EFFECT OF DIETARY PROTEIN LEVELS ON GROWTH AND PROTEIN UTILIZATION IN JUVENILE ARCTIC CHAR (*Salvelinus alpinus*)

Yaa Tiwaah Amoah
Ministry of Food and Agriculture
Fisheries Commission, Ashanti Region – Ghana
madamyat@yahoo.com

Supervisors:

Professor Helgi Thorarensen
Hólar University College
helgi@holar.is
Mr. Olafur Sigurgeirrson, Associate Professor
Hólar University College
olisig@holar.is

ABSTRACT

A six-week trial was conducted with juvenile Arctic char reared in 20 l flow-through buckets to study the effect of dietary protein level on protein retention in the fish. At the end of the study, growth parameters and conversion efficiencies were all sensitive to the increased dietary protein levels in feed. Specific growth rate (SGR%) and live weight gain (LWG%), increased with increased dietary protein levels. The highest SGR (2.75±0.05) and LWG (285.41±9.62) were recorded from fish fed 39.89% Crude Protein (CP). The SGR and LWG values recorded from fish fed 29.30% CP performed remarkably well. Statistically, the FCR values recorded were not significantly different from each other except the value recorded at CP level 30.61% (1.78), but it was not different from that at CP level 29.91% (1.71). The body protein content increased with increased dietary protein content. The highest body protein content was recorded at CP level 39.89%. There were generally no significant differences in body protein content among the other feeds. Protein efficiency ratio (PER) and protein productive value (PPV) were not different among treatments except at CP level 29.31%. Dietary protein level in feed was found to be directly proportional to growth rate but inversely proportional to PPV and PER.

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TABLE OF CONTENTS

LIST OF FIGURES **3**

LIST OF TABLES **4**

1 INTRODUCTION **5**

1.1 GOAL AND OBJECTIVES 7

2 LITERATURE REVIEW **7**

2.1 GROWTH OF FISH 7

2.2 FEEDING AND NUTRITION OF FISH POPULATIONS 7

2.2.1 Protein requirements *8*

2.2.2 Lipid requirements of fish *8*

2.3 DIGESTIBILITY AND DIGESTIBILITY STUDIES IN FISH 8

2.4 NUTRIENT RETENTION AND FEED EFFICIENCY IN FISH 9

2.5 WATER QUALITY IN AQUACULTURE 10

2.5.1 Temperature *10*

2.5.2 Ammonia (NH_3) *11*

2.5.3 Dissolved Oxygen *11*

2.5.4 pH *11*

3 MATERIALS AND METHODS **12**

3.1 FEED 12

3.2 EXPERIMENTAL DESIGN, DATA COLLECTION AND ANALYSIS 12

4 RESULTS **14**

4.1 TEMPERATURE AND DISSOLVED OXYGEN 14

4.2 PROXIMATE ANALYSIS OF FEEDS 14

4.3 GROWTH AND CONVERSION VARIABLES 15

4.4 BODY TISSUE ANALYSIS 18

5 DISCUSSION **19**

LIST OF REFERENCES **22**

ACKNOWLEDGEMENTS **26**

LIST OF FIGURES

Figure 1. Aquaculture Production in Ghana from 2004 - 2010 (MOFA 2010)...........................6
Figure 2. Map of Iceland indicating the study areas..12
Figure 3. Experimental set-up..14
Figure 4. Temperature (^oC) and Dissolved Oxygen (mg/l). 14
Figure 5. Final average weight of juvenile arctic char (n = 4 replicates) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa. 16
Figure 6. Specific growth rates of juvenile arctic char (n = 4 replicates) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa. 16
Figure 7. Thermal growth co-efficient of juvenile arctic char (n = 4 replicates) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa. 17
Figure 8. Feed conversion ratio of juvenile arctic char (n = 4) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa...17
Figure 9. Protein efficiency ratio of juvenile arctic char (n = 4 replicates) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa. 18
Figure 10. Protein productive value of juvenile arctic char (n = 4 replicates) fed different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa. 19

LIST OF TABLES

Table 1. Projected fish demand and supply for Ghana from 2007 to 2012 (Agbo 2008) 5
Table 2. Estimated crude protein content of experimental feeds..12
Table 3. Formulated composition of experimental feeds. 13
Table 4. Results from proximate analysis of experimental feeds. 15
Table 5. Estimated growth and conversion variables of juvenile arctic char fed different 15
Table 6. Body tissue composition of juvenile arctic char fed different dietary protein levels. .18

1 INTRODUCTION

The total fisheries production from both capture fisheries and aquaculture was about 142 million tonnes in 2008 out of which aquaculture contributed 52.5 million tonnes accounting for 46% of the total production. Aquaculture has continued to grow in contrast to the stagnating capture fisheries. It is the fastest growing animal food-producing sector with an annual growth rate of 6.6% from 1970 to 2008 (FAO 2010). The average annual per capita supply of food fish from aquaculture for human consumption increased from 0.7 kg in 1979 to 7.8 kg in 2008 (FAO 2010).

The intensification of aquaculture in recent years has led to the use of formulated feeds. Feeding in aquaculture is very important because it usually accounts for about 30-50% of the variable operating costs in a production cycle (Miles and Chapman 2011).

Protein is the most expensive bulk raw material in fish feed regardless of its source and the most essential nutrient for growth performance, survival and yield of fish. It also influences the economics of a fish farming operation by determining the feed cost. In the process of amino acid breakdown, ammonia (NH_3) is formed and excreted to the water, affecting the water quality and growth condition of the fish. Recognition of the optimal protein requirement for each farming species therefore has both economical and biological aspects.

Fish is a preferred source of animal protein in Ghana and about 70% of the total production is consumed locally with an annual per capita fish consumption of 25kg. The total national fish requirement is estimated to be about 968,000 mt out of which only 390,000 mt (54%) is produced locally leaving a deficit of about 580,000 mt. (MOFA 2010). The projected fish supply and demand for Ghana is shown in Table 1.

Table 1. Projected fish demand and supply for Ghana from 2007 to 2012 (Agbo 2008)

Year	Demand (mt)	Supply (mt)
2007	913,992	511,836
2012	1,044,226	584,767
2017	1,193,017	668,090
2022	1,363,010	763,286

The shortfall in fish supply combined with the relatively expensive alternate sources of animal protein (mutton, pork, poultry, small ruminants, beef etc.) makes it imperative for measures to be taken to increase fish supply to bridge the widening gap between demand and supply. Aquaculture and culture-based fisheries provide the best hope for Ghana to achieve fish food sufficiency for a growing population (1.82% growth rate), which has a clear preference for fish as its principal source of animal protein (Manu 2004).

