EFFECTS OF STOCKING DENSITY ON PERFORMANCE, PROXIMATE COMPOSITION AND PIGMENTATION OF CULTURED ARCTIC CHARR (Salvelinus alpinus)

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EFFECTS OF STOCKING DENSITY ON PERFORMANCE, PROXIMATE COMPOSITION AND PIGMENTATION OF CULTURED ARCTIC CHARR *(Salvelinus alpinus)*

by

©Metusalach, B.Sc. Hon. (Fishery)

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ABSTRACT

The effects of stocking density on performance, proximate composition and pigmentation of cultured Arctic charr *(Salvelinus alpinus)* were studied. Results indicated that specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) of fish were significantly ($p<0.05$) influenced by stocking densities, but no significant ($p>0.05$) effects were evident in the hepatosomatic index (HSI) of fish. These performance parameters of fish correlated inversely with stocking density at 40, 50 and 75 kg/m^3 ($r = -0.9522$ for SGR, -0.9696 for FCR, -0.9886 for PER, and -0.9059 for HSI). Although SGR, FCR and PER of fish from different stocking densities varied significantly ($p<0.05$), their final weights did not.

Results also indicated that while the moisture content decreased, protein and lipid content increased as fish grew; the mineral content remained relatively unchanged over this period. Analyses indicated that moisture, protein and lipid content varied significantly ($p<0.05$) among density groups while ash content did not. Regression analysis showed that moisture and ash content were directly related to stocking density ($r = 0.9440$ and 0.9994, respectively), whereas protein and lipid content were not ($r = -0.5394$ and -0.7030, respectively).

The total amino acid content of fish varied significantly ($p<0.05$) according to stocking density and sampling dates. Aspartic acid, glutamic acid, leucine and lysine were the most abundant amino acids present, whereas hydroxyproline was the least abundant.

The free amino acid content of the fish also varied significantly ($p<0.05$) and inversely with stocking density ($r = -0.9441$) and sampling dates. The major free amino acids were anserine, taurine, glycine and alanine.

Lipid fatty acid composition of charr flesh was relatively unchanged over the course of the experiments. Among density groups, fatty acid contents of fish flesh were also relatively similar. Unsaturated fatty acids were the dominant fatty acids, accounting for up to 75% of the total lipids. Saturated and monounsaturated fatty acids correlated inversely with stocking density ($r = -0.9914$ and -0.9963, respectively), whereas polyunsaturated fatty acids correlated directly with stocking density ($r = 0.9984$).

The Hunter L^*, a^*, b^* values varied significantly ($p<0.05$) among density groups. For belly skin, these colour parameters decreased with increasing stocking density. No correlation was observed between the total carotenoid contents of fish skin and these colour parameters. On the other hand, the Hunter L^* values of fillets and homogenized tissues were inversely correlated with their carotenoid contents ($r = -0.9245$ to -0.9844, respectively), whereas their Hunter a^* and b^* values were directly correlated with total carotenoid contents ($r = 0.9040$ to 0.9824 for a^* values and 0.9527 to 0.9924 for b^* values, respectively).

The content of carotenoid pigments in flesh and skin of charr increased with duration of feeding on pigmented diets. After 11 to 16 weeks of feeding on a canthaxanthin-pigmented diet, fish flesh attained the required level of carotenoids (3-4 mg/kg wet tissue) considered sufficient for providing a satisfactory colour impression.

However, stocking density did not correlate with flesh carotenoid concentration ($r = -0.6034$). Canthaxanthin, echinenone, 4′-hydroxyechinenone, lutein and its esters, isocryptoxanthin and β-carotene were identified in different organs of charr. Canthaxanthin was the main carotenoid in charr flesh, whereas β-carotene, lutein esters and 4′-hydroxyechinenone, and echinenone were the dominant carotenoids in charr skin, gonads and liver, respectively. Canthaxanthin was not detected in the liver of fish. Therefore, dietary carotenoids may be deposited in different organs as such or may undergo reductive changes.

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CHAPTER 1. INTRODUCTION

1.1 Background

Unlike pure chemical compounds with definite and unchangeable composition, the musculature of a fish enfolds a variety of constantly changing interactive systems. The balance between these systems can vary widely without causing the death of the fish but, after capture and killing, these factors influence the acceptability of fish meat for human food and its suitability for processing **(Love, 1992).**

The assessment of nutritional status of fish is important in both culture and in wild populations. Numerous methods have been described and those which depend upon the relationship between body protein, moisture and lipid appear to be strongly indicative of nutritional status of fish; the measurements to be performed are comparatively simple **(Shackley *et al*, 1993).**

The overall quality of fishery products as food depends on a number of factors which determine their acceptance by consumers. Among these are safety, nutrition, flavour, texture, colour, appearance and suitability of the raw material for processing and subsequent storage. The relative importance of any of these characteristics depends on the specific product and its intended use **(Connell, 1990; Pigott and Tucker, 1990; Haard, 1992a).**

One of the advantages of seafoods is their superior nutritional value compared with other competing protein foods. Fish is low in calories and fat, high in protein, low in sodium and very low in cholesterol. In addition, fish and shellfish serve as an excellent source of ω-3 fatty acids which are thought to have important therapeutic and protective effects against heart disease, diabetes, cancer and other major illnesses. Consequently, the consumer perception of high nutritional value of seafoods is growing. Fish marketers, therefore, now routinely use nutritional data in their advertising and promotional materials **(Dore, 1990).**

The sensory properties of fish muscle may change as the animal ages. In the wild, the diet of fish may change because of feed availability or due to changes in their habitat or ability to capture prey as they grow. The diet and other factors can influence the free amino acid composition, mineral and fatty acid profile of fish flesh. These constituents may, in turn, influence the eating quality and post-harvest changes of the meat **(Haard, 1992a).**

Maturation of gonads and spawning of fish may have a profound influence on the quality of fish fillets as food **(Love, 1988; Yamashita and Konagaya, 1991). Bilinski *et al* (1984)** found that during sexual maturation of Pacific salmon *(Oncorhynchus kisutch)* the moisture content of fillets increased significantly with a concurrent decrease in their lipid and protein contents. The

non-protein nitrogen content remained constant, but the amount of free fatty acids increased significantly. **Papoutsoglou *et al* (1987)** found that body lipid and protein content of rainbow trout *(Oncorhynchus mykiss)* increased, their moisture content declined, and their ash content remained constant with age. Fish at higher stock densities also had higher moisture and lower fat content. **Fagerlund *et al* (1981)** found that increasing the population density of coho salmon raised in hatchery ponds was associated with a significant decrease in weight, length, condition factor, and feed conversion efficiency; elevated moisture content; reduced lipid and protein content; and increased mortality.

Colour of food plays an important role in their acceptability. In many cases, the price of seafoods is directly related to their colour. Salmon, for example, is often priced according to the intensity of its hue, therefore, the seafood marketing procedures must ensure that the colour is maintained both in terms of quality and quantity. Fish farmers must provide the right coloration for their cultivated fish in order to satisfy the consumer's expectation.

Arctic charr *(Salvelinus alpinus)* is closely related to salmon and trout. Arctic charr is utilized commercially in the USSR and Northern Canada, especially in Labrador. It is a high quality fish, regarded by some experts as superior to even the best salmon **(Dore, 1990).** However, the culture of this species is a relatively new in Canada as compared to that of salmon and rainbow

trout. In the Atlantic provinces, total production of charr for 1992 was around 32 tonnes (**1993 Aquaculture Association of Canada Bulletin, 93-2**), most of which was in New Brunswick and Prince Edward Island. In Newfoundland, there is a hatchery/grow out operation in Daniel's Harbour and a grow out facility in Grand Lake (Valley charr farm).

Due to high growth rates and tolerance of high densities, there are high expectations for the aquaculture of Arctic charr (**Jobling, 1983; Wallace *et al*, 1988; Baker and Ayles, 1990; Brown *et al*, 1991; Brown *et al*, 1992**). **Jobling (1983)** found that under farming conditions (in tanks), the growth rates of young charr were amongst the highest reported for salmonid species. Arctic charr have become recognized as a food delicacy with high consumer acceptability in North America (**Iredale, 1983**). In recent years there has been increased interest in examining the aquaculture potential of Arctic charr in northern temperate areas. Accordingly, there has been an increased interest in the culture of Arctic charr in Canada (**Papst and Hopky, 1983**), although the species has not been widely cultured (**MacCrimmon and Gots, 1980**). Arctic charr are successfully grown in Iceland and Norway. Production is still relatively small, but the fish has a highly-priced gourmet market which encourages expansion of its production (**Dore, 1990**).

Arctic charr *(Salvelinus alpinus* L*)* is a gregarious species in the wild **(Noakes, 1980).** It is the major fish species of the Canadian Arctic and it is an important food source for the indigenous people **(Yurkowski, 1986).** Although attempts to rear Arctic charr in sea cages under Norwegian fish farming conditions have not proven promising **(Gjedrem and Gunnes, 1978),** the species appears suitable for production by intensive culture **(Papst and Hopky, 1983). Wandsvik and Jobling (1982)** reported that Arctic charr (size range 25-78 g) reared at 13°C in fresh water had a specific growth rate of 1.4% per day. **Wallace *et al* (1988)** observed that Arctic charr from initial weight of 16 g/fish grew extremely well after being stocked at an initial density of 110 kg/m^3. However, little attention has been paid to the quality of the edible portion of Arctic charr. A better understanding of quality changes may contribute to a more efficient utilization of Arctic charr resource. Thus, defining the parameters such as stocking density and their effects on the body composition of Arctic charr is essential for clear understanding of the effects of diet and feeding rate on body composition.

1.2 Objectives

The objectives of this study were to examine the effect of stocking density on the flesh composition and pigmentation of Arctic charr. The initial stocking

densities were chosen within the optimal ranges suggested by **Baker and Ayles (1990).** Some aspects of Arctic charr performance, especially the growth rate, feed conversion, protein utilization efficiency, and hepatosomatic index (HSI) were also examined.

CHAPTER 2. LITERATURE REVIEW

2.1 Fish performance

2.1.1 Growth

Different fish species may vary in their potential growth rate. Some species are capable of growing faster than the others. However, considerable variation in growth also exists among individuals or groups within the same species. The stocking density at which maximum growth (weight gain) is achieved is likely to be dependent upon a number of biotic and abiotic factors such as the degree of domestication, ability of fish to make behavioural adjustment, physiological status, feed availability, and water quality **(Symons, 1968; Fenderson and Carpenter, 1971; Refstie and Kittelsen, 1976; Milensky, 1988; Poston and Williams, 1988).**

Physiological studies of growth in animals predict that growth rates should decrease with increasing size. Social interactions are known to influence both growth and sexual maturation in a number of aquatic organisms **(Borowsky, 1973, 1978; Sohn, 1977; Nelson and Hedgecock, 1983; Nelson *et al*, 1983; Ra'Anan, 1983).** Several authors have described a negative correlation between fish rearing density and growth **(Keenleyside and Yamamoto, 1962; Brown, 1957; Fenderson *et al*, 1968; Symons, 1968; Refstie and Kittelsen, 1976;**

Fagerlund *et al*, 1981). However, when Arctic charr were reared in large groups there was often a positive correlation between initial body size and the growth rate of an individual fish (**Jobling, 1985**). Similarly, **Wallace *et al* (1988)** observed that growth rate in Arctic charr was positively correlated with the stocking density. Likewise, **Kjartansson *et al* (1988)** found that Atlantic salmon *(Salmo salar)* did not experience much chronic stress at either intermediate or high stocking densities as compared to a low stocking density.

Mazur *et al* (1993) suggested that the significant effect of rearing density on mean weight of fish may have been due to energy allocation to what **Schreck (1982)** termed "resistance and compensation during stress", i.e., an effect due to increased behavioural interactions at a higher stocking density. Lower mean weights may also have resulted from depression of feeding rates in fish reared at very high densities. **Fenderson and Carpenter (1971)** found social interaction to be the main factor that depresses feeding rates in salmonids.

Wallace *et al* (1988) observed that growth rate of Arctic charr was positively correlated with stocking density. Arctic charr *(Salvelinus alpinus)* of initial weight of 16 g grew extremely well after being stocked at an initial density of 110 kg/m^3.

2.1.2 Feed Conversion Ratio

Feed conversion ratio (FCR) is defined as the ratio of the weight of the feed consumed to that gained by the fish. FCR often serves as a measure of efficiency of the diet. The more suitable the diet for growth, the less feed is required to produce a unit weight, i.e., a lower FCR. One of the problems regarding the FCR is that, the diet and the weight gain may comprise varied amounts of moisture and fat, which in turn, complicate the evaluation of protein level in the diets.

The partial efficiency of feed utilization for growth depends on many factors; the composition of the diets and its compatibility with the requirements for growth is a major factor. When the diet is deficient in any of the essential nutrients for growth, such as an essential amino acid, fatty acid, vitamin or mineral, a larger amount of feed is required to supply this deficient element and the efficiency of the feed utilization decreases. Also the fate of food in the body is important, whether it is converted into proteinaceous tissue or accumulated as lipid. The maximum theoretical efficiency of conversion of glucose to fat is about 70% of the metabolizable energy. For the conversion of amino acids to protein, if amino acids are present in the right proportion, the maximum theoretical efficiency is about 80% of the metabolizable energy. However, the actual efficiencies of utilization of food for growth are much lower (**Hepher,**

1988).

Environmental factors such as temperature also affect the FCR, since temperature affects the growth of fish. **Andrews and Stickney (1972)** have shown that FCR of channel catfish declined with increasing water temperature over the range of 18 to 30°C. This may be due to the fact that while growth rate increases with the increase in temperature, energy expenditure associated with maintenance is relatively low at higher temperatures. Therefore, energy is allocated more for growth than for maintenance **(Hepher, 1988).**

The gradual increase in FCR with increasing weight has been observed by many researchers **(Parker and Larkin, 1959; Davis and Warren, 1968; Brett and Groves, 1979).** This may also be associated with the different rates of increase in energy requirement for maintenance and growth with the increase in weight. While maintenance metabolism increases at a power of 0.8 of the weight, growth rate, and therefore the energy required for it, increases only by a power of 0.66 of the body weight. This means that the ratio between these two functions changes in favour of maintenance, which leads to an increase in FCR **(Hepher, 1988). Yoshida (1970a,b)** tried to formulate the relationship between weight and FCR in the form of a regression, and showed that the regression coefficient was directly dependent on temperature; it was higher at higher temperatures, but at low temperature, when growth is small, the effect of body

weight was small. **De Silva and Balbontin (1974)** observed that at 14°C, the FCR drastically decreased with increasing weight, however, at 6.5°C no such trend was observed. They also reported that the mean feed conversion of young herring *(Clupea harengus)* fed at 1.3% of its body weight/day was higher than that of fish fed to satiation.

The lower conversion rate in fish during winter months was probably due to reduced energy requirements, caused by lower water temperatures **(Smith, 1989)**. It has been shown that feed intake in sockeye salmon decline with decreasing water temperature, and that it declined at a faster rate than did the decrease in energy requirement **(Brett *et al*, 1969)**. Since the energy for growth as well as other factors in the energy budget of animals must be found in the difference between maintenance energy requirements and the feed energy consumed, the difference in the rates of decline of these two parameters would likely result in reduced growth **(Mazur *et al*, 1993)**. According to **Hepher (1988)**, the use of FCR for comparing the performance of diets of different composition is even more complicated than its use for evaluating the performance of the same diet under different conditions. Moreover, there is no common denominator for comparing diets of different composition, since they can differ in various nutrients (energy, protein, vitamins, minerals) which have, sometimes, very little in common, and which become important at different levels of

stocking density. However, since the main purpose of the fish farmer is to evaluate the economic efficiency of the feed, FCR can be expressed in terms of cost, where the cost of each feed is multiplied by the FCR. This expresses the cost of feed required by fish to gain one unit of weight.

2.1.3 Protein Efficiency Ratio

Protein efficiency ratio (PER) is defined as the ratio of the weight gain of fish to the amount of protein consumed. PER is probably the most widely used method for evaluating protein quality in fish. This ratio corrects the error due to variability of the moisture contents in the diets. However, this proposition also has some drawbacks: (1) It evaluates the protein in the diet rather than the diet itself. The higher the PER, the more efficient the dietary protein, but not necessarily the level of protein in the diet. (2) Similar to FCR, PER does not consider differences in the composition of the weight gained and the accumulation of lipids. Therefore, PER may increase as feeding level increases. (3) It is assumed that all protein in the diet is utilized for the synthesis of new tissues, whereas in fact, part of it is utilized for maintenance **(Hepher, 1988)**; the latter drawback may be corrected by adding the weight gain of fish fed on experimental diets with the body weight loss when the fish are fed on a protein-free diet **(Nose, 1971)**.

