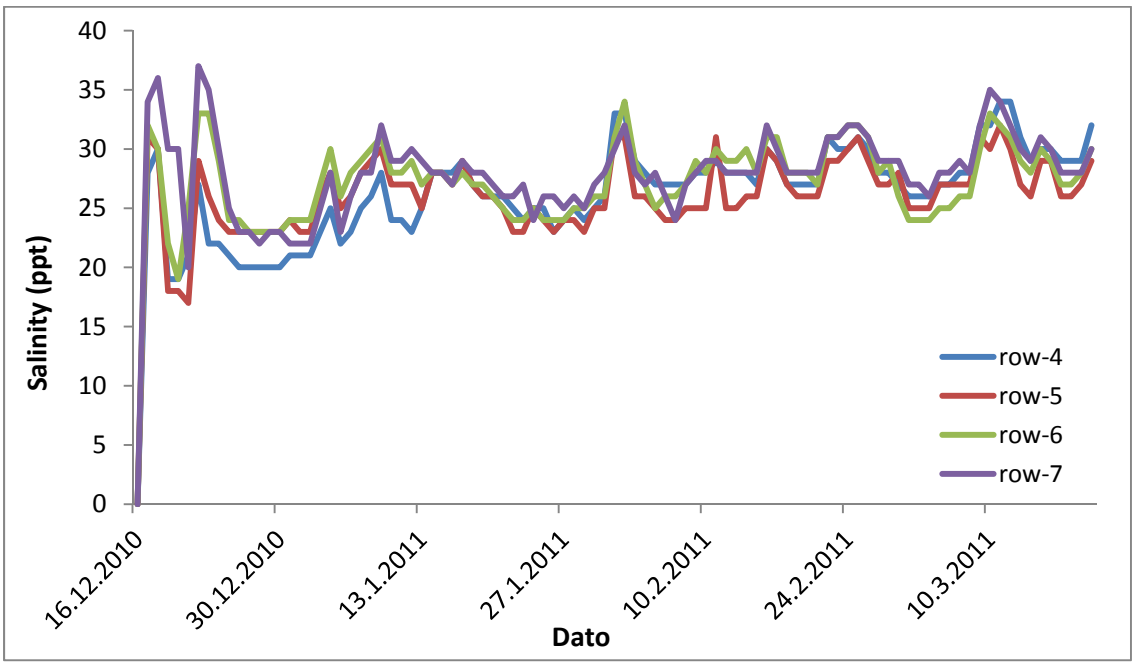

Fig. 3 Salinity in the experimental tank system during the trial 1b period (fish in sw, initial weight 100g).

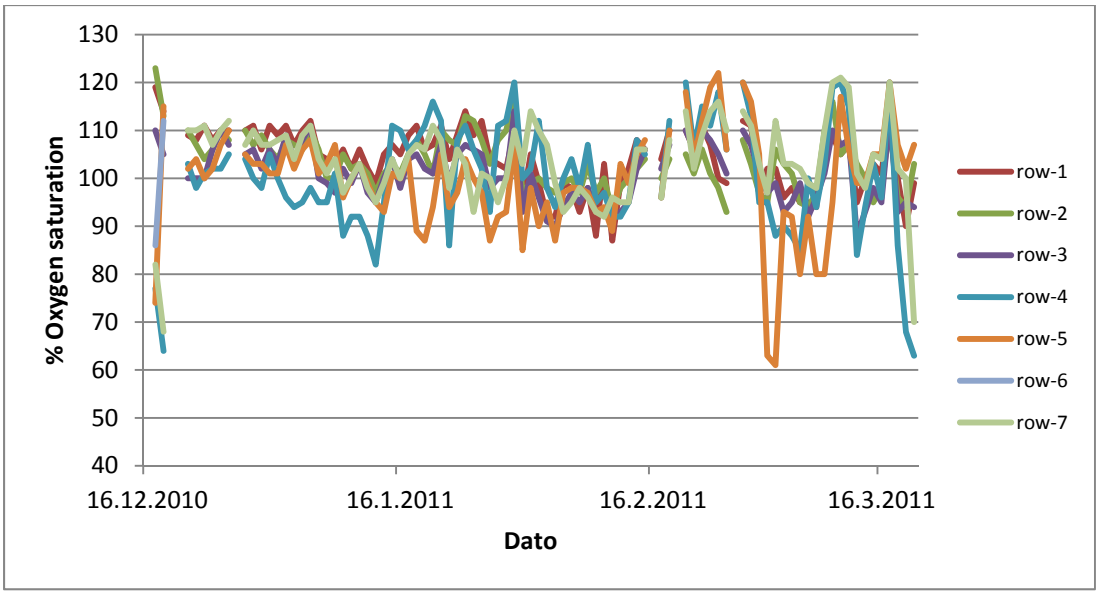

Fig. 4. Minimum oxygen saturation (%) during trial period for trial 1a &1b. (initial fish size 100g). Row 1-3 in FW, row 4-7 in SW.

Experimental fish

The fish in all trials was of the Holar strain. Initial number of fish in each tank was generally 60-66.

In Trial 1a (fresh water) 768 fish, with an average weight of 100 grams, were divided into 12 tanks of 0,8 cubic meters.

In Trial 1b (saline water) 960 100gram fish were divided into 15 tanks of the same volume as in Trial 1a.

In Trial 2a (fresh water) 720 fish, with an initial average weight of 588 grams, were divided into 12 tanks of 1,6 cubic meters.

In Trial 2b (saline water) 795 fish, with an initial average weight of 609 grams, were divided into 12 tanks of 1,6 cubic meters.

Experimental diets

Five experimental diets were manufactured using a commercial extruder at Laxá Feed Mill in Akureyri, Iceland. The diets were formulated and produced to be iso-energetic (~22 MJ/kg) but different in protein levels. The major protein source was fishmeal, followed by soya- and canola meal, formulated in different combinations (table 1). The feeds were produced in two different pellet sizes of 4mm and 6mm in diameter.

Table 1. Ingredient composition of the five experimental diets used feeding trials for Arctic charr.

Diet nr.	**2973**	**2974**	**2975**	**2976**	**2977**
Raw-materials %:					
Wheat	30,8	26,7	28,4	21,8	17,8
Corn gluten meal	2,8	8,5	10,0	10,0	10,0
Wheat gluten meal	0,0	0,0	2,6	5,6	9,8
Hypro soya	10,0	10,0	10,0	10,0	10,0
Canola meal	8,5	4,8	2,1	3,2	0,4
Fish meal NSM 705/109	18,4	21,3	24,1	27,0	29,8
MonoCalcium phosphate	0,3	0,2	0,1	0,0	0,0
Fish oil (Capelin)	28,0	24,6	21,6	21,4	21,1
Carop. Red	0,0	0,0	0,0	0,0	0,0
Carop. Pink	0,0	0,0	0,0	0,0	0,0
Premix	1,0	1,0	1,0	1,0	1,0
Yttrium oxid	0,015	0,015	0,015	0,015	0,015

Diets were tested in triplicate in all trials. In trial 1b (small fish in saline water) all diets were tested, diets 2973 – 2976 in trial 1a (small in fresh water) and diets 2974 – 2977 were tested for the bigger initial fish size both in fresh- and saline water (trials 2a and 2b).

The fishes were fed in excess and feeding volume adjusted daily to feed intake. Uneaten feed pellets were collected from the effluent water in a pellet trap, similar as described by Helland et al. (1996), for estimation of amount of feed eaten and FCR.

Chemical analysis of fillets and feed

The moisture content was determined by drying a 5 g sample at 110°C overnight followed by cooling it in a desiccator before reweighing (AOAC, 2000). Crude protein was calculated from total nitrogen content from a 0.5 g sample which was determined in a Kjeldahl system following acid digestion and titration of sample distillate according to the ISO standard (ISO 5983, 2005). Crude lipid was determined gravimetrically following ethyl-ether extraction from a dried sample according to Ba 3-38 (AOCS, 1998) in a Soxhlet extractor. Ash content was determined as total inorganic matter by incineration of a 10 g sample at 550°C overnight followed by cooling in a desiccator before reweighing according to ISO standard (ISO 5984, 2002). Energy content in 0.2 g dried sample was determined by combustion to ash in a bomb calorimeter (IKA C200) with the aid of pure oxygen, cotton thread and oil according to the calorimeter instruction manual. Yttrium oxide, phosphorus and zinc were determined following NMKL method number 186 published in 2007.

Sensory evaluation of Arctic charr

The sensory evaluation for this project was split in two parts. Firstly, four sample groups of arctic charr, grown in freshwater were evaluated the 5th. of October. The four groups were grown on feed

which differentiated in the content of protein. The samples were evaluated with profiling method (generic descriptive analysis, DA) where the intensity of sensory attributes was evaluated to describe sensory characteristics in flavour, odour, appearance and texture (Stone and Sidel, 1985). Thirteen trained and experienced panellists participated in the evaluation (ISO, 1993). Ten panellists evaluated all samples for freshwater grown Arctic charr and eight panellists evaluated all samples for seawater grown Arctic charr. Five panellists evaluated all samples for both freshwater grown and seawater grown Arctic charr. Sensory attributes used in this evaluation had been defined in earlier projects for Arctic charr. The scale consisted of 24 attributes which are listed in Table 2. The panel was experienced in sensory evaluation of Arctic charr but prior to this evaluation the panel had one reviewing session where they evaluated three samples from different feed groups, The intensity of each attribute for a given sample was described using an unstructured scale (from 0-100). The samples were 30-40g of skinned Arctic charr fillets. The samples were placed into small aluminium boxes and boiled in steam for five minutes. They were presented warm and with a lid to the panellists. Four samples were evaluated in each session. All samples were coded with three digit numbers and a duplicate was evaluated for each sample group. The sensory evaluation program FIZZ (2.10c, 1994-2005, Biosystémes) was used to collect sensory data.

In the Sensory evaluation the statistical program NCSS 2000 (NCSS, Utah, USA) was used to calculate analysis of variance (ANOVA) to compare sample groups and calculate p – values. Duncan´s test was used to calculate multiple comparisons between sample groups. The significance level was set at 5%, if not stated elsewhere.

Table 2. Sensory attributes, short names, scale anchors and attribute definition for GDA analysis of arctic charr.

Sensory attribute	short name	scale	definition
ODOUR			
sweet characteristic	O-sweet	none ‖ much	Sweet characteristic odour of boiled trout
metallic	O-metallic	none ‖ much	Metallic odour
fresh fishoil	O-fishoil	none ‖ much	Odour of fresh unspoiled fishoil
acidic	O-acidic	none ‖ much	citric acid, not spoilage sour
earthy	O-earthy	none ‖ much	Earthy odour
spoilage sour	O-sour	none ‖ much	Spoilage sour odour, spoilage characteristic
rancid	O-rancid	none ‖ much	Rancid odour, spoilage characteristic
APPEARANCE			
white precipitation	A-precipit.	none ‖ much	White precipitation on the sample surface and/or between flakes in sample
heterogenous colour	A-heterog.	none ‖ much	On the sample surface, how heterogenous is the colour
colour	A-colour	white ‖ orange	Inside sample; white / orange colour
yellow liquid	A-yellow liq.	colourless ‖ yellow	How yellow is the liquid in the box
fat droplets	A-fat dropl.	none ‖ much	Amount of fat in liquid surface.
FLAVOUR			
sweet characteristic	F-sweet	none ‖ much	Sweet characteristic flavour of boiled trout
metallic	F-metallic	none ‖ much	Metallic flavour
fresh fishoil	F-fresh fishoil	none ‖ much	Flavour of fresh unspoiled fishoil
acidic	F-acidic	none ‖ much	Citric acid flavour, not spoilage sour
earthy	F-earthy	none ‖ much	Earthy flavour
spoilage sour	F-sour	none ‖ much	Spoilage sour flavour, spoilage characteristic
rancid	F-rancid	none ‖ much	Rancid flavour spoilage characteristic
TEXTURE			
soft	T-soft	firm ‖ soft	Evaluated in first bite
juicy	T-juicy	dry ‖ juicy	Dry- draws liquid from mouth
tender	T-tender	tough ‖ tender	Evaluated while chewing
mushy	T-mushy	little ‖ much	Mushy texture, puree
sticky	T-sticky.	none ‖ much	sticky texture, force needed to pull teeth apart after biting

Harvesting output and yield

The effect of different feed composition on harvesting output and yield was estimated for fish in trial 2a. Nine fish from each group were taken for harvesting yield and output evaluation (table xx). The fish vas killed with a sharp blow to the head, bled by cutting all gill racers, gutted and filleted by hand. The condition factor (K) was calculated by the formula: 100*weight (g)/(length (cm))^3. Gutting yield, HSI and fillet output estimated by weighing ratio (liver weight/whole bled fish). The fillet yield is calculated as a ratio from whole bled fish.

Calculations

Specific growth rate (SGR) was calculated by following formula:

SGR%= 100*(LN(final weight) – LN(start weight)/number of feeding days

Feed conversion ratio (FCR) was calculated according to following formula:

FCR = kg feed eaten/ kg growth

Nitrogen free extractives (NFE) were estimated by difference calculation:

$$\boldsymbol{NFE = DM - (protein + lipid + ash)}$$

Apparent digestibility co-efficient (ADC) of nutrients and elements in each diet were calculated according to the following equation (Barrows et al., 2008):

ADC (%) = $100 - 100\left(\frac{\%Yt\ in\ diet \times \%\ nutrient\ in\ faeces}{\%\ Yt\ in\ faeces \times \%\ nutrient\ in\ diet}\right)$

For estimation of energy content the gross energy in MJ/kg; for fat (39,5), protein (23,6) and NFE (17,3) is used.

Statistical analysis

Data was analysed using general lineal model and one-way ANOVA in SPSS statistical program to determine existing significant difference of the measured variable to the different protein level in diet. The significance level was set at 5%.

Results

Diet composition

The composition of the experimental diets is shown in table 3.

Table 3. Analysed composition of experimental diets

Diet	2973	2974	2975	2976	2977
As is %:					
Water	7,87	8,30	7,89	5,48	5,12
Dry matter	92,13	91,70	92,11	94,52	94,88
Crude protein	29,01	30,06	32,71	37,79	40,84
Lipid (Soxlet)	21,61	20,42	20,52	21,91	22,24
NFE	36,09	35,08	32,64	28,72	25,83
Ash	5,41	6,13	6,24	6,10	5,96
Calculated brutto energy (MJ/kg)	21,6	21,2	21,5	22,5	22,9
In DM %:					
Crude protein	31,49	32,78	35,51	39,98	43,05
Lipid (Soxlet)	23,46	22,27	22,28	23,18	23,45
NFE	39,18	38,26	35,43	30,39	27,22
Ash	5,87	6,68	6,77	6,46	6,28
Calculated brutto energy (MJ/kg)	23,5	23,2	23,3	23,8	24,1

Smaller fish in fresh water (Trial 1a)

Growth

This trial lasted for 94 days. Growth curves of the feed groups (means of triplicates ± SEM) are shown in Figure 5. The average mean weight of the fish in all groups more than doubled during the period. On the second measuring day,- after 64 days of feeding, the average weight of the groups are different, where the group getting diet highest in protein content (40% CP in DM) is gaining most weight and are significantly heavier than other groups. The groups getting 33 or 36 % CP (in DM) are not statistically different in weight. The group getting the lowest CP content is lowest in average weight. This group is not statistically different from the group getting 36 % CP but the group getting 33% CP is statistically heavier. The differences between groups are similar on the final measuring day (after 94 days of feeding).

The growth rate (SGR) in this trial was reasonably good with an overall average of 0,9% per day (fig. 6). When SGR is calculated through the entire growing period no statistical difference between groups fed different protein content is detected. With closer look on SGR in the periods between measuring days (fig. 7), one can see a drop of growth rate in the group fed the lowest CP and increased variation within groups (tank effect). This also shows generally increased SGR during the growing period, although the culture conditions are fairly constant (temperature and oxygen level).