Over the last ten years, aquaculture has been given much publicity because it is perceived to be an important contributor to increase fish production in Ghana. This publicity has led to a steady rise in production from aquaculture from 950 mt in 2000 to 10,000 mt in 2010 (Figure 1) (MOFA 2010). The annual growth rate of fish farming in Ghana has been estimated to be 16% since 2000 (Asmah 2008).

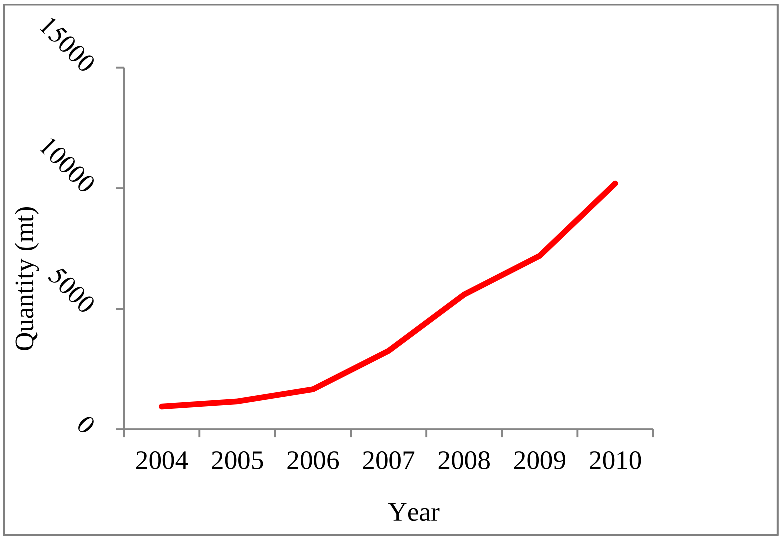

Figure 1. Aquaculture Production in Ghana from 2004 - 2010 (MOFA 2010).

Fish farming in Ghana is characterised by semi-intensive systems in ponds and tanks, and intensive systems in cages. Culture practices range from monoculture to polyculture practices and the major cultured species are tilapia and the African catfish.

A major challenge currently for fish farmers especially pond farmers in Ghana is high cost of fish feed and its constant supply. Currently most pond farmers feed their fish with imported formulated feeds for the first month of the production period and thereafter use local agricultural by- products; rice bran, maize bran mixed with groundnut peels for the rest of the culture period. This is because the imported feeds are expensive and the average pond farmer cannot support its use throughout a production cycle. There are sometimes feed shortages on the market due to delays in shipping and clearing of the feeds from the harbour. This compels farmers to fall on the local agricultural by-products whose availability is assured and are relatively cheaper.

However, few studies have been conducted on the use of the local agricultural by-products in Ghana. Studies on the nutritional composition of mixing two by-products e.g. rice bran and groundnut peels and the quantities to mix them to achieve optimum nutritional balance have not been conducted. Therefore, further studies need to be conducted on these feeds to increase their efficiency and subsequent production from fish farming in Ghana.

Arctic char (*Salvelinus alpinus*) is a temperate fish cultured in Europe, especially, Iceland, Sweden and Canada and cannot be cultured in the tropics. But nutrition and feeding of cultured fish is similar and therefore, methods for nutritional studies of one species can be applied to other species. This study uses arctic char as a tool for further studies on feeds in Ghana.

1.1 Goal and objectives

The main goal of this study is to assess the effect of dietary protein levels in feed on growth and protein utilization in juvenile arctic char.

Objectives

- Measure the effect of dietary protein level in the diet on the growth of Arctic char.
- Measure the protein retention in Arctic char.
- Estimate optimum protein levels in feeds for juvenile Arctic char.

2 LITERATURE REVIEW

2.1 Growth of fish

Growth of fish in aquaculture is dependent on many factors such as the type of feed and the conditions of the water. Various variables have been proposed to describe the growth process in fish in numerical terms and to quantify the influence of biotic and abiotic factors on growth based on either theoretical considerations of biogenetics or empirical studies (Ricker 1979). Where feed supply to fish is not a limiting factor, two factors that have great influence on fish are size and temperature (Corey *et al.* 1983).

The Specific Growth Rate (SGR) is the most frequently used numerical description of growth in fish, and is calculated as:

$$\text{SGR (\%)} = \frac{[(\ln(\text{Final body weight}) - \ln(\text{Initial body weight})]}{\text{Number of days}} \times 100$$

SGR varies in different species and sizes of fish and decreases with increasing body size. SGR seems to be affected by different dietary protein levels. The SGR of brown trout (*Salmo trutta)* (Arzel *et al.* 1995) and young *Heteropneustes fossilis* (Siddiqui and Khan 2009) increased with increased dietary protein levels. Growth of the Arctic char has been reported to be similar to other salmonids. Arctic char grows rapidly during the early fresh water rearing stages (Jobling 1983).

Another variable used to estimate growth in fish is the Thermal Growth Co-efficient (TGC). This quantifies and predicts the growth potential of a given species and stock of fish in relation to diet, husbandry, fish size and temperature (Cho 1992). This inter-relates to SGR. SGR increases with increased temperature.

$$\text{TGC} = \frac{[(\text{Final body weight})^{1/3} - (\text{Initial body weight}^{1/3})] \times 1000}{\text{Sum of degree days } (^{o}\text{C})}$$

2.2 Feeding and nutrition of fish populations

Feeding in fish is greatly influenced by factors such as the behaviour of fish, quality of feed, daily ration and the size of feed (Bascinar *et al*. 2007). Studies have revealed that the economic viability of a culture operation is dependent on the feed quality and feeding frequency. It means that nutritionally well-balanced diets and their adequate feeding are the main requirements for successful culture operations (Anderson *et al.* 2007).

Every species has its particular food preferences and feeding behaviours. The best feeding practice should be based on the fish's appetite. Ultimately the fish should be fed to satiation every day, and the feed should be evenly distributed throughout in water volume during feeding. It has been recommended to feed the Arctic char daily due to its relatively short gastrointestinal system and limited storage capacity in the stomach (Dalen 1998). The optimum feed particle size is 1.6-1.7% of fork length for 73-110 mm Arctic char, and 2.0-2.4% of fork length for 121-400 mm Arctic char (Tabacheck 1993).
Arctic char feed from both the bottom and from the water column which makes it better suited for tank culture rather than net pens, where a large proportion of the feed would be lost (Jesse, 2006).