For maintenance level, fish require protein for replacement of degenerative tissues and proteinaceous products such as intestinal epithelial cells, enzymes and hormones, which are vital for proper function of the body, and are recycled very quickly. The requirement of protein for the synthesis of new tissues is obvious, since proteins make up 45 to 75% of the tissue, on a dry weight basis. The capacity of the fish to synthesize proteins *de novo* from carbon skeletons is limited; therefore, most of the protein must be supplied through the diet **(Hepher, 1988)**.

2.1.4 Hepatosomatic Index

The hepatosomatic index (HSI), the weight ratio of liver to the whole fish, is one of the parameters used to evaluate the nutritional status of fish. Hepatosomatic index is quite useful for expressing the nutritional status of fish, especially those which store much of their energy in the liver **(Love, 1992).** Therefore, much information may be extracted from primary data, especially when studying nutritional status of lean fish.

2.2 Stocking density

Stocking density may be expressed either as biomass (i.e., in kg/m^3) or as the number of fish per unit volume of water (or bottom area of culture media).

However, to keep densities within desired biomass limits the number of fish within rearing units are regularly reduced as fish grow **(Kincaid *et al*, 1976; Refstie, 1977; Westers and Pratt 1977; Soderberg and Krise 1986; Kjartansson *et al*, 1988).**

Rearing density is one of the most important factors influencing controlled fish cultivation **(Papoutsoglou *et al*, 1987; Refstie and Kittelsen, 1976).** This has been described for almost all cultured fish species, and for all types of production systems **(Haskell, 1955; Kilambi *et al*, 1977; Refstie, 1977; Carr and Aldrich, 1982).** In optimising production, a number of factors related to stocking density must be considered. Of these, the physicochemical condition of water, the production system, the type and size of rearing tanks, the water exchange rate, the size of fish and quality of the ration have been particularly emphasized **(Trzebiatowski *et al*, 1981).**

Rearing conditions such as rearing density can have a significant effect, at the production level, on parameters of economic importance to fish farmers. In intensive aquaculture, the density at which a fish species can be stocked is an important factor in determination of production costs in relation to capital investment. The higher the stocking density, the lower will be the production cost of fish, provided that satisfactory survival and growth are maintained **(Wallace *et al*, 1988).** Earlier investigations have shown that growth of cultured

fish is influenced by stocking density **(Keenleyside and Yamamoto, 1962; Refstie and Kittelsen, 1976; Refstie, 1977; Trzebiatowski *et al*, 1981; Pickering and Stewart, 1984)**. As might be expected, optimal stocking densities vary from species to species. Age and/or size within a species, and exogenous factors, such as temperature and feeding rate, can also influence the stocking densities which give optimal production results. In salmonid culture, there is a remarkable uniformity of technology, at least with regard to hatchery rearing, however, little attention has been paid to possible interspecific differences in husbandry requirements **(Wallace *et al*, 1988).**

Many authors have studied the effect of density of fish on behaviour, growth, aggression, and migration in rivers and lakes **(Fenderson *et al*, 1968; Keenleyside and Yamamoto, 1962; Fenderson and Carpenter, 1971; Symons, 1970, 1971).** However, all these studies have used low densities and the results cannot be directly related to crowded rearing tanks and ponds. Other researchers have dealt with density and carrying capacities in ponds, tanks, and cages **(Swingle, 1951; Andrews *et al*, 1971; Hackney, 1974; Brown *et al*, 1992)** which used warm water species where food and water turnover were limiting factors.

Confinement of salmonids at the unnaturally high stocking densities employed in commercial rearing units may have direct effects on both behavioral

and physiological variables **(Pickering and Stewart, 1984; Shreck *et al,* 1985; Laidley and Leatherland 1988; Vijayan and Leatherland, 1990). Patino *et al* (1986)** reported that stocking density clearly affected the physiological status of coho salmon *(Oncorhynchus kisutch).* An alternative approach may be adopted, in that the number of fish per unit volume of water is kept constant throughout the experiment. Consequently, stocking density in terms of biomass increases with time as the fish grows. This approach is adopted because regular removal of individuals could disturb the social relationships within the treatment group **(Laidley and Leatherland, 1988; Baardvik and Jobling, 1990).**

An increase in population density generally resulted in increased aggression among salmon as a result of territorial defense and competition for available food **(Keenleyside and Yamamoto, 1962; Fenderson and Carpenter, 1971).** In culture facilities, the stress on fish may be increased by a concomitant deterioration of water quality, due to decreased oxygen and increased ammonia contents **(Wedemeyer *et al,* 1976).** A moderate degree of crowding over extended periods induces a general state of stress in salmonids, which manifests itself in reduced growth rate **(Kawanabe, 1969; Refstie and Kittelsen, 1976; Li and Brocksen, 1977; Refstie, 1977),** feed consumption **(Fenderson and Carpenter, 1971),** feed utilization, body lipid content **(Li and Brocksen, 1977)** and physiological changes associated with the general adaptation syndrome

(Selye, 1950) such as interrenal hypertrophy and increased plasma cortisol, blood glucose, and liver glycogen concentrations **(Wedemeyer *et al*, 1970; Noakes and Leatherlandd, 1977; Ejike and Schreck; 1980).**

Crowding stress has adverse consequences on the health of hatchery fish **(Schreck, 1981)**. An inverse relationship between rearing density and plasma cortisol in rainbow trout *(Oncorhynchus mykiss)* has been reported **(Leatherland and Cho, 1985)**. However, the relative importance of this factor as a mediator of the deleterious action of high rearing density on coho salmon **(Fugerlund *et al*, 1983; Schreck *et al*, 1985)** is unclear. Identification of major factors by which rearing density affects the eating quality of fish is desirable since it may be used to improve present hatchery practices.

2.3 Proximate composition

The content of moisture, protein, lipid and ash (proximate composition) is routinely measured for experimental fish at the end of feeding trials. Knowledge of the proximate composition of fish and factors affecting it allows assessment of fish health, determination of efficiency of transfer of nutrients from feed to fish, and prediction of carcass composition **(Shearer, 1994)**.

The nutritive and commercial value of various fish depends on flesh structure and other edible parts and their proportion in the total mass of the

specimen. In addition, the chemical composition of the meat, gonad, liver and factors related to fishing and handling procedures are important factors affecting the commercial value of fish. The main chemical components of fish meat are moisture, protein and lipid. Together, they constitute up to about 98% of the total mass of the flesh. These components have the largest impact on the nutritive value, the functional properties, the sensory quality, and the storage stability of the meat. The other constituents, namely carbohydrates, vitamins and minerals, although minor in quantity, play a significant role in the biochemical processes occurring in the post-mortem tissue. These minor constituents are also coresponsible for the sensory properties, nutritive value, and wholesomeness of the product **(Sikorski, 1990a)**.

Physiological condition of fish has been defined as the gross nutritional state **(Love, 1970)** and the level of reserve nutrients, particularly fat, present in the body **(Gershanovich *et al*, 1984)**. Consequently, chemical composition of an individual fish carcass should characterize its physiological condition and, in general, its health. Furthermore, this physiological status determines the individual's ability to compete successfully (e.g. through optimal foraging and reproduction), sustain growth, maintain and repair tissues, and cope with stresses induced by environmental changes. Variations in body composition generally reflect storage or depletion of energy reserves **(Brown and Murphy, 1991)**.

Quantitative analysis of primary body constituents of numerous marine and fresh-water fish species has been reported **(Jaquot, 1961).** Generally, whole-body composition of fish is 70-80% moisture, 12-26% protein, 2-12% lipid, and 1-2% ash; however, extreme values of these components may fall outside these ranges **(Weatherley and Gill, 1987).** Several studies have shown significant changes in whole-body composition or specific organs or muscle tissues due to age, diet, feeding frequency, migration, ration, season, sex, starvation and temperature **(Chang and Idler, 1960; Brett *et al*, 1969; Groves, 1970; Savitz, 1971; Niimi, 1972; Elliot, 1976; Craig, 1977; Grayton and Beamish, 1977; Millikin, 1982; Weatherley and Gill, 1983).**

Available evidence indicates that the protein content of salmonids is determined solely by fish size (is endogenously controlled); lipid level is affected by both endogenous and exogenous factors; ash content is homoestatically controlled, and the whole body moisture is inversely related to body lipid. **Shearer (1994)** divided the effects of endogenous factors on proximate composition as given below.

1. Where nutrition is adequate, the relative sizes of the tissues and organs are dependent on the size and life cycle stage of the fish.
2. Protein, amino acids and ash levels are life-cycle and size-dependent.
3. Dietary energy intake in excess of the maintenance requirement results in

lipid storage.

4. Lipid and moisture contents are inversely related.

In cultured fish, whole body protein and ash are size-dependent and lipids increase with increasing fish size but are affected by life-cycle stage and energy intake. Furthermore, the moisture and lipid levels are inversely related. When experimental treatments create size differences between treatments, differences in proximate composition may be due to endogenous factors, such as size, life-cycle stage, and exogenous factors (treatments), or both. It is therefore necessary to remove the endogenous effects before treatment effect can be examined **(Shearer, 1994)**.

Exogenous factors, both environmental and dietary, have been reported to affect the proximate composition of cultured fish. These include temperature, salinity, exercise, intraspecific differences, interspecific differences, diet, time and frequency of feeding, among others. **Shearer (1994)** drew the following conclusions from the effects of exogenous factors on the proximate composition of cultured fish.

1. The proximate composition of salmonids is determined by endogenous and exogenous factors that operate simultaneously.
2. The primary determinants of proximate composition in growing fish are size, life-cycle stage and energy intake.

3. For a particular species, at a given weight and life stage, organ and tissue, weight, whole body protein, amino acid and ash contents are controlled within narrow limits.
4. The amount of whole body lipid is dependent on dietary energy input and the metabolic energy demands of the fish.
5. Whole body moisture is inversely related to whole body lipid and decreases or increases as lipid is stored or utilized.

Numerous authors have reported marked variability of proximate composition within a species due to age, sex, season, diet, and combination of these factors. Fat appears to be most affected because of energy demands associated with overwintering starvation in juvenile and mature fish and eventual gonad maturation in sexually mature fish. Protein and ash are less dynamic as compared to fat **(Brown and Murphy, 1991).**

2.3.1 Moisture

The moisture content of fish is usually expressed on a wet-weight basis, i.e. the mass of water in unit mass of fish. The moisture content of fish is usually determined by oven drying at 100 to 120°C for 16 to 18 hours. The loss of mass is equivalent to the mass of water in the original sample **(Doe and Olley, 1990).**

Muscles of marine fish contain from about 60 to 80% moisture, depending on the species and the nutritional status of the animal. Starvation, which is common in many fish species during spawning, depletes the energy reserves of the tissues and consequently increases the water content of the flesh. In muscles and other tissues, water plays the important part of a solvent for a host of organic and inorganic solutes, provides the environment for biochemical reaction in the cells, is an active partner in many reactions, and has a large impact on the conformation and reactivity of proteins. The hydration of proteins is responsible for the rheological properties and juiciness of muscle foods **(Sikorski, 1990a)**. The amount of water affects both the quality and processing of fish **(Kent, 1985)**. Moisture normally represent about 80% (w/w) in low fat species and is less in species which store fat in their muscles. The lipid and water contents normally add up to about 80% of the weight of the muscle **(Pigott and Tucker, 1990; Haard, 1992a)**. A number of factors may influence the moisture content of muscle tissues. In particular, the nutritional status and sexual maturity of fish can have a marked effect on the moisture content of the flesh **(Connell, 1990; Haard, 1992a)**. Depletion of nutrients during sexual maturation or starvation may increase the moisture content of lean muscle. The moisture content of muscle of cultured fish is less than that of the wild fish **(Saeki and Kumagai, 1984)** which may reflect their better nutritional status. It has been suggested that

the moisture content of raw flesh is an important quality indicator for determining the acceptability of salmon for canning during sexual maturation **(Bilinski *et al*, 1984).**

The state of water in the fish flesh depends upon various interactions of water structures with different solutes and especially with proteins. The hydrophilic amino acid residues participate in H-bonding with water molecules, while the hydrophobic groups in proteins and lipids act as structure makers, i.e., they induce around themselves layers of highly ordered water clathrates. Thus, in fish meat, only a part of aqueous medium can be regarded as intercellular water; the rest is involved in water-protein-lipid-solute interactions **(Sikorski, 1990a).**

Moisture content is clearly a good parameter for identifying undernourished fish and it has been long recognized that body moisture varies inversely with fat content **(Jacquot, 1961; Gardiner and Geddes, 1980).** Water moves into the extracellular space as fat and carbohydrate are utilized and muscle proteins are catabolized **(Shackley *et al.* 1993).** In chronically-starved fish, body fat, and later, body protein decreases **(Love, 1980).** These are replaced by water and the proportion of body moisture increases.

2.3.2 Protein

Protein is one of the most important nutrients obtainable from consumption of muscle foods and fish **(Nettleton, 1990; Gorga and Ronsivalli, 1988)**. The crude protein content of fish muscle normally ranges from 11 to 24% **(Sikorski *et al*, 1990b)**; however. in extreme cases, may be less than 6% or greater than 25% of the tissue weight **(Haard, 1992; Pigott and Tucker, 1990)** depending on the species of animal, nutritional condition, and the type of muscle **(Sikorski *et al*, 1990b)**. The total content and nutritional quality of protein of meat from farmed and wild fish appear to be similar **(Nettleton, 1990)**. In Ayu *(Plecoglossus altivelis)*. the protein content of muscle decreased slightly from summer to autumn with no appreciable difference between cultured and wild fish **(Hirano *et al*, 1980)**. The amino acid score for essential amino acids was similar for wild (89) and cultured (83) bastard halibut *(Paralichthys olivaceus)* **(Sato *et al*, 1986)** and coho salmon *(O. kisutch)* **(Hata *et al*, 1988)**. **Saeki and Kumagai (1982)** reported that the protein content in puffer muscle did not change during the growth of both wild and cultured fish.

The amino acid composition of total muscle proteins, i.e. of the whole skinned fillets, of various fish species do not differ much from one another. The nutritive value of fish proteins which constitute 65 to 75% of the total body weight, on a dry weight basis **(Wilson, 1989)**, is very high because of their

favourable essential amino acid pattern. The *in vivo* digestibility of the proteins of raw fish meat is in the range of 90-98% **(Sikorski *et al*, 1990a).**

Finfish are a good source of protein (11.40-21.90%) and are relatively low (0.58-10.24%) in fat content (Table 1). The percentage of ash (total minerals) in the fish ranged from 0.80 to 4.66%, while the moisture ranged from 70.16 to 81.50%. In tables of food composition, the content of protein usually refers to the crude protein (% Kjeldahl nitrogen x 6.25). This represents proteins and other nitrogenous compounds such as nucleic acids, nucleotides, trimethylamine (TMA) and its oxide (TMAO), free amino acids and urea, among others **(Sikorski *et al*, 1990b)**. Based on the solubility in salt solutions of increasing concentration, proteins may be classified into three distinct groups: the components of the sarcoplasmic fraction which perform biochemical tasks in the cell (18-20%): the myofibrillar proteins of the contractive system (65-80%); and the protein of the connective tissues, responsible mainly for the integrity of the muscles (3-5%) **(Hall and Ahmad, 1992a; Sikorski *et al*, 1990b)**. The relative amounts of different groups of proteins in fish depend upon the sexual development and depletion of the body protein of fish and may fluctuate in the annual cycle by a few percent **(Sikorski *et al*, 1990b).**

The term sarcoplasmic proteins usually refers to the proteins of the sarcoplasm, the component of the extracellular fluid, and the proteins contained

Table 1. Proximate composition (%) of some selected finfish fillets.[1]

Species	Moisture	Protein	Lipid	Ash
Anchovy[b]	81.50	11.40	6.10	0.80
Barracuda[d]	79.50	18.6	0.98	1.01
Bluefish[a]	70.16	19.56	2.00	1.12
Croaker[a]	79.37	18.13	1.90	1.08
Flounder[a]	77.00	21.23	1.19	1.23
Giant perch[b]	78.20	16.00	0.70	1.70
Greasy grouper[d]	77.70	19.20	0.58	1.29
Mackerel[b]	80.00	16.10	1.80	1.60
Mackerel[d]	74.90	21.90	1.49	2.95
Mullet[b]	79.10	15.30	2.20	1.10
Mullet[c]	74.23	17.61	2.96	4.66
Pink perch[b]	79.50	15.30	1.30	1.50
Red snapper[d]	78.69	19.30	1.10	1.36
Sardine[b]	77.60	17.10	1.20	1.60
Sea Bass[a]	79.80	18.46	2.08	1.09
Sea Trout (Gray)[a]	76.98	18.62	3.41	1.13
Spot[a]	70.23	19.70	10.24	1.20
Yellow tail[d]	75.05	20.3	2.95	1.49

[1]Source: [a]Anthony *et al.* 1983; [b]Chandrashekar and Deosthale, 1993; [c]Marais and Erasmus, 1977; [d]El-Faer *et al.* 1992.

in the small particles of the sarcoplasm. The truly intracellular soluble fraction makes up to 90-95% of the total proteins of the extract obtained by homogenizing the muscle tissue with water or solutions of neutral salts of ionic strength below 0.15. The sarcoplasmic proteins are also soluble in more concentrated salt solutions. Generally, the sarcoplasmic fraction makes up to about 30% of the total amount of proteins in fish muscles. The content of sarcoplasmic proteins is higher in pelagic than in demersal fish muscles (**Sikorski *et al*, 1990b**).