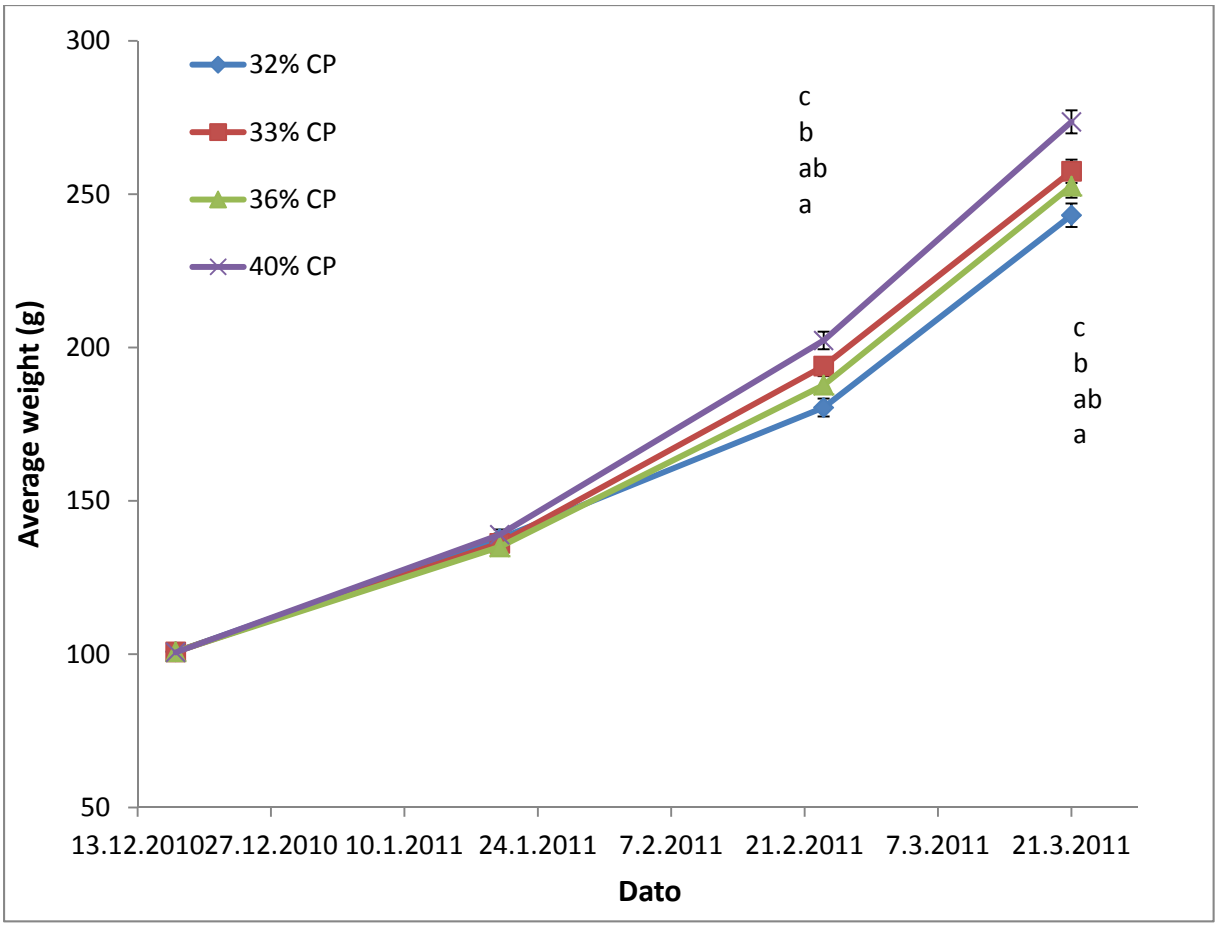

Figure 5. Mean weight (g ± SEM) of Arctic charr fed with diet of different protein content (32-40% CP) in fresh water (trial 1a) for 94 days. Initial weight is 100 g. n=3. Different letter is expressing statistical (p>0,05) difference between groups.

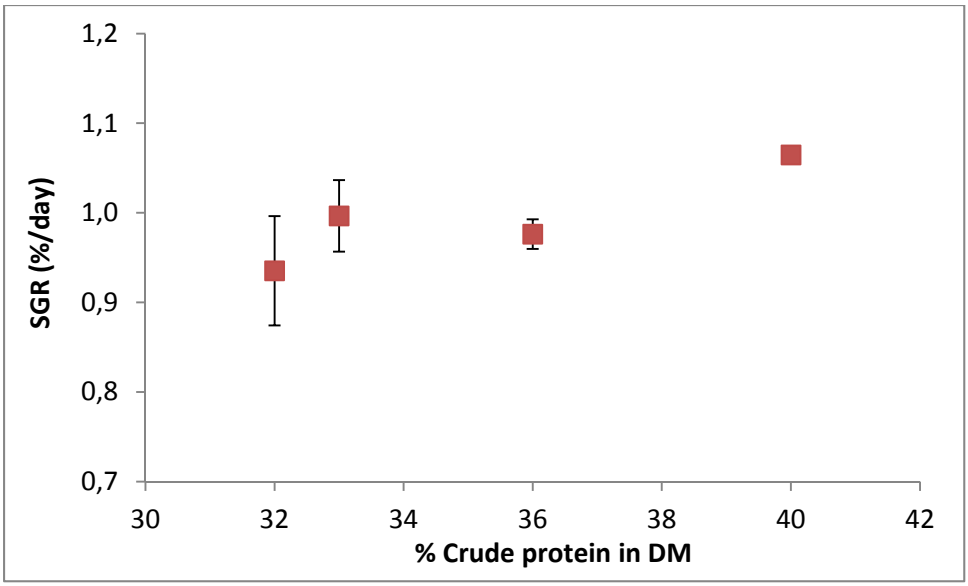

Figure 6. Average daily growth rate (SGR± SEM) of Arctic charr fed different protein content (% CP) during 94 days trial period in fresh water (Trial 1a).

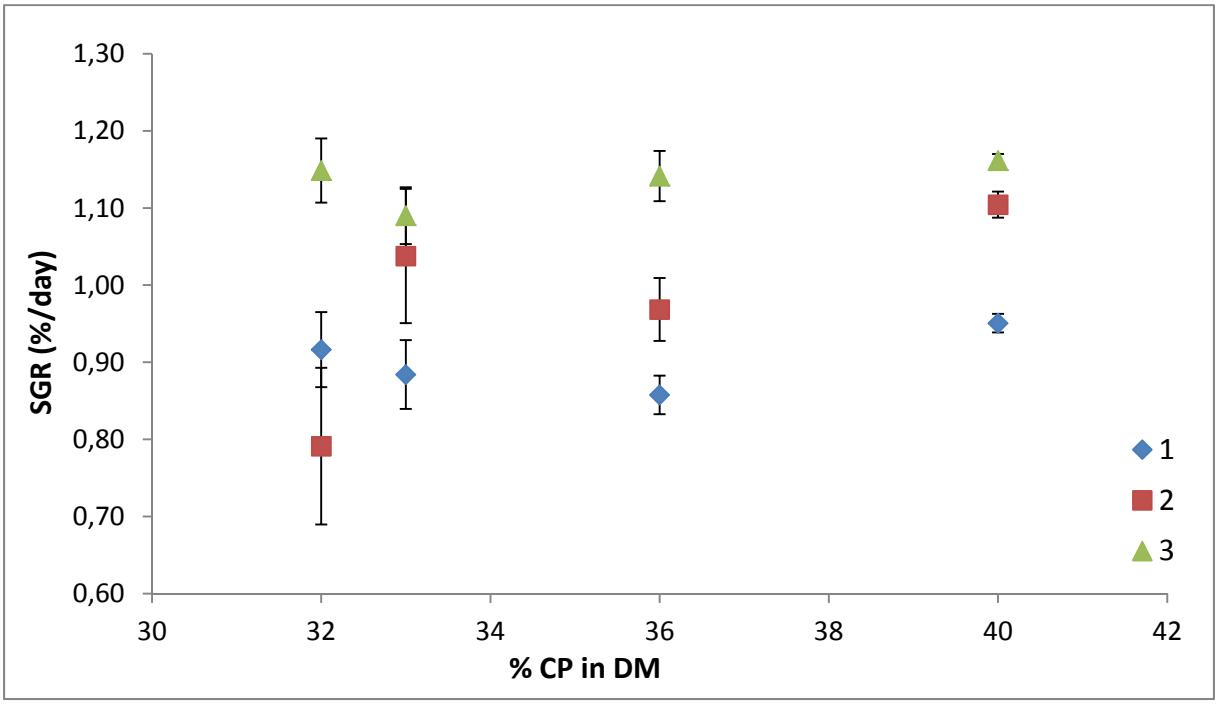

Figure 7. Average daily growth rate (% SGR± SEM) of replicates of A. charr, in separated growing periods (1= day 0-34; 2= day 34-68; 3 = day 68-94) for groups in fresh water (trial 1a) fed on different protein content in diet (Trial 1a).

Feed utilization

The average FCR in this trial is in the range of 0,96 to 1,14 (fig. 8). The FCR is significantly lover in the group getting 40% CP compared to the other three groups. There is no difference in FCR between groups getting 32, 33 or 36 % CP in diet.

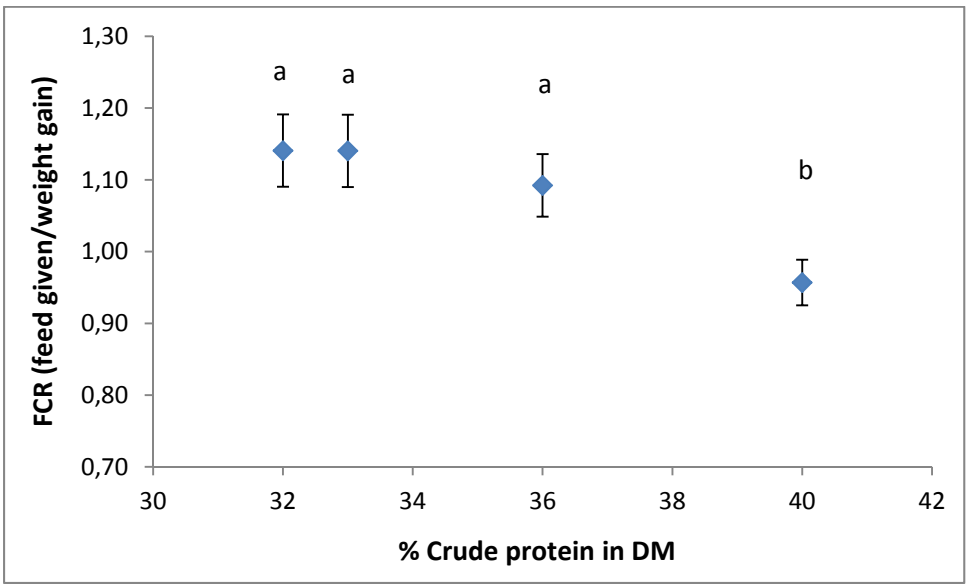

Figure 8. Average feed conversion ratio (FCR± SEM) in groups in fresh water (trial 1a), fed different protein content in the diet. n=3. Different letter is expressing statistical (p>0,05) difference between groups.

Sea water (Trial 1b)

Growth

In this 94 day trial the weight of the fish increased from 100 grams to an average of 200-250g grams (Figure 9) On the third measuring day the two groups getting the highest protein content in diet (40 & 43 % CP in DM) are significantly heavier than the other three groups, which are not different form each other. This difference is similar on the final weigh day.

The SGR in this trial varied from 0,5 to 1,05. There was a considerable variation between replicates within groups, but the average means between groups are not statistically different. There is, although an overall tendency to increased SGR with increasing protein content in the diet. (figure 10).

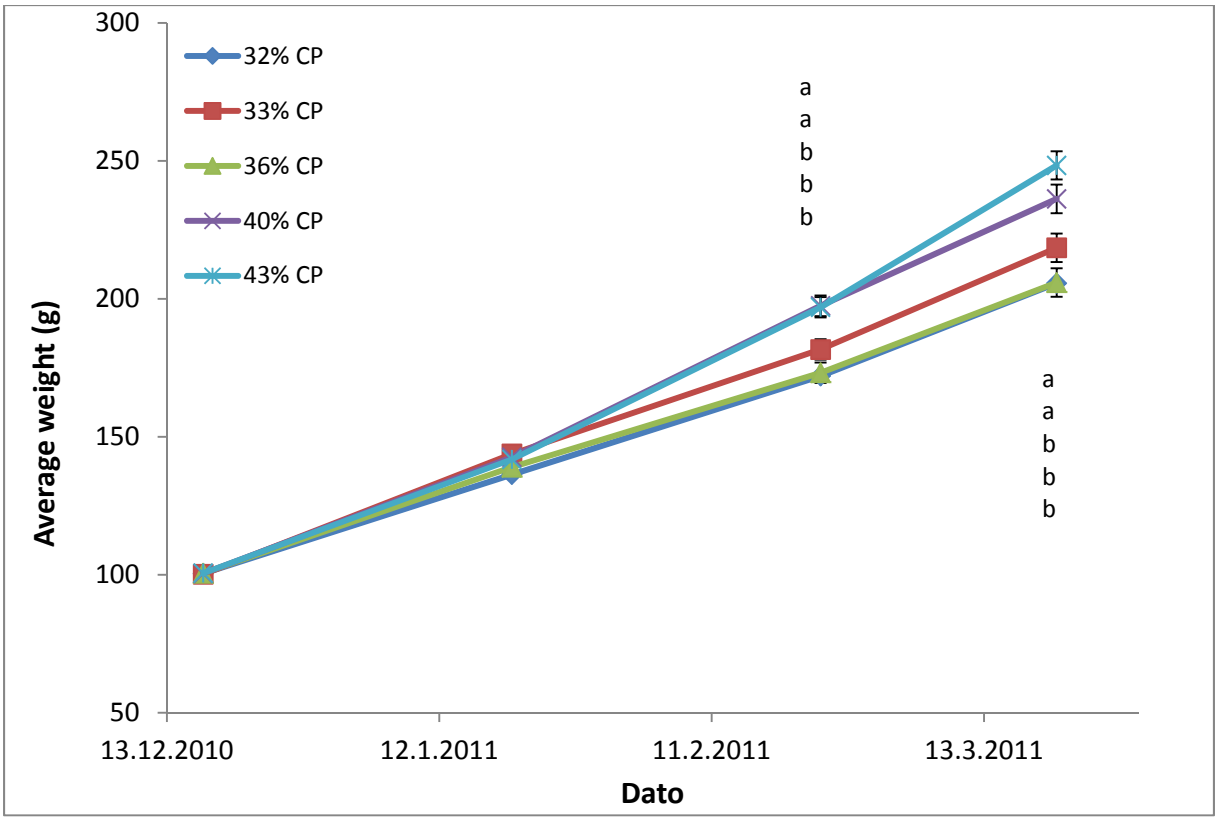

Figure 9. Average weight of Arctic charr (g ±SEM) fed on diet with different protein content (Trial 1b) in saline water for 94 days. n=3. Different letter is expressing statistical (p>0,05) difference between groups.

