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 contributes the highest cost in aquafeeds. This study was done to estimate nutrient digestibility in aquafeeds, prepared at various 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 (647g) 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 was significantly high (>89%) in all treatments and was 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 was 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 digestibility; however proteins can be reduced to 36% without hampering nutrient digestibility.

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ABBREVIATIONS

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

1 INTRODUCTION

1.1 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 and Matthews 1999, Msangi and 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 fishmeal 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 utilized (Shiau and 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 contributes towards environmental pollution, which can manifest itself as eutrophication or algal blooms (Pitcher and 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 and 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 and 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 and 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 and 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 anti-nutritional 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).

1.2 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.

1.3 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.

1.4 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?

1.5 Expected outcomes

It is expected to understand the 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.

1.6 Arctic charr aquaculture literature

Arctic charr are the most abundant fish species in lakes that 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 and Black 1998). Currently Iceland is the world's leader in Arctic charr production with around 3000 MT. 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).

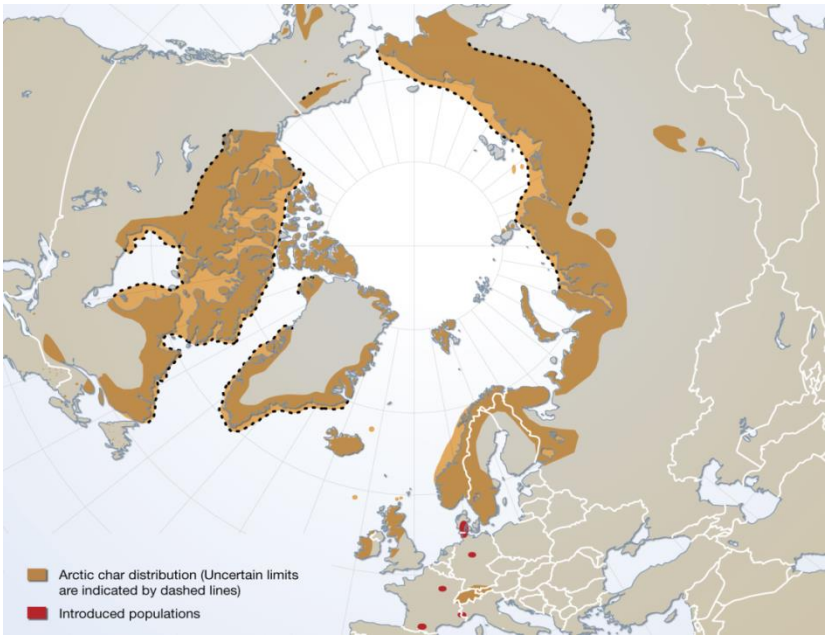

Figure 1. Distribution of Arctic charr (UNEP/GRID-Arendal 2010)

There are a good number of studies that 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 and 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 that 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 seawater 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 and Gunnarsson 2011), feeding frequency (Malcolm 1985), feed intake (Jørgensen and Jobling 1989), fish oil replacements (Jónasson 2008), PUFA levels and temperature (Olsen and 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.

2 MATERIALS AND METHODS

2.1 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.

2.2 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.

2.3 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 (
Table 1). 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. Ingredient 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

2.4 Faeces collection

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.

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

2.5 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.

2.6 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:

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

2.7 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.

3 RESULTS

The proximate composition of the experimental diets is presented in Table 2. Analyzed 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 (Figure 6). 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 (Figure 7). 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 Figure 8. 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 Figure 9 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 analyzed 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 (Figure 10). 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 (Figure 11). The total inorganic matter ADC is summarized on Figure 12 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%).