The myofibrillar proteins can be extracted form the comminuted fish meat with neutral salt solutions of ionic strength above 0.15, usually ranging form 0.30 to 1.0. The fraction of the myofibrillar proteins, which can be precipitated by tenfold dilution of the centrifuged supernatant with distilled water, makes up 40-60% of the total amount of N x 6.25 of fish meat. The myofibrillar proteins participate in the post-mortem stiffening of the tissues (rigour mortis). Changes in these proteins later lead to the resolution of stiffness, while during long-term frozen storage they may cause toughening of the meat. The myofibrillar proteins are also responsible for the water holding capacity of fish, for the characteristic texture of fish products, as well as for the functional properties of fish minces and homogenates, especially their gel-forming ability. The residue after extraction of sarcoplasmic and myofibrillar proteins, is known as stroma and is

composed of the main connective tissue proteins namely collagen and elastin of reticulin, and of denatured aggregated myofibrillar and possibly sarcoplasmic proteins, which might have lost their characteristic solubility. The stroma which is insoluble in dilute solutions of hydrochloric acid or sodium hydroxide constitute about 3% of the total muscle proteins of teleosts and up to 10% of elasmobranchs (**Sikorski *et al*, 1990b**).

The technological value of fish flesh depends mainly on its proteins. The functional properties attributed to proteins of fish flesh comprise hydration, which is reflected in solubility, dispersibility, water retention, swelling, and gel-forming ability, as well as interaction with lipids, i.e. emulsifying capacity and emulsion stability. These properties depend on the composition and conformation of proteins. They change during storage and processing of the fish (**Sikorski *et al*, 1990b**).

2.3.3 Lipid

Fish display a particularly diverse anatomical pattern of lipid storage. In elasmobranchs, lipid may comprise 90% of the weight of the liver. Much of the lipid is squalene or diacylglyceryl ether, both of which have lower densities than triacylglycerols, contributing to the buoyancy of these swimbladderless fish (**Malins and Wekell, 1970**). Different teleosts deposit fat in a variety of

locations, including liver, muscle, and bone marrow **(Phleger *et al*, 1976)**. The primary fat storage type in higher bony (teleost) fish is triacylglycerol (TG), however 1-0-alkyl diacylglyceryl ethers (long chain hydrocarbons linked to glycerol by an ether bond plus two fatty acids linked by ester bonds) have been reported in certain tissues of some species. The major storage sites of lipids in fish are mesenteric fat, muscle and liver. Compositional data of various organs in several teleostean species are shown in Table 2. The skeletal muscles of cod contain little lipid, but considerable amounts of fat are stored in their liver **(Sheridan, 1988)**. Whether the liver or the muscle serves as the predominant site varies with the species of fish. In general, the liver serves as the main storage depot for lipid in sluggish bottom dwelling fish, whereas the skeletal muscle serves this function in more active species **(Robinson and Mead, 1973)**. The latter statement corresponds with the findings of **Sheridan (1988)** who observed that rainbow trout have substantial amounts of lipid stored in their skeletal muscles.

Marine lipids are composed of phospholipids, sterols, triacylglycerols, wax esters, minor quantities of their metabolic products, as well as small amounts of unusual lipids, such as diacylglyceryl ethers (DAGE), glycolipids, sulpholipids, and hydrocarbons **(Sikorski *et al*, 1990a)**. Phospholipids and sterols occur in small, but relatively constant amounts of 0.6 to 1.2% based on the wet weight

Table 2. Lipid composition (mg/g wet weight) in different organs of some fish species.

Fish organ	Lipid class						
	PL	TG	CH	FFA	CE	WE	Total
	Trout, *Salmo gairdnerii* (Juvenile: FW parr)						
MF	6.2	529.0	-	-	-	-	527
DM	26.0	113.0	5.5	-	-	-	137
LM	20.0	46.0	3.0	-	-	-	62
L	22.0	17.0	1.2	0.06	0.72	1.23	46
S	7.5	11.0	2.3	1.5	1.6	1.2	25
	Trout, *Salmo gairdnerii* (Juvenile: FW smolt)						
MF	5.8	535.0	-	-	-	-	540
DM	39.0	10.0	0.2	-	-	-	47
LM	21.0	10.0	0.2	-	-	-	27
L	25.0	5.0	0.6	0.03	0.08	0.10	30
S	5.2	7.0	1.1	0.5	0.26	0.66	14
	Trout, *Salmo gairdnerii* (Adult: FW resident)						
MF	10.0	34.0	5.0	2.0	-	-	51
DM	4.0	16.0	2.0	1.5	-	-	23
L[a]	9.6	2.9	6.2	4.4	3.1	-	26
	Salmon, *O. kisutch* (Juvenile: FW parr)						
MF	19.0	590.0	17.0	-	-	-	625
DM	37.0	53.0	11.0	-	-	-	107
L	19.5	22.0	13.0	5.0	tr	tr	60
	Salmon, *O. kisutch* (Juvenile: FW smolt)						
MF	17.0	525.0	18.0	-	-	-	580
DM	27.0	41.0	9.0	-	-	-	62
L	12.0	10.0	7.5	3.0	tr	tr	41
	Whitefish, *Coregonus albula* (Adult)						
MF	10.0	<----------20.0[b]---------->					30
Roe	22.5	62.7	1.4	.02	<---1.7[c]--->		98
	Tilapia, *Oreochromis mossambicus*						
LM	3.3	-	1.7[d]	17.8	-	-	66.5
L	31.6	-	15.6[d]	47.6	-	-	164.4
Gill	3.8	-	3.5[d]	8.5	-	-	76.9
Brain	80.4	-	25.5[d]	35.0	-	-	212.3
	Bogue, *Boops boops*						
LM	5.3	12.1	0.3	tr	-	-	17.7
L	28.2	48.7	0.9	tr	-	-	77.8
Head	12.0	144.0	2.0	tr	-	-	158
Skin	8.0	169.3	1.0	tr	-	-	178

[a]Includes serum; [b]Data for all neutral lipids combined; [c]Data for CE and WE fractions combined; [d]Data for WE and alcohols; [e]Data for total CH; includes both CH and CH esters; [f]Includes unidentified components and hydrocarbons; PL-phospholipid, TG-triacylglycerol, CH-cholesterol, FFA-free fatty acid, CE-cholesteryl ester, WE-wax ester, MF-mesenteric fat, DM-dark muscle, LM-light muscle, L-liver, S-serum, FW-freshwater, tr-trace, -, data not available. *Adapted from Phleger *et al* (1976).

of tissues. They play an important structural role in biomembranes and participate in basic cellular functions. The remaining lipids are essentially energy stores and are important for buoyancy.

The reserve lipids are used in various parts of the body when needed during starvation, overwintering, fast movements, reproduction, and growth. During gonad maturation, lipids are transported from the liver and muscles to the gonads. After spawning, the fish resumes intensive feeding and the lipid content in the flesh and liver increases, while it decreases in the gonads **(Sikorski *et al*, 1990b)** if gonads are resorbed. Otherwise, eggs/sperm are lost.

Since reports related to the health-promoting effects of seafood were published, considerable attention has been focused on the nutritional value of fishery products **(Kayama, 1986; Nettleton, 1985, 1987; Pigott and Tucker, 1990; Gordon and Ratliff, 1992)**. Comparison of the nutritional value of farmed and wild fish has been monitored by examining their lipids **(Nettleton, 1990, 1992)**. Wild fish tend to contain a higher percentage of long chain ω-3 fatty acids than farmed fish **(Vliet and Katan, 1990; Nettleton, 1990)** although the absolute amount of fatty acids is often similar **(Haard, 1992a)**. Diet influences the amount of nutritionally important ω-3 fatty acids in the muscle of farmed fish **(Lovell, 1988; Nettleton, 1990)**. However, the final fatty acid composition of cultured fish can be adjusted through dietary changes over a four- to six-weeks period prior to harvest **(Nettleton, 1990)**.

Due to the reported benefits of ω-3 polyunsaturated fatty acids (PUFA) in reducing the risk of coronary heart diseases (**Bradlow, 1986; Kinsella, 1987**), lipids in fish muscles have received much interest as a source of unsaturated fat in the human diet (**Barlow and Stansby, 1982; Kinsella, 1987**). As a consequence, the nutritional quality of aquacultured products is a possible factor in determining the consumer acceptance of these products (**Erickson, 1993**). The nutritional benefits of consuming ω-3 fatty acids has clearly increased the marketability of salmon (**Carroll, 1986; Herald and Kinsella, 1986; Kinsella, 1986; Lands, 1986; Hearn *et al*, 1987; Stansby, 1990; Holub, 1992; Nettleton, 1992**). However, some concern has been expressed as most cultured fish species have higher levels of ω-6 fatty acids, lower levels of ω-3 fatty acids, and thus lower ω-3/ω-6 ratios compared to the wild stocks (**Ackman and Takeuchi, 1986; Chanmugam *et al*, 1986; Suzuki *et al*, 1986**). It should be noted that some fish such as farmed catfish are naturally rich in ω-6 fatty acids, and lack EPA and DHA, while salmonids are naturally rich in ω-3 fatty acids even when C18:2ω-6 is supplied (**Polvi and Ackman, 1992**).

Polyunsaturated fatty acids in fish tissues predominantly belong to the ω-3 series (**Cowey and Sargent, 1977**). Fatty acids of the ω-3 series are essential for fish. Some species have the ability to convert linolenic acid (C18:3ω-3) rapidly to longer chain PUFAs (C22:5ω-3, C22:6ω-3) that have full essential fatty acid activity. Other species lack this ability and the ω-3 PUFA must be

supplied in their diet for maximal growth and freedom from pathogens.

The long-term consumption of PUFAs of the ω-3 class by humans leads to decreased incidence of coronary arterial diseases, and alleviation of symptoms of breast cancer, rheumatoid arthritis, multiple sclerosis, and psoriasis **(Goodnight, *et al*, 1982; Kinsella, 1988)**. Their mode of action is twofold. First, they inhibit production of the bioregulatory 2-series eicosanoid family from ω-6 PUFAs; second, they produce the bioactive 3-series eicosanoids from the precursor eicosapentaenoic acid (EPA, C20:5ω-3) **(Dyerberg, 1986; Fischer and Weber, 1983)**. As a result of joint roles of ω-3 and ω-6 PUFAs in eicosanoid production, the ratio is usually calculated along with the level of EPA when nutritional evaluation of lipids is made.

At present, with the exception of the addition of carotenoids to the diet for flesh coloration, little attempt has been made to modify carcass composition to meet the consumer preference for a specific characteristic in cultured salmonids **(Shearer, 1994)**. Numerous reports indicate that carcass lipid is directly related to dietary energy intake, and that the profile of stored lipid reflects that of the diet **(Sargent *et al*, 1989)**. Given the growing awareness of the importance of ω-3 and ω-6 fatty acids in human diet, it is only a matter of time before demand for ω-3 enhanced cultured salmon increases **(Austreng and Krogdahl, 1987)**. The fatty acid composition of the fish also appears to affect its organoleptic properties. Studies of **Spinelli (1979)** on rainbow trout and **Burtle (1989)** on

catfish indicate that fish containing long-chain (18 or more carbon atoms) PUFAs show less desirable flavour and texture after frozen storage when compared to fish containing short-chain (12-14 carbon atoms) saturated fatty acids.

However, when discussing the beneficial effect of fish lipids, it must be remembered that the proportions of the various PUFAs in fish muscles are not constant. We are again dealing with a dynamic system. The lipid composition of fish food intake has probably the most important influence on the lipid composition of its tissues **(Lovern, 1935; Hall, 1992). Worthington and Lovell (1973)** reported that the lipid composition of the food accounted for 93% of the variance in the fatty acid composition of channel catfish *(Ictalurus punctatus)*; the genetic and other factors accounted for the rest.

The lipids in the edible part of fish are important to the food scientists in three respects. Firstly, any oily deposits noticeably influence the sensation of the cooked flesh in the mouth of the consumer. Secondly, fish lipids, as is now widely recognized, posses beneficial health effects. Thirdly, flesh lipids contribute to the flavour of the fish. Although lipids themselves have a slight taste, their propensity to develop an off-flavour through oxidation in the frozen state is of greater importance **(Love, 1992).**

2.3.4 Ash

The mineral components are contained in food as macro and microelements. The macroelements are present in quantities of several hundreds of milligrams per 100 grams wet weight. The microelements are present in the flesh in quantities not larger than that of iron, i.e. from below 0.1 to a few tens of micrograms per gram. The components of both groups are important in human nutrition. Some of them are highly desirable in large quantities, while others are required in small amounts since they may be toxic in higher concentrations. Nutritional requirements for humans depend upon the biological state of the organism and efficiency of utilization of the elements of the diet **(Sikorski *et al*, 1990b)**.

In teleosts, the water environment may contribute significantly to the mineral requirements of both the adult and young fish. Minerals may be absorbed and become a part of the tissues, or they may serve important functions in osmoregulation. **Hayes *et al* (1946)** found that calcium and sodium were absorbed from, and phosphorus and potassium were lost to, the environment by eggs of Atlantic salmon *(Salmo salar)*. The amount of ion movement is undoubtedly influenced by ionic concentration in the water. Quantitative measurement of net effects indicates that the water medium may contribute significant amounts of calcium, sodium, potassium and iron **(Zeitoun *et al*, 1976)**.

Marine foods serve as a rich source of minerals, as their content in the raw flesh ranges from 0.6 to 1.5% of wet tissue weight (**Sikorski *et al*, 1990a**). Fish muscle normally contains practically all the mineral elements occurring in the water habitat. Minerals are important constituents of fish flesh because of their nutritive value, safety considerations, and their contribution to taste. The ash content in the muscle of cultured and wild fish is often similar (**Saeki and Kumagai, 1982; Date and Yamamoto, 1988; Morishita *et al*, 1988; Jahnke *et al*, 1988**). However, **Kunisaki *et al* (1986)** reported that wild horse mackerel *(Trachurus japonicus)*, and bastard halibut (*Paralichthys olivaceus*) (**Sato *et al*, 1986**) tend to have slightly higher ash contents than their cultured counterparts. **Date and Yamamoto (1988)** suggested that considerable seasonal variations may exist in the content of specific minerals such as Ca, Na, and K in muscles of cultured yellowtail *(Seriola quinqueradiata)* muscle.

2.4 Total and free amino acids

2.4.1 Total amino acids

The actual amino acid composition of fish muscle is roughly the same as that in terrestrial animals, although the proportions of different protein types vary, reflecting the environment in which these animals live (**Hall and Ahmad, 1992a**). The amino acid composition is important in two respects: nutrition and flavour (**Hall and Ahmad, 1992b**). The composition of total amino acids affects

nutritional value of the food, while the free amino acids affect its flavour. Within the body, most amino acids are present as constituents of proteins (**Cowey and Walton, 1989**). There is little difference in the amino acid composition of body proteins among fish species or even in the whole versus gutted fish (**Connell and Howgate, 1959**).