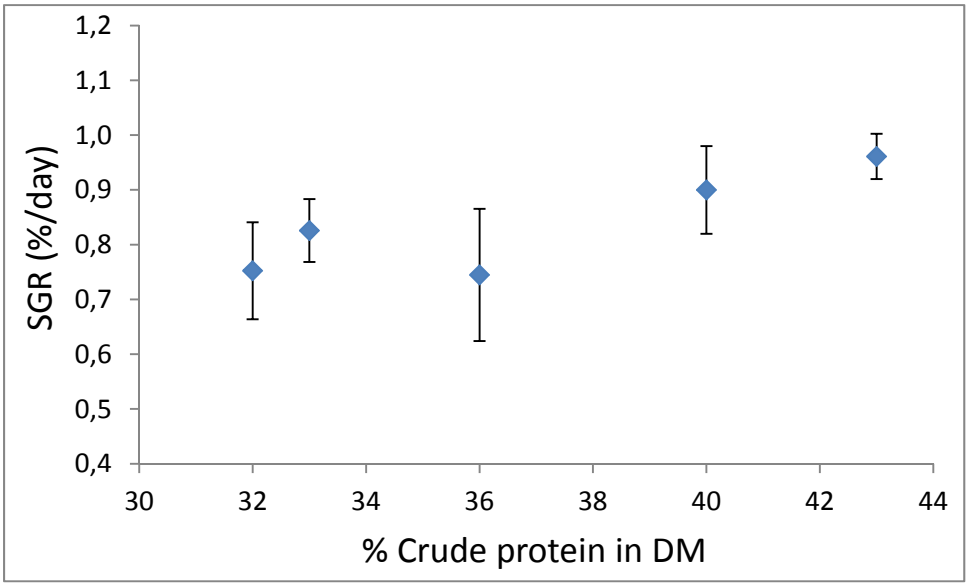

Figure 10. Average daily growth rate (% SGR ± SEM) of Arctic charr fed diet with different protein content in feed for 94 days in sea water (trial 1b). n=3.

Feed utilization

There is no statistical difference detected in FCR for the feeding groups in this trial (fig. 11). There are some variations within groups, particularly in the group fed with 36% CP in diet, where one of the three replicate is an outsider. These unexplained tank-effects are reflected in the results for growth and feed conversion for this group respectively. There is tendency towards lower FCR with increasing CP in the test diets.

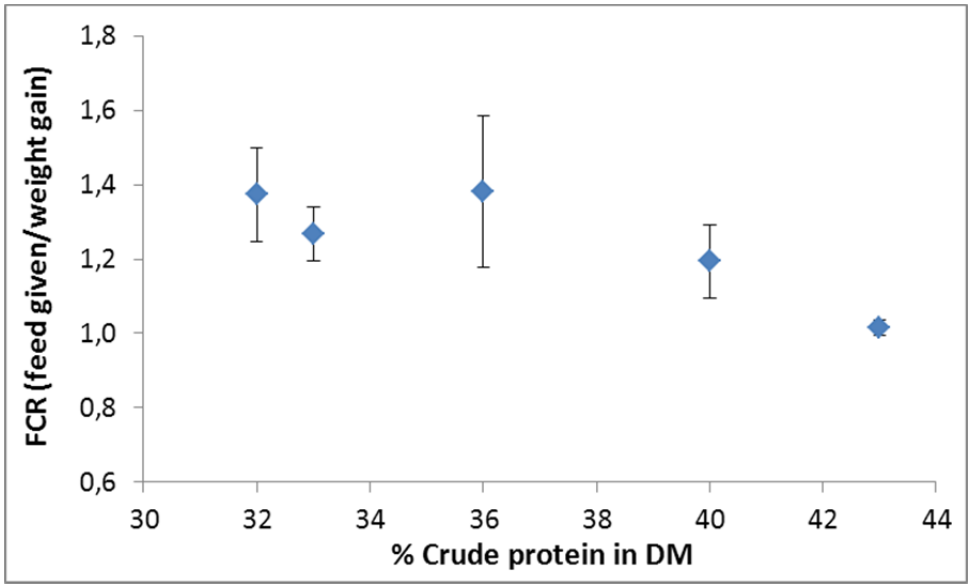

Figure 11. Average feed conversion ratio (FCR: kg feed/kg growth ± SEM) of Arctic charr fed with different protein content in diet for 94 days in sea water (Trial 1b). n=3

Fresh water (Trial 2a)

Maturation and mortality

Table 4. % of mature fishes in all experimental tanks at the final weighing day and average maturation ratio of each feed group.

Feed no (%CP in DM)	2974 (33)	2975 (36)	2976 (40)	2977 (43)
% mature fish: replicate-1	10,5	15,3	15,1	15,8
% mature fish: replicate-2	18,3	22	5	8,3
% mature fish: replicate-3	33,3	20,3	21,4	11,9
% Average maturation	20,7	19,2	13,8	12,0

Mortality: Mortality was negligible in general during this trial (21 of 720) i.e. less than 2% in average, but seven fishes died in one single tank (11,7%) and 6,7% in other, both in feed group getting fed 2976.

Development of weight and growth

The initial weight of the groups in this trial was around 590g but the final weight was from 774-943g. Even though the fish did not double the weight during the three months duration of this trial there is a significant effect of CP content in the feed on weight in both the intermediate and final weight (Figure 12). The two groups getting the highest protein content in diet (40 &43% CP in DM) are not significantly different from each other but are significantly heavier than the other two groups (33 & 36 % CP in DM). The group getting the lowest protein content (33%) is showing the lowest growth response and the average final weight is significantly lower than the 36% PC group.

The SGR in this trial was in the range of 0,23-0,57%, with an average of 0,28-0,49 in the feeding groups respectively. The SGR is showing similar trend as the weight gain, where the group getting the lowest protein content in diet has the lowest SGR, the two highest %CP group have significantly higher SGR and the 36% CP group has an intermediate SGR value (fig.13).

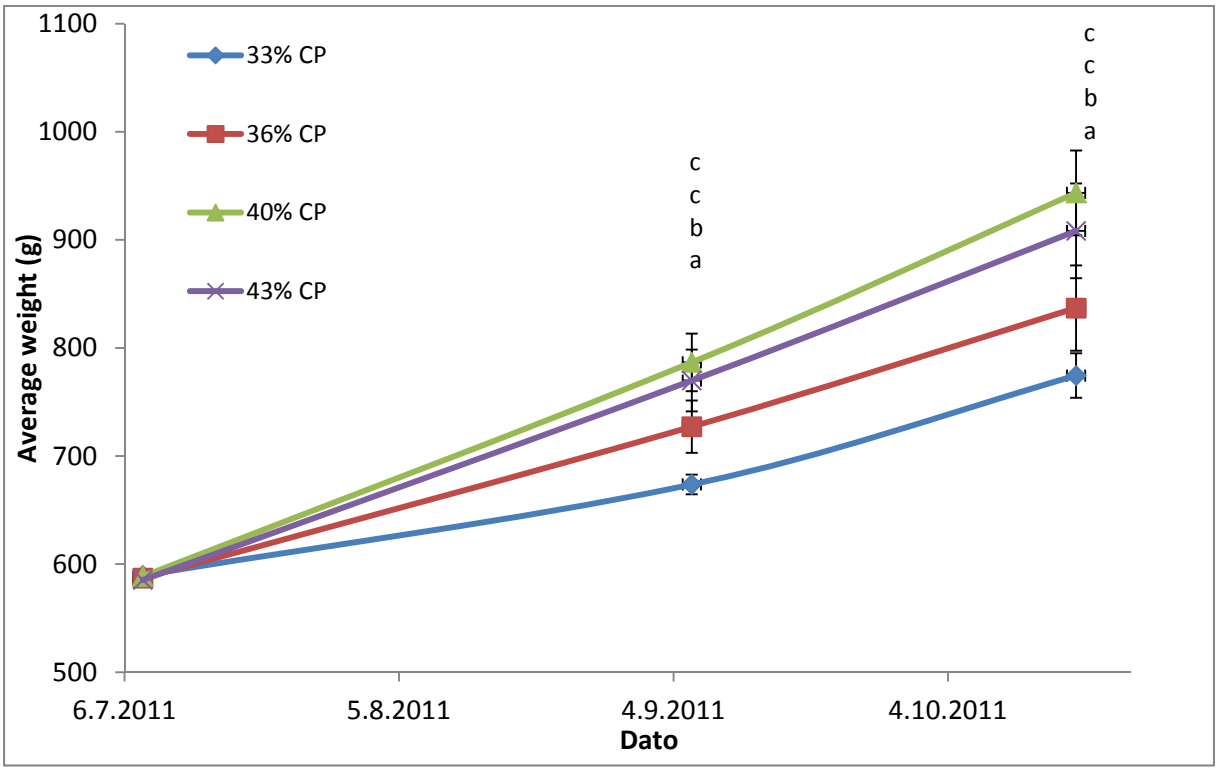

Figure 12: Average growth of Arctic charr in fresh water, fed with different protein content (33-43%C) in diet (trial 2a). n=3. Different letters showing statistical difference ($p<0,05$) at particular measuring day.

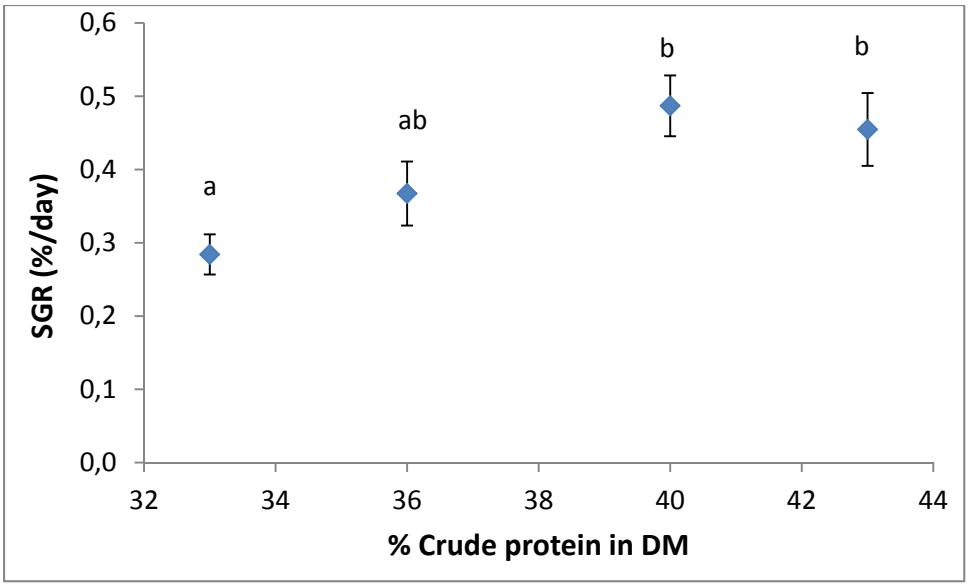

Figure 13: Average daily growth rate (%SGR) of the fish fed on different protein content in feed for 94 days (trial 2a). n=3. Different letter is expressing statistical (p>0,05) difference between groups.

Feed utilization

The FCR values in this trial were in the range of 1,82-1,09, and in average between groups in the range of 1,58-1,13. There is an overall tendency for reduction in FCR with increasing protein in the diet (fig.14). The group getting the lowest CP% diet has the highest average FCR and there is considerable variance within this group (1,36-1,82). However there is minimal effect of protein content higher than 36% in the dry matter.

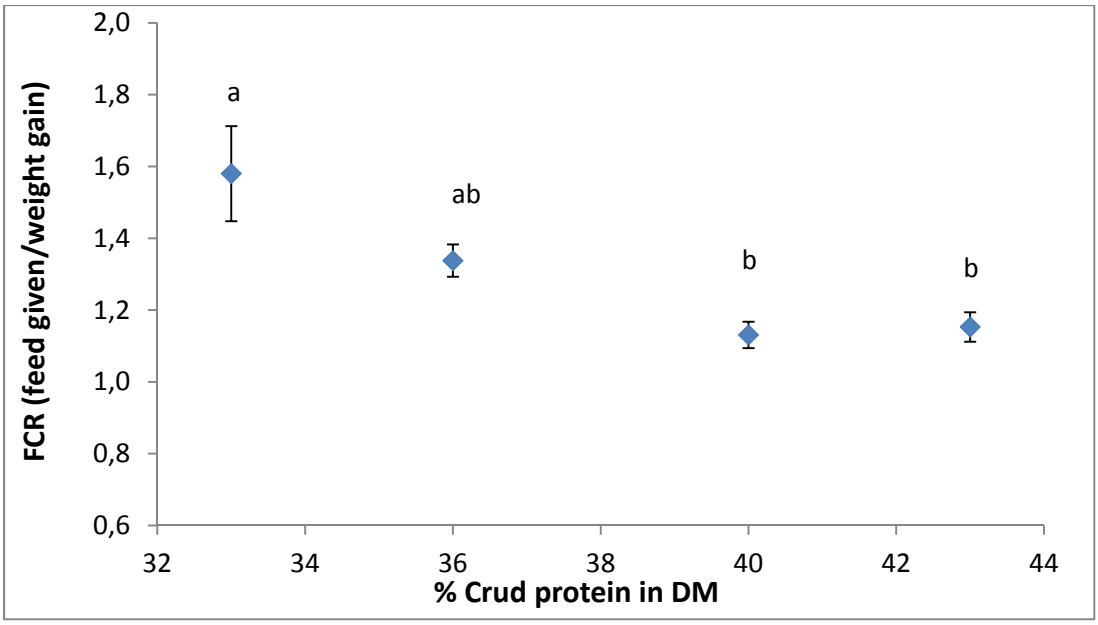

Figure 14. Average feed conversion ratio (FCR± SEM (kg feed/kg growth)) in Arctic charr fed different protein content (33-43%CP) in feed for 94 days (trial 2a). n=3. Different letter is expressing statistical (p>0,05) difference between groups.

Harvesting output and yield

The results on harvesting output and yield are shown in table 5. There are no statistical differences between feed groups in K-factor, % gutting weight loss or hepatosomatic index (HSI). Fillet yield is lowest in the 30%CP group, probably as an effect of slightly smaller fish in the sample and a tendency to lower K- factor with decreasing CP content.

Table 5: Average harvesting output at final weighing day (± SEM), condition factor and hepatosomatic index (HSI) of fish grown in fresh water, fed on different %CP í feed. n= 10

	Feed type (%CP in dry matter)			
	30	33	40	43
Length (cm ± SEM)	405,3± 6,2	411,3±7,1	414,4±5,9	423,5±8,3
Weight (g)	973,3 ± 73,7	1047,5 ± 87,8	1136,3 ± 59,9	1174,6 ± 75,9
Condition factor (K)	1,44 ± 0,08	1,48 ± 0,08	1,58 ± 0,04	1,52 ± 0,03
Guttet weight (g)	853,1±63,6	924,4± 75,2	1000 ± 52,4	1051,7 ± 73,1
% gutting weight loss	12,1 ± 0,69	11,5 ± 0,48	11,9 ± 0,72	10,7 ± 0,70
HSI (%)	2,29 ± 0,22	2,03 ± 0,17	2,08 ± 0,11	1,90 ± 0,12
% Fillet yield (untrimmed)	62,4 ± 0,42 a	64,4 ± 0,81 b	65,7 ± 0,85 b	65,6 ± 1,1 b

Fillet composition

Chemical compositions of the fillets in the groups are shown in table 6. The composition (DM, %CP, % lipid and % ash) seems to be unaffected by %CP in the diets, although some variations are recognized between groups.

Table 6. Chemical composition of fillet DM in trial 2a.