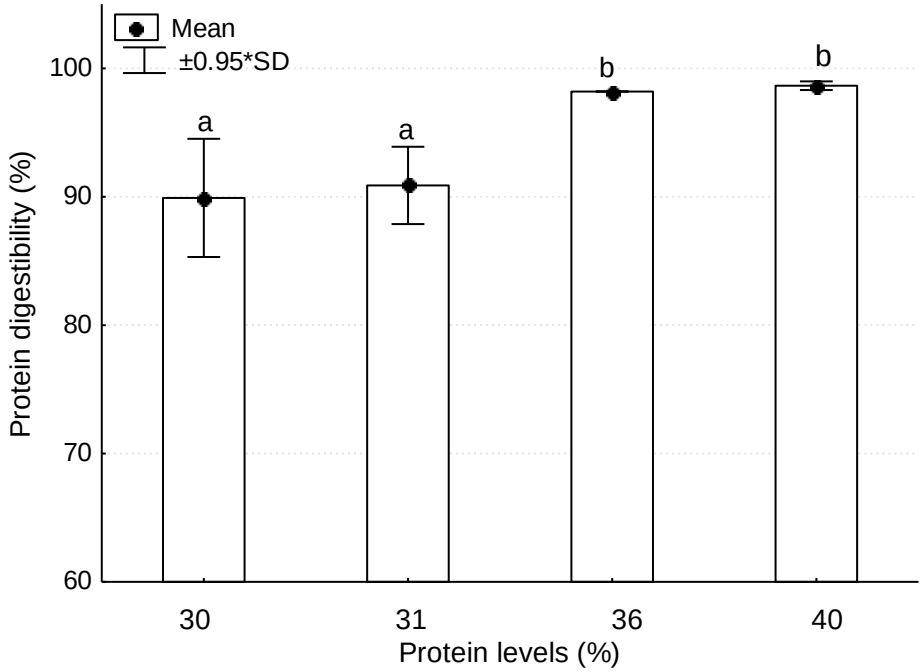

Figure 6. Apparent digestibility co-efficient of crude protein in experimental diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

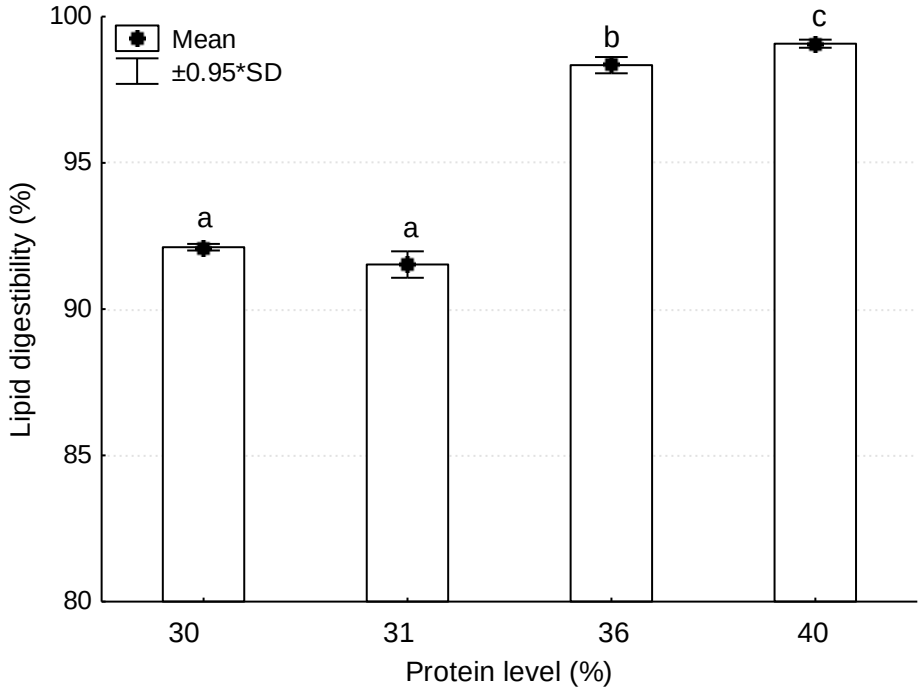

Figure 7. Apparent digestibility co-efficient of crude lipid in experimental diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