Relatively little information is available on differences in specific protein fractions as a function of rearing conditions. There is, however, sufficient information to suggest that rearing conditions can influence the content of specific protein(s) which are related to one or more food quality attributes (**Haard, 1992a**).

2.4.2 Free amino acids

The pools of free amino acids in the body are relatively small and are derived from the diet and the catabolism of proteins. The concentration of free amino acids depends on many factors, such as species, dietary history, and time and site of sampling after feeding (**Cowey and Walton, 1989**). Amino acids, peptides, nucleotides, guanidine compounds, and quarternary ammonium compounds are the major contributors to the taste of seafoods. Glycine, alanine, serine and threonine are sweet in taste, while arginine, leucine, valine, methionine, phenylalanine, histidine, and isoleucine possess a bitter taste. Histidine and taurine are the most abundant free amino acids in some species of

fish. Migratory fish such as tuna *(Thunnus sp.)* and mackerel *(Trachurus sp.)* are high in free histidine. Taurine in fish muscle is probably derived from cysteine. TMAO (trimethylamine oxide) is one of the most abundant non-protein nitrogenous constituents in fish. Its concentration ranges from 156 mg/100 g in puffer fish *(Fugu sp.)*, about 400 mg/100 g in flathead flounder *(Platycephalus fuscus)*, to around 1000 mg/100 g in Alaska pollack *(Theragra chalcogramma)* and cod *(Gadus callarias)*, and 750 to 1450 mg/100 g in the muscle of sharks and rays **(Sikorski *et al*, 1990a).**

The content of free amino acids in the muscles of aquatic species is normally higher than that in land-based animals. The free amino acid content in muscles of aquatic organisms normally ranges from about 0.5% to 2% of the muscle weight. Cultured fish tend to contain less free amino acids than their wild counterparts. **Hata *et al* (1988)** reported that free amino acids in cultured coho salmon *(O. kisutch)* account for 315 mg/100 g of white muscle, whereas in wild coho salmon they constitute 439 mg/100 g of white muscle. **Morishita *et al* (1989)** reported that cultured sea bream *(Chrysophrys major)* contain 310-400 mg free amino acids/100 g dorsal white muscle, while those of wild sea bream contained 528 mg free amino acids/100 g sample. The main free amino acid in both salmon and red bream was taurine. Free amino acids, notably anserine, glycine, alanine, and lysine, tend to be higher in wild than in cultured ayu *(P. altivalis)* **(Suyama *et al*, 1977)**. Free amino acids and related compounds play

an important role as taste- and flavour-active components **(Haard, 1992a).**

2.5 Lipid fatty acid composition

The fatty acid composition of marine lipids is much more complex than that of lipids of terrestrial plant and animals. The carbon chain length is generally form C14 to C24, although C12 and C26 are found as well. The marine fatty acids are particularly highly unsaturated. Even C14 and C16 acids have unsaturated ethylenic bonds, while C20 and C22 acids contain four, five and six ethylenic double bonds. Most of the PUFAs of fish lipids occur as ω-3 type. The ω-6 acids make up only a few percent of the total. Fish lipids contain also some odd carbon number fatty acids (C15, C17 and C19). The content of these unusual fatty acids is 1 to 3%, but occasionally in mullet *(Mugil cephalus)*, it may be even above 10% **(Sikorski *et al*, 1990a).**

The distribution of fatty acids in lipids is far from uniform. The polyenoic acids occur predominantly in phospholipids, while monunsaturated acids are dominant in triacylglycerols. Thus, at least 50% of the fatty acids in phospholipids are polyenoic, basically the 20:5ω-3 and 22:6ω-3 (Table 3), the former occurring mainly in phosphatidylcholine and the latter in phosphatidylethanolamine. The triacylglycerols are composed of 50% monoenoic fatty acids and the rest are more or less equally divided between saturated and polyenoic acids. The content of different fatty acids in fish lipids depends on

Table 3. Fatty acid composition (w/w %) of total muscle phospholipids of some species of Pacific (Japanese) salmon.[1]

Species	*O. gorbus-cha*	*O. keta*	Fall *O.keta* (female)	Fall*O.keta* (male)	*O. nerka*
14:0	1.5	0.9	2.7	2.0	1.4
15:0	0.4	0.4	0.2	0.5	0.5
16:0	19.7	21.6	21.0	22.7	25.7
17:0	0.7	0.8	0.5	0.5	1.0
18:0	2.5	2.7	2.5	1.2	3.6
∑SFA	**25.2**	**27.0**	**27.4**	**27.0**	**32.9**
16:1	0.8	0.8	2.5	1.9	1.2
18:1	4.8	5.3	8.8	6.6	7.2
20:1	3.4	1.5	1.1	0.5	1.6
∑MUFA	**9.8**	**8.2**	**12.6**	**9.1**	**10.4**
18:2ω-6	0.5	0.4	0.4	0.4	0.5
20:4ω-6	0.9	0.8	0.6	0.7	0.8
20:5ω-3	6.5	8.1	19.9	16.3	6.7
22:5ω-3	1.5	1.6	3.3	2.3	1.8
22:6ω-3	51.1	50.5	33.6	42.1	43.2
∑PUFA	**63.0**	**63.4**	**58.5**	**63.3**	**55.2**
ω-3	**61.0**	**61.8**	**57.3**	**61.8**	**53.4**
ω-6	**1.7**	**3.1**	**1.4**	**1.3**	**1.6**

[1]Adapted from Takama *et al*, 1994. Symbols are SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids.

numerous factors such as the diet, geographic location, temperature, season of the year, body length and lipid content among others **(Sikorski *et al*, 1990a).**

The lipids of cultured fish contain more ω-6 and less ω-3 PUFAs than the lipids of wild fish. Tropical fish are richer in ω-6 acids than fish from the northern hemisphere. The two most important ω-3 PUFAs are the 20:5 and 22:6 which are typical of marine algae and zooplanktons, respectively. The proportion of these two acids in lipids depends on the feeding habit of marine organisms. Most pelagic fish feed on zooplankton or are predators on other fish; thus, their lipids contain more 22:6 than 20:5. The Antarctic fish living under the sea ice and feeding on algae contain more 20:5 than 22:6 **(Sikorski *et al*, 1990a).**

Polyenoic acids are recognized as important dietary components. These fatty acids are essential since the double bonds at the third and the sixth carbon atoms from the methyl end group cannot be synthesized in the animal body and have to be derived from food. The ω-6 acids are essential for man as they serve to generate the eicosanoids which are modulators of important metabolic functions **(Sikorski *et al*, 1990a)**. Fish lipids are rich in the ω-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and have been recognized as being effective in preventing hyperlipidemia, thrombosis, atherosclerotic cardiopathy, myocardial infarction, and cerebral embolus (**Dyerberg *et al*, 1978; Herold and Kinsella, 1986; Kinsella, 1988; Nordoy and Goodnight, 1990)**. Work on physiological effects of ω-3 PUFA has also shown

stimulation of brain function and suppression of breast cancer **(Yamamoto *et al*, 1987; Yazawa and Kageyama, 1991)**.

The fatty acid composition of lipids is important due to its influence on flavour, odour and storage characteristics of fish **(Ackman, 1967; Worthington *et al*, 1972; Gathlin and Stickney, 1982). Worthington and Lovell (1973)** showed that 98% of the variance in fatty acid composition of channel catfish in their experiment was accounted for by the diet. Fish size and age were also found to significantly influence their fatty acid profiles **(Tidwel and Robinette, 1990).**

During digestion and absorption, dietary lipids (mainly triacylglycerols; TG) are acted on by lipase, which causes the hydrolysis of triacylglycerols to free fatty acids. If not required metabolically, these free fatty acids can be re-esterified to form depot triacylglycerols. Through this process, a significant portion of the fatty acids present in tissue lipids of fish can be derived from dietary sources without any alteration **(Stickney and Andrews, 1972; Dupree *et al*, 1979)**. However, profiles of deposited lipids can differ from those of the dietary lipids depending on species, genetic variation **(Yingst and Stickney, 1979)**, metabolical need **(Lovern, 1964; Dupree *et al*, 1979)**, season **(Gruger *et al*, 1964)** and possibly the age of the animal **(Yingst and Stickney, 1979).**

Mobilization of depot lipids in fish proceeds via the activation of lipolytic enzymes (triacylglycerol lipase) and results in the hydrolysis of stored TG and

subsequent release of fatty acids. The mobilized lipids generally remain unesterified. Fatty acids are carried in the plasma of fish by one, or perhaps more albumin-like binding proteins. Lipase activity has been observed in the liver, dark muscle and mesenteric fat of coho salmon, *O. kisutch* **(Sheridan *et al*, 1985).**

The major contribution of C20 and C22 PUFA's in the human diet is from fish. However, food, location and season of catch are known to exert significant effects on fatty acid composition **(Gruger, 1967)** of fish; therefore, all such factors must be considered when evaluating lipid quality of a fish species.

2.6 Pigments

Colour plays an important role in the overall acceptability of food products. It is considered as one of the man's basic experiences that a particular foodstuff must have a distinct colour in order to be edible. Colour also has an important function in cooking and thermal processing. Natural, fresh colour provokes the appetite, enhances the enjoyment of food and also acts as an optical seasoning.

The colour of a seafood is the first characteristic noted by the consumer and is directly related to the subsequent acceptance or rejection of it. Carotenoids contribute to the yellow, orange, and red colours which are found in the skin, shell, or exoskeleton of aquatic species. Carotenoids are indeed the

most widespread pigments in nature as they occur in bacteria, yeasts, molds, all green plants, and many animals and perform various functions. They are biosynthesized by photosynthesizing organisms, some heterotrophic bacteria and fungi, but not by animals **(Bjerkeng, 1992)**. From an anthropocentric consideration, the most significant aspect of carotenoids is the colour they impart to the food and environment and the fact that they represent the major source of vitamin A precursor in the diet. The particular shade and intensity of coloration differs substantially, depending on species, habitat, feed and physiological status **(Dore, 1990)** of the species. In animals, the carotenoids are also associated with reproductive organs and hence the hatching success and survival of alevins **(Srivastava, 1991)**.

Carotenoids are a group of fat-soluble pigments **(Fox and Vevers, 1960; Matsuno and Hirao, 1989; Gross, 1991; Ikan, 1991)** which are also soluble in non-polar organic solvents such as acetone, alcohol, diethyl ether, and chloroform. While the carotenes are soluble in non-polar solvents such as petroleum ether and hexane, their oxygenated derivatives, xanthophylls, dissolve best in polar solvents such as alcohols **(Gross, 1991)**. Carotenoids are known to be indispensable cellular components in microorganisms, fungi, algae, higher plants, animals, and humans **(Goodwin, 1976, 1980, 1984; Britton, 1976; Liaaen-Jensen, 1978)** and are one of the most important group of natural marine pigments **(Matsuno and Hirao, 1989)**. They are also one of the main natural

food colorant groups with widespread application **(Sigurgisladottir *et al*, 1994).**

Although it is generally accepted that animals are unable to synthesize carotenoids *de novo*, they apparently are able to modify dietary plant carotenoids **(Buchecker, 1982).** The carotenoids are either dissolved in fats or combined with proteins in the aqueous phase. In the higher plants, they are found in the leaves together with chlorophyll. In living organisms, these photosensitive compounds play important roles, such as colouring the body, tissues, or biological fluids, and therefore, allowing chromatic adaptation to environments, and also by assuming various physiological functions, as in respiration and vision **(Ghidalia, 1985).** Biosynthetically, they are derived from the acyclic lycopene through hydrogenation, dehydrogenation, cyclization, and oxygenation reactions **(Britton, 1976).** The biochemistry of carotenoids in plants and animals has been thoroughly reviewed by **Goodwin (1980, 1984)** in a two-volume series.

Carotenoids are responsible for the colour of many important fish and shellfish products. Many of the more expensive seafoods, such as shrimp, lobster, crab, crayfish, trout, salmon, redfish, red snapper, and tuna have orange-red integument and/or flesh containing carotenoid pigments **(Haard, 1992b).** For species such as shrimp, salmon, rockfish, and snapper, the grading or pricing is directly related to the intensity of red hue **(Sacton, 1986).** According to **Ostrander *et al* (1976)**, consumers consider colour as the most important factor distinguishing salmon and trout. Pen-reared salmon, which lacked typical salmon

colour, was not considered to be different from trout by sensory panels.

Haard (1992b) divided pigments in seafood into three categories:

1. Biological pigments such as carotenoids and haemoproteins which are synthesized or otherwise accumulated in the living animal.
2. Postharvest pigments which are formed in the postmortem tissue as a result of reaction to injury from improper handling or processing conditions. These include enzymatic and non-enzymatic browning reactions.
3. Additive colorants which are added to fish or fish products.

Pigments may be applied antemortem by including them in the diets of cultivated animals or may be administered postmortem, e.g., by dipping in a solution of canthaxanthin or apocarotenoid, or by postharvest injection of food dyes **(Simpson *et al*, 1981)**.

Carotenoids are less widely distributed in muscle than in integument. Economically-important fish species, which typically exhibit flesh pigmentation, include salmon *(Oncorhynchus sp)* and trout *(Salmo sp, Salvelinus sp)*. Carotenoids are also found in the eggs, sperm, liver, eyes, brain, intestine, and mouth mucus of fish **(Haard, 1992b)**. Table 4 lists the carotenoid distribution of some selected fish and shellfish of economical importance as seafoods.

The ovaries and eggs of fish and shellfish are almost always pigmented. The colours range from yellow, orange and red to green, blue and purple **(Miki *et al*, 1982)**, mainly due to the presence of carotenoids and/or carotenoproteins.

Table 4. Carotenoid pigments in some important seafoods.[a]

Species	Common name	Carotenoids
CRUSTACEA:		
Procambarus clarkii	Red crayfish	β-carotene, astaxanthin, echinenone, phoenicoxanthin
Pleuroncodes planipes	Red crab	β-carotene, astaxanthin
Portunus trituberculatus	Blue crab	Canthaxanthin, 4-hydroxyechinenone, 3-hydroxycanthaxanthin, echinenone, astaxanthin, isocryptoxanthin, β-carotene.
Penaeus japonicus	Shrimp	β-carotene, canthaxanthin astaxanthin, lutein, echinenone, phoenicoxanthin, dihydroxypirardixanthin, zeaxanthin.
Penaeus setiferus	White shrimp	Astaxanthin, cryptoxanthin.
Crangon crangon	Sand shrimp	β-carotene, astaxanthin, zeaxanthin, lutein, canthaxanthin.
Geryon quinquidens	Red crab	Astaxanthin, β-doradexanthin, zeaxanthin.
Carcinus maenas	Green crab	β-carotene, lutein, γ-carotene, zeaxanthin, astaxanthin, canthaxanthin, echinenone.
Homarus gammarus	Lobster	Astaxanthin.
Panulirus japonicus	Spiny lobster	Astaxanthin, echinenone, 4-hydroxyechinenone, isocryptoxanthin, β-zeacarotene, 3-hydroxycanthaxanthin, β-carotene, β-doradexanthin.
MOLLUSCA:		
Mytilus edulis	Mussel	Mytiloxanthin, isomytiloxanthin, astaxanthin, β-carotene, zeaxanthin, lutein.
Veneus japonicus	Short-necked clam	β-carotene.
Meretrix lusoria	Hard clam	β-carotene, zeaxanthin, lutein.
Octopus vulgaris	Octopus	Tunaxanthin.
O. bimaculatus	Octopus	Xanthophylls.

Table 4. continued .....