	Diet	Fillet			
Diet	Crude protein % in DM	DM	CP % in DM	Lipid % in DM	Ash % in DM
2974	33	34,8	52,3	22,7	3,2
2975	36	34,3	54,2	21,6	3,2
2976	40	35,0	53,1	22,9	3,1
2977	43	35,3	54,1	22,4	3,4

Sensory evaluation

Very little difference was seen between sample groups (Figure 15). No difference was observed in odour between groups. Difference was seen in colour and heterogeneous colour. Group 2976 had a more heterogeneous and orange colour than group 2977. No difference was seen in flavour or texture between groups. (For details see Appendix 2: Table 1)

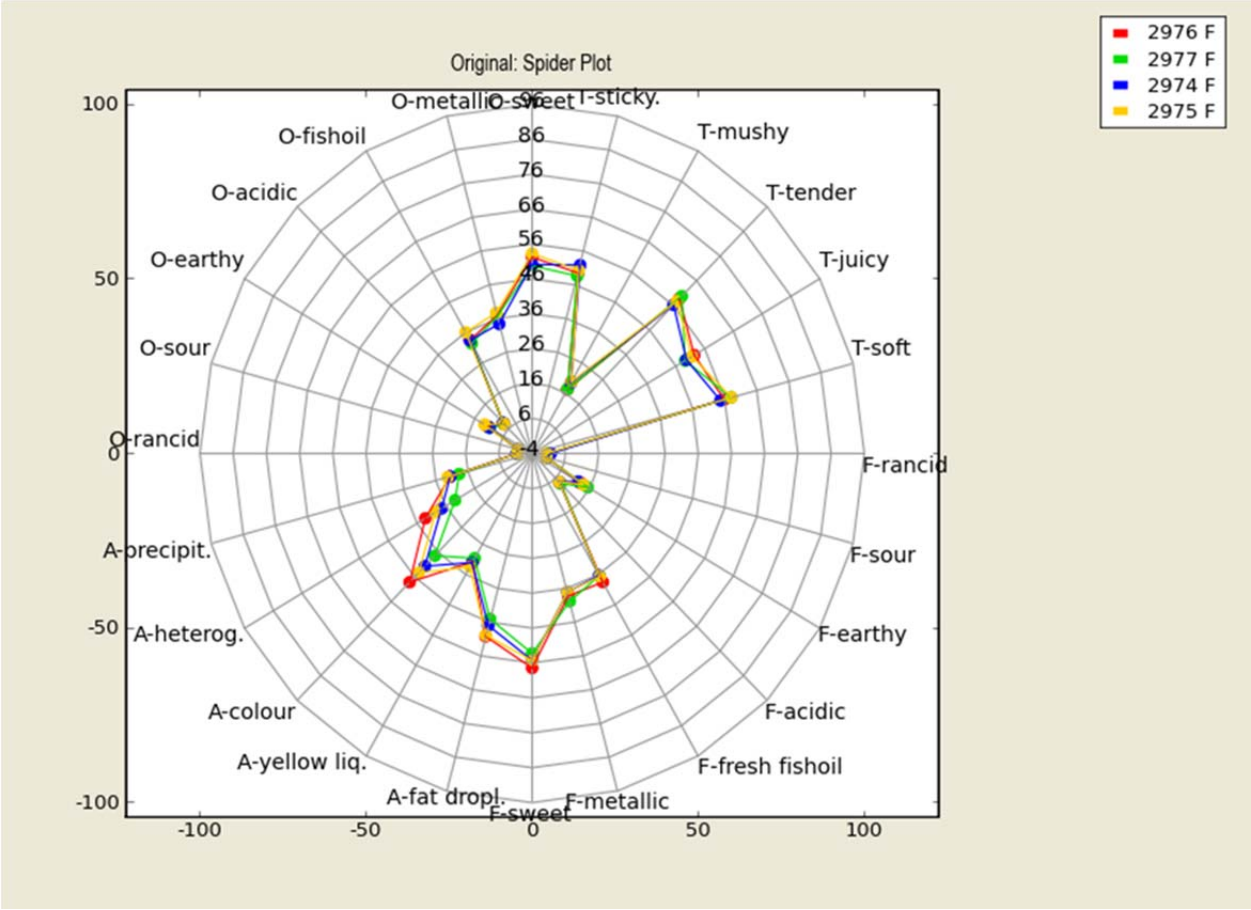

Figure 15. Mean values of sensory attributes (scale 0-100) for four different feed groups of arctic charr grown in freshwater.

Sea water (Trial 2b)

Growth

During this three month growth trial the average weight of the fish is nearly double and the SGR is reasonably good, related to fish size and water temperature. Both growth and growth rate (SGR) is unaffected of protein content in feed in this trial and no statistical difference is detected between groups (figure 16 and 17). Although it is a trend for a better growth in the group fed with the highest protein content in the diet (43% CP).

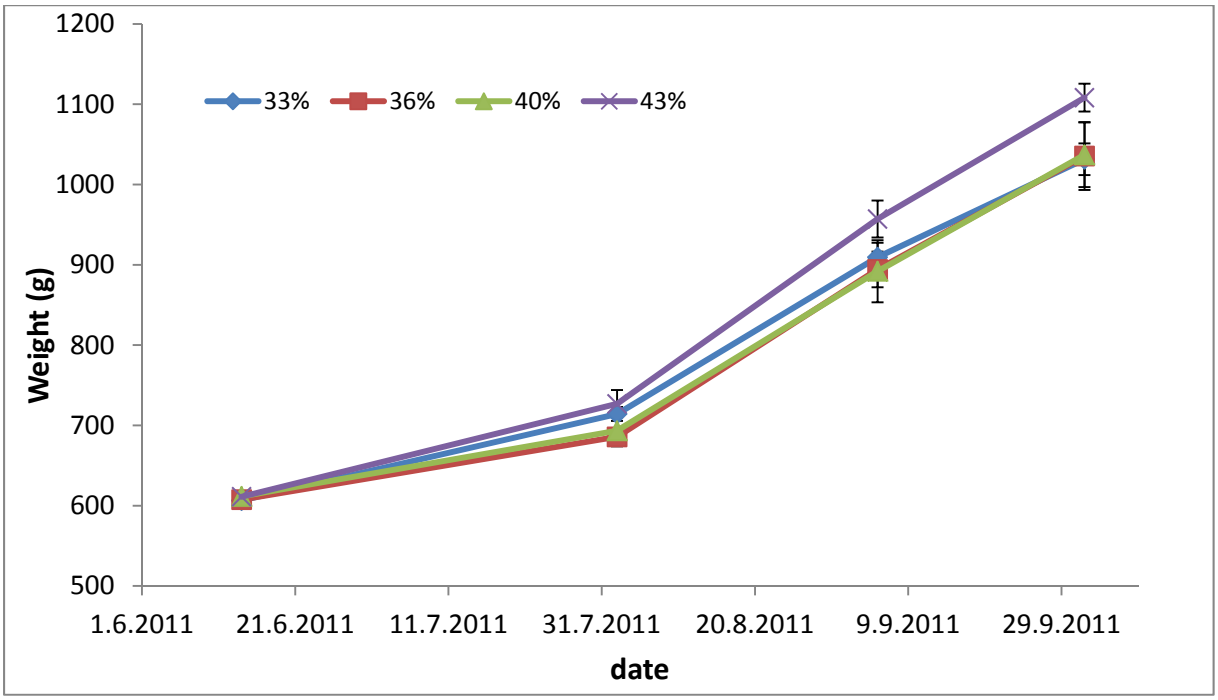

Figure 16. Average weight of Arctic charr (g±SEM) fed with different protein content in feed (33, 36, 40 & 43 %CP in DM) in 94 days in sea water (trial 2b). n=3.

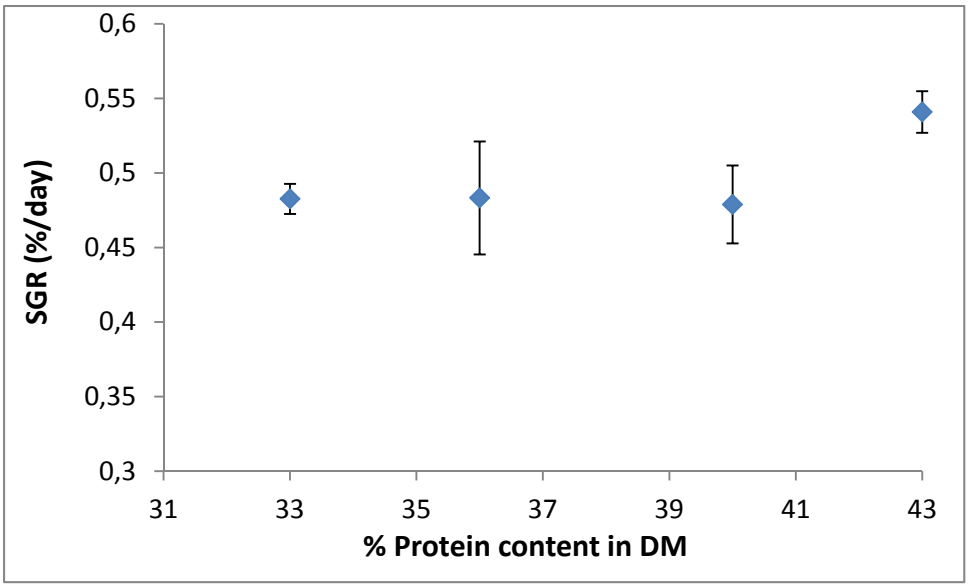

Figure 17. Average daily growth rate (%SGR) of Arctic charr fed different protein content in feed (33-43%CP) during the 94 days trial period in sea water (trial 2b). n=3.

Feed utilization

The FCR is a bit high and is in the range of 1,55-1,31 for the groups in this trial. There appears to be a decline in FCR with increasing protein in feed in this trial (figure 18). The FCR for the groups getting the lowest CP% is similar and statistically higher than in the other two groups. The 40%CP group has also significantly higher FCR than the 43% CP group, which has the lowest FCR. There are small variations in FCR within groups in this trial.

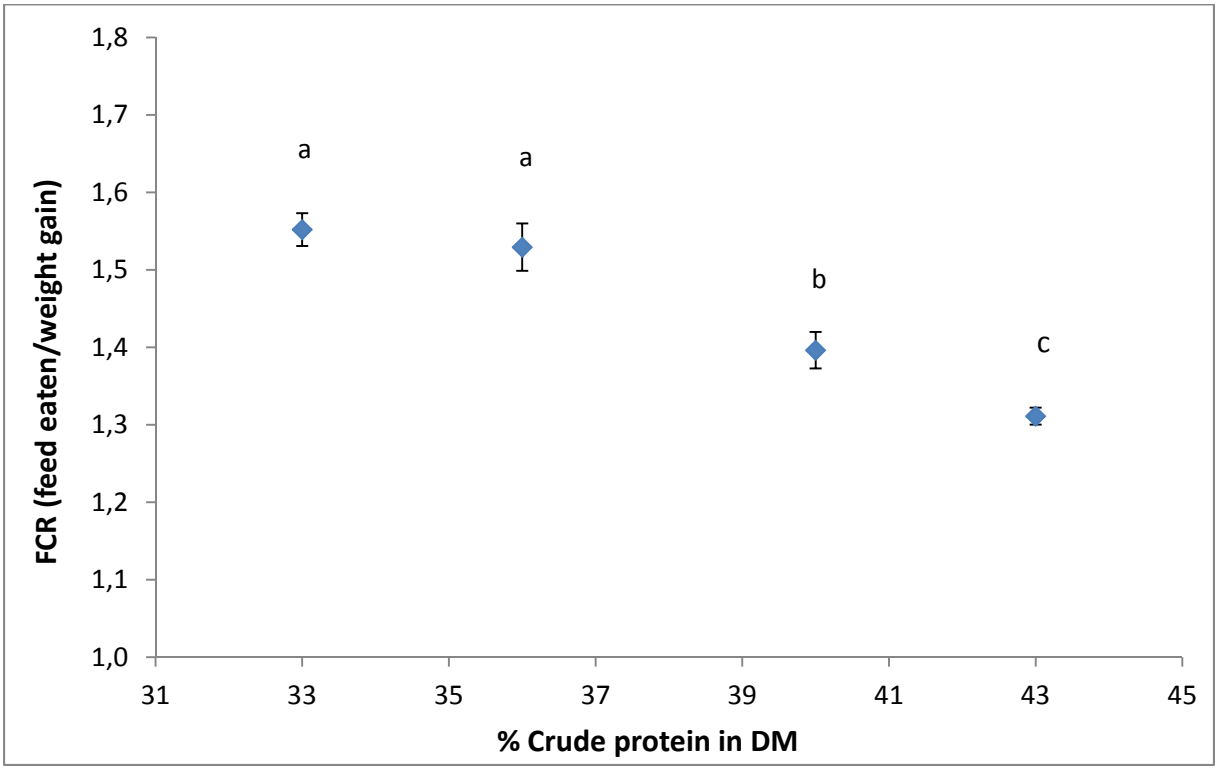

Figure 18. Average feed conversion ratio (FCR ± SEM: (kg feed/kg growth)) in Arctic charr fed with different protein content in feed (33-43%CP) for 94 days in sea water (trial 2b). n=3. Different letter is expressing statistical (p>0,05) difference between groups.

Harvesting yield

A sample of harvesting weight and yield is shown in table 7. The gutting loss is between 11-12,9% (bled fish) and the condition factor is 1,45-1,49. Fillet yield, taken by hand-filleting, is around 67% on untrimmed fillets. There were no differences between groups on these parameters. A slide difference is in the hepatosomatic index, where the liver is slightly heavier in the group fed on the lowest protein feed.

Table 7: Average harvesting output at final weighing day (± SEM), condition factor and hepatosomatic index (HSI) of fish grown in saline water, fed on different %CP í feed. n= 11.

	Feed number			
	2974	2975	2976	2977
% CP in dry matter	33	36	40	43
Length (cm)	432,8±3,2	442,2±4,2	442,9±4,5	437,5±4,0
Whole weight (g)	1219,5±56	1283,3±43	1298,9±63	1214,0±48
Condition factor: K	1,49±0,04	1,48±0,02	1,48±0,03	1,45±0,04
Gutted weight (g)	1071,1±48	1131,6±43	1132,9±59	1078,9±40
Gutted loss (%)	12,1±0,6	11,9±0,7	12,9±0,8	11,0±0,5
HSI (%)	2,6±0,2 [a]	2,2±0,1[b]	2,0±0,1[b]	2,0±0,1[b]
Fillet yield (%)-untrimmed	67,0±0,3	67,8±0,6	66,9±0,8	67,8±0,5

Fillet composition

The chemical composition of the fillets is shown in Table 8. There is a certain variation in the composition between the groups with lowest water content and highest protein and lipid content in the fillets of fish consuming the highest protein diet.

Table 8. Chemical composition of fillets in experiment 2 b.

	Diet	Fillet			
Diet	Crude protein % in DM	DM	CP % in DM	Lipid % in DM	Ash % in DM
2974	33	33,9	51,9	22,1	2,9
2975	36	34,0	53,5	21,5	3,2
2976	40	35,0	51,7	24,6	3,1
2977	43	36,2	50,8	26,2	3,0

Sensory evaluation

The mean values of sensory attributes and p-values for statistical differences are shown in table 5. Very little difference was seen between sample groups (Figure 19). Most difference was seen in odour attributes. Group 2976 had more metallic odour and odour reminding of fresh fish oil than other groups. Groups 2975 and 2977 had the lightest colour but group 2976 the darkest orange colour. Difference in heterogeneous colour and white precipitation on sample surface was marginally significant. No difference was seen in flavour or texture between sample groups. (For details see Appendix 2: Table 2).