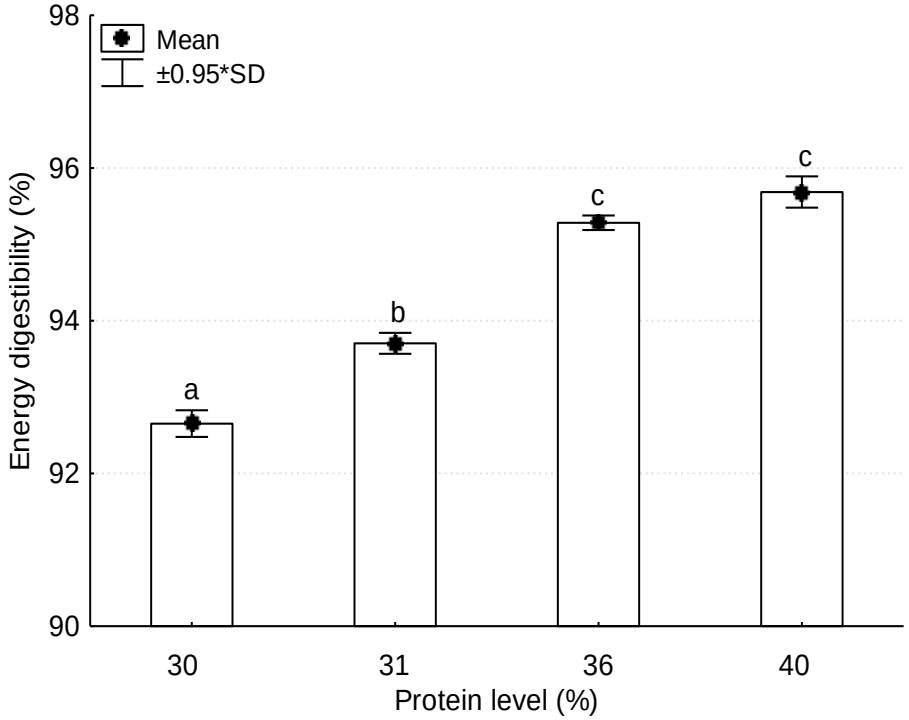

Figure 8. Apparent digestibility co-efficient of energy in diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

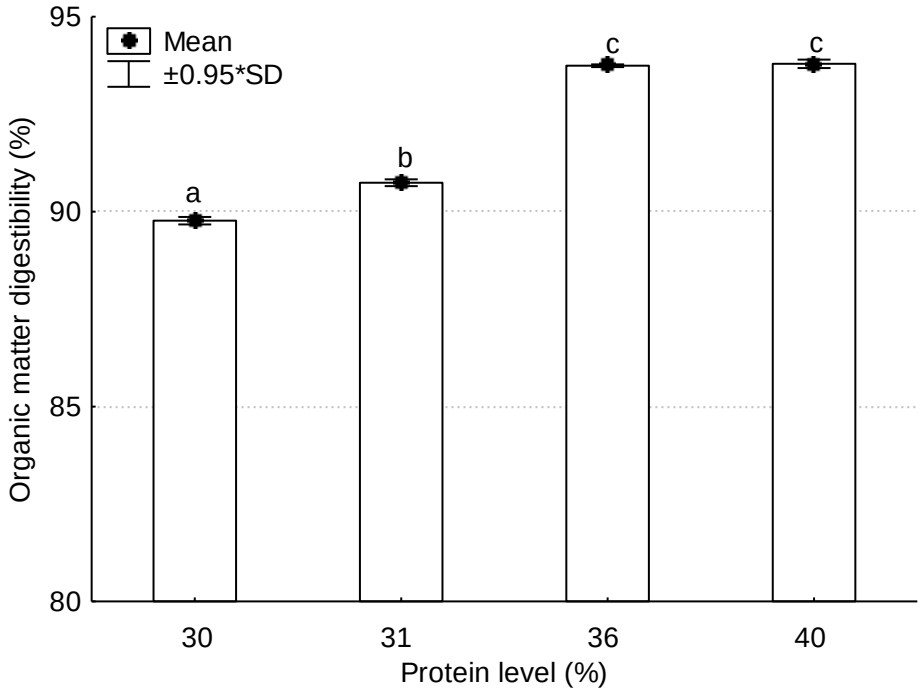

Figure 9. Apparent digestibility co-efficient of organic matter in diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

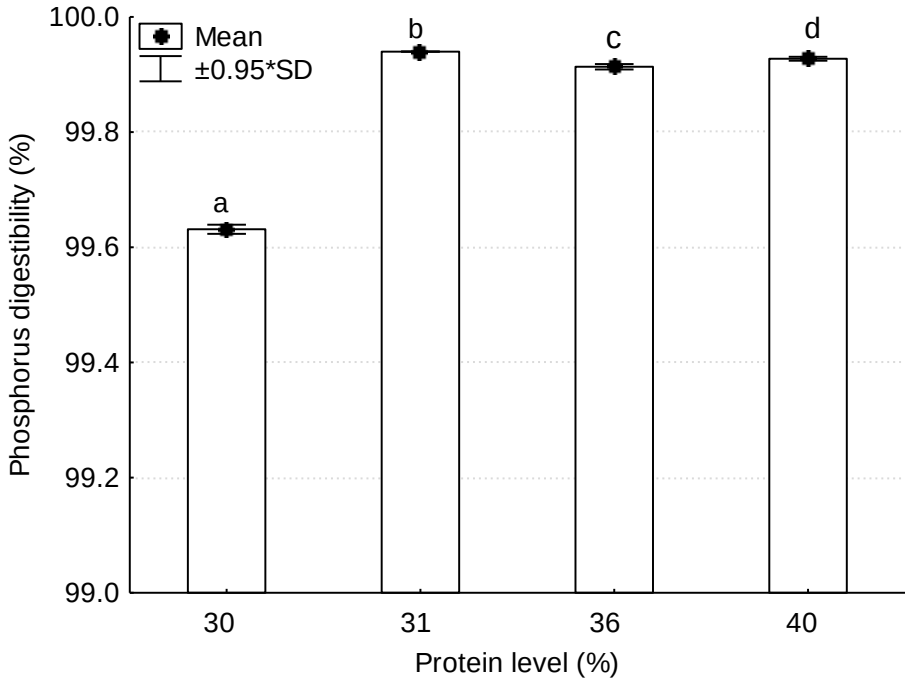

Figure 10. Apparent digestibility co-efficient of phosphorus in diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