Loligo vulgaris	Squid	α-, β-, γ-carotene, α-cryptoxanthin, isocryptoxanthin, isorenieratene, capsanthin, copsorubin, triophaxanthin, zeaxanthin, astataxanthin, 4-hydroxy-α-carotene,4-keto-α-carotene.
Stichopus tremula	Sea cucumber	Echinenone.
Cucumaria lubrica	Sea cucumber	Astaxanthin.
Holothuria leucospilota	Sea cucumber	β-carotene, echinenone, canthaxanthin, astaxanthin.
FISH:		
Auxis thazard	Mackerel	Tunaxanthin, zeaxanthin.
Chrysophrys major	Sea bream	Zeaxanthin, tunaxanthin, astaxanthin,α-carotene, α-doradecin,β-carotene, lutein.
Clupea harengus	Atlantic herring	Astaxanthin, canthaxanthin, lutein.
Clupea pallasii	Pacific herring	Tunaxanthin, zeaxanthin, lutein, α-cryptoxanthin
Engraulis japonicus	Anchovy	Zeaxanthin, cynthiaxanthin, diatoxanthin, lutein.
Evynnis japonica	Red sea bream	Astaxanthin, canthaxanthin, lutein.
O. gorbuscha	Pink salmon	Astaxanthin.
O. keta	Chum salmon	Astaxanthin.
O. kisutch	Coho salmon	Astaxanthin.
O. masou	Cherry salmon	Astaxanthin.
O. nerka	Sockeye salmon	Astaxanthin.
O. tschawytscha	Chinook salmon	Astaxanthin.
Parasilurus asotus	Catfish	Lutein, astaxanthin.
Salmo gairdneri	Rainbow trout	Astaxanthin, β-carotene, canthaxanthin, lutein.
S. trutta	Sea trout	Astaxanthin, canthaxanthin, β-carotene,lutein
S. salar	Atlantic salmon	Astaxanthin, canthaxanthin, zeaxanthin,lutein
Salvelinus fontinalis	Brook trout	Lutein, astaxanthin, canthaxanthin.
Sardinops melanosticta	Sardine	Tunaxanthin, lutein, zeaxanthin.
Theragra chalcogramma	Alaskan pollock	Tunaxanthin, lutein, zeaxanthin.
Thunnus albacares	Yellowfin tuna	Tunaxanthin.

[a]Source: Simpson (1982); Goodwin (1984).

Major carotenoproteins present in the ovaries of marine invertebrates have been well characterized **(Cheesman *et al*, 1967; Ke, 1971; Thommen, 1971; Zagalsky, 1976)**; however, only in a few cases have qualitative and quantitative analyses of the whole carotenoids have been performed **(Zagalsky *et al*, 1967; Campbell, 1970; Gilchrist and Lee, 1972; Griffiths and Perrott, 1976).**

Unlike most fish species, salmonids deposit ingested carotenoids in their muscle tissues. Interest in salmonids flesh coloration has increased and is among the most important quality criteria used by consumers and processing industries. An important quality deficiency concerning farmed salmonids is poor and variable pigmentation **(Bjerkeng, 1992)**. The fish farming industry is working hard to develop strains of fish which have redder flesh and which readily take the dietary pigments. In time, fish farmers will be able to supply quite precise colours as the market requires **(Dore, 1990)**.

The colour and appearance of the skin and meat of cultured and wild fish are significantly different for some species. Colour may be the prime reason for consumer acceptance of some species **(Ostrander and Martinsen, 1976; Scurman *et al*, 1979; Love, 1988)**. The consumer normally expects the sensory characteristics of cultured fish to be similar to those wild fish. Notably, farmed salmonids often have a less red hue when compared to wild salmonids **(Haard, 1992b; Shahidi *et al*, 1992).**

Flesh pigmentation is particularly important in salmonid species that normally accumulate the oxycarotenoid astaxanthin from their natural diet **(Gentle and Haard, 1991)**. Skin colour depends on diverse array of pigments and chromatophore cells. As noted earlier, fish, like other animals, are unable to synthesize carotenoid pigments *de novo* and absorb them from the diet **(Shahidi *et al*, 1992)** and deposit them in the flesh in the unesterified form **(Ingemansson *et al*, 1993)**, where they bind to actomyosin **(Henmi *et al*, 1989)**. **Boyer and Toever (1993)** suggested that one of the primary retail market requirements in selling Arctic charr *(Salvelinus alpinus)* concerns the level of flesh pigmentation. Highly pigmented, red-fleshed fish command a high price.

Carotenoid pigments must be included in the diets of cultured salmonid fish for improved coloration and market acceptability. Because of inadequate dietary carotenoids, the skin appearance of cultured striped jack *(Caranx delicatissimus)* **(Okada *et al*, 1991)** and sea bream *(C. major)* **(Katayama *et al*, 1973)** is inferior to that of the wild fish. **Stroud and Dalgarno (1982)** reported that farmed lobster with adequate carotenoids in their diet are similar in odour, flavour, and texture to wild lobster. **Howell and Mathews (1991)** found that lack of sufficient dietary carotenoids is the cause of blue disease in farmed tiger prawn, affecting both appearance and the stress susceptibility of the live animal.

It is generally accepted that carotenoids are mobilized from the muscle of salmonid females during sexual maturation and accumulate in the ripening eggs

(Steven, 1949; Goodwin, 1950; Crozier, 1970; Kitahara, 1983). However, this assumption is based on observations made on wild fish caught in nature and whose feeding activity was possibly reduced or even interrupted **(Choubert and Blanc, 1989).** For farmed female Atlantic salmon *(S. salar)*, the mobilization of astaxanthin from the flesh to the ovaries was shown to be dependant on the level of astaxanthin in the flesh and also on the availability of dietary astaxanthin **(Torrissen and Torrissen, 1985)** though a large portion of the astaxanthin would be lost **(Torrisen and Naevdal, 1988).** **Torrissen and Torrissen (1985)** observed that the level of astaxanthin in flesh and ovaries decreased significantly during sexual maturation, but the total amount in the ovaries continued to increase. The level of astaxanthin in the plasma was also influenced by time and stage of sexual maturation.

Mobilization of body carotenoids and their deposition in skin and ovaries during maturation of various salmonid species and their quantification has been reported **(Steven, 1949; Crozier, 1970; Sivtseva and Dubrovin, 1981; Kitahara, 1983).** The depletion of flesh pigments has economic consequences because it is one of the factors limiting the acceptability of maturing salmonids for food consumption.

In Atlantic salmon *(S. salar)*, astaxanthin was found as the dominant carotenoid **(Khare *et al*, 1973; Torrissen and Torrissen, 1985)**, but small amounts of canthaxanthin were also identified **(Torrissen and Torrissen, 1985).**

There was no significant difference in the astaxanthin level between males and females, either in the plasma or in the flesh. Mobilization of the flesh astaxanthin was detected by a reduction in the level of astaxanthin in maturing as compared to immature fish **(Torrissen and Torrissen, 1985)**. The level of carotenoids in plasma is probably influenced mainly by the absorption of dietary carotenoids **(Torrissen and Torrissen, 1985)**. The significant difference between immature and maturing fish shows that the plasma level of carotenoids is influenced by the process of maturation **(Torrissen and Torrissen, 1985)**. Smaller fish do not take up pigment readily, accordingly pan-sized salmon are generally much paler than fully grown fish of the same species **(Dore, 1990)**. Astaxanthin and cantaxanthin or a mixture are generally used to enhance the colour of salmonids **(Bjerkeng, 1992)**.

The mobilization of flesh astaxanthin, and transportation and deposition in the ovaries during the period of sexual maturation, supports the hypothesis that carotenoids play a role in reproduction or early life. **Tacon (1981)** argued that, so far, no adequate data have shown a specific function of carotenoids in reproduction: however, **Torrissen (1984)** found a growth-promoting effect of astaxanthin or canthaxanthin supplementation in the start-feeding diet to Atlantic salmon fry.

2.6.1 Pigmentation of fish

The most important aspect of carotenoids for pigmentation of cultured fish species relates to the quantity of pigmenting carotenoids in the feed, their digestibility. the composition of feed and the type of feed processing. The pigmenting effect of a carotenoid is influenced by its own colour and its colour effects in a specific tissue. its chemical structure, absolute configuration, binding form. and solubility **(Latscha, 1991).**

The comparable flesh carotenoid concentration between trout fed either 50 ppm astaxanthin or 100 ppm canthaxanthin confirms that astaxanthin is more efficiently utilized for flesh pigmentation than canthaxanthin **(Foss *et al*, 1984, 1987; Torrissen, 1986, 1989; Bjerkeng *et al*, 1990).** Astaxanthin-pigmented trout had a higher redness intensity at comparable feed carotenoid concentrations **(Skrede and Storebakken, 1986a,b; Skrede *et al*, 1990; No and Storebakken, 1991).**

The amount of carotenoids deposited in the flesh is related to the content of dietary pigments and the size and sex of the fish. After 120 days of feeding, fish fed on diets containing 60 and 90 mg/kg carotenoids and weighing over 215 g possessed good to excellent colouration **(Spinelli and Mahnken, 1978).** Carotenoid concentration in rainbow trout weighing between 100 and 500 g may reach 6 to 7 mg/kg in the flesh. while larger trout may contain as much as 25 mg/kg. Carotenoid uptake is positively influenced by the content of dietary

lipids. Apparent digestibility coefficients for astaxanthin and canthaxanthin increase with increasing dietary lipid levels and this results in higher content of carotenoids in flesh of rainbow trout **(Storebakken and No, 1992).** The carotenoid-coloured extract, containing some 15.5 mg pigment/kg oil, is well utilized by trout and Arctic charr when it is fed either alone or incorporated into a solid diet at a level of 60-90 mg/kg **(Spinelli and Mahnken, 1978; Shahidi *et al*, 1993).**

Generally, Atlantic salmon *(S. salar)* and sea trout *(*anadromous *S. trutta)* utilize dietary carotenoids less efficiently than rainbow trout **(Storebakken *et al*, 1986; Foss *et al*, 1987).** This enables the fish farmer to produce satisfactorily pigmented trout for the market within a relatively short period. **Choubert and Storebakken (1989)** and **Torrissen (1985, 1986, 1989)** reported that the mean retention coefficient for astaxanthin was 1.3 to 1.5 times higher than that for canthaxanthin. However, **Torrissen (1989)** found that a combination of free astaxanthin and canthaxanthin in the diet gave a higher level of total carotenoid in the flesh than either of the two carotenoids alone.

Shahidi *et al* (1993) observed that feeding Arctic char with diets containing 75 mg/kg carotenoids for 15 weeks increased the pigment content in flesh and skin from 1.35 and 10.73 mg/kg wet tissue to 5.56 and 41.21 mg/kg, respectively (Table 5). However, adequate colour impression was achieved after 9 to 12 weeks of feeding on astaxanthin-containing diets. Table 6 summarizes

the deposition of carotenoids from various sources in the flesh of salmonid fish.

The distribution pattern of carotenoids are different at various life-stages. Fry and fingerlings deposit carotenoids mainly in their skin. Post juvenile fish in a rapid phase of growth deposit carotenoids mainly in their flesh. Salmonids undergoing sexual maturation mobilize carotenoids from the flesh and selectively transfer them to the skin and gonads **(Crozier, 1970; Sivtseva and Dubrovin, 1981; Kitahara, 1983).**

Studies on rainbow trout in freshwater **(Choubert and Storebakken, 1989)** and saltwater **(Bjerkeng *et al*, 1990)** have shown that carotenoid concentration in the flesh of immature trout does not increase when the dietary pigment concentrations were increased above 50 mg/kg. The lack of a proportionate response to doses higher than 50 mg/kg was noted for both astaxanthin and canthaxanthin. Carotenoid digestibility was depressed when their dietary concentrations were increased **(Torrissen *et al*, 1990)**. Astaxanthin was found to be deposited unchanged in the free form in the flesh of Atlantic salmon **(Khare *et al*, 1973; Schiedt *et al*, 1981; Storebakken *et al*, 1985)** and Arctic char **(Shahidi *et al*, 1994)**. Besides astaxanthin, yellow xanthophylls were also detected in minor amounts (2-3% of total carotenoids) in the wild salmon **(Schiedt *et al*, 1981)** and Arctic char **(Shahidi *et al*, 1994).**

Haard (1992b) has suggested that carotenoids ingested by an animal may be passed from the animal via feces in the unchanged form, assimilated and

Table 5. Carotenoid content of Arctic charr fillet and skin during a 15-week feeding experiment.[1]

Sampling time	Carotenoid content, mg/kg wet tissue	
	Fillet	Skin
Before pigmentation	1.35 ± 0.06	10.73 ± 0.32
After pigmentation:		
5 weeks	2.55 ± 0.42	14.86 ± 0.18
9 weeks	4.10 ± 0.06	36.01 ± 1.57
12 weeks	4.69 ± 0.31	24.85 ± 2.51
15 weeks	5.56 ± 0.11	41.21 ± 2.27

Adapted from Shahidi *et al* (1993).

Table 6. Deposition of pigment (mg/kg tissue) in rainbow trout, brook trout, coho salmon and Arctic charr flesh fed different source of carotenoids.[1]

Species	Source of carotenoids in the diet	Total muscle carotenoids	Reference
Arctic charr	75 mg/kg astaxanthin	5.65 wb	Shahidi *et al.* 1993
Arctic charr	75 mg/kg canthaxanthin	6.93 wb	Shahidi *et al.* 1993
Brook trout	20% crab waste	1.7 db	Saito & Regier, 1971
Brook trout	20% shrimp waste	14.3 db	Saito & Regier, 1971
Brook trout	control, commercial feed (no shells)	1.4 db	Saito & Regier, 1971
Coho salmon	90 mg/kg carotenoids from red crab waste extracted with soy oil	3.5	Spinelli & Mahnken, 1978
Rainbow trout	100 mg/kg astaxanthin	6-8	Foss *et al.* 1984
Rainbow trout	50 mg/kg canthaxanthin	5-7	Torrissen, 1989
Rainbow trout	100 mg/kg canthaxanthin	3-4	Foss *et al.* 1984
Rainbow trout	25% red crab pulp	13.0 db	Spinelli *et al.* 1974
Rainbow trout	25% shrimp waste	10.3 db	Spinelli *et al.* 1974
Rainbow trout	20% red crab meal	0.06	Kuo *et al.* 1976
Rainbow trout	45 ppm carotenoid extracted from craw-fish waste by soy oil	9.8	Chen *et al.* 1984
Rainbow trout	Commercial feed	6.4 db	Spinelli *et al.* 1974
Rainbow trout	30% shrimp meal	11.6 db	Choubert & Luquet, 1983
Rainbow trout	Commercial feed + 90 mg/kg canthaxanthin	15.0 db	Spinelli *et al.* 1974
Rainbow trout	Commercial feed + 10% red crab shells	11.6 db	Spinelli *et al.* 1974

[1]db, dry weight basis; wb, wet weight basis.

stored as such, assimilated and converted to other storage forms, or assimilated and completely catabolized. In migrating fish, for example, during spawning migration, carotenoid content in the muscle decreases, whereas in the skin and gonads increases. In particular, in the skin, not only the quantity increases, but the quality is also changed **(Kitahara, 1983).** Mobilizing of astaxanthin from muscle, where it is stored in the free form, to the skin, where it is stored in the ester form, occurs via the high density lipoprotein (HDL) in the blood serum **(Nakamura *et al*, 1985).** In trout, the extent to which pigment is stored in the integument and the flesh of fish can differ considerably **(Schiedt *et al*, 1986).** The distribution of red colour in the skin and flesh of trout appears to be independent of the configurational isomers of the dietary astaxanthin **(Schiedt *et al*, 1985).**

CHAPTER 3. MATERIALS AND METHODS

3.1 Fish and rearing conditions

Two-year old Arctic charr *(Salvelinus alpinus* L.) Fraser river strain grown in Daniel's Harbour hatchery in Newfoundland, were used in these studies. Charr, each weighing approximately 150 g, from the same population, were divided into three density groups of 40, 50, and 75 kg/m^3. Each density was stocked into two 2.4 m^3 tanks (i.e., in duplicate) and reared for approximately 24 weeks. Tanks were supplied with fresh water at a temperature of 5.5 to 6.6°C and an exchange rate of approximately 15 l/min. All groups were hand-fed a commercial feed pigmented with 65 mg/kg canthaxanthin (Moore-Clark Co., St. Andrews, NB) (see Table 7 for details). Rations were set at a standard hatchery level of 4.1% of biomass/day (**Fagerlund *et al.* 1981**) and were provided three times a day, six days a week. The amount of feed provided was found to be sufficient since some feed were usually remained in the bottom of tanks when the next portion of meal was served. Feed level calculation was performed separately for each tank. Feed level was adjusted by weighing fish from each tank every three weeks for a total of 21 weeks and water volume was changed in order to maintain the original densities. This approach was adopted because regular removal of individuals could disturb the social relationship within the treatment groups, if adjustments were not made (**Laidley and Leatherland,**

1988; Baardvik and Jobling, 1990).