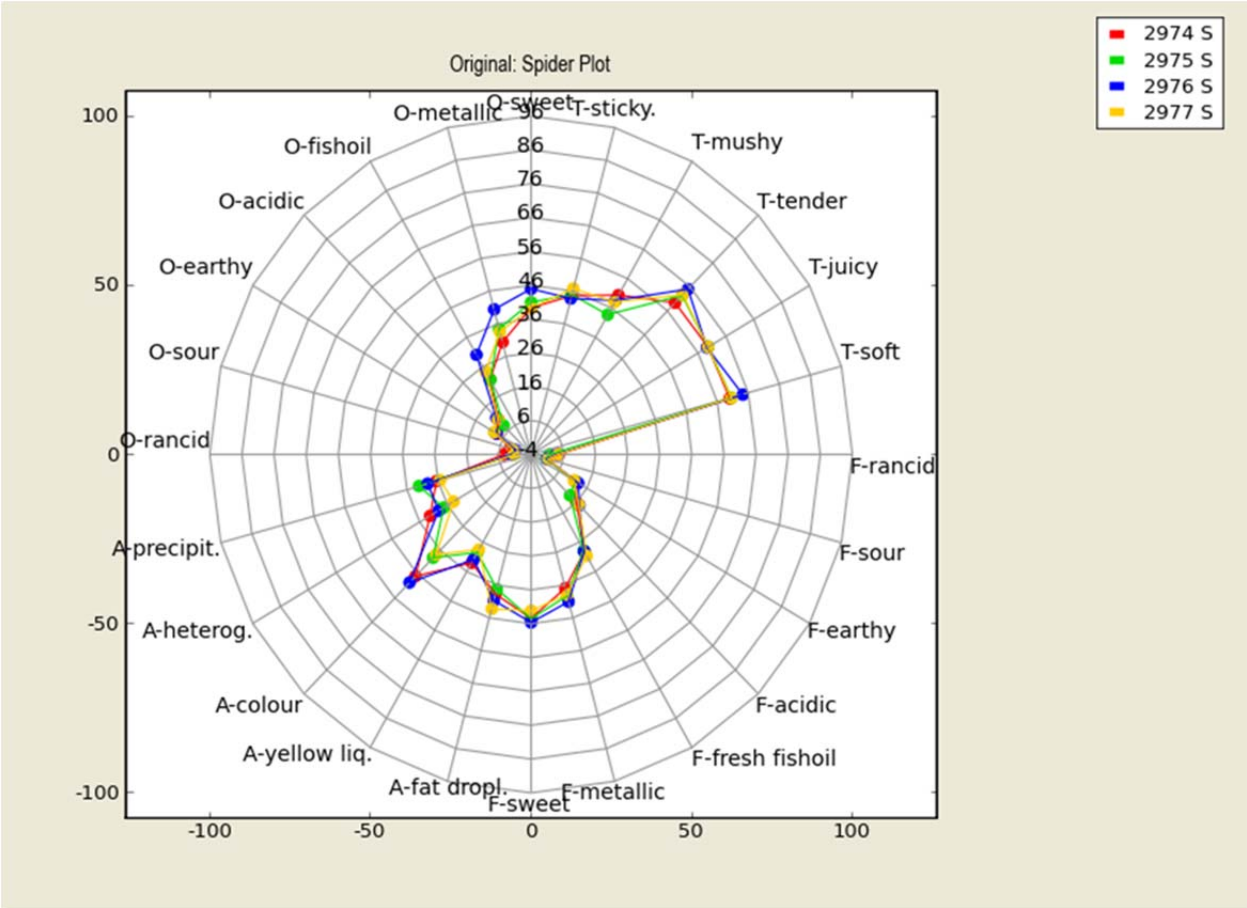

Figure 19. Mean values of sensory attributes (scale 0-100) for four feed groups of arctic charr reared in seawater. No difference was seen in flavour or texture between sample groups.

Digestibility of the experimental diets

This study was done to estimate nutrient digestibility in aquafeeds prepared to vary in protein inclusion levels on Arctic charr (Salvelinus alpinus). The four experimental diets were prepared to be iso-energetic and contain 30%, 34%, 38% and 42% protein levels. The fish (647 g) were fed in quadruplicates for 7 weeks under partial recirculation. Digestibility of protein, lipid, energy, organic matter, inorganic matter, phosphorus and zinc was estimated at the end of the feeding period following chemical and calorimetric methods using yttrium oxide as an inert marker.

The protein ADC were significantly high (>89%) in all treatments and were positively correlated to protein levels in diet. Both lipid and energy ADC followed the same trend of being significantly high in higher protein diets. The overall ADC of organic matter were high (>89%) and positively correlated to protein level in diet. The ADC of both summed and individual minerals were positively correlated to protein content in diet. Protein content in feeds for Arctic charr should be kept between 36% and 39% to achieve optimal nutrient and energy digestibility; however proteins can be reduced to 36% without hampering nutrient digestibility.

For further details see separate paper in Appendix 1.

Discussion

The present study was a continuation of a previous study on the same or similar topic (AVS project: R040-07-08), testing CP concentrations ranging from 37 – 52% in the DM. In the previous study, fish meal was the main protein source, supplying about 90% of the CP in each diet (volume ranging 35 – 67% of the raw materials, varying on CP inclusion ratio). In the present study the protein inclusions tested was in the range of 31 – 43% in DM with 18 -30% of the raw materials as fish meal (depending on protein content in the feed) supplying 50% of the CP in each diet. The rest of the CP in the feed formulation was supplied by different plant protein raw materials.

Growth and feed utilisation

The previous study concluded that the minimum CP content (37% in DM), supported maximum growth in Arctic charr from 100 grams until harvest size ((Sigurgeirsson et al 2009). The present study supports these findings that minimum protein requirement for Arctic charr over 100 grams is about 37% in the DM, corresponding to 35% in a diet with 95% DM. Lower protein content in feed for the smaller fish resulted in a tendency to reduced final weight and growth rate (SGR) and increased feed conversion ratio (FCR). In the trials for bigger fish the results were not as clear as for the smaller initial fish size. In the trial 2a (bigger fish in fw), substantial ratio of the fish became mature, which is most probably affecting the growth and the feed intake. In the group of bigger fish in seawater (trial 2b) maturation was not a problem. In this trial no difference in the growth development or the growth rate was detected between groups fed diet with 33-43% CP. Therefore, looking at the both bigger fish trials, the minimum CP content for bigger fish might be close to or even below 37% CP in DM.

The FCR was higher when the fish was fed lower CP content. These results indicate that the fish can compensate for lower protein in diet by increase the consumption. Other explanation might be less utilization of feed with lower CP, kept in mind that diets with lower protein content in this experiment contained relatively higher inclusion of plant-originated raw material. The results on the diets digestibility show lower digestibility (protein & lipid) in the low CP diets and this might affect the FCR and the growth of these groups in all trials.

In both studies and all trials the diets were formulated to ensure sufficient amounts of all essential amino acids. The results of the two studies show that as long as the diets are formulated according to the needs of essential amino acids a substantial part of the protein in the diet for Arctic charr can originate from plant protein.

Bendiksen et al (2005), concluded that 41%,35% and 32% CP in the diet was supporting maximum growth of 300, 1000 and 1600 gram Atlantic salmon, respectively, without any negative effect on FCR. In the present study the reaction to the protein content in the diet was very similar at initial weight 100 gram and 600gr indicating that the protein need is the same or identical for the two size classes of Arctic charr, i.e. about 37% CP in DM.

Present study included trials run in fresh water as well as in salt water (26, 6 ppt). In general the results do not show pronounced consistent effect of salinity on either SGR or FCR or on the effect of protein content in the diet. The trials on smaller fish (initial size 100g) were run simultaneously in fw and saline water with fish from the same pool and quite similar culture conditions except salinity.

The trials for bigger fish were not as comparable and the fish was of different origin and in different culture conditions. The substantial maturation ratios in the fw groups are most probably affecting the results.

Fillet yield and composition

The CP content in the diets is not having effect on gut loss or fillet yield. In general the fillet yield of untrimmed fillet is very high (67% from whole bled fish), also indicating high condition factor of the grown A. charr. Analyses of the filets sampled at the end of the trials with the 600 gram fish show small if any effect of CP in the diet on dry matter in the fillet. It is an tendency of increased lipid content in relation to CP content in the trial run in seawater but no such effect in the freshwater trial. This is indicating higher fat content in relation to good growth in seawater and probably also indicating the general poor growth of the fish in fw. The maturation process might also affect these results in the fw fish. There is only minor effect of CP in diet on CP in fillet, which is in agreement with the findings in the previous series ((Sigurgeirsson et al 2009).

Digestibility

See Appendix 1.

Sensory evaluation

Little differences were found between fish fed on different feed in the sensory evaluation. The only difference seen between groups of freshwater bred arctic charr was in colour and heterogeneous colour but differences in those attributes between individual fillets within groups were big. Differences between feed groups for seawater grown arctic charr was also minor but group 2976 getting diet with 40% CP in DM was characterised more than other groups by metallic odour and odour of fresh fish oil. Some differences were also seen in colour, heterogeneous colour and white precipitation. Some differences were observed between freshwater grown and seawater grown arctic charr. The most obvious difference was in mushy texture but seawater grown arctic charr had more mushy texture than freshwater grown arctic charr. These results are interesting and should be investigated further.

In general, differences between feed groups were minor. All groups were characterised by descriptions indicating freshness and no spoilage characteristics were seen.

Conclusion

The main conclusion of this experiment can be shown in fig. 20. The results of minimal protein content in diet are consistent to earlier findings and the results are adding to our knowledge of minimal protein content requirement for optimal growth of Arctic charr.

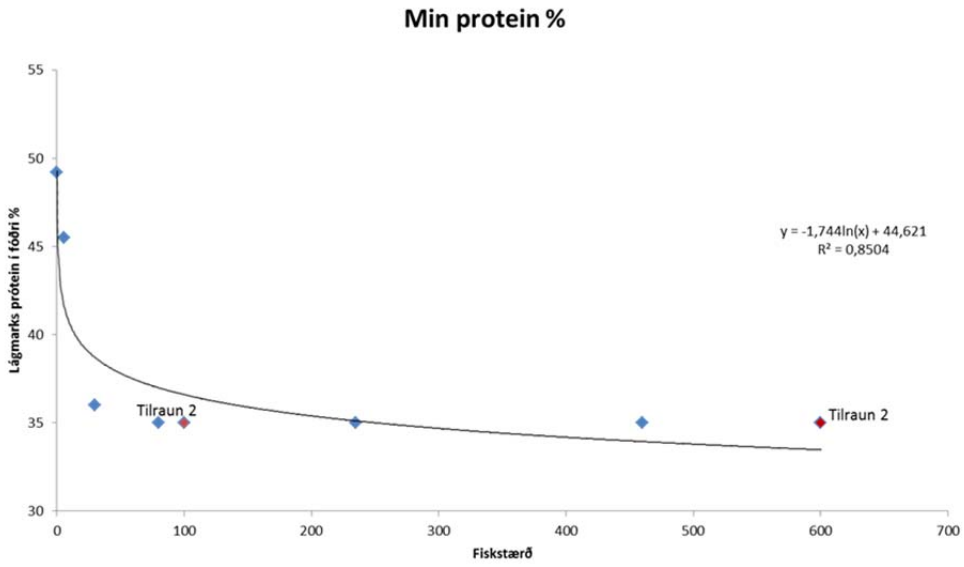

Fig. 20. Minimum protein requirement of Arctic charr in relation to fish size.

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Appendix 1:

EFFECT OF PROTEIN LEVELS ON NUTRIENT AND ENERGY DIGESTIBILITY IN DIET OF ARCTIC CHARR (*SALVELINUS ALPINUS*)

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Abstract

Alternative protein sources and inclusion levels need to be optimized in aquafeeds to make aquaculture production efficient and cost-effective. As feed costs contribute the most to operational costs, the nutrient input and utilization need to be balanced more especially proteins because it contribute the highest cost in aquafeeds. This study was done to estimate nutrient digestibility in aquafeeds prepared to vary in protein inclusion levels on Arctic charr (*Salvelinus alpinus*). The four experimental diets were prepared to be iso-energetic and contain 30%, 34%, 38% and 42% protein levels. The fish (647 g) were fed in quadruplicates for 7 weeks under partial recirculation. Digestibility of protein, lipid, energy, organic matter, inorganic matter, phosphorus and zinc was estimated at the end of the feeding period following chemical and calorimetric methods against yttrium oxide as an inert marker.

The protein ADC were significantly high (>89%) in all treatments and were positively correlated to protein levels in diet. Both lipid and energy ADC followed the same trend of being significantly high in higher protein diets. The overall ADC of organic matter were significantly high (>89%) and positively correlated to protein level in diet. The ADC of both summed and individual minerals were positively correlated to protein content in diet. Protein content in feeds for Arctic charr should be kept between 36% and 39% to achieve optimal nutrient and energy digestibilities; however proteins can be reduced to 36% without hampering nutrient digestibility.

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ADC	Apparent digestibility coefficient
FAO	Food and Agricultural Organisation of the United Nations
PUFA	Polyunsaturated fatty acids
RAS	Recirculation Aquaculture System
UNEP	United Nations Environment Programme

Introduction

Background

There is a high demand for seafood throughout the world as captures from many stocks are declining. This has resulted in a rapid expansion of aquaculture production (Hammond & Matthews, 1999; Msangi & Rosegrant, 2005). The decreasing availability from fisheries, increasing demand for seafood and economic diversification has been the driving force behind aquaculture growth. The increasing demand for seafood is the result of increasing world population, healthier diets, more expendable income and food security (Cunningham, 2005). Some other factors fuelling this growth are the search for lower costs of production and higher net revenues. Farmers and scientists can work to improve both the fish and the production methods used. However, limitations like the impact on aquatic ecosystems and human health still need to be considered (FAO, 2002).

Feeding costs make up a large percentage (50-60%) of the total expenses in intensive aquaculture; and for those trying to culture new species, achieving a competitive economic performance is a high priority (Hernández et al., 2007). The most expensive component in fish feed is the protein while the other nutrients like lipids, carbohydrates, minerals and vitamins are relatively cheaper. Fishmeal is an excellent but costly protein source for fish feed formulation (Wang et al., 2006), and there is no known plant material which can compare with fish meal in terms of balanced nutrient source and thus serve as a sole replacement in finfish diet (Barrows *et al.*, 2008; Gomes *et al.*, 1995). Efforts have been made to reduce or replace fishmeal levels in fish feeds with the objective of achieving similar growth performance by trying to understand the nutrient requirements of most economically important species (Viola *et al.*, 1982). In doing these replacements or reduction there is a need to understand nutrient utilization in order to minimize cost, hence studies were and still are conducted to evaluate protein inclusion levels in diets. The ultimate objective in fish feed research is to have minimal input cost and have maximal or optimal production outputs in terms of nutrient utilization (Hardy *et al.*, 2011).

Nutrient utilization primarily depends on the functionality of the gastrointestinal tract. The gastrointestinal tract is a metabolically active organ that processes foodstuff, and feed in the stomach and intestine is not properly in the body because the lining of these organs is merely an extension of the outer skin (Solis de los Santos *et al*., 2005). The condition of the gut determines the extent to which nutrients will be utilised (Shiau & Yu, 1999). Impaired digestion and absorption increases the amount of undigested substrate and may cause rapid growth of bacteria in the gut (Dirkzwager *et al*., 2005). High levels of carbohydrates and proteins in faeces decreases faecal integrity and as such increases the dissolution of the faecal matter when expelled into the water column, and thereby deteriorating water quality where fish are kept (Glencross *et al*., 2004). High nutrient load in water contribute towards environmental pollution which can manifest itself as eutrophication or algal blooms (Pitcher & Gilbert, 2005).

Best aquafeeds are not defined by nutritional composition, but the degree to which a fish can digest, absorb and assimilate the nutrients. Simple sugars can be absorbed as eaten, but complex components such as fats, proteins and complex carbohydrates must be digested to simpler components before they can be absorbed (Lovell, 1989). Development of aquafeeds generally requires the description of potential ingredients, their digestibility, palatability, nutrient utilization or interferences and functionalities in the fish (Glencross *et al.*, 2007).