2.2.1 Protein requirements

Protein is the single most important nutrient that fish need to grow. On a dry-weight basis, it makes up the maximum weight in their body structure (Craig and Helfrich, 2009). Fish are very adept at converting food to body tissues and they require between 40-60% protein in dietary feeds. In carnivorous fish optimum growth occurs when protein supplies about 40-50% of the energy requirements (Jobling 1994). Many studies have been carried out to determine protein requirements for arctic char.

The optimum protein content in juvenile Arctic char feeds have been estimated to be between 45-49% crude protein (CP) and that the requirement decreases with increasing size (Sigurgeirsson *et al*. 2008). These results are in accord with the results from other studies on fish (Gao *et al.* 2005).

2.2.2 Lipid requirements of fish

Lipids serve as an important source of dietary energy for all fish. Fish use lipids mainly to store energy and as components of cell membranes. From a nutritional standpoint, the quality and type of fatty acids in feed is very important with respect to consumer preference for the product.
Arctic char requires at least 15 to 20% of dietary lipids that are high in polyunsaturated fatty acids (PUFAs) particularly 18:3(n-3) for good growth (Johnston 2002).

2.3 Digestibility and digestibility studies in fish

Digestibility of fish diets is important because it can influence energy and nutrient availability, absorption and utilisation. Naturally occurring inert markers are incorporated in fish feeds for digestibility studies. These markers should not affect the digestion or palatability of the fish feed (Belal 2005). The percentage of the indicator is measured in the feed and a sample of the faeces to estimate the Apparent Digestibility Coefficient (ADC) by the equation:

$$\text{ADC (\%)} = 100 - 100 \times \frac{\text{\% indicator in food}}{\text{\% indicator in faeces}}$$

Yittrium oxide (Y_3O_2) is a suitable option as an inert digestibility marker. This is because it has the advantage of not affecting the metabolism of fish and can be included in feeds at low concentrations (mg/kg range) (Reis *et al.* 2008).

The digestibility of proteins, lipids, energy components and individual amino acids are normally estimated by the apparent digestibility co-efficient and should be considered as the basis for feed formulation (Reis *et al.* 2008). A careful selection of ingredients in fish feed is thus essential to improve apparent digestibility of a feed (Gudmundsson *et. al*. 1998).

Digestibility seems to be affected by increased dietary protein levels in fish feed. Apparent digestibility in *Chanos chanos* increased dietary protein levels (Jana *et al.* 2006). It is also affected by temperature. The apparent digestibility of nutrients in arctic char was higher at 10^{o}C than the digestibility of arctic char at 0.6^{o}C (Olsen and Ringo1998).

2.4 Nutrient retention and feed efficiency in fish

If fish utilises all the protein in the diet for growth then the protein content should be reflected in the protein content of the muscle. When food waste is high and nutrient retention and assimilation is poor, a major portion of the nitrogen is added to the culture system, which may ultimately pollute the environment (Jindal *et al.* 2010).

The body composition and feed utilisation for Arctic char fed nine experimental diets of varying protein levels (34, 44 and 54%) at each of three lipid levels (10, 15 and 20%) showed that increasing either dietary protein and/or lipid resulted in improved weight gain, feed efficiency and energy retained. Protein efficiency ratio and protein retention were directly related to dietary lipid but inversely proportional to dietary protein (Tabacheck 1986).

A number of studies have been conducted on protein utilization and retention in fish in recent years. The carcass protein content of *Channa punctatus* fingerlings fed low protein diets was reduced compared with fish fed with high protein diets (Jindal *et al.* 2010).

The body protein content of young *Heteropneustes fossilis* increased with increased dietary protein levels. The body protein content levelled off when the optimum levels were reached. In the same study, the body fat content of fish increased with increased dietary protein levels until the optimum levels were reached (Siddiqui and Khan 2009).

The body composition of Piracanjuba (*Brycon orbignyanus*) a Brazilian migratory fast-growing omnivore was also affected when it was fed with different protein and lipid content feeds in a study conducted by De Borba *et al.* (2003). As with proteins, the dietary lipid level of feeds have been found to be directly proportional to the deposition of lipid in the body of Arctic char (Lin 1997).

These studies show that nutrient retention in different fish species increases with increased dietary nutrient levels in feed. When the optimum levels of nutrients are reached any further increase does not affect deposition in the body of the fish.

FCR is a measure of how efficiently a feed is utilised for the purpose of growth (Johnston 2002). It indicates the quantity of feed required to produce one unit of wet fish. It is expressed as;

FCR = Feed consumed / Weight gain

The lower the FCR of a feed the more efficient it is and vice versa. Salmonids exhibit good feed conversion ratios, but this declines with increase in body size. FCR in Arctic char falls between 1.0 and 2.0. FCR rates recorded from juvenile Arctic char held in net pens and a commercial hatchery were found to range between 1.8-3.5 and 0.9 -1.6 respectively (Johnston 2002).

The quality and utilisation of protein in a diet is measured by the protein efficiency ratio (PER) and the protein productive value (PPV) (Albrektsen *et al.* 2006). PER is the ratio of weight gain to the quantity of protein fed. PER increases with the quantity of protein fed.

PER = Wet weight gain / Protein fed

Productive protein value (PPV) also known as 'efficiency of protein utilization' (Gerking 1971), evaluates the protein in the diet by the ratio between the protein retained in fish tissues and the dietary protein fed. PPV is determined by carcass analyses of samples of fish taken before and after feeding with the evaluated protein, and generally expressed as a percentage of the protein retained from the feed fed.

$$PPV = \frac{[(\%CP \text{ in fish of } t_2/100)\times W_2] - [(\%CP \text{ in fish of } t_1/100)\times W_1]}{\text{Gram feed} \times (\%CP \text{ in feed}/100)} \times 100$$

where t_1 and t_2 are the initial and final protein content of the fish and W_1 and W_2 are the initial and final weight of the fish

2.5 Water quality in aquaculture

Water quality refers to all the physical, chemical and biological factors that influence its use (FAO 1994). Fish performs all its bodily functions such as eating, growth, respiration, excretion and reproduction in water. Water quality in aquaculture can affect these functions; therefore, in order to maintain the success of an aquaculture operation, water quality parameters should be monitored and controlled.

2.5.1 Temperature

Temperature is one of the most important environmental factors affecting fish. The effects of increased temperatures on fish mortality, feeding, growth and maturation have been widely studied (Jobling 1994). The thermal niche and the ability to tolerate thermal stress vary among fish species (Reist *et al*. 2006a). Salmonid species mostly have optimum growth at 12–17^{o}C.