3.2 Sampling of fish

Fish were sampled every 4-6 weeks from the onset of rearing experiments. Five to six fish from each tank were taken, slaughtered, and immediately frozen and stored at approximately -20°C until used for biochemical analyses.

3.3 Fish performance

3.3.1 Specific growth rate

Specific growth rate (SGR) was calculated according to the formula given below (Jobling, 1985; Wallace *et al*, 1988; Olsen *et al*, 1991; Palsson *et al*, 1992).

$$SGR = \frac{\ln w_1 - \ln w_0}{t_1 - t_0} \times 100$$

where w_0 = initial weight (g), w_1 = final weight (g), t_1-t_0 = duration of experiments starting on day t_0 and ending on day t_1 (days).

3.3.2 Feed conversion ratio

Feed conversion ratio (FCR) was calculated as dry feed intake per unit body weight gain (Papoutsoglou *et al*, 1987; Steffens, 1989).

Table 7. Composition of Arctic charr feed used during the experiments[1].

Feed components	Proportion, %
Moisture[a]	6.1
Crude protein[a]	45.3
Crude lipid[a]	22.6
Ash[a]	8.5
Crude carbohydrate[b]	12.5
Calcium, actual[c]	2.2
Phosphorus, actual[c]	1.5
Sodium, actual[c]	0.3
Vitamin premix, actual[c]	0.5
Mineral premix, actual[c]	0.5
Canthaxanthin pigment[d]	66.87 mg/kg

[1]Ingredients: Fish meal, canola meal, soybean meal, feather meal, whole wheat, fish oil, cane molasses, sugar beet extract. A vitamin premix containing: Retinyl acetate (A), Retinyl palmitate (A), Vitamin D3, dl-Alpha-tocopheryl acetate (E), Calcium D-pantothenate, Riboflavin, Nicotinic acid, Thiamine mononitrate, Pyridoxin hydrochloride (B6), Vitamin B12, D-biotin, Folic acid, Inositol, Menadione dimethyl-pyrimidinol bisulphate (K), Ascorbyl polyphasphate (C). A mineral premix containing: Manganese sulphate; Zinc sulphate; Calcium Iodate; and Betaine. [a]Determined during proximate composition analyses of experimental fish. [b]Calculated by difference. [c]Based on data provided by the feed manufacturer. [d]Determined during carotenoid analyses of experimental fish.

$$FCR = \frac{\text{dry feed intake (g)}}{\text{weight gain (g)}}$$

3.3.3 Protein efficiency ratio

Protein efficiency ratio (PER) was determined as wet weight gain per gram of protein intake **(Papotsouglou *et al*, 1987)**.

$$PER = \frac{\text{wet weight gain (g)}}{\text{protein intake (g)}}$$

3.3.4 Hepatosomatic index (HSI)

The hepatosomatic index of fish was determined by calculating the ratio of the weight of liver (g) to that of the whole fish body on a wet weight basis **(Love, 1992).**

3.4 Biochemical analyses

For determination of proximate composition, three fish from each group were randomly selected, eviscerated, filletted, skinned, and thoroughly homogenized. Sub-samples were taken, at least in duplicate, from the homogenized fillets for further analyses.

3.4.1 Moisture determination

Moisture content was determined by weighing exactly 2 to 3 g of the homogenized sample into a pre-weighed aluminium pan. The sample was then dried in a forced-air convection oven (Fisher Isotemp 300, Fair Lawn, NJ) at 105°C overnight or until a constant weight was obtained **(AOAC, 1990)**. The moisture content was calculated from the weight difference data.

3.4.2 Crude protein determination

The crude protein content of charr fillets was determined by the Kjeldahl method. Approximately 0.2 to 0.3 g of each sample was digested in concentrated H_2SO_4 (Fisher Scientific Co., Fair Lawn, NJ) in the presence of a catalyst (Kjeltab catalyst tablets, Profamo, Dorval, PQ). During digestion, the nitrogen was converted to ammonia in the form of ammonium sulphate. Upon the addition of base (25% NaOH) and distillation (Büchi 321 distillation unit, Büchi Laboratories, Switzerland), the released ammonia was collected into a 4% (w/v) boric acid solution (Fisher Scientific Co., Fair Lawn, NJ) and subsequently titrated with a standard 0.1 N H_2SO_4 solution to a red end point. The nitrogen content was calculated and reported as crude protein content (N % x 6.25) **(AOAC, 1990).**

3.4.3 Total lipid determination

Lipids were extracted into chloroform-methanol-water **(Bligh and Dyer, 1959).** Approximately 25 g of fish tissue were homogenized with 25 ml chloroform (Fisher Scientific Co., Fair Lawn, NJ) and 50 ml methanol (J.T. Baker Chemical Co., Phillipsburg, NJ) for about 2 min using a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON) homogenizer. To the mixture, 25 ml of additional chloroform were added and after blending for 30 s, 25 ml of distilled water were also added followed by blending for another 30 s. The homogenate was then filtered through Whatman No. 1 filter paper (Fisher Scientific Co., Fair Lawn, NJ) on a Büchner funnel with slight suction. The slurry was re-extracted with 35.5 ml of chloroform and filtered as described above. The filtrate was then transferred to a 250 ml separatory funnel, and after complete separation and clarification, the chloroform layer containing total lipids was separated. The volume of the lipid-containing chloroform layer was recorded. As much as 10 ml of the lipid-containing chloroform were transferred into a pre-weighed aluminium pan and then evaporated to dryness. The dried matters were weighed and the content of total extracted lipids were calculated.

3.4.4 Ash determination

Ash content of charr flesh was determined by charring 3 to 5 g of the exactly weighed sample into a pre-weighed crucible over a Bunsen burner and

then heating in a muffle furnace (Blue M Electric Co., Blue Island, IL) at 550°C overnight or until the ash had a white appearance (**AOAC, 1990**). The ash content was calculated from the weight difference data.

3.5 Total and free amino acid determination

3.5.1 Total amino acids

Total amino acids of charr fillets were determined as described by **Shahidi *et al* (1990a)**. Samples were freezed-dried and then hydrolysed for 24 h at 110°C with 6 N HCl (**Blackburn, 1968**). The HCl was removed under vacuum, and dried samples were reconstituted using a lithium citrate buffer at pH 2.2. The hydrolysed amino acids were then determined using a Beckman 121 MB amino acid analyzer (Beckman Instruments, Inc., Palo Alto, CA) (**Shahidi *et al*, 1990a**). Tryptophan was determined separately by hydrolysis of the sample under vacuum in 3M mercaptoethanesulphonic acid at 110°C, as described by **Penke *et al* (1974)**. Cysteine and methionine were determined after performic acid oxidation prior to hydrolysis in 6 N HCl, and were measured as cysteic acid and methionine sulphone, respectively (**Blackburn, 1968**).

3.5.2 Free amino acids

For determination of free amino acids, 10 g of fish flesh were homogenized using a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON)

homogenizer in a 50 ml centrifuge tube with 20 ml of ice-cold 6% perchloric acid for 2 min in an ice bath **(Yamanaka, 1989)**. The homogenized samples were then incubated in ice for 30 min before centrifugation (IEC Centra MP4 centrifuge, International Equipment Co., Needham Heights, MA) at 2000xg for 15 min. The residue was then re-extracted with 20 ml perchloric acid and centrifuged, as described above. The supernatants from the first and second extraction were combined and filtered through a Whatman No.4 filter paper. The pH of the filtrate was adjusted (Accumet pH meter, Model 810, Fisher Scientific Co., Fair Lawn, NJ) to 7.0 using a 20% (w/v) KOH solution and then centrifuged at 2000xg for 10 min to remove precipitates of potassium perchlorate. The supernatant was then acidified to pH 2.2 using a 10 N HCl solution, and diluted to 50 ml with distilled water. One millilitre of the extract was taken into a clean tube and 3.0 ml of lithium citrate buffer (pH 2.2; Beckman Instruments, Inc., Palo Alto, CA) were added to it. Samples were then analyzed on a Beckman 121 MB amino acid analyzer using Benson D-X 8.25 resin and a single column employing three-buffer lithium method as per Beckman 121 MB-TB-017 application notes. Results were calculated and reported as μg/g tissue.

3.6 Lipid fatty acid composition

Fatty acid composition of lipids was determined using gas chromatography (GC) (Hewlett Packard Series II, Type 5890, with an HP-7673 GC/SFC

autosampler and an HP-7673 controller; Hewlett-Packard (Canada) Ltd., Mississauga, ON) according to the procedure described by **Keough and Kariel (1987)**. Thirty to sixty milligrams of lipids were placed into a transmethylation vial and transmethylated overnight in an oven (Thelco, Model 2, Precision Scientific Co., Chicago, IL) at 61.8°C in 6% (v/v) H_2SO_4 in 99.9 mol% methanol containing 15 mg BHA (butylated hydroxyanisole) as an antioxidant. After incubation, 1.0 ml of distilled water was added and then extracted three times with 1.5 ml of pesticide-grade hexane. During the first extraction, a few more crystals of BHA were added. The hexane layer was removed into a clean tube and then washed twice with 1.5 ml of H_2O by vortexing. On the first wash, the H_2O layer was discarded. On the second wash, the hexane layer was transferred into a clean tube. The hexane was then evaporated under N_2 in a fumehood. The dried matters were dissolved in CS_2 prior to GC analysis. Methyl esters were separated on a 30 m HP-Innowax fused silica capillary column with an internal diameter of 0.25 mm and a 0.25 μm film thickness (Hewlet-Packard (Canada) Ltd., Mississauga, ON). The injector and detector temperatures were maintained at 250°C. Column oven temperature was programmed from 220°C to 240°C at 30°C/min with an initial hold of 10.25 min and a final hold of 9 min. Helium was used as carrier gas at a flow rate of 2 ml/min. Fatty acid methyl esters were identified by comparison of their retention times with those of standard (Supelco, Inc., Toronto, ON). Peak areas of identified fatty acids were

used to determine relative content of fatty acids in the sample.

3.7 Pigmentation

3.7.1 Colour measurement

Hunter colour parameters (Hunter L^*, a^*, b^*) of the belly skin of fish, the fillet and the homogenized tissues were measured colorimetrically using a Colormet colorimeter (Instrumar Engineering Ltd., St. John's, NF) as described by **Shahidi *et al* (1992b).** The unit was standardized with a B-143 white calibration tile having a Hunter L^* value of 94.5±0.2, a^* value of −1.0±0.1, and b^* value of 0.0±0.2.

3.7.2 Pigment determination

Determination of carotenoid pigments, both total and individual, was performed for the flesh, skin, gonads, and the liver of fish as detailed below.

3.7.2.1 Total pigment

a. Flesh pigment

The method employed for determination of total flesh pigments was that of **Saito and Regier (1971).** Approximately 10 g of the pre-homogenized fish tissues were extracted three times with 50 ml of acetone (BDH Inc., Toronto, ON) for 2 min. The homogenized sample was then filtered through a Whatman

No.4 filter paper. The filtrate was transferred into a 50 ml tube and centrifuged (IEC Centra MP4 centrifuge, International Equipment Co., Needham Heights, MA) for 5 min at 4000xg. Carotenoid pigments in acetone were then transferred to 40 ml petroleum ether (Fisher Scientific Co., Fair Lawn, NJ) in a 250 ml separatory funnel. To maximize the transfer of carotenoids, 100 ml of distilled water containing approximately 0.5% sodium chloride were added to the mixture. The petroleum ether layer was then separated and transferred into a 50 ml volumetric flask, and made up to volume. The absorption spectrum was recorded at 400-600 nm using a diode array spectrophotometer (Hewlett Packard, Model 8452A, Hewlett-Packard (Canada) Ltd., Mississauga, ON). Total carotenoids in samples were calculated according to the equation given below **(Simpson *et al*, 1981)**, using an extinction coefficient $E^{1\%}_{1cm}$ of 2200 for canthaxanthin in petroleum ether as provided by **Bauernfeind (1981)**.

$$C\ (\mu g/g) = \frac{A_{466nm} \times V_{extract}}{E^{1\%}_{1cm} \times W_{sample}}$$

where C = total carotenoid concentration; A = absorbance at 466 nm; V = volume of the extract (ml); E = extinction coefficient of 1% standard canthaxanthin in petroleum ether in 1 cm cell; and W = weight of tissue extracted (g wet weight).

b. Skin pigment

The carotenoid pigments in fish skin were extracted into chloroform-methanol-water **(Bligh and Dyer, 1959)**. All skin, except the head skin, including all fins, were cut into small pieces using a pair of scissors before extraction was performed. Approximately 10 g of the cut skin were extracted with 25 ml chloroform and 50 ml methanol for about 2 min using a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON) homogenizer at 16,000 rpm. To the mixture, 25 ml of additional chloroform were added and after blending for about 1 min, 15 ml of distilled water were added followed by blending for another 30 s. The homogenate was then filtered through a Whatman No.1 filter paper on a Buchner funnel with slight suction. The slurry was washed with 35 ml of chloroform and filterred as described above. The filtrate was transferred to a 250 ml separatory funnel, and after complete phase separation, the chloroform layer was removed and the solvent evaporated under vacuum. The carotenoids were then dissolved in 50 ml of petroleum ether. The absorption spectrum of the pigment was recorded at 400-600 nm. The concentration of carotenoids in the skin was calculated using the equation used for quantitation of flesh carotenoids.

c. Gonad pigment

Pigments in gonads were determined using the same protocols employed for determination of flesh carotenoids. Although deposition of pigments in gonads might be affected by stocking density, gonads of fish from all density groups were pooled since they were too small to allow determination on density-group bases. In addition, pigments in gonads are not as important as those in flesh as far as consumer's acceptance is concerned. Therefore, determination was aimed primarily to identify the type and proportion of carotenoids present in the gonads.

d. Liver pigment

Carotenoids in liver were determined using the same protocol as that described for fish flesh. Although the weight of liver collected from each group allowed for determination on density-group basis, analysis was performed for only one group since pigments in the liver were not as important as those in flesh with respect to the sensory performance of the fish. As for the gonads, determination of carotenoid pigments in liver was also aimed to identify the type and proportion of carotenoids present.

3.7.2.2 Individual pigments

Individual pigments were separated on an aluminium oxide (J.T. Baker Chemical Co., Phillipsburg, NJ) column (15 cm x 1.0 cm i.d.). For a better separation of 4'-hydroxyechinenone, it was necessary to use a silica gel G (Selecto Inc., Kennesaw, GA) column (15 cm x 1.0 cm i.d.). The pigment extract for total pigment determination was evaporated under vacuum (Büchi 461 Water Bath), and then applied to the column. A mixture of acetone and hexane was used for elution; the proportions of acetone in hexane were 0, 4, 10, 15, 20, 30, and 50% (v/v). Finally, an acetone-methanol-hexane (1:1:8, v/v/v) solvent system was used to elute the yellow non-carotenoid fraction. Each fraction was collected and the solvent evaporated under nitrogen to dryness. The dried carotenoids were then dissolved in a known volume of petroleum ether, the absorbance of which at 400-600 nm was recorded. The type of carotenoid in each fraction was identified according to its absorption maximum, and its concentration was determined using the above equation. To confirm the type of carotenoids present, the R_f values on thin layer chromatographic (TLC) plates of separated pigments were compared to those of standard carotenoids (Hoffman-La-Roche, Etobicoke, ON) and also the absorption maxima as given by **Bauernfeind (1981)**. The carotenoid extract was spotted on a silica gel G TLC plate (Uniplate, Analtech Inc., Newark, DE) and then developed in a benzene-petroleum ether-acetone (10:3:2, v/v/v) solvent system. Each fraction band was

then compared with the band of the standard pigment.

3.8 Statistical analysis

For statistical analysis, mean values of experimental data were subjected to analysis of variance (One-way ANOVA) using SigmaPlot Scientific Graphing Software version 5.0 **(Jandel Corporation, 1986-1994)**. For comparison between treatments, when necessary, unpaired-student's t-test was employed. Significance was considered at 5% level of probability. Linear regression analysis was used to examine the relationship between different parameters.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Fish performance

4.1.1.1 Growth

After 24 weeks, the weight of fish reached 623.43 ± 96.64 g, 563.13 ± 123.83 g, and 514.31 ± 58.47 g for densities of 40, 50, and 75 kg/m^3, respectively (Table 8) from an initial weight of 154.52 ± 29.49 g. ANOVA results found no significant ($p>0.05$) differences existed in mean weights of fish among density groups. Although no statistical differences found, it was clear that there was a remarkable difference in the final weights of fish, particularly between densities of 40 and 75 kg/m^3 (over 100 g difference) (Fig. 1). Gonad maturation (0.3-0.5 g each) was noticeable after 8 weeks of feeding. At the end of the experiments (24 weeks), the weight of gonads did not exceed 2.4 g. Therefore, none of the experimental fish would spawn following the feeding trials.