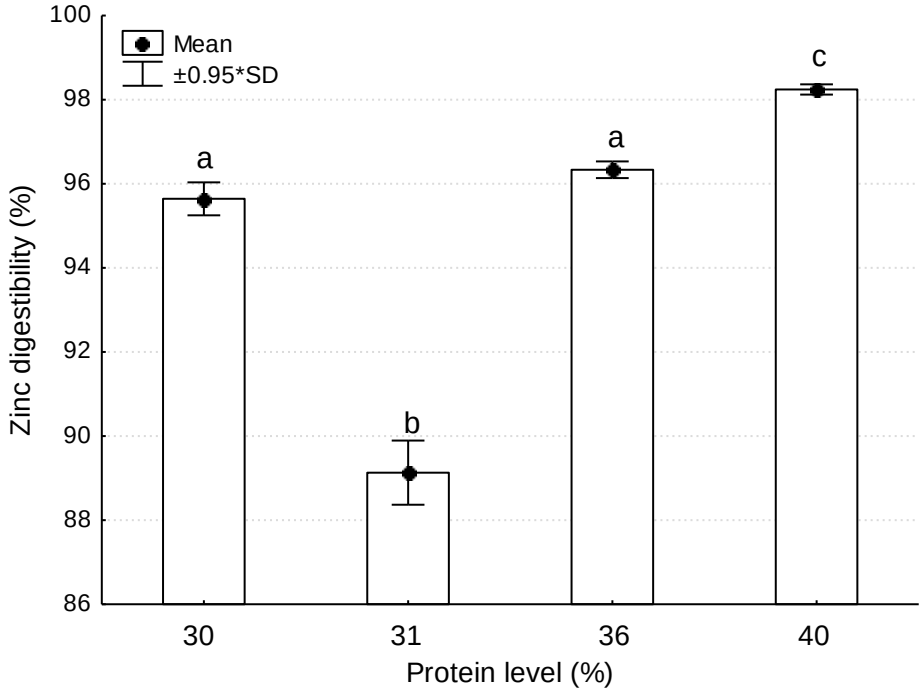

Figure 11. Apparent digestibility co-efficient of zinc in diets fed to Arctic charr.

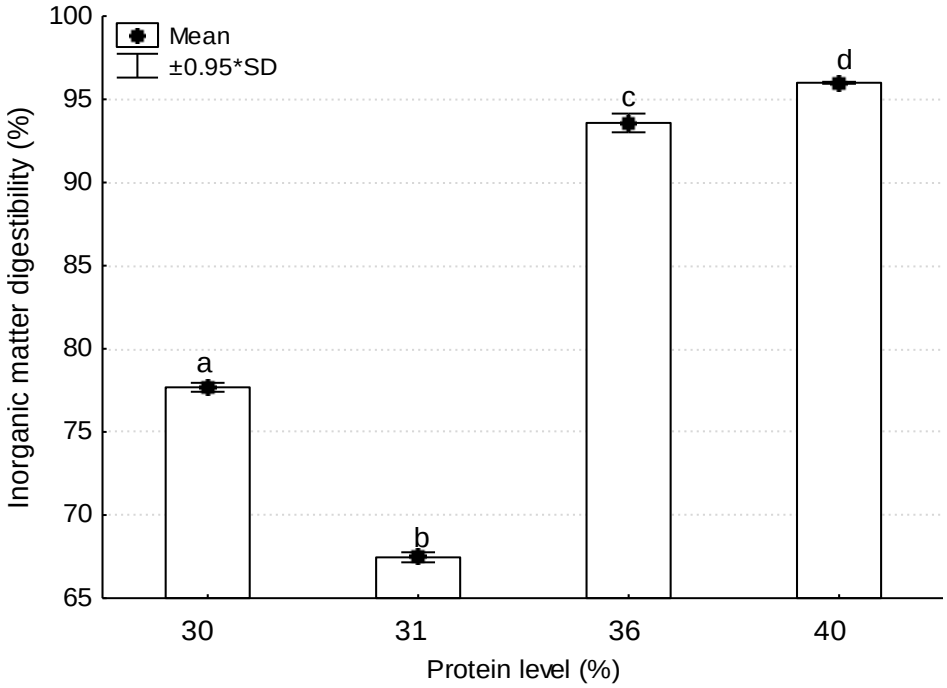

Figure 12. Apparent digestibility co-efficient of inorganic matter in diets fed to Arctic charr. Different letters above the error bars indicate significant difference.