Arctic char are capable of maintaining growth at temperatures approaching zero (Brannas and Wiklund 1992). Thus, Arctic char have lower thermal limits for growth than other salmonids. However, it has been discovered that the rates of growth of Arctic char, in common with other fish species, is temperature dependent. The growth of 0-group and yearling Arctic char increased with increased temperature. It is maximal at 12–15°C, and then reduces at higher temperatures while adult Arctic char grow well at temperatures of 7–8°C. Brood stock should not be exposed to high temperatures to avoid compromising egg development and spawning (Jobling *et al*. 1993). Considerable growth was achieved from rearing juvenile Arctic char at 15ºC compared to lower temperatures. In this study juveniles reared at constant 15ºC weighed 44% and 78% more compared to fish reared at either constant 12 or 9ºC respectively (Gunnarsson *et al*. 2011).

2.5.2 Ammonia (NH_3)

Ammonia (NH_3) is the principal nitrogen excretory product of fish. NH_3 is more toxic to fish and it is highly soluble in water. Its toxicity in culture water increases with high pH. The rate of nitrogen excretion increases with feeding rate and metabolism.

There is very little ammonia present in natural lakes and rivers (less than 0.2mg/l), which poses no threat to fish health. However, in aquaculture settings ammonia and its by-products become a concern to fish health because fish is often stocked in high density with heavy feed rations and protein rich diets (Johnston 2002).

The concentration of ammonia in rearing water is usually the by-product of biological processes related with fish metabolism, which is excreted mostly through the gills. Smaller quantities are also excreted in the urine. It can also arise from the biological degradation of protein in waste feed and faeces. High concentrations in the culture water affect the diffusion gradient, which can cause a subsequent increase or build-up in the blood of the fish. When the concentration of ammonia reaches acute toxicity in the fish, it can be manifested in hyperventilation, irregular swimming, convulsions and even death. This is a phenomenon that cannot be under estimated.

Safe levels of NH_3 in the water for Arctic char is still not known, however it has been found to tolerate high concentrations (Eriksson 1991). Arctic char reared in water with NH_3 level of 0.0015m/l at a pH of 7.9 and stocking density of 150kg/m^3 (100-200g) had normal growth and good health (Ricks 1991).

Current studies in salmonid culture have suggested that maximum safe levels for NH_3 in salmonid aquaculture to support maximum growth and welfare range between 0.012 and 0.030 mg NH_3–N L^{-1} (Thorarensen and Farrell 2011).

2.5.3 Dissolved Oxygen

Dissolved oxygen (DO) is the key water quality parameter in aquaculture systems. This is because oxygen is poorly soluble in water. There is approximately 30 times less oxygen in a litre of water than in air (Thorarensen and Farrell 2011).

Low levels of DO may inhibit feed intake that could subsequently lead to poor growth in fish. This may be due to the reduced oxygen availability to support the energy demands of the fish (Jobling 1994). However, it is difficult to specify critical dissolved concentrations because the response to low DO is not always fatal, but rather, a range of physiological effects (Harmon, 2009). The DO level needed for salmonid aquaculture is estimated at 7.0 mg/l (Pillay and Kutty 2005). Arctic char should be kept in water with sufficient oxygen concentration, between 70% to 100 % saturation to avoid reduced appetite and growth (Johnston 2002).

The oxygen consumption of a fish varies with factors such as body mass, temperature, growth rate, feeding rate, swimming velocity and stress (Thorarensen and Farrell 2011).

2.5.4 pH

The acidity or alkalinity of culture water is one of the key parameters for attaining proper functioning of fish metabolism. High alkalinity (p>9) results in the conversion of ammonium to ammonia, which is toxic to fish. Fish in general, are sensitive to changes in water pH within the range of 5-9. High productivity occurs however at pH ranging from 6.5-8.5. Arctic char has however been found to be less sensitive to pH changes than other salmonids (Jobling 1994).

3 MATERIALS AND METHODS

The study was conducted in two institutions; feeding trials was conducted in Verid located in Saudarkrokur and all chemical analysis in Matis located in Akuryeri. Feeds used for the study were all formulated by Laxa Feed Company – Akureyri (Figure.2).

Figure 2. Map of Iceland indicating the study areas.

3.1 Feed

Five different types of feeds of varying expected crude protein (CP) content levels were formulated (Table 2). The formulated composition of experimental feeds is outlined in Table 3.

Table 2. Estimated crude protein content of experimental feeds.

Feed number	**CP (%)**
2973	26
2974	30
2975	34
2976	38
2977	42

3.2 Experimental design, data collection and analysis

Juvenile fish of average weight 0.8 g were obtained from Verid and stocked in 20 l buckets at 50 g per bucket. The set up was fitted with a continuous water flow-through system (Figure 3). Four buckets were randomly selected for each of the five feeds such that each feed was tested in quadruplicates. Fish were fed ad libidum daily by means of an automatic feeder for 6 weeks. Samples of fish were taken before and after the feeding trial for carcass analysis. All weight measurements were recorded by means of a Kern K8 electronic balance.

The total amount of feed given in each bucket was recorded but it was not possible to account for lost feed. Daily temperature and weekly dissolved oxygen measurements in the buckets were taken.

Table 3. Formulated composition of experimental feeds.

g/100g diet	Diet 2977	Diet 2976	Diet 2975	Diet 2974	Diet 2973
Fishmeal	29.79	26.95	24.11	21.28	18.44
Wheat	17.82	21.79	28.57	26.65	30.83
Wheat gluten	9.79	5.63	2.64	0.00	0.00
Maize gluten	10.00	10.00	10.00	8.50	2.84
Soya HIPRO	10.00	10.00	10.00	10.00	10.00
Canola meal	0.42	3.16	2.05	4.76	8.48
Mono Cal	0.00	0.00	0.061	0.20	0.33
Fish oil	21.11	21.4	21.64	24.55	28.02
Carophyll Red	0.027	0.027	0.027	0.027	0.027
Caropyll Pink	0.027	0.027	0.027	0.027	0.027
Premix	1.00	1.00	1.00	1.00	1.00
Yttrium oxide	0.015	0.015	0.015	0.015	0.015
Proximate composition (g/kg)					
Crude protein	420.0	380.0	340.0	300.0	260.0
Crude lipid	250.0	250.0	250.0	275.0	305.0
Ash					
Starch	136.0	159.0	195.0	200.0	200.0
GE ($MJ.kg^{-1}$)	21.60	21.00	21.0	21.00	21.00
Phosphorus					

Figure 3. Experimental set-up.

Proximate analyses of feeds and fish were conducted by drawing sub-samples from the samples taken from the feeds and fish replicates respectively.