Specific growth rate (SGR) of fish, in general, decreased as the size of fish increased (Table 8). At the end of the experiment, the SGR decreased from 1.25 ± 0.01 to 0.76 ± 0.02, 1.10 ± 0.04 to 0.70 ± 0.05, and 1.21 ± 0.22 to 0.66 ± 0.02 for densities of 40, 50 and 75 kg/m^3, respectively. Despite the fact that the final mean weights among stocking densities were not significantly ($p>0.05$)

Table 8. Weight gain, SGR, FCR, PER, and HSI of Arctic charr reared at different stocking densities over a 24-week feeding period.

Feeding period, weeks	Stocking density, kg/m^3		
	40	50	75
Weight gain[1]:			
5	242.25 ± 46.74^{ax}	229.42 ± 22.96^{ax}	239.62 ± 37.07^{ax}
11	371.43 ± 52.26^{ax}	357.99 ± 59.87^{bx}	335.71 ± 25.98^{bx}
16	503.03 ± 52.53^{bx}	477.71 ± 39.46^{cx}	450.24 ± 54.42^{cx}
24	623.43 ± 96.64^{bx}	563.13 ± 123.83^{cx}	514.31 ± 58.47^{cx}
Initial	154.52 ± 29.49		
SGR[1]:			
5	1.25 ± 0.01^{ax}	0.91 ± 0.16^{ay}	1.21 ± 0.22^{axy}
11	1.11 ± 0.09^{bx}	1.06 ± 0.10^{ax}	0.98 ± 0.04^{ax}
16	0.99 ± 0.04^{cx}	0.94 ± 0.06^{bxy}	0.89 ± 0.00^{by}
24	0.76 ± 0.02^{dx}	0.70 ± 0.05^{cxy}	0.66 ± 0.02^{cy}
FCR[1]:			
5	2.45 ± 0.08^{ax}	4.10 ± 1.05^{ay}	4.94 ± 1.08^{ay}
11	1.77 ± 0.25^{bx}	1.92 ± 0.65^{by}	3.23 ± 0.23^{bz}
16	1.25 ± 0.05^{cx}	1.53 ± 0.15^{by}	2.16 ± 0.04^{cz}
24	1.05 ± 0.02^{dx}	1.40 ± 0.20^{by}	2.05 ± 0.16^{cz}
PER[1]:			
5	1.03 ± 0.03^{ax}	0.51 ± 0.10^{ay}	0.54 ± 0.12^{ay}
11	1.47 ± 0.21^{bx}	1.15 ± 0.15^{bcx}	0.79 ± 0.06^{ay}
16	2.08 ± 0.08^{cx}	1.68 ± 0.16^{cy}	1.18 ± 0.03^{bcz}
24	2.31 ± 0.07^{dx}	1.86 ± 0.27^{cy}	1.25 ± 0.10^{byz}
HSI[2,3]:			
5	1.22 ± 0.18^{ax}	1.24 ± 0.44^{ax}	1.14 ± 0.20^{ax}
11	1.68 ± 0.19^{bx}	1.50 ± 0.18^{ax}	1.31 ± 0.15^{ay}
16	1.59 ± 0.16^{bx}	1.68 ± 0.17^{ax}	1.45 ± 0.11^{ax}
24	1.43 ± 0.29^{abx}	1.42 ± 0.16^{ax}	1.37 ± 0.31^{ax}

[1,2]Results are mean values of 26-30 and 10 determinations ± standard deviation, respectively. [3]Values are multiplied by 10^2. Values in each row with the same superscript (x,y,z) are not significantly different ($p>0.05$) from one another. Values in each column for each parameter with the same superscript (a,b,c,d) are not significantly different ($p>0.05$) from one another. SGR, specific growth rate (% of body weight/day); FCR, feed conversion ratio; PER, protein efficiency ratio; HSI, hepatosomatic index.

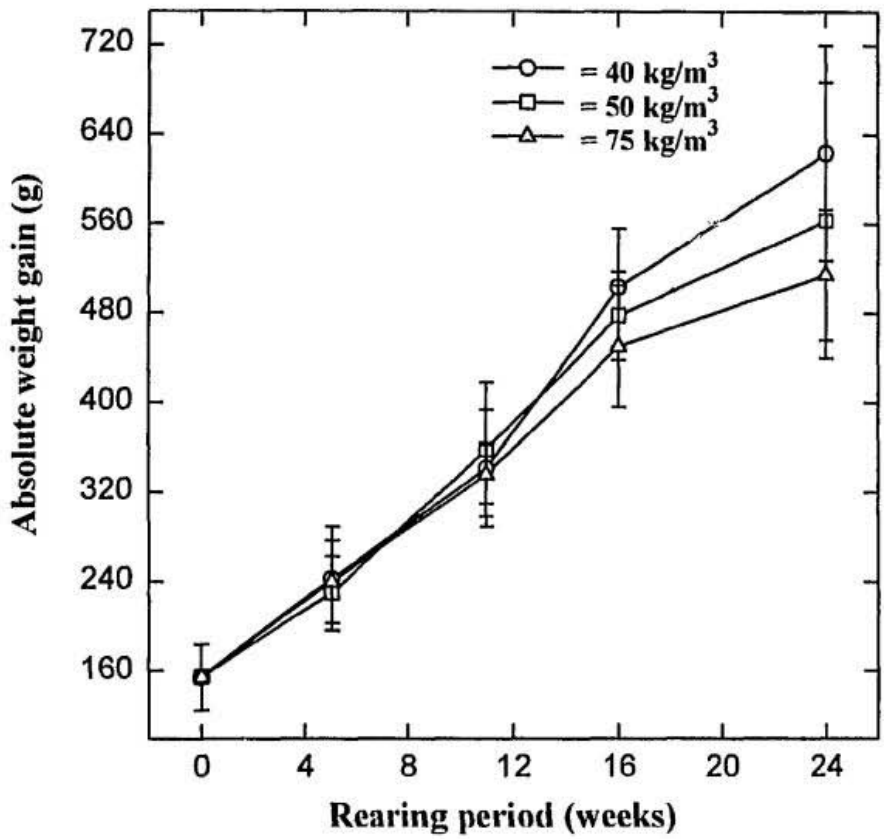


Fig. 1. Absolute weight gain of Arctic charr reared at different stocking densities over a 24-week period. n = 26-30 samples per period. Vertical bars = standard deviation.

different, their SGR were otherwise ($p<0.05$), especially between fish at densities of 40 and 75 kg/m^3 (Fig 2A). Linear regression analysis also revealed a fairly good inverse relationship between stocking density and the SGR of fish ($r = -0.9522$).

4.1.1.2 Feed Conversion Ratio

The feed conversion ratio (FCR) of fish from all density groups is given in Table 8. At the beginning of the experiment, fish at densities of 50 and 75 kg/m^3 showed a very poor FCR as compared to fish at a density of 40 kg/m^3 (4.01 ± 1.05 and 4.94 ± 1.08 compared to 2.49 ± 0.08). At the end of the experiment, the best FCR value was attained by the fish at a density of 40 kg/m^3 (1.05 ± 0.02), followed by the fish at densities of 50 (1.40 ± 0.20) and 75 kg/m^3 (2.05 ± 0.16). Results of statistical analysis (t-test) indicated significant ($p<0.05$) differences in FCR values among density groups, except between densities of 40 and 50 kg/m^3. Figure 2B shows that FCR improved significantly during the feeding period. A very strong inverse relationship also existed between density and FCR values ($r = -0.9696$).

4.1.1.3 Protein Efficiency Ratio

Protein efficiency ratio (PER) of the experimental fish is presented in Table 8. The PER values of fish increased throughout the experimental period

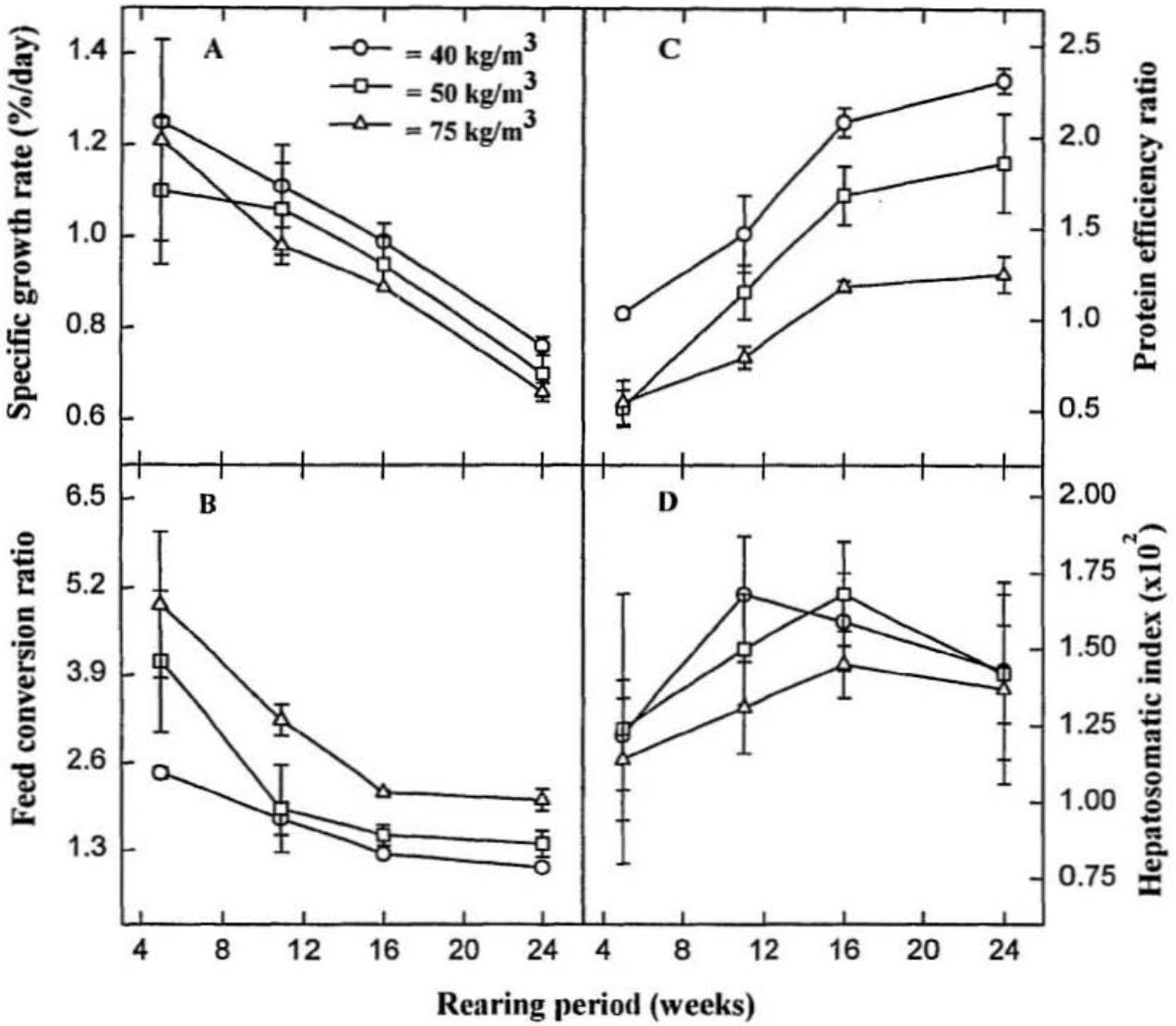


Fig. 2. Specific growth rate (A), feed conversion ratio (B), protein efficiency ratio (C), and hepatosomatic index (D) of Arctic charr reared at different stocking densities over a 24-week period. n = 26-30 for A,B,C and 10 determinations per period for D, respectively. Vertical bars = standard deviation.

(Fig. 2C). As in the case of FCR, fish at a density of 40 kg/m^3 attained the best PER (2.31 ± 0.07), followed by the fish at densities of 50 (1.86 ± 0.27) and 70 kg/m^3 (1.25 ± 0.10). Statistical analysis indicated that significant ($p<0.05$) differences in PER values were present among density groups, except between densities of 40 and 50 kg/m^3. Regression analysis also showed that there was a strong inverse relationship between density and PER values ($r = -0.9886$).

4.1.1.4 Hepatosomatic Index

It appears that neither fish size nor stocking density has any effect on hepatosomatic index (HSI) of fish. Table 8 shows that although some variations existed within groups, especially for fish at a density of 40 kg/m^3, the HSI values at the end of the experiment were not different ($p>0.05$) as compared to that of other sampling dates. For fish at densities of 50 and 75 kg/m^3, their HSI values were relatively similar throughout the experimental period and no significant ($p>0.05$) differences existed for HSI of fish (Fig. 2D). Regression analysis also indicated a moderate relationship between HSI and stocking density ($r = -0.9059$).

4.1.2 Proximate composition

4.1.2.1 Moisture

Moisture content of fish flesh from all density groups decreased from an

initial level of 73.00 ± 1.11% to a lowest level of 67.40 ± 1.00% on week-16 (Table 9). Although moisture of fish at densities of 40 and 50 kg/m^3 increased slightly after 11 weeks, the amount was not significantly ($p>0.05$) different after 5 weeks of feeding experiments. At the end of the experiment (week-24), the moisture content of fish at densities of 40 and 75 kg/m^3 increased significantly ($p<0.05$) as compared to week-16, whereas that of fish at a density of 50 kg/m^3 did not ($p>0.05$). Nonetheless, changes in moisture contents of fish flesh for all density groups followed a similar pattern (Fig. 3A). Significant ($p<0.05$) differences in moisture contents of fish at different stocking densities, especially during the early stages (week-5) and at the end of the experiment were observed. On week-11 and week-16, however, no significant ($p>0.05$) differences were observed. The correlation coefficient between the two parameters was r = 0.9940.

4.1.2.2 Crude Protein

Crude protein contents of flesh of experimental fish are given in Table 9. The crude protein content of fish increased significantly ($p<0.05$) from an initial amount of 15.57 ± 0.24% at the beginning of the experiment to a maximum level of 19.02 ± 1.48% at the end of the study. The fish at densities of 50 and 75 kg/m^3 attained a maximum protein content on week-16 (19.42 ± 0.66% and 20.22 ± 1.28%, respectively), and then experienced a slight decrease at the end of the

Table 9. Proximate composition (%) of flesh of Arctic charr reared at different stocking densities.

Feeding period, weeks	Moisture[1]	Protein[2]	Lipid[1]	Ash[2]
	Stocking density of 40 kg/m^3			
5	69.18 ± 1.35^{abx}	16.55 ± 0.90^{ax}	8.74 ± 0.92^{ax}	1.12 ± 0.03^{ax}
11	70.17 ± 1.68^{ax}	18.76 ± 0.95^{bx}	9.81 ± 1.00^{bx}	1.11 ± 0.01^{bcx}
16	67.40 ± 1.00^{cx}	18.91 ± 0.52^{bx}	12.11 ± 0.25^{cx}	1.09 ± 0.01^{bx}
24	68.99 ± 0.31^{bx}	19.02 ± 1.48^{bx}	11.03 ± 0.70^{dx}	1.12 ± 0.00^{ax}
	Stocking density of 50 kg/m^3			
5	69.63 ± 1.56^{acx}	17.52 ± 0.71^{ay}	8.16 ± 0.66^{ay}	1.18 ± 0.06^{ay}
11	70.16 ± 1.06^{ax}	19.17 ± 1.58^{bx}	8.75 ± 0.09^{by}	1.10 ± 0.00^{bx}
16	67.83 ± 1.26^{bdx}	19.42 ± 0.66^{bx}	11.31 ± 0.13^{cx}	1.13 ± 0.03^{by}
24	68.87 ± 0.81^{cdx}	18.71 ± 0.75^{bx}	12.38 ± 0.88^{dy}	1.14 ± 0.02^{by}
	Stocking density of 75 kg/m^3			
5	71.45 ± 0.63^{ay}	17.40 ± 0.87^{ay}	8.49 ± 0.33^{ayz}	1.20 ± 0.05^{ay}
11	70.99 ± 0.74^{ax}	18.44 ± 1.03^{ax}	8.67 ± 0.39^{ayz}	1.12 ± 0.03^{bx}
16	67.61 ± 1.29^{bx}	20.22 ± 1.28^{by}	11.22 ± 0.18^{bx}	1.09 ± 0.02^{bx}
24	70.73 ± 1.15^{ay}	18.79 ± 2.36^{abx}	9.61 ± 1.17^{cz}	1.17 ± 0.06^{ay}
Initial	73.00 ± 1.11	15.57 ± 0.24	5.39 ± 0.23	1.27 ± 0.01

[1,2]Results are mean values of 18 and 12 replicates ± standard deviation. Values in each column for each density group with the same superscript (a,b,c,d) are not significantly different ($p>0.05$) from one another. Values in each column for the same feeding period and with the same superscript (x,y,z) are not significantly different ($p>0.05$) from one another.