4 DISCUSSION

The digestibility of feeds is a complex process that has to hydrolyze 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 and 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 Figure 6 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 and 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 (Figure 7) 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 require 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 (Figure 8) 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 and 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 and 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 and Reigh 1995). Although the organic matter ADC (Figure 9) of this study was 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 (Figure 10) 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 and 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 (Figure 11). 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 (Figure 12), 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.

5 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.

LIST OF REFERENCES

Ai, Q., Mai, K., Zhang, W., Xu, W., Tan, B., Zhang, C. and Li, H. (2007). Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology* 147(2):502-508.

Alanärä, A. and Brännäs, E. (1996). Dominance in demand-feeding behaviour in Arctic charr and rainbow trout: the effect of stocking density. *Journal of Fish Biology* 48(2): 242-254.

Albrektsen, S., Mundheim, H. and Aksnes, A. (2006). Growth, feed efficiency, digestibility and nutrient distribution in Atlantic cod (*Gadus morhua*) fed two different fish meal qualities at three dietary levels of vegetable protein sources. *Aquaculture* 261 (2):626-640.

AOAC International **(**2000). *Official methods of analysis of AOAC International.* (17 ed). Horwitz, W. eds. 2200 pp. Gaithersburg, Maryland: AOAC International.

American Oil Chemists Society (AOCS) **(**1998). Official method Ba 3-38. In Firestone, D. eds. *Official Methods and Recommended Practices of the American Oil Chemists' Society*. pp. 1-3. Champaign, Illinois: American Oil Chemists Society.

Arndt, R.E., Hardy, R.W., Sugiura, S.H. and Dong, F.M. (1999). Effects of heat treatment and substitution level on palatability and nutritional value of soy defatted flour in feeds for Coho Salmon, *Oncorhynchus kisutch*. *Aquaculture* 180(1-2):129-145.

Arnesen, A.M., Jørgensen, E.H. and Jobling, M. (1993a(. Feed intake, growth and osmoregulation in Arctic charr, *Salvelinus alpinus* (L.), following abrupt transfer from freshwater to more saline water. *Aquaculture* 114(3-4):327-338.

Arnesen, A.M., Jørgensen, E.H. and Jobling, M. (1993b). Feed intake, growth and osmoregulation in Arctic charr, *Salvelinus alpinus* (L.), transferred from freshwater to saltwater at 8°C during summer and winter. *Fish Physiology and Biochemistry* 12(4): 281-292.

Banwell, J.G. (1979). Environmental Contaminants and Intestinal Function. *Environmental Health Perspectives* 33:107-114.

Barrows, F.T., Gaylord, T.G., Sealey, W.M., Haas, M.J. and Stroup, R.L. (2008). Processing soybean meal for biodiesel production; effect of a new processing method on growth performance of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 283:141-147.

Bendiksen, E.A., Berg, O.K., Jobling, M., Arnesen, A.M. and Måsøval, K. (2003). Digestibility, growth and nutrient utilisation of Atlantic salmon parr (*Salmo salar* L.) in relation to temperature, feed fat content and oil source. *Aquaculture* 224(1-4):283-299.

Biswas, A.K., Seoka, M., Inoue, Y., Takii, K. and Kumai, H. (2005). Photoperiod influences the growth, food intake, feed efficiency and digestibility of red sea bream (*Pagrus major*). *Aquaculture* 250(3-4):666-673.

Brett, J.R. and Higgs, D.A. (1970). Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka*. *Journal of the Fisheries Research Board of Canada* 27(10):1767-1779.

Brown, G.E., Brown, J.A. and Srivastava, R.K. (1992). The effect of stocking density on the behaviour of Arctic charr (*Salvelinus alpinus* L.). *Journal of Fish Biology* 41(6): 955-963.

Cheng, Z., Ai, Q., Mai, K., Xu, W., Ma, H., Li, Y. and Zhang, J. (2010). Effects of dietary canola meal on growth performance, digestion and metabolism of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture* 305(1-4):102-108.