Protein content in both the experimental feeds, initial and final fish carcasses were analysed by the Kjedahl method (N-Kjeldahl × 6.25 using Kjeltec Tecator^{TM} – 1002 distilling unit). Fat content in all samples was extracted by the Soxlet method (Soxlet distillation equipment) with di-ethyl ether as extraction solvent for 6 hours. 10g of samples to be used for ash content determination were weighed into oven dried (104^{o}C) crucibles and incinerated at 550^{o}C for about 6 –8hours. For moisture content, samples were mixed with sand, weighed, oven dried (104^{o}C) for about 2 hours and reweighed. All methods are in accordance with AOAC (2000).

From the data gathered from the feeding trial, growth and conversion efficiencies (SGR, TGC, LWG, FCR, PER, and PPV) of the fish were estimated.

All data collated were coded into SPSS software and subjected to a mixed model one-way analysis of variance (ANOVA) with replicate tanks nested under feed types.

4 RESULTS

4.1 Temperature and Dissolved oxygen

Weekly temperature and dissolved oxygen (DO) measurements were taken during the study period. Recorded weekly averages were 12.0 – 12.82^{o}C and 7.3 – 9.0mg/l for temperature and dissolved oxygen respectively (Figure 4). Lowest DO concentration was recorded in the fourth week of the study.

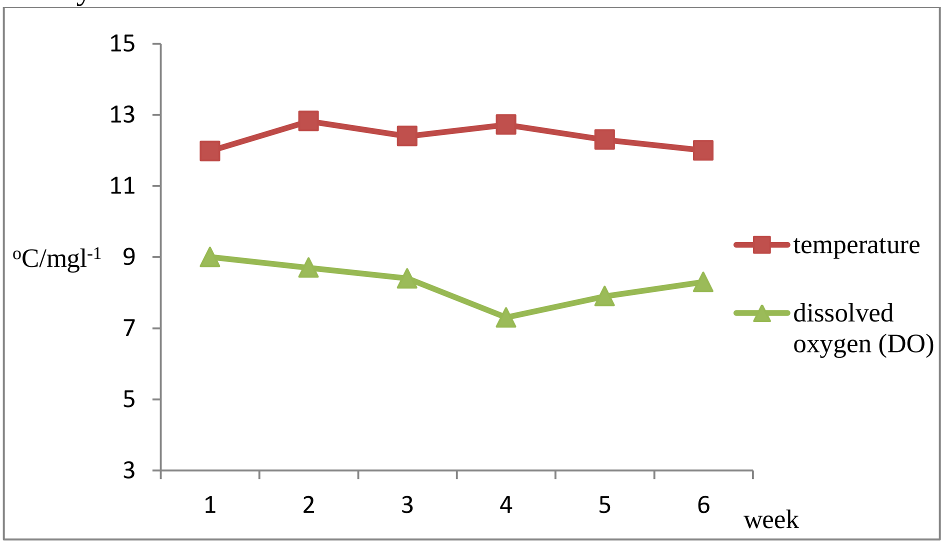

Figure 4. Temperature (^{o}C) and Dissolved Oxygen (mg/l).

4.2 Proximate analysis of feeds

Proximate analysis conducted on all the feeds showed some degree of variation in the protein content from the expected percentages. The expected crude protein percentages for the feeds were 26%, 30%, 34%, 38% and 42% (Table 4) but the proximate analysis showed the feeds as having in the same order, 29.30%, 29.91%, 30.67%, 36.18% and 39.89% indicating a significant deviation from the expected figures. The dietary protein level hierarchy was, however, maintained.

Table 4. Results from proximate analysis of experimental feeds.

Parameter/feed	2973	2974	2975	2976	2977
Protein	29.30[a]	29.91[a]	30.67[b]	36.18[c]	39.89[d]
Moisture	8.19	9.11	8.80	8.29	8.30
Ash	5.94	7.53	6.42	6.49	5.91
Fat	20.65	24.87	23.34	22.34	22.53

Mean values sharing the same superscript are not significantly different from each other (p>0.05)

4.3 Growth and conversion variables

Growth and conversion variables; final average weight, live weight gain (LWG%), specific growth rate (SGR%), protein efficiency ratio (PER) and feed conversion ratio (FCR) were calculated for juvenile Arctic char at the end of the study. All growth variables estimated were sensitive to the different dietary protein levels fed the fish (Table 5).

Table 5. Estimated growth and conversion variables of juvenile arctic char fed different dietary protein levels.

	CP levels (%)				
	29.30	29.91	30.67	36.18	39.89
Initial biomass	50.18±0.07	50.14±0.08	50.14±0.06	50.21±0.08	50.10±0.30
Initial average weight	0.8±0.00	0.8±0.00	0.8±0.00	0.8±0.00	0.8±0.00
Final biomass	178.85±5.22[b]	166.94±4.44[a]	162.58±3.65[a]	183.76±0.46[b,c]	192.94±4.74[c]
Final average weight	2.77±0.07[b]	2.60±0.06[a,b]	2.52±0.03[a]	3.01±0.07[c]	3.27±0.07[d]
Weight gain	128.67±5.16[b]	116.80±4.37[a,b]	109.59±3.63[a]	133.56±0.45[b,c]	142.88±4.77[c]
LWG(%)	256.41±10.01[b]	232.94±8.36[a,b]	218.56±7.15[a]	266.06±1.00[b,c]	285.41±9.62[c]
FCR	1.55±0.07[b]	1.71±0.06[a,b]	1.78±0.06[a]	1.50±0.01[b]	1.50±0.05[b]
SGR(%)	2.59±0.06[b]	2.45±0.05[a]	2.40±0.05[a]	2.65±0.01[b,c]	2.75±0.05[c]
PER	2.19±0.09[b]	1.95±0.07[a]	1.83±0.06[a]	1.85±0.01[a]	1.79±0.06[a]
TGC	3.13±0.09[b]	2.93±0.07[a,b]	2.85±0.007[a]	3.21±0.01[b,c]	3.37±0.08[c]
Feed given (g)	200±0.00	200±0.00	200±0.00	200±0.00	200±0.00

Mean values of four replicates ± SEM. Mean values sharing the same superscripts in a row are not significantly different (P>0.05)

The final average weight of fish increased with increased dietary protein levels (Figure 5). Weight gain of fish fed feeds CP levels 29.30%, 29.91% and 30.67% were not significantly different from each other but different from CP levels 36.18% and 39.89%. Feeds 2976 and 2977 were significantly different from each other (p<0.05).