Digestibility and metabolizability of nutrients are key measures of the value of feedstuff. The first measures how much of the eaten feed is digested while the latter measures how much of the digested nutrients remain in the tissue and not are excreted as waste. Digestibility is generally more used than metabolizability to evaluate nutrient's value as it is less complicated to measure (NRC, 1993).

Over the years methods to estimate digestibility of nutrients have been improved and new methods developed. These methods work on the basis of incorporating a known amount of a marker into the diet, then the amount of marker in the faeces is measured together with assayed nutrients. All this relies on the collection of faeces in the water or handling fish to collect faeces. Systems have been designed to ease the collection of faeces without stressing the fish, for example by increasing the slope of the tanks to aggregate faeces or by a special stand-pipe that can be controlled to siphon faeces without much interference with the fish (Choubert *et al.*, 1979; Lovell, 1989). The methods of faecal collection and markers directly affect the digestibility coefficient of major nutrients (Vandenberg & De La Noüe, 2001). The stripping method is notorious for yielding lower digestibility values due to mixing of digested faecal matter with undigested food, while collection of faeces from the water body leads to overestimation of digestibility because of loss of soluble nutrients in the water column (Fernandez *et al.*, 1998).

Besides the technical effects on digestibility, feed quality and application also influence nutrient digestibility. A study done on rainbow trout showed that protein digestibility and feed efficiency ratio is better in extruded feeds than pelleted feeds (Fenerci & Sener, 2005). In red drum apparent digestibility coefficient (ADC) of dry matter was positively influenced by protein and lipid content of the ingredient and negatively influenced by crude fibre content (McGoogan & Reigh, 1996). Fishmeal quality and replacement levels with plant based nutrients have been shown to affect nutrient digestibility as well. Low quality fishmeal lowered nutrient digestibility in Atlantic cod (Albrektsen *et al.*, 2006). Some studies have attributed feeding frequency and feeding ratio to affecting digestibility through impacts on gastric evacuation because some fish ingest beyond what they require, thus shortening the evacuation time (Hardy *et al.*, 2011). In general most fish regulate their food intake based on their capacity to utilize nutrient.

For most fish an increase in temperature results in an increase in enzymatic secretion, which thus increase nutrient digestibility. In some fish this results in high gastric motility which passes food through the gut without being thoroughly digested, thus lowering their digestibility (Elliott, 1972; Hertrampf, 2006). This is not always the case because in fingerlings of sockeye salmon complete digestion was achieved when retention time decreased from 147 hours at 3°C to 18 hours at 23°C (Brett & Higgs, 1970). Some studies found no significant differences in protein, energy and lipid digestibility when varying temperature like in Atlantic salmon (Bendiksen *et al.*, 2003; Ng *et al.*, 2004) and rainbow trout (Ng *et al.*, 2003). Although light regimes are often overlooked, in red sea bream longer light exposure increased nutrient digestibility (Biswas *et al.*, 2005).

The physiological state of fish determines the overall performance of the fish. The age of fish has a direct effect on nutrient digestibility. Young fish tend to need live food due to underdeveloped digestive tract and hence most marine fish are fed live prey in the early stages to make nutrients easily digestible (Sagiv, 2001). It is widely known that sick fish to not perform optimally, some do not even eat. Stress associated with handling, infection and infestation changes the hormonal profile which in turn affects enzymatic secretions. The

antinutritional factors in plant-based nutrients are mitogenic in organs and have a binding affinity which may initiate pathogenesis of the gastrointestinal tract and may also impair digestion and absorption of nutrients (Banwell, 1979; Gilani, 2005). A major challenge facing the development of plant-based feeds is that their nutritional contents differ significantly from those of natural food and they may also contain anti-nutritional factors which decrease digestion, nutrient utilization and growth (Olsen et al., 2007). Treating (e.g heat treatment and acid fermentation) plant-based nutrients have been shown to improve nutrient digestibility in Coho salmon (Arndt *et al.*, 1999), rainbow trout (Barrows *et al.*, 2008), haddock (Kim *et al.*, 2007), channel catfish (Peres *et al.*, 2003) and Atlantic salmon (Refstie *et al.*, 2005).

Motivation of study

South Africa's marine finfish aquaculture has not made a significant contribution to either Africa's or global fish production because it is still at a developmental stage and there are still research gaps which need to be filled before production can reach competitive levels. Most of the husbandry research has been conducted; however most research has heavily relied on either imported feeds or by improvising with trout feeds. The use of these two feeds is highly costly and nutritionally incorrect as nutritional requirements of fish species differ.

The first step towards feed development for a species requires determining the nutritional requirements and then optimizing the use of alternative ingredients to replace the costly fishmeal based diets. In general, measurements of intake and digestibility are among the most critical estimates needed to determine the nutrient content of different raw materials to match with the nutrient requirements and feed utilization in fish. Many methods have been devised to study these parameters, but all have their shortcomings and disadvantages, as direct measurements are not applicable. One of the most common methods used to measure nutrient intake of fish is based on relative content to an inert marker in the feed and compare it to the relationship in the faeces produced from the ingested feed.

Aim

The study primarily aims to establish the digestibility of key nutrients in aquafeeds formulated to measure the effect of protein levels in the diet of Arctic charr.

Key questions

1. What is the protein digestibility in four artificial diets with varying protein content fed to Arctic charr?
2. What is the lipid digestibility in four artificial diets with varying protein content fed to Arctic charr?
3. What is the energy digestibility in four artificial diets with varying protein content fed to Arctic charr?
4. What is the phosphorus digestibility in four artificial diets with varying protein content fed to Arctic charr?
5. What is the overall nutrient loss in Arctic charr fed diets varying in protein levels?

Expected outcomes

Understanding of nutrient digestibility in Arctic charr fed different protein levels and possible impact to the environment based on the amount of nutrients that ends up as a waste. The techniques and skills gained during this experiment will be applied in South Africa when assessing different diets for aquaculture use. The study is also expected to yield a manuscript and the results will be presented at a forum related to aquaculture nutrition.

Arctic charr aquaculture literature

Arctic charr are the most abundant fish species in lakes which were formed after the ice age in the Nordic countries and other high latitude lakes in the subarctic regions (Figure 1). They are largely found in lakes, and also to a lesser extent in rivers and marine environment. Research into its culture dates back as far as the late 1970s and Arctic charr is now mostly cultured in the Nordic region as well as Canada (DFO, 2004). It is a strong species for aquaculture as it grows well under culture conditions when environmental, nutritional and handling conditions matches closely to its biology (Arnesen *et al.*, 1993a; Duston *et al.*, 2007; Heasman & Black, 1998). Currently Iceland is the world's leader in Arctic charr production with around 3000 MT production. Most of the eggs and juveniles are produced at Holar University College and Stofnfiskur, and are grown out in about 15 farms located around Iceland. The highest importers are United States of America and Switzerland. Although Iceland is the leader, efforts have recently intensified to find cheap production ways in order to maximise profit (Gunnarsson, 2010).

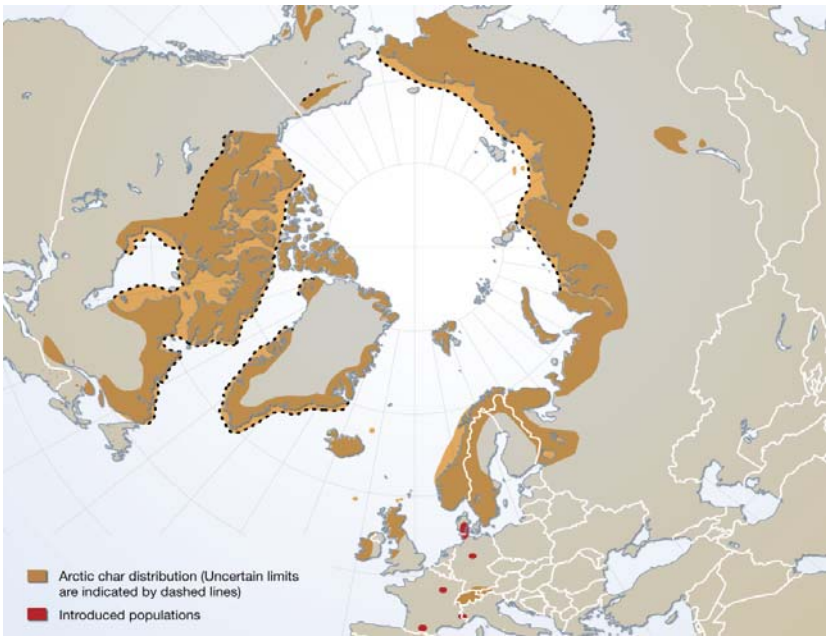

Figure 1. Distribution of Arctic charr (UNEP/GRID-Arendal, 2010)

There are a good number of studies which have been conducted towards the culture of Arctic charr ranging from culture systems to quality of final product. In Northern Norway a recirculation aquaculture system (RAS) was developed specifically for cold water culture of Arctic charr and similar designs are now used in other parts of the world (Skybakmoen *et al.*, 2009). When using demand-feeders, the bite activity of charr was observed to increase with increasing stocking density and incidences of monopoly were not observed (Alanärä & Brännäs, 1996). Charr reared at higher densities grew much better than those at lower density (Brown *et al.*, 1992). This was perhaps informed by their schooling behaviour in the wild, and hence was the underlying argument to evaluate different stocking densities in trials (Grünbaum *et al.*, 2008). Similar results were obtained where growth rates were similar for charr stocked at the medium and high densities, while the lowest stocking density performed poorly and there was no correlation between densities and feed intake and growth (Jørgensen *et al.*, 1993).

Another study compared the performance of hatchery raised and wild-caught charr, and it became apparent that hatchery raised charr outperforms wild caught in RAS (Siikavuopio *et al.*, 2009). Grünbaum *et al.*, (2008) showed that growth performance of newly hatched Arctic charr can be greatly improved by creating water current in holding tanks that will stimulate exercise which will result in growth improvements. Arctic charr larvae can be stocked at high densities as well; provided there is enough supply of oxygen and there is sufficient water replacement to prevent the build-up of dissolved wastes. The build up of waste is a major concern as it stimulates growth of bacteria which can wipe the entire stock (Johnston, 2002). To add on this, lower stocking densities of fries results in significantly slower growth and higher mortality than in populations held at higher densities (Wallace *et al.*, 1988).

Several studies have looked at the effect of salinity on performance of charr based on the knowledge that Arctic charr is anadromous wherein both sexually mature and immature fish perform seasonal migrations between river systems and coastal areas (Klemetsen *et al.*, 2003). After 30 days of rearing in salinities ranging from freshwater to 35‰, Arnesen *et al.*, (1993a) found normal plasma osmolality and comparably high growth rates. In another study both growth and feed intake was significantly reduced when charr was directly transferred to sea

water during winter (Arnesen *et al.*, 1993b). After finding poor food intake and conversion in higher salinities, Duston *et al*., (2007) concluded that direct transfer of charr from freshwater to seawater does not appear viable for commercial aquaculture.

Culture of a new species requires a lot of research before it can be produced at commercial scale. In aquaculture, nutrition is the back-bone of grow-out considerations. There are numerable studies that have looked at defining nutritional requirements and feed application of charr. These studies have covered feeding rates for maturing charr (Imsland & Gunnarsson, 2011), feeding frequency (Malcolm, 1985), feed intake (Jørgensen & Jobling, 1989), fish oil replacements (Jónasson, 2008), PUFA levels and temperature (Olsen & Henderson, 1997), fishmeal replacement (Sigurgeirsson *et al.*, 2010), protein and lipid requirements (Gurure *et al.*, 1995; Tabachek, 1986), pigmentation (Hatlen *et al.*, 1995) and feed size (Tabachek, 1988). This study seeks to determine digestibility of nutrients in diet with different protein levels.

Materials and methods

Experimental system

The experiment was conducted in a partial re-circulation system (Figure 2) at Verið in Sauðakrókur. The rearing conditions were maintained at 17.8±1.8 ‰, 12.7±0.9 mg/ℓ oxygen, 9.3±0.6°C at a flow-rate ca 0.2 $\ell.kg^{-1}.min^{-1}$. Constant oxygenation and lighting was maintained throughout the trial. Uneaten feeds were cleared from the system by a combination of stand-pipe outlet fitted with a sieve and a mechanical filter.

Figure 2. Experimental system that was used during the feeding phase of the digestibility experiments.

Experimental fish

A total of 336 fish (Figure 3) with an average of mass of 647.75±13.77 g (quadruplicate: 21 fishes/tank) were equally distributed into the experimental tanks (volume 800 ℓ) and acclimatised to the experimental diets. The fish were anaesthetized in 2-phenoxyethanol (0.15

mℓ/ℓ) and weighed to the nearest 0.1 g to determine the starting biomass. Faeces were stripped 16–18 h post-feeding.

Figure 3. Arctic charr that was fed experimental diets.

Experimental diets

The 4 experimental feeds (Figure 4) were manufactured using a commercial extruder at Laxá Feed Mill in Akureyri, Iceland. The feeds were produced to contain same amount of energy (~22 MJ/kg) but different protein levels. The protein levels were made up of different ingredient combinations; however the major contributor of protein was fishmeal, followed by soya and canola meal (**Error! Reference source not found.**). The fishmeal difference between the feeds was ~3g/100 and the canola meal decreased with increasing fishmeal content. The fish were fed twice per day to apparent satiation until they were fully acclimatized to the diet before being fed with a belt-feeder. The feeding trial was run for 7 weeks.

Figure 4. Experimental diets which were tested on Arctic charr.

Table 1. Ingredients composition of the four experimental diets fed to Arctic charr for estimating nutrient digestibility.

g/100g diet	2974	2975	2976	2977
Fishmeal	21.28	24.11	26.95	29.79
Wheat	26.65	28.57	21.79	17.82
Wheat gluten	0.00	2.64	5.63	9.79
Maize gluten	8.50	10.00	10.00	10.00
Soya HIPRO	10.00	10.00	10.00	10.00
Canola meal	4.76	2.05	3.16	0.42
Mono Cal	0.20	0.061	0.00	0.00
Fish oil	24.55	21.64	21.40	21.11
Carophyll Red	0.03	0.03	0.03	0.03
Caropyll Pink	0.03	0.03	0.03	0.03
Premix	1.00	1.00	1.00	1.00
Yttrium oxide	0.02	0.02	0.02	0.02

Faeces collection

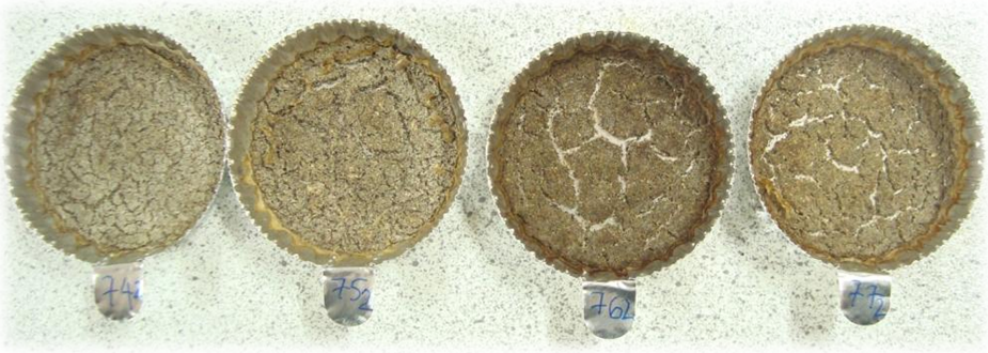

Figure 5. Dried faeces of Arctic charr from the digestibility experiment.

To collect faeces, fish were anaesthetized in 2-phenoxyethanol (0.15 mℓ/ℓ) and pressed along the abdomen closer to the anus to initiate defecation without starvation. Faeces (Figure 5) were collected at the end of the feeding period and each replicate was pooled. All samples were frozen until further analyses. All fish were returned to their respective tanks after handling to allow recovery.