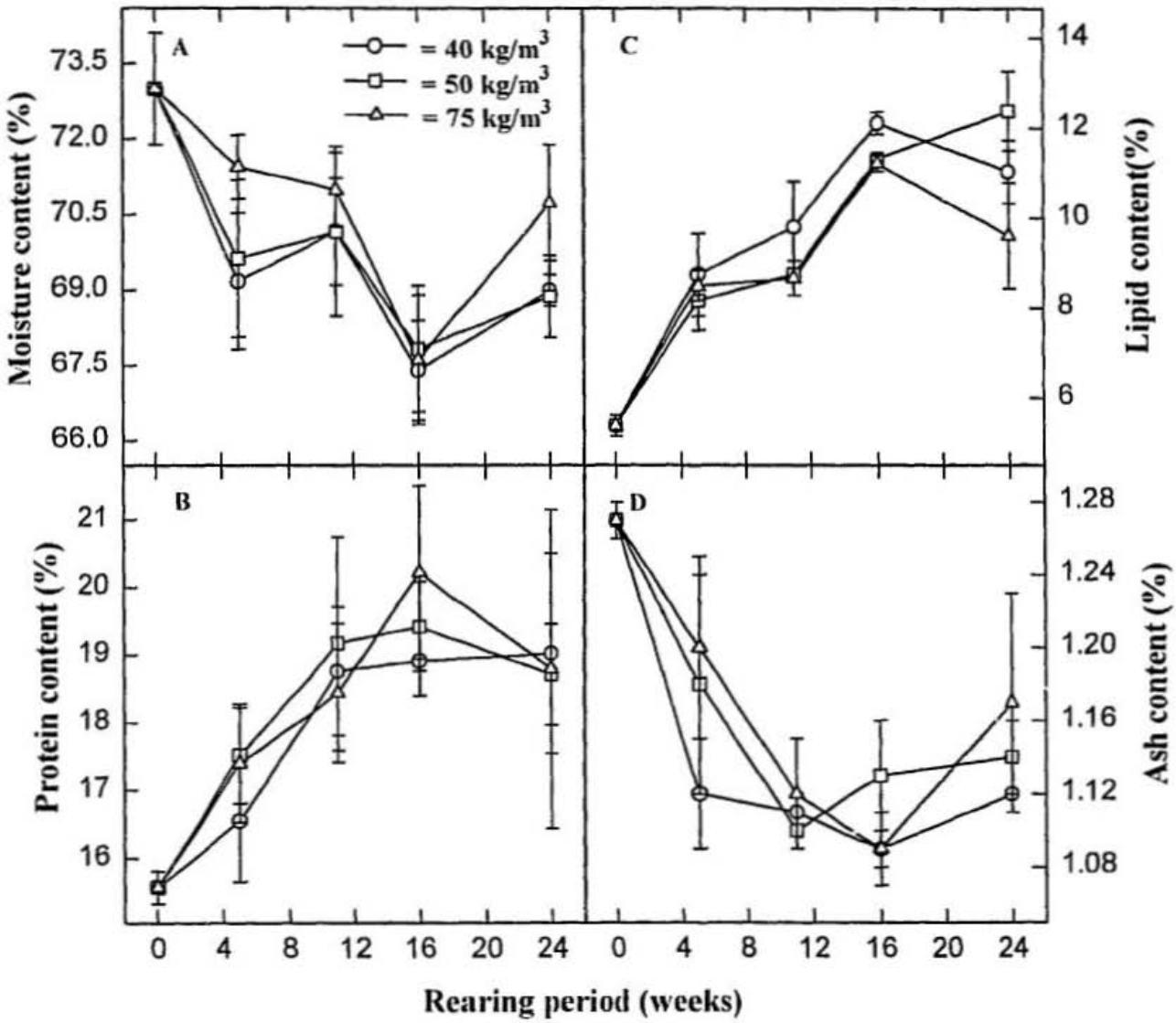


Fig. 3. Moisture (A), crude protein (B), total lipid (C), and ash (D) contents of Arctic charr reared at different stocking densities over a 24-week period. n for A and C = 18; and B and D = 12 determinations per period. Vertical bars = standard deviations.

experiment, but not to any significant ($p>0.05$) extent. On the other hand, the protein content of fish at a density of 40 kg/m^3 continued to increase throughout the experimental period (Fig. 3B). However, this increase was not significantly ($p>0.05$) different from that on week-16. At the end of the experiment, protein contents of fish flesh were 19.02 ± 1.48%, 18.71 ± 0.75%, and 18.76 ± 2.36% (wet weight basis), for densities of 40, 50 and 75 kg/m^3, respectively. Statistical analysis showed that there were no significant ($p>0.05$) differences among the mean protein contents of fish at the end of the experiment. Furthermore, regression analysis suggested that no relationship ($r = -0.5394$) existed between protein content and stocking densities.

4.1.2.3 Total Lipid

The total lipid contents of fish flesh increased significantly ($p<0.05$) during the course of the experiment from an initial amount of 5.39 ± 0.23% to a maximum of 12.38 ± 0.88% (Table 9). Within density groups, significant ($p<0.05$) variations were observed for different sampling dates. The lipid content of fish at densities of 40 and 75 kg/m^3 reached maxima on week-16 (12.11 ± 0.25% and 11.22 ± 0.18%, respectively), whereas that for fish at a density of 50 kg/m^3 continued to increase (to 12.38 ± 0.88%). At the end of the experiment, the total lipid contents of fish at densities of 40 and 75 kg/m^3 decreased significantly ($p<0.05$) as compared to that on week-16, while that of fish at a

density of 50 kg/m^3 increased significantly ($p<0.05$) (Fig. 3C). Lipid contents of fish among different densities also varied significantly from the beginning to the end of the experiment. It is evident from regression analysis that stocking density and lipid content were not well correlated ($r = -0.7030$). This suggests that stocking density has little, if any, effect on the total lipid content of fish.

4.1.2.4 Ash

The ash content of fish flesh from all groups is presented in Table 9. The initial ash content was 1.27 ± 0.01%. Within groups, it appeared that there was a slight, but significant ($p<0.05$), decrease in ash content from the beginning to the end of the experiment. Fish at a density of 75 kg/m^3 showed a significant ($p<0.05$) progressive decrease in their ash content ($p<0.05$) until week-16 (1.09 ± 0.02% from 1.20 ± 0.05% on week-5), and then increased significantly ($p<0.05$) at the end of the experiment (to 1.17 ± 0.06%). On the other hand, fish at a density of 50 kg/m^3 exhibited a significant ($p<0.05$) decrease in their ash content up to week-11 (to 1.10 ± 0.00% from 1.18 ± 0.06% on week-5), and then increased, but not to any significant ($p>0.05$) extent, at the end of the experiment.

Statistical analysis indicated some variations in ash content of fish among density groups for the same sampling dates, except for week-11 when fish from all density groups had a similar ash content (Fig. 3D). At the end of the

experiment, only fish at densities of 40 and 75 kg/m^3 showed a significant ($p<0.05$) difference. Regression analysis, however, revealed a very strong positive relationship between stocking density and ash content of fish ($r = 0.9994$).

4.1.3 Total and free amino acids

4.1.3.1 Total amino acids

Total amino acid compositions of flesh of reared Arctic charr prior to and after feeding on pigmented diets for 24 weeks are presented in Table 10. The amino acid contents of fish varied according to stocking density. Of the amino acids determined, only cysteine, methionine and histidine had a similar concentration ($p>0.05$) before and after the feeding experiments. However, small, but significant ($p<0.05$) differences existed between experimental values for some amino acids. The most abundant amino acids were aspartic acid, glutamic acid, leucine and lysine, whereas the least abundant was hydroxyproline (Figs. 4-7). Within density groups, amino acid composition of fish flesh also varied significantly ($p<0.05$) according to sampling dates. For fish at a density of 40 kg/m^3, only hydroxyproline, cysteine and tyrosine had a similar concentration ($p>0.05$), whereas fish at densities of 50 and 75 kg/m^3 had a similar cysteine content at different sampling dates. The content of some of the amino acids of fish from all density groups decreased significantly ($p<0.05$) on

Table 10. The contents of total amino acid (mg/g protein) of flesh of Arctic charr reared at different stocking densities at the end of the experiment.[1]

Amino acid	Fish prior to the experiments	Density, kg/m^3		
		40	50	75
Alanine	65.04 ± 0.26^{ac}	67.11 ± 0.18^{b}	64.60 ± 0.78^{c}	69.75 ± 0.53^{d}
Arginine	60.82 ± 0.27^{a}	63.16 ± 0.35^{b}	68.53 ± 0.67^{c}	66.03 ± 0.16^{d}
Aspartic acid	90.35 ± 0.31^{a}	82.85 ± 0.50^{b}	85.98 ± 0.40^{c}	82.63 ± 0.36^{b}
Cysteine	22.48 ± 0.18^{a}	21.53 ± 2.95^{a}	23.98 ± 3.61^{a}	22.36 ± 2.97^{a}
Glutamic acid	105.92 ± 0.50^{a}	124.12 ± 3.00^{b}	103.69 ± 1.20^{a}	132.88 ± 1.43^{c}
Glycine	54.96 ± 0.18^{a}	58.68 ± 0.11^{b}	56.19 ± 0.27^{c}	50.54 ± 0.52^{d}
Histidine	31.87 ± 0.15^{a}	30.02 ± 0.11^{a}	30.36 ± 1.96^{a}	30.19 ± 0.17^{a}
Hydroxyproline	1.39 ± 0.10^{a}	1.01 ± 0.05^{b}	1.31 ± 0.10^{a}	1.24 ± 0.12^{a}
Isoleucine	58.07 ± 0.17^{a}	50.70 ± 0.27^{b}	55.92 ± 0.33^{c}	52.06 ± 0.18^{d}
Leucine	84.89 ± 0.24^{a}	80.38 ± 0.40^{b}	87.94 ± 0.63^{c}	82.74 ± 0.36^{d}
Lysine	105.05 ± 0.41^{a}	101.77 ± 0.28^{b}	105.94 ± 0.42^{a}	103.11 ± 0.22^{c}
Methionine	42.16 ± 0.29^{a}	41.50 ± 0.30^{a}	41.81 ± 0.26^{a}	43.05 ± 0.83^{a}
Phenylalanine	45.95 ± 0.28^{a}	47.00 ± 0.11^{b}	45.81 ± 0.58^{a}	48.99 ± 0.24^{c}
Proline	42.09 ± 0.14^{a}	37.32 ± 0.10^{b}	40.80 ± 0.95^{c}	38.53 ± 0.23^{d}
Serine	35.96 ± 0.14^{a}	42.34 ± 0.15^{b}	36.23 ± 0.38^{a}	34.18 ± 0.49^{c}
Threonine	47.76 ± 0.12^{a}	45.84 ± 0.22^{b}	47.73 ± 0.46^{a}	44.14 ± 0.66^{c}
Tryptophan	14.28 ± 0.15^{a}	12.33 ± 0.14^{b}	13.68 ± 0.15^{c}	12.79 ± 0.10^{b}
Tyrosine	34.81 ± 0.23^{a}	39.98 ± 0.18^{b}	33.06 ± 1.16^{ac}	31.76 ± 0.13^{c}
Valine	67.10 ± 0.20^{a}	59.98 ± 0.40^{b}	66.58 ± 0.87^{a}	61.52 ± 0.30^{b}

[1]Results are mean values of triplicate determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

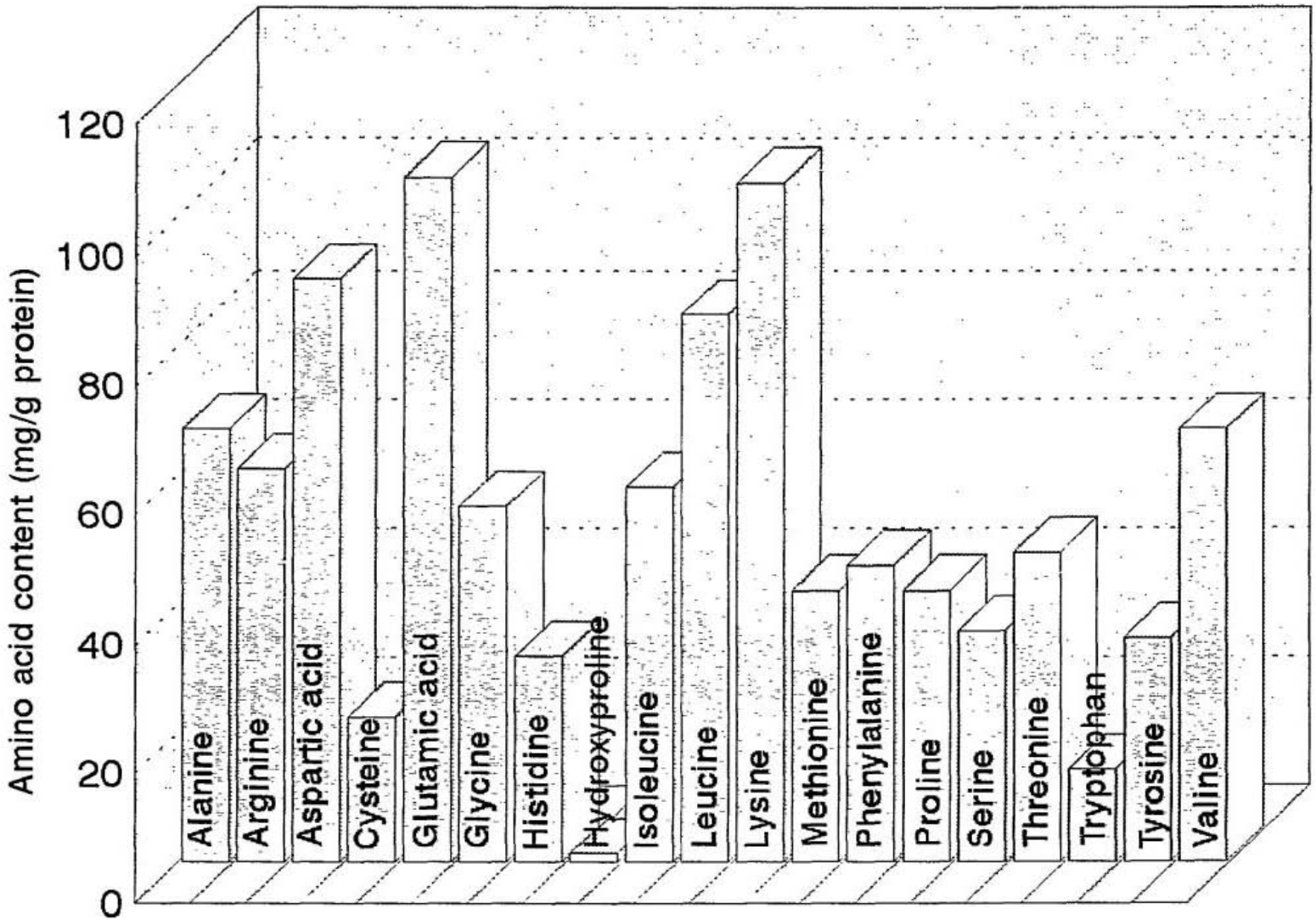


Fig. 4. The contents of total amino acids (mg/g protein) of flesh of Arctic