Choubert, G., Noue, J.D.L. and Luquet, P. (1979). Continuous quantitative automatic collector for fish feces. *Progressive Fish-Culturist* 41(2):64-67.

Cunningham, L. (2005). *Assessing the contribution of aquaculture to food security: a survey of methodologies.* FAO Fisheries Circular – 1010. Rome: Food and Agriculture Organization of the United Nations.

De Silva, S.S., Turchini, G. and Francis, D. **(**2012). Nutrition. In Lucas, J.S. and Southgate, P.C. eds. *Aquaculture: Farming Aquatic Animals and Plants*. pp. 164-186. Oxford: Blackwell Publishing.

DFO (2004). Cambridge Bay Arctic Char, 2004/010 pp. 15. Ottawa: Canadian Science Advisory Secretariat.

Dirkzwager, A., Veldman, B. and Bikker, P. (2005). A nutritional approach for the prevention of post weaning syndrome in piglets. *Animal Research* 54(3):231-236.

Duston, J., Astatkie, T. and Murray, S.B. (2007). Effect of salinity at constant 10°C on grow-out of anadromous Arctic charr from Labrador. *Aquaculture* 273(4):679-686.

Elliott, J.M. (1972). Rates of gastric evacuation in brown trout, *Salmo trutta* L. *Freshwater Biology* 2(1):1-18.

Food and Agricultural Organization of the United Nations FAO **(**2002). World review of fisheries and aquaculture The State of World Fisheries and Aquaculture - 2002 (SOFIA). Rome: FAO.

Fenerci, S. and Sener, E. (2005). In vivo and in vitro protein digestibility of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1972) fed steam pressured or extruded feeds. *Turkish Journal of Fisheries and Aquatic Sciences* 5:17-22.

Fernandez, F., Miquel, A.G., Guinea, J. and Martinez, R. (1998). Digestion and digestibility in gilthead sea bream (*Sparus aurata*): the effect of diet composition and ration size. *Aquaculture* 166(1-2):67-84.

Forster, I., Higgs, D.A., Dosanjh, B.S., Rowshandeli, M. and Parr, J. (1999). Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11°C fresh water. *Aquaculture* 179(1-4):109-125.

Gaylord, T.G. and Gatlin III, D.M. (1996). Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). *Aquaculture* 139(3-4):303-314.

Gilani, G.S., Cockell, K.A. Sepehr, E. (2005). Effects of antinutritional factors on protein digestibility and amino acid availability in foods. *J. AOAC Int.* 88(3):967-987.

Glencross, B.D., Booth, M. and Allan, G.L. (2007). A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition* 13(1):17-34.

Glencross, B.D., Carter, C.G., Duijster, N., Evans, D.R., Dods, K., McCafferty, P., Hawkins, W.E., Maas, R. and Sipsas, S. (2004). A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 237(1-4):333-346.

Gomes, E.F., Rema, P. and Kaushik, S.J. (1995). Replacement of fish meal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. *Aquaculture* 130(2-3):177-186.

Grünbaum, T., Cloutier, R. and François, N.R.L. (2008). Positive effects of exposure to increased water velocity on growth of newly hatched Arctic charr, *Salvelinus alpinus* L. *Aquaculture Research* 39(1):106-110.

Gunnarsson, V.I., (2010). Arctic charr. [December 2011] <http://www.fisheries.is/aquaculture/species/arctic-charr>.

Gurure, R.M., Moccia, R.D. and Atkinson, J.L. (1995). Optimal protein requirements of young Arctic charr (*Salvelinus alpinus*) fed practical diets. *Aquaculture Nutrition* 1(4): 227-234.

Hammond, A. and Matthews, E. (1999). *Critical consumption trends and implications: Degrading Earth's ecosystems*. Washington D.C.: World Resource Institute pp. 51-71.

Hardy, R.W., Gatlin-III, D.M., Bureau, D.P., D'Abramo, L.R., Davis, D.A., Halver, J.E., Krogdahl, Å., Médale, F., Shiau, S.-Y., Tocher, D.R. *et al.* 2011. *Nutrient Requirements of Fish and Shrimp*. Washington, D.C. The National Academies Press, pp. 376.

Hatlen, B., Aas, G.H., Jørgensen, E.H., Storebakken, T. and Goswami, U.C. (1995). Pigmentation of 1, 2 and 3 year old Arctic charr (*Salvelinus alpinus*) fed two different dietary astaxanthin concentrations. *Aquaculture* 138(1-4):303-312.