Chemical analysis of feeds and faeces

The moisture content was determined by drying a 5 g sample at 110°C overnight followed by cooling it in a desiccator before reweighing (AOAC, 2000). Crude protein was calculated from total nitrogen content from a 0.5 g sample which was determined in a Kjeldahl system following acid digestion and titration of sample distillate according to the ISO standard (ISO 5983, 2005). Crude lipid was determined gravimetrically following ethyl-ether extraction from a dried sample according to Ba 3-38 (AOCS, 1998) in a Soxhlet extractor. Ash content was determined as total inorganic matter by incineration of a 10 g sample at 550°C overnight followed by cooling it in a desiccator before reweighing according to ISO standard (ISO 5984, 2002). Energy content in 0.2 g dried sample was determined by combustion to ash in a bomb calorimeter (IKA C200) with the aid of pure oxygen, cotton thread and oil according to

the method that came with the calorimeter. Yttrium oxide, phosphorus and zinc were determined following NMKL method number 186 published in 2007.

Calculations

Apparent digestibility co-efficient (ADC) of nutrients and elements in each diet were calculated according to the following equation (Barrows *et al.*, 2008):

$$\text{ADC (\%)} = 100 - 100\left(\frac{\%Yt\ in\ diet \times \%\ nutrient\ in\ faeces}{\%\ Yt\ in\ faeces \times \%\ nutrient\ in\ diet}\right)$$

Dietary carbohydrate was estimated by difference calculation:

$$carbohydrates = DM - (protein + lipid + ash)$$

Statistical analysis

Data was analysed using a one-way ANOVA followed by a post-hoc test where significant difference existed and regression to determine the relationship of the measured variable to the protein level in diet. All percentage data was arcsine transformed before analysis. A significance level of 95% was considered to indicate statistical differences ($p < 0.05$). Where there was significant difference, Tukey's test was done to determine where the difference was. All statistical analyses were run on SPSS® 14.0 software. Results are presented as mean ± standard deviation.

Results

The proximate composition of the experimental diets is presented in **Error! Reference source not found.**. Analysed crude protein and crude lipid content were lower than the calculated values, while gross energy content was slightly above the calculated values. The ADC of crude protein in Arctic charr ranged from 89.1± 4.84% to 98.7% with the 2977 diet having the highest ADC, while 2974 diet had the lowest (**Error! Reference source not found.**). There were significant differences observed between these treatments and a linear relationship was observed showing a strong positive correlation (r = 0.95) with increasing protein level in the diet. Crude lipid ADC was significantly affected by protein content in diet and was highest (99.1%) in the 2977 diet while lowest digestibility (91.5%) was observed in a 2975 diet (**Error! Reference source not found.**). There was a strong positive correlation (r = 0.95) showing a linear relationship between crude lipid digestibility and protein levels in diet. The 2977 diet had a significantly high (99.1%) energy ADC while the 2975 diet had the lowest (91.5%) as shown on **Error! Reference source not found.**. Energy ADC was strongly correlated (r = 0.95) to protein content in diet in a linear relationship The total organic matter ADC is summarized on **Error! Reference source not found.** where differences were significantly different between treatments. Organic matter ADC correlated linearly (r = 0.93) to an increase in protein content in diet where the lowest ADC (89.8%) was observed in the 2974 treatment while both the 2976 and 2977 feed were equally high (93.8%).

Table 2. Calculated and analysed proximate composition of the four experimental diets fed to Arctic charr

g/100g diet	Diet 2974	Diet 2975	Diet 2976	Diet 2977
Crude protein	30.0	34.0	38.0	42.0
Crude lipid	27.5	25.0	25.0	25.0
GE ($MJ.kg^{-1}$)	21.0	21.0	21.0	21.6
Proximate composition (g/100g diet) as analysed				
Crude protein	29.91±0.25	30.67±0.54	36.18±0.15	39.89±0.26
Crude lipid	22.61±4.79	21.29±0.77	20.50±3.85	20.66±0.64
Ash	7.53±0.04	6.42±0.01	6.49±0.70	5.91±0.02
Carbohydrates*	30.85±4.74	32.83±1.16	28.53±4.15	25.24±0.84
Moisture	9.11±0.15	8.80±0.16	8.29±0.22	8.30±0.13
Phosphorus	1.03±0.01	1.05±0.00	1.05±0.01	0.98±0.02
Zinc	0.05±0.00	0.03±0.00	0.02±0.00	0.02±0.00
GE ($MJ.kg^{-1}$)	20.38±0.20	21.25±0.03	21.47±0.06	21.62±0.04

* : value was calculated from the analysed nutrients

Phosphorus ADC was significantly high in all treatments, and the lowest ADC (99.96%) was observed in the 2974 treatment while the other treatments had equally high (99.99%) digestibility co-efficient (**Error! Reference source not found.**). There was a positive correlation (r = 0.57) between phosphorus ADC and protein levels in diet. Zinc ADC was significantly different between treatments and it increased linearly (r = 0.68) with an increase in protein inclusion levels in diet (**Error! Reference source not found.**). The total inorganic matter ADC is summarized on **Error! Reference source not found.** where differences were significantly different between treatments. Inorganic matter ADC correlated (r = 0.88) to an increase in protein content in diet where the lowest ADC (67.5%) was observed in the 2975 treatment while 2977 had the highest (96.0%).

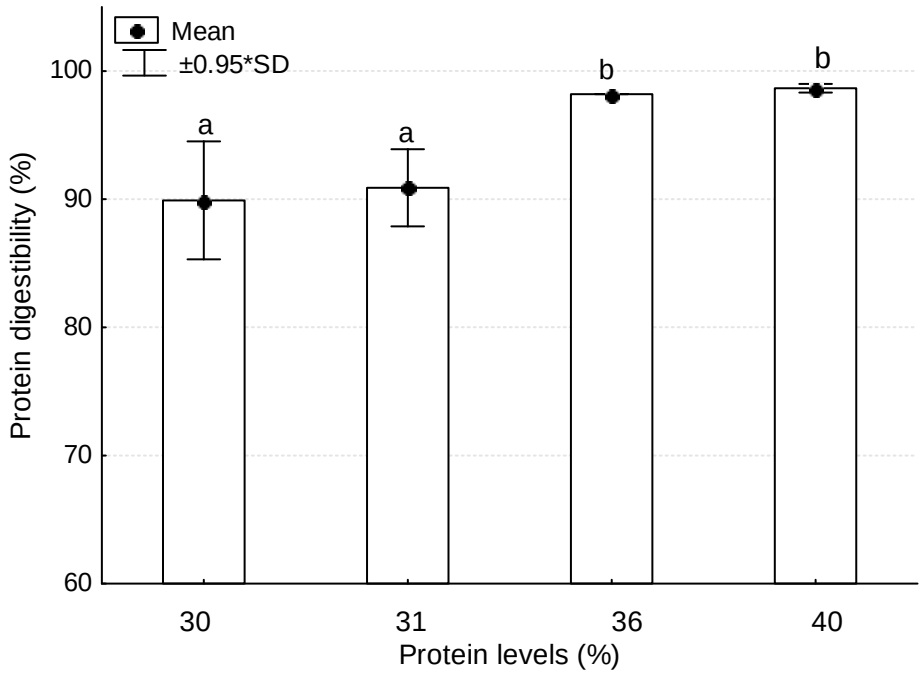

Figure 6. Apparent digestibility co-efficient of crude protein in experimental diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

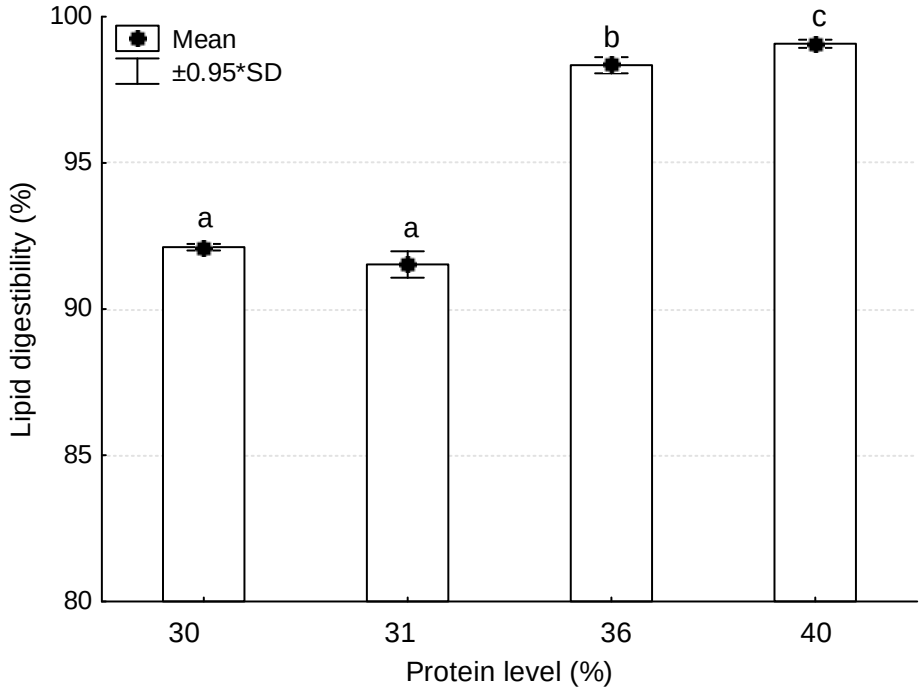

Figure 7. Apparent digestibility co-efficient of crude lipid in experimental diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

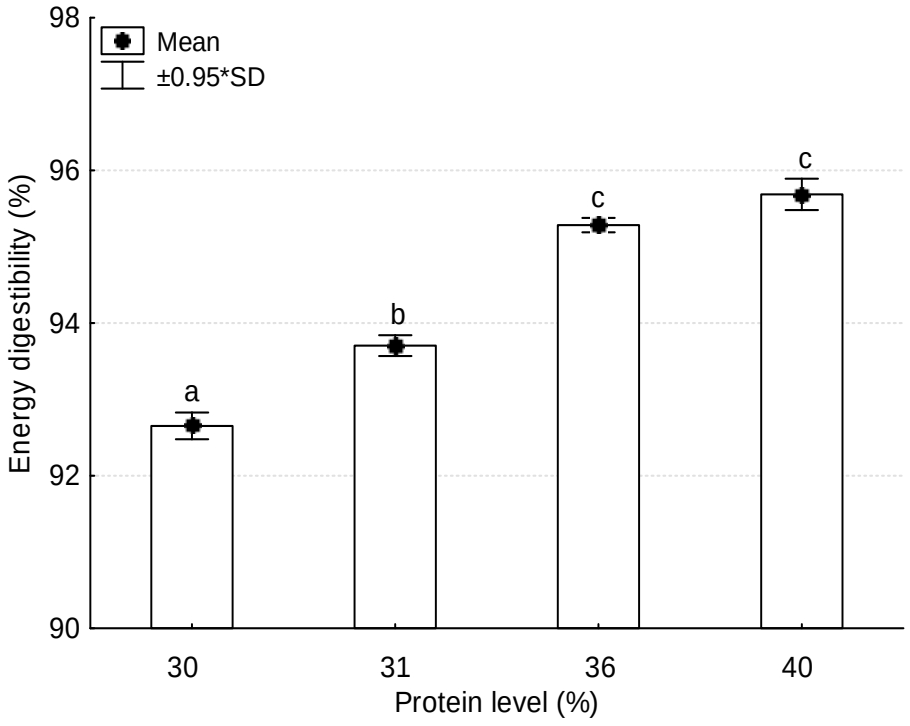

Figure 8. Apparent digestibility co-efficient of energy in diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

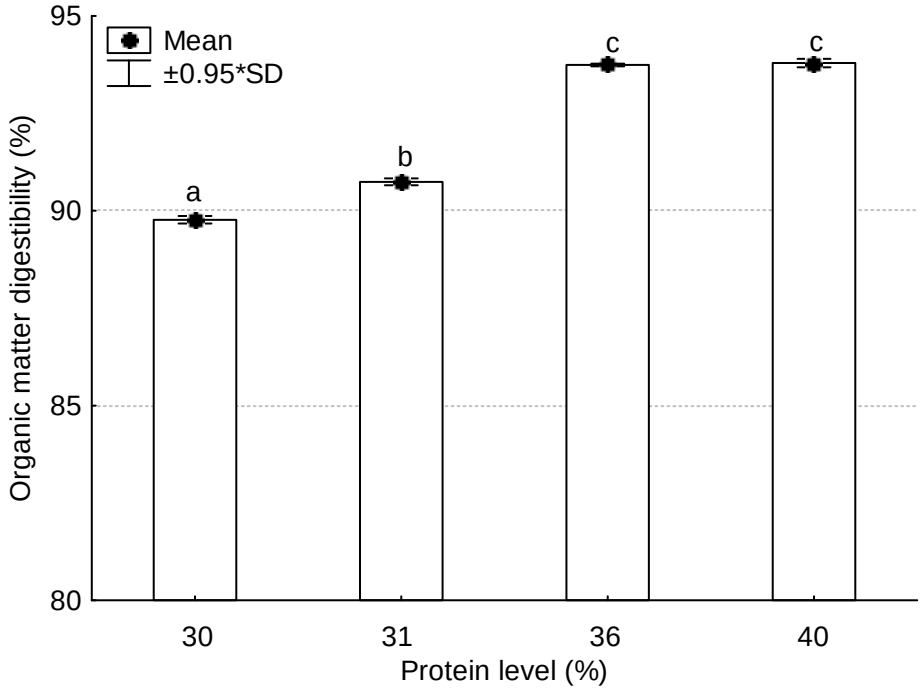

Figure 9. Apparent digestibility co-efficient of organic matter in diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

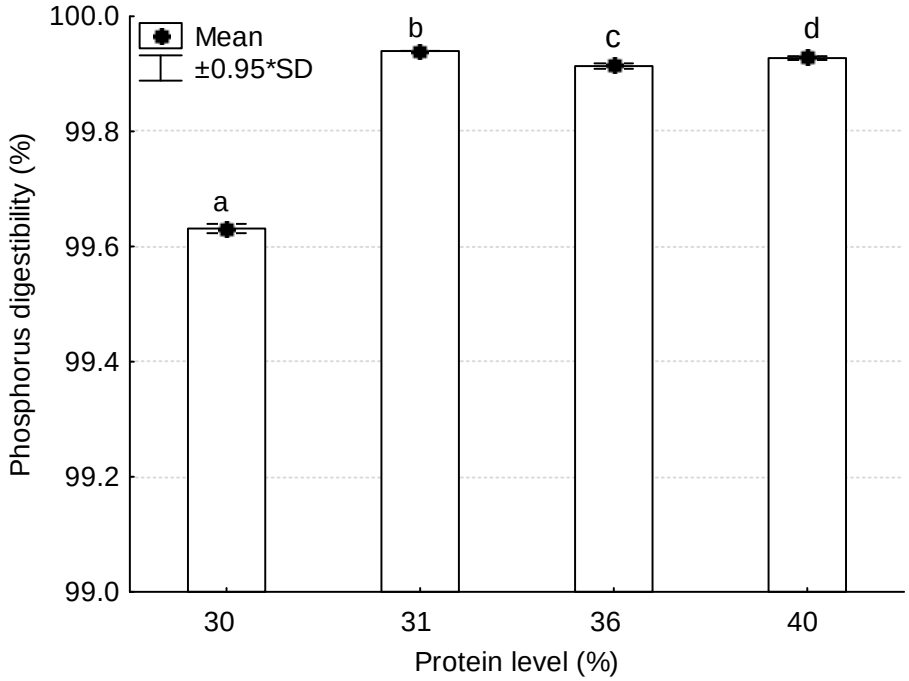

Figure 10. Apparent digestibility co-efficient of phosphorus in diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

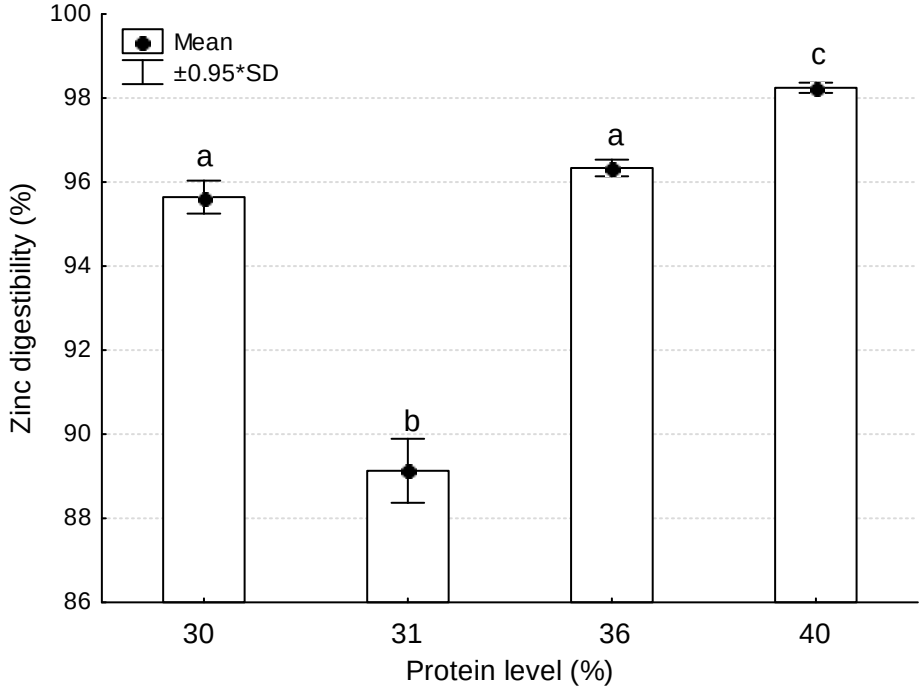

Figure 11. Apparent digestibility co-efficient of zinc in diets fed to Arctic charr.