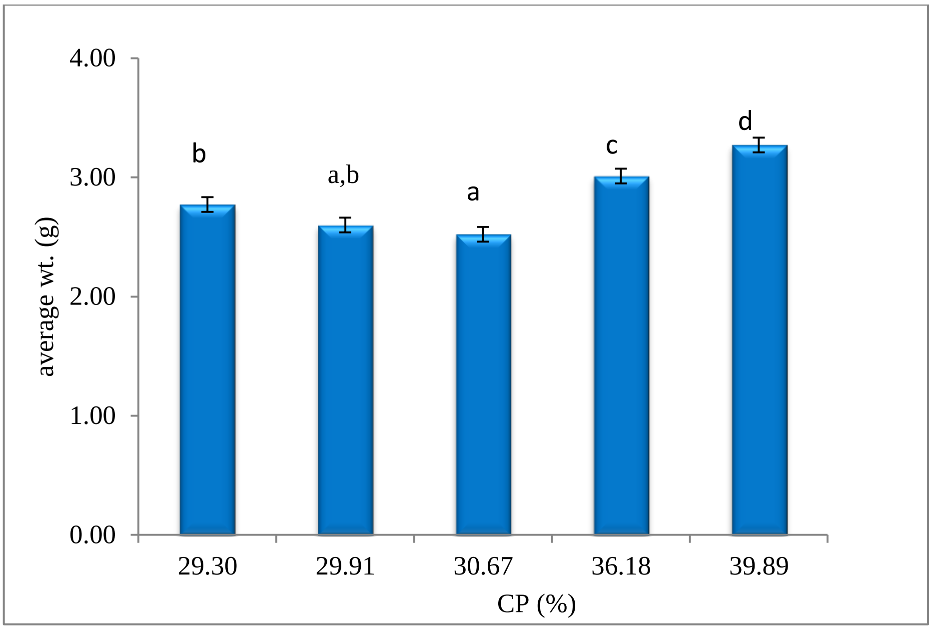

Figure 5. Final average weight of juvenile arctic char (n = 4 replicates) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa.

The SGR of fish also increased with increased dietary protein levels (Figure. 6). The lowest SGR was recorded at CP level 30. 67% while the highest growth rate was attained at CP level 39.89%. Fish fed with CP 29.30% however performed better as it was not significantly different from fish fed 36.18% CP (p>0.05).

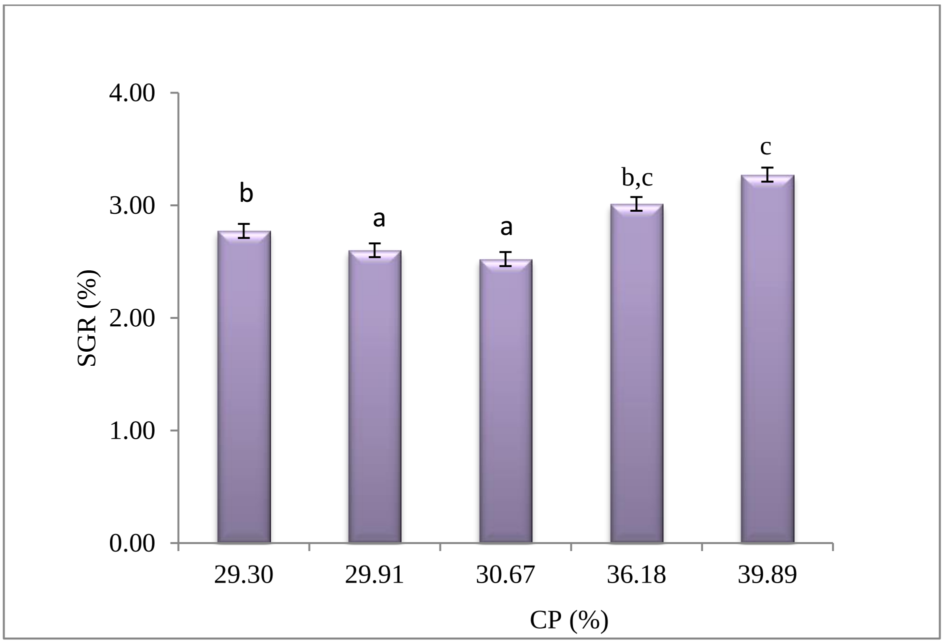

Figure 6. Specific growth rates of juvenile arctic char (n = 4 replicates) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa.

The thermal growth coefficient (TGC) followed a similar trend as the SGR (Figure. 7). It increased with dietary protein levels and again the lowest TGC was recorded at CP level 30.67% and the highest value at CP level 39.89%.

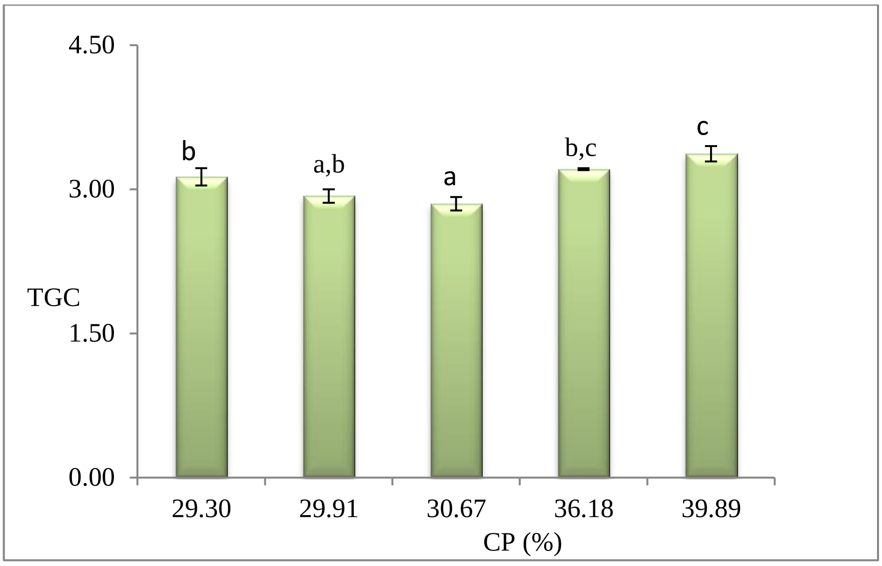

Figure 7. Thermal growth co-efficient of juvenile arctic char (n = 4 replicates) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa.

The highest mean value of FCR, 1.78±0.1 was recorded from fish fed CP level 30.67% but it was not signigicantly different from the value recorded from fish fed CP level 29.91%. The lowest FCR value of 1.40±0.09 was attained from fish fed CP level 39.89% (Figure 8).