Heasman, M.S. and Black, K.D. (1998). The potential of Arctic charr, *Salvelinus alpinus* (L.), for mariculture. *Aquaculture Research* 29(1):67-76.

Hernández, M.D., Martínez, F.J., Jover, M. and García García, B. (2007). Effects of partial replacement of fish meal by soybean meal in sharpsnout seabream (*Diplodus puntazzo*) diet. *Aquaculture* 263(1-4):159-167.

Hertrampf, J. (2006). Factors influencing feed digestibility. *Feed Tech* 10(6):31.

Imsland, A.K. and Gunnarsson, S. (2011). Growth and maturation in Arctic charr (*Salvelinus alpinus*) in response to different feed rations. *Aquaculture* 318(3-4):407-411.

ISO 5983. **(**2005). Determination of nitrogen content and calculation of crude protein content. In: *Animal feeding stuffs* pp. 1-10. Geneva: The International Organization for Standardization.

ISO 5984. **(**2002). Determination of crude ash. In: *Animal feeding stuffs* pp. 1-14. Geneva: The International Organization for Standardization.

Jobling, M. and Davies, P.S. (1980). Effects of feeding on metabolic rate, and the Specific Dynamic Action in plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* 16 (6):629-638.

Johnston, G. **(**2002). *Arctic charr aquaculture*. Cornwall: Fishing News Books pp. 266.

Jónasson, B. (2008). Replacing fish oil in Arctic charr diets. MSc Thesis, Department of Business and Science, University of Akureyri, Iceland 80 pp.

Jørgensen, E.H. and Jobling, M. (1989). Patterns of food intake in Arctic charr, *Salvelinus alpinus*, monitored by radiography. *Aquaculture* 81(2):155-160.

Jørgensen, E.H., Christiansen, J.S. and Jobling, M. (1993). Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Salvelinus alpinus*). *Aquaculture* 110(2):191-204.

Karalazos, V., Bendiksen, E.Ã. and Bell, J.G. (2011). Interactive effects of dietary protein/lipid level and oil source on growth, feed utilisation and nutrient and fatty acid digestibility of Atlantic salmon. *Aquaculture* 311(1-4):193-200.

Kim, J.D., Tibbetts, S.M., Milley, J.E. and Lall, S.P. (2006). Effect of the incorporation level of herring meal into test diet on apparent digestibility coefficients for protein and energy by juvenile haddock, *Melanogrammus aeglefinus* L. *Aquaculture* 258(1-4):479-486.

Kim, J.D., Tibbetts, S.M., Milley, J.E. and Lall, S.P. (2007). Effect of the incorporation level of dehulled soybean meal into test diet on apparent digestibility coefficients for protein and energy by juvenile haddock, *Melanogrammus aeglefinus* L. *Aquaculture* 267(1-4):308-314.

Klemetsen, A., Amundsen, P.A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F. and Mortensen, E. (2003). Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish* 12(1):1-59.

Lovell, T. **(**1989). *Nutrition and feeding of fish*. New York: Van Nostrand Reinhold pp. 1-260.

Malcolm, J. (1985). Physiological and social constraints on growth of fish with special reference to Arctic charr, *Salvelinus alpinus* L. *Aquaculture* 44(2):83-90.

McGoogan, B.B. and Reigh, R.C. (1996). Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets. *Aquaculture* 141(3-4):233-244.

Mohanta, K.N., Mohanty, S.N., Jena, J.K. and Sahu, N.P. (2008). Optimal dietary lipid level of silver barb, *Puntius gonionotus* fingerlings in relation to growth, nutrient retention and digestibility, muscle nucleic acid content and digestive enzyme activity. *Aquaculture Nutrition* 14(4):350-359.

Msangi, S. and Rosegrant, M.W. (2005). *Fish to 2020: Supply and demand in changing global markets.* In: Global Aquaculture Meeting. Seattle, June 20, 2005.

Mwachireya, Beames, Higgs and Dosanjh. (1999). Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of commercial canola meal in rainbow trout *Oncorhynchus mykiss* (Walbaum) held in fresh water. *Aquaculture Nutrition* 5(2):73-82.

Ng, W.K., Campbell, P., Dick, J. and Bell, J. (2003). Interactive effects of dietary palm oil concentration and water temperature on lipid digestibility in rainbow trout, *Oncorhynchus mykiss*. *Lipids* 38(10):1031-1038.

Ng, W.K., Sigholt, T. and Gordon Bell, J. (2004). The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (Salmo salar L.) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. *Aquaculture Research* 35(13):1228-1237.

NRC. **(**1993). *Nutrient Requirements of Fish*. Washington, D.C.: National Academy Press pp. 1-116.