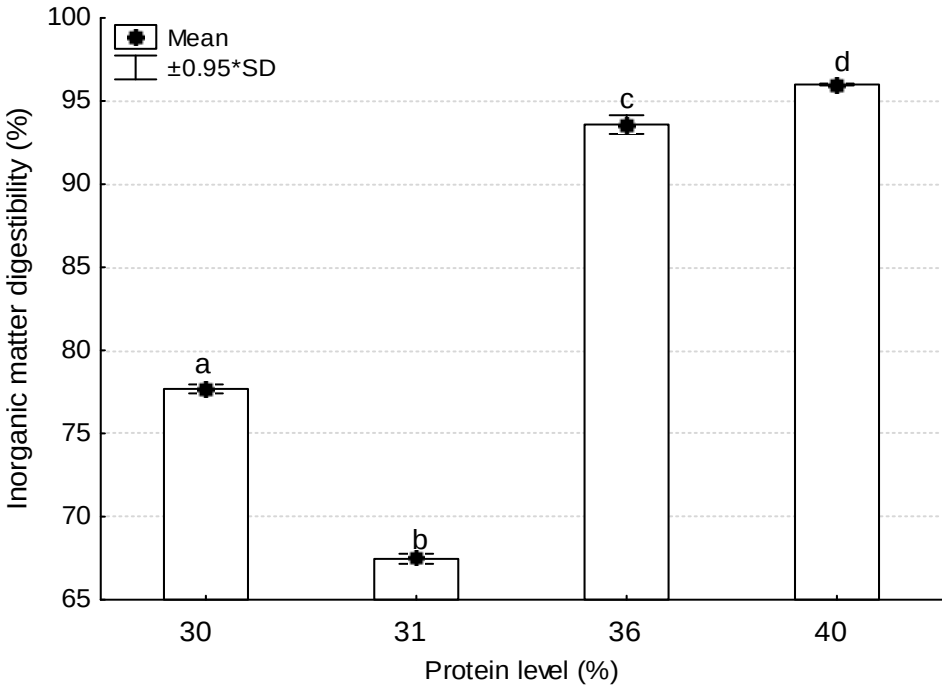

Figure 12. Apparent digestibility co-efficient of inorganic matter in diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

Discussion

The digestibility of feeds is a complex process that has to hydrolyse each ingredient in the feed to smaller components that will be available for absorption and assimilation. Bioavailability of nutrients is the primary determiner of nutrient requirements in fish species, and this has to be understood in order to produce low-pollution aqua feeds. Fishmeal is a well-studied and best protein source that has a high digestibility co-efficient in most carnivorous fish; however its cost motivated its reduction in aqua feeds while maintaining the same protein levels from plant sources. Although fishmeal-replacement studies on salmonids are well-documented, there are still some gaps on various combinations of potential inclusion levels and sources of plant-based nutrients that can closely match full fishmeal diets.

Ingredients play a major role on whether proteins in feed will be partially hydrolysed or completely hydrolyzed. Studies on striped bass (Sullivan & Reigh, 1995), Coho salmon (Sugiura *et al.*, 1998) and rainbow trout (Mwachireya *et al.*, 1999), showed that salmonids are capable of digesting proteins in plant-based nutrient sources to the same levels or better than in fishmeal. The high protein digestibility on **Error! Reference source not found.** shows that Arctic charr is capable of efficiently digesting protein components of the diet in all treatments, although proteins were more digestible with an increase in protein inclusion. This better digestibility with increase in protein content was also observed in red drum (McGoogan & Reigh, 1996), gilthead sea bream (Fernandez *et al.*, 1998) and Atlantic salmon (Karalazos *et al.*, 2011). In contrast on haddock, dietary inclusion levels from 10% and 50% of protein did not affect ADC values for protein and energy as they remained constant (Kim *et al.*, 2006). This may be due to that fishmeal has less interference with digestibility as compared to plant-based meals which may have higher carbohydrate contents. The chemical composition of the feed shows that the diets low in protein are rather high in carbohydrates, and studies have shown that higher carbohydrate suppresses protein digestibility (NRC, 1993). This may also be attributed to the high levels of fibre in canola meal which is poorly digestible in carnivorous fish because they do not secrete cellulase. This reduces the value of the feed as nutrients will pass undigested. A study on rainbow trout concluded that high levels of fibre, either alone or together with phytate, have adverse effects on digestibility of proteins (Mwachireya *et al.*, 1999). After all, the protein digestibility levels are high in all treatments. This may indicate that the anti-nutritional factors in both the canola meal and soybean meal did not have an effect on the capacity of proteolytic enzymes to hydrolyze proteins in feeds.

Lipids are required by fish as a source of available energy, as structural components of bio-membranes, carriers of fat-soluble vitamins, precursors to eicosanoids, hormones and vitamin D, and as enzyme co-factors (Lovell, 1989). They are highly digestible in fish and are a preferred nutrient source for energy as compared to carbohydrates (Mohanta et al., 2008), however other components in feed may interfere with their digestibility. The ADC of lipid in this study (**Error! Reference source not found.**) shows that digestibility of lipids increase with an increase in protein levels. Contrasting results were obtained in Atlantic salmon (Karalazos *et al.*, 2011) where high protein resulted in lower lipid ADC. The ADC of lipids in the 2974 and 2975 diets are much lower than in other studies, and can be explained by the study on Japanese seabass that found a negative correlation between canola meal levels in diet and ADC of lipids (Cheng *et al.*, 2010). According to Johnston (2002), Arctic charr have to be fed lipids in the range of 20% to 22% between growing and finishing stages, nevertheless in this study the supplied lipids were above that level. This suggests that when there is an adequate supply of lipids in feeds, lower protein content may limit the capacity of fish to digest lipids maximally as compared to the higher protein treatments.

Energy requirements differ between fish strains and species, and is affected by a variety of factors more especially those which relate to ontogenetic developments. It is generally known that herbivorous fish have low ADC of feeds high in energy, while carnivorous fish may display higher ADC (Lovell, 1989). Arctic charr requires 15.5 $MJ.kg^{-1}$ digestible energy (De Silva *et al.*, 2012), and the results of feed analysis shows that there was adequate supply of energy in feeds. The energy digestibility on this study (**Error! Reference source not found.**) suggests there is an increase in energy digestibility when protein levels are increased. The complete metabolism of proteins has a higher energy demand compared to other nutrients and the demand increases with an increase in protein levels in diet (Jobling & Davies, 1980; NRC, 1993). This explains why there was an increase in energy digestibility with increasing protein levels in diet and may also mean that there is an increase in energy expenditure with an increase in protein levels. No relationship was found between energy digestibility and protein levels in Atlantic salmon (Karalazos *et al.*, 2011) and haddock (Kim *et al.*, 2006). It can therefore be deduced that an increase in protein content in diet increases energy digestibility in Arctic charr.

Organic matter digestibility gives an overall estimate as to what degrees all organic nutrients from the different ingredients are digestible. Organic matter digestibility is influenced by composition of ingredients. Higher inclusion levels of complex carbohydrates like starch and fibre, reduces the capacity for the fish to digest nutrients (Gaylord & Gatlin III, 1996). A study on hybrid striped bass showed that organic matter ADC in feeds with ingredients from plants and animals were negatively related to fibre and starch content (Sullivan & Reigh, 1995). Although the organic matter ADC (**Error! Reference source not found.**) of this study were high, the higher carbohydrate level in both the 2974 and 2975 diet may as well explain the slightly lower digestibility on these groups. Higher ash content in diet also lowers nutrient digestibility as observed in gilthead sea bream studies (Fernandez *et al.*, 1998). This argument also fits well as an explanation for the reduced organic matter digestibility on the 2974 diet.

Canola meal, just like soybean meal contains phytic acid and glucosinolates which can drastically reduce phosphorus and protein digestibility, thus reducing the overall performance of the fish (Forster *et al.*, 1999). ADC of dietary phosphorus was very high in all the test diets (**Error! Reference source not found.**) suggesting a complete digestion and absorption irrespective of protein content in diets. This does not necessitate inclusion of phytase in diets to improve phosphorus digestibility which counters the effect of phytic acid as observed in Japanese seabass (Ai *et al.*, 2007). Phosphorus absorption is regulated by blood phosphorus concentrations (Sajjadi & Carter, 2004). Once the required phosphorus levels are met, additional phosphorus in feeds will not be used but excreted in faeces thus reducing digestibility. The supplied phosphorus level in feeds was adequate and maximally digested, thus implying a negligible effect on the environment from all the test diets. Although there were significant statistical differences in phosphorus ADC, in practical terms the differences were not substantial, thus implying that protein levels in diet has minimal influence on phosphorus digestibility.

The digestibility of zinc and other minerals is directly affected by their form in the diet and level of anti-nutritional factors in diet more especially if high in plant nutrients (NRC, 1993). Minerals bound to organic compounds are more digestible than those bound to inorganic compounds (Hardy *et al.*, 2011). Phytic acid may react with cations like zinc in the stomach to form complexes more especially in the presence of calcium. The formed molecules therefore reduce the availability of zinc, thus reducing its digestibility. The results of this study show

that zinc digestibility increases with increasing protein content in the diet (**Error! Reference source not found.**). Lowered digestibility of zinc and other minerals is generally expected when fish are fed diets that may contain phytic acid as observed in juvenile Chinook salmon (Richardson *et al.*, 1985). The lowered zinc digestibility in the 2974 and 2975 diets can be attributed to either lower protein content or possibly higher phytic acid levels from canola meal.

Inorganic matter (also known as ash) is the measure of total minerals in feed. Mineral digestibility is generally high in fish that has stomach because of the low acidic media that is available than in stomach-less fish (Hardy *et al.*, 2011). Inorganic matter ADC in this study showed a positive correlation with protein levels in diet (**Error! Reference source not found.**), thus indicating that the fish in the 2976 and 2977 treatment were better equipped to digest and absorb minerals than the fish in the lower protein levels. Higher phytic acid and other anti-nutritional factors lower the digestibility of most minerals. At the same time minerals interact with each other thus creating an antagonistic effect in terms of digestibility as shown in rainbow trout (Sugiura *et al.*, 2000). The lower digestibility in the low protein diets maybe due to the higher carbohydrates or possibly together with phytic acid.

Conclusion

The apparent digestibility values obtained in this study are relatively high, particularly for protein and phosphorus which are of great importance in feed formulation as they are the backbone of growth and nutrient utilization. The best ADC of all the measured variables (organic, inorganic and energy) are not far from the recommended 37% - 42% crude proteins levels in diet, and further show a possibility of reducing protein levels to about 36% without compromising nutrient and energy digestibility.

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Appendix 2

Table 1. Results of sensory evaluation of fillets from trial 2a

Skynmatsþáttur		2974 A	2975 A	2976 A	2977 A	p-gildi
LYKT						
sæt, einkennandi		50	53	52	50	0,562
málmlykt		34	38	37	37	0,492
fersk fiskolía		33	36	33	32	0,485
sýrulykt		8	8	8	8	0,989
moldarlykt		11	13	11	11	0,653
skemmdarsúr		0	0	0	0	0,974
þráalykt		1	0	1	0	0,670
ÚTLIT						
hvítar útfellingar		22	22	22	19	0,542
mislitur	**	27	30	33 **a**	23 **b**	0,009
litur	ms	42	44	48 **a**	37 **b**	0,053
gulur vöki		32	33	32	31	0,944
fitudropar í vatni		47	50	50	45	0,415
BRAGÐ						
sætt, einkennandi		55	55	57	53	0,493
málmbragð		37	37	38	40	0,567
fersk fiskolía		36	37	39	36	0,875
sýrubragð		7	7	8	8	0,941
moldarbragð		12	14	13	15	0,438
skemmdarsúrt		1	0	1	1	0,885
þráabragð		2	0	1	1	0,522
ÁFERÐ						
mýkt		55	58	56	58	0,748
safi		50	52	52	49	0,877
meyrni		56	58	59	60	0,838
maukkennt		19	20	17	17	0,595
viðloðun		52	50	50	49	0,564

Table 2. Sensory evaluation of filets from trial 2b

Skynmatsþáttur		2974 B		2975 B		2976 B		2977 B		p-gildi
LYKT										
sæt, einkennandi		39		41		45		39		0,270
málmlykt	*	30	b	34		40	a	34		0,021
fersk fiskolía	**	22	b	21	b	30	a	24	b	0,004
sýrulykt		10		8		11		11		0,508
moldarlykt		8		9		9		9		0,852
skemmdarsúr	*	3	a	2		1	b	2		0,029
þráalykt	*	5	a	1	b	2	b	1	b	0,020
ÚTLIT										
hvítar útfellingar	ms	27		32		29		25		0,054
mislitur	ms	32		27		29		24		0,080
litur	*	47		39		50		38		0,046
gulur vöki		33		29		32		28		0,298
fitudropar í vatni		39		37		41		43		0,178
BRAGÐ										
sætt, einkennandi		44		45		46		42		0,710
málmbragð		37		39		41		39		0,445
fersk fiskolía		29		29		29		31		0,901
sýrubragð		16		13		17		17		0,134
moldarbragð		12		12		13		11		0,888
skemmdarsúrt		1		1		1		2		0,427
þráabragð		5		2		4		4		0,617
ÁFERÐ										
mýkt		60		61		64		61		0,401
safi		60		60		59		60		0,999
meyrni		59		63		65		63		0,397
maukkennt		50		44		49		48		0,576
viðloðun		45		45		44		47		0,782