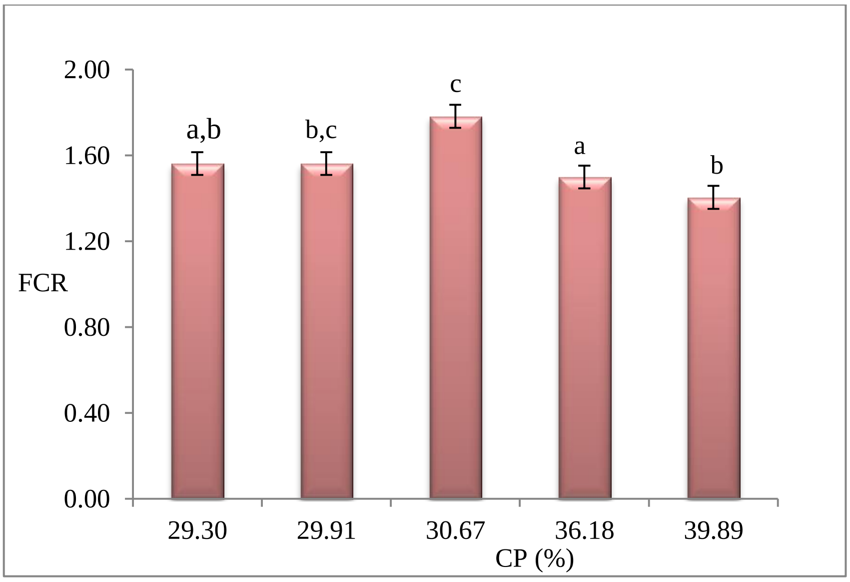

Figure 8. Feed conversion ratio of juvenile arctic char (n = 4) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa.

The protein efficiency ratios (PER) of the feeds decreased with increasing protein content in the feed (Figure 9). PER value recorded from fish fed 29.30% CP was significantly different ($p<0.05$) from all the other values recorded from the four CP levels which were not different from each other ($p>0.05$).

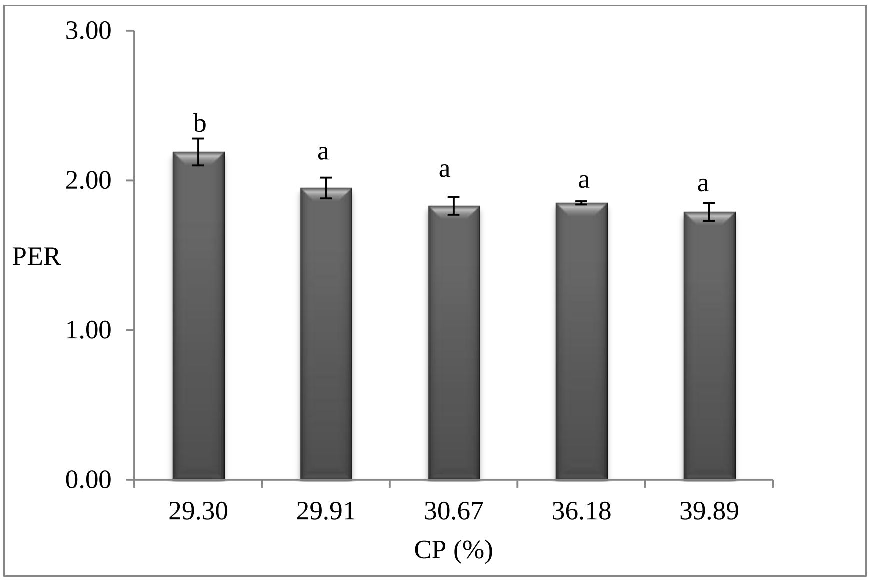

Figure 9. Protein efficiency ratio of juvenile arctic char (n = 4 replicates) fed at different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa.

4.4 Body tissue analysis

Results of body composition analysis of initial and final fish samples are shown in Table 6. The percentage protein retained in the fish increased significantly from that of the initial fish population in all the feeds. However, the protein content in fish fed 29.31% CP was significantly different from all the other CP levels.

The moisture content reduced significantly from that of the initial fish but there is no significant difference among all the feeds. The ash content in the fish increased at all dietary protein levels. Fish fed CP level 29.30% had the highest ash content followed by fish at CP level 30.67%. Ash content values from the other CP levels were not significantly different from each other. In a similar context, high body fat content was recorded at CP level 29.30%. this was however similar to the fat content of fish fed at 29.91% CP level. The fat content of fish from CP level 29.91% to 39.89% were not different from each other ($p>0.05$).

Table 6. Body tissue composition of juvenile arctic char fed different dietary protein levels.

	CP levels (%)					
	Initial	29.30	29.91	30.67	36.18	39.89
Protein	10.50±0.05	12.13±0.07[a]	11.83±0.11[a]	11.95±0.08[a]	12.08±0.19[a]	12.55±0.05[b]
Moisture	80.91±0.05	76.23±0.08[a]	77.27±0.09[a]	76.12±1.24[a]	76.90±0.15[a]	76.95±0.43[a]
Ash	1.47±0.01	1.72±0.04[b]	1.54±0.01[a]	1.61±0.03[c]	1.54±0.01[a]	1.50±0.01[a]
Fat	4.88±0.23	11.32±1.02[b]	9.99±0.15[a,b]	9.35±0.41[a]	9.48±0.43[a]	9.49±0.24[a]
Protein productive value (%)	-	28.01±1.07[b]	24.20±0.86[a]	23.11±0.71[a]	23.77±0.75[a]	24.49±0.51[a]

Mean values of three replicates ± SE. Mean values sharing the same superscripts in a row are not significantly different ($P>0.05$)

The highest protein productive value was recorded from fish fed the least CP level (29.30%) and this was significantly different from the other feeds ($p<0.05$). PPV values recorded from fish fed the other dietary protein levels are not different from each other ($p>0.05$) (Figure 10).

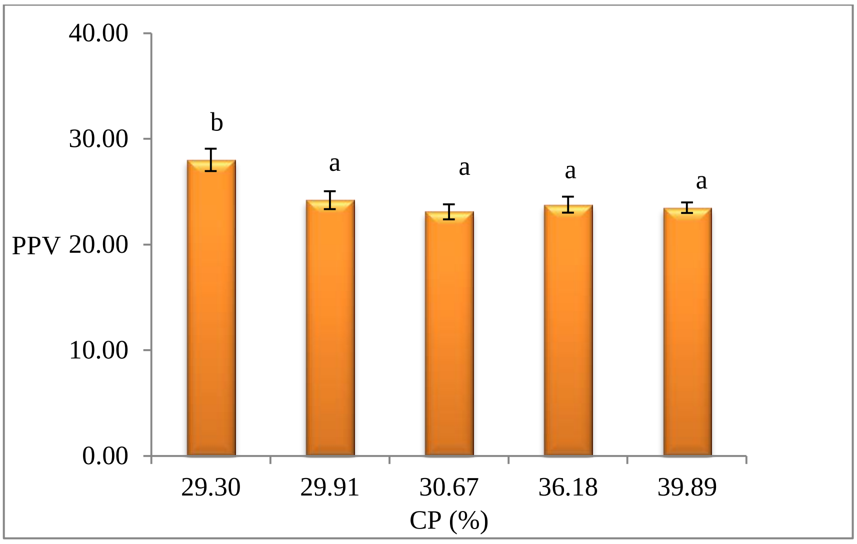

Figure 10. Protein productive value of juvenile arctic char (n = 4 replicates) fed different dietary protein levels. Bars sharing the same alphabets are not significantly different from each other and vice versa.