Olsen, R.E. and Henderson, R.J. (1997). Muscle fatty acid composition and oxidative stress indices of Arctic charr, *Salvelinus alpinus* (L.), in relation to dietary polyunsaturated fatty acid levels and temperature. *Aquaculture Nutrition* 3(4):227-238.

Olsen, R.E., Hansen, A.-C., Rosenlund, G., Hemre, G.-I., Mayhew, T.M., Knudsen, D.L., Tufan Eroldogan, O., Myklebust, R. and Karlsen, Ø. (2007). Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) II -- Health aspects. *Aquaculture* 272(1-4):612-624.

Peres, H., Lim, C. and Klesius, P.H. (2003). Nutritional value of heat-treated soybean meal for channel catfish (*Ictalurus punctatus*). *Aquaculture* 225(1-4):67-82.

Pitcher, G.C. and Gilbert, P.M. (2005). Special issue on harmful algal blooms. *Oceanography* 18(2):134-135.

Refstie, S., Sahlström, S., Bråthen, E., Baeverfjord, G. and Krogedal, P. (2005). Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture* 246(1-4):331-345.

Richardson, N.L., Higgs, D.A., Beames, R.M. and McBride, J.R. (1985). Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *The Journal of Nutrition* 115:553-567.

Sagiv, K. (2001). Digestive enzymes in fish larvae and juveniles - implications and applications to formulated diets. *Aquaculture* 200(1-2):181-201.

Sajjadi, M. and Carter, C.G. (2004). Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (*Salmo salar* L.) fed a canola-meal-based diet. *Aquaculture* 240(1-4):417-431.

Shiau, S.Y. and Yu, Y.P. (1999). Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus×O. aureus*. *Aquaculture* 179(1-4): 439-446.

Sigurgeirsson, Ó.I., Árnason, J., Thorarensen, H. and Ólafsdóttir, A. (2010). Effect of replacing fish meal in Arctic charr diet on growth, and feed utilization, R031-08, Holar University, Iceland. 80 pp.

Siikavuopio, S.I., Skybakmoen, S. and Sæther, B.S. (2009). Comparative growth study of wild- and hatchery-produced Arctic charr (*Salvelinus alpinus* L.) in a coldwater recirculation system. *Aquacultural Engineering* 41(2):122-126.

Skybakmoen, S., Siikavuopio, S.I. and Sæther, B.S. (2009). Coldwater RAS in an Arctic charr farm in Northern Norway. *Aquacultural Engineering* 41(2):114-121.

Solis de los Santos, F., Farnell, M.B., Tellez, G., Balog, J.M., Anthony, N.B., Torres-Rodriguez, A., Higgins, S., Hargis, B.M. and Donoghue, A.M. (2005). Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. *Poultry Science* 84(7):1092-1100.

Sugiura, S.H., Dong, F.M., Rathbone, C.K. and Hardy, R.W. (1998). Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* 159(3-4):177-202.

Sugiura, S.H., Babbitt, J.K., Dong, F.M. and Hardy, R.W. (2000). Utilization of fish and animal by-product meals in low-pollution feeds for rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* 31(7):585-593.

Sullivan, J.A. and Reigh, R.C. (1995). Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone saxatilis* X *Morone chrysops*). *Aquaculture* 138(1-4):313-322.

Tabachek, J.A.L. (1988). The effect of feed particle size on the growth and feed efficiency of Arctic charr *[Salvelinus alpinus* (L.)]. *Aquaculture* 71(4):319-330.

Tabachek, J.L. (1986). Influence of dietary protein and lipid levels on growth, body composition and utilization efficiencies of Arctic charr, *Salvelinus alpinus* L. *Journal of Fish Biology* 29(2):139-151.

UNEP/GRID-Arendal (2010). Arctic char species complex, distribution map. [December 2011] <http://maps.grida.no/go/graphic/arctic-char-species-complex-distribution-map>.

Vandenberg, G.W. and De La Noüe, J. (2001). Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. *Aquaculture Nutrition* 7(4):237-245.

Viola, S., Mokady, S., Rappaport, U. and Arieli, Y. (1982). Partial and complete replacement of fishmeal by soybean meal in feeds for intensive culture of carp. *Aquaculture* 26(3-4):223-236.

Wallace, J.C., Kolbeinshavn, A.G. and Reinsnes, T.G. (1988). The effects of stocking density on early growth in Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* 73(1-4): 101-110.

Wang, Y., Kong, L.-J., Li, C. and Bureau, D.P. (2006). Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (*Nibea miichthioides*). *Aquaculture* 261(4):1307-1313.

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