5 DISCUSSION

Dissolved oxygen is a very important water quality parameter in aquaculture. Low levels of DO inhibit feed intake, which could subsequently lead to poor growth in fish. This may be due to the reduced oxygen availability to support the energy demands of the fish (Jobling 1994). The recorded DO value during the study period was between 7.3 – 9.0 mg/l. This is above the estimated value of 7.0 mg/l (Pillay and Kutty 2005). Therefore, DO levels in the experimental buckets is unlikely to have had adverse effect on the growth of fish in this study.

Temperature is also very vital for optimum growth to be achieved in aquaculture facilities because it affects feeding, growth and maturation of fish. The mean temperatures recorded over the study period were between 12.0 – 12.8^{o}C. The growth of juvenile arctic char is highest at temperatures between 12-15^{o}C (Jobling 1993). The temperature in the culture water was within the optimum range for juvenile arctic char and this is likely to have an effect on the growth of the fish.

The level of protein in fish feed is of fundamental importance because it influences growth. Inadequate protein level in fish feed results in a reduction or cessation of growth due to withdrawal of protein from less vital tissues for body maintenance. Increase in dietary protein levels is often associated with higher growth rate in many species until it reaches the level beyond which further growth is not supported and may even decrease.

The growth rate of fish is high at the juvenile stage and decreases with increased body size. From this study, there was growth in juvenile Arctic char at all dietary protein levels but highest growth rate was observed in fish fed high dietary protein level (39.89% CP). Similar trends have been observed in similar studies with increased dietary protein levels (Jindal *et. al.* 2010, Siddiqui and Khan 2009, De Borba *et al.* 2003, Tabacheck 1986). The lower growth rate and weight gain values recorded from CP level 30.6% could be due to underfeeding or a tank effect on the fish, which reduced their appetite. The fish may also have been acclimatized to the system later than those in the other treatments. Since there was no growth plateau observed in fish at the different dietary protein levels it is not possible to estimate the optimum protein level required for growth.

In general, the protein requirement at the start of feeding salmonids is high. It is common to use feed containing 48–55% protein in the initial phase. The protein content in this experimental feed is far below that level. Therefore, one could expect that the growth of fish here is not optimal. Growth model (tables) and results from other experiments show that one can expect a SGR of 4-6% for arctic char of this size reared at 12°C, well above the 2.7% for the highest protein diet in the present study.

Due to practical constraints in experiments with fish, it was not possible to ensure that all food presented was ingested nor was it possible to collect uneaten food from the experimental tanks. Therefore for calculation of FCR, PER and PPV, the amount of feed fed (instead of feed consumed/intake) was used and no correction was made for any wastage. This could actually lead to overestimation of FCR and underestimation of the PER and PPV.

The lower the FCR of a feed, the higher the efficiency of the feed and vice versa. Relatively high FCR also reflects underfeeding of fish, i.e. the growth is less than expected due to lack of feed. This indicates that a relatively less energy is available for growth since energy for fundamental metabolism (ground metabolism) and motion is prioritized. The lowest FCR in this study was recorded from fish fed feed 2977 that contained the highest protein content but this was not significantly different from what was recorded from feeds 2973 and 2976. The highest value was recorded from feed 2975 but it was also not different from the value recorded in feed 2974. The high FCR in feed 2975 is manifest in the low growth rate recorded. The FCR range for Arctic char falls typically between 1.0 and 2.0 (Johnston 2002). All FCR values recorded from the study are therefore within the range specified indicating high efficiency in the feeds in the fish.

Usually, when fish are fed protein levels below optimum requirements, the fish needs to consume more feed to make up for protein required for growth and metabolism. At high and optimum protein levels, less feed is required to maintain a balance between the energy for growth and metabolism. This is an indication that protein efficiency ratio (weight gain/protein fed) is reduced with increased dietary protein levels. It is also possible that the feed intake is regulated by the energy requirement of the fish. Fish might stop feeding when it has consumed enough energy but not enough protein for growth. It thus becomes important to utilize the protein eaten, as reflected in the trend of PER and PPV in relation to protein content in the feed. From this study, the protein efficiency ratio (PER) decreased with increased dietary protein levels. The highest value was recorded in fish fed 29.3% CP. There was generally no significant difference in the PER values recorded in fish among treatments. This could be due to the fact that the actual amount of feed consumed by the fish was not taken into consideration but rather fixed amount of feed that was given in all the treatments because it was not possible to collect excess feed from the water outflow since the feed particles are small for this size of fish.

The Protein productive value recorded from the fish was not different at all the dietary protein levels except CP level 29.31%. PPV decreased with increased dietary protein levels. In earlier studies, the PPV has been estimated to be 40-45% for bigger fish (initial wt. 460g) and tend to decrease with increased protein content in feed. The PER was 2.2-2.7 for that same size of fish as being the highest PER on the lowest protein content in the feed (Sigurgeirsson *et al*. 2008)

The body protein content of the fish increased with respect to the initial body composition. The protein content of fish fed the higher CP level was higher and significantly different from the body protein content of fish fed the lower CP levels. This is in accordance with similar studies with different fish species where body protein level increased with increased dietary protein levels (Ruohonen *et al.,* 1999, De Borba *et al*. 2003, Siddiqui and Khan 2009).

Dietary lipids are a major provider of energy to all fish species especially carnivorous fish. It can be used to spare proteins in fish feeds by minimising the use of protein as energy sources through oxidation and conversion of amino acids. The fat content recorded from the body content analysis of the fish in this study, reflected the amount of fish oil inclusion in the feeds. Fish fed high lipid inclusion level at CP level 29.31% had the highest body content of fat. The fat inclusion levels of the other feeds were similar and this accounts for the lack of variation in the body fat content of fish fed with those diets. This is in accordance with the findings of Lin (1997) where body lipid level was directly proportional to the lipid levels in feed.

In conclusion, the rate of growth and body protein composition of juvenile arctic char was directly proportional to increased dietary protein levels while PER and PPV is inversely proportional to dietary protein levels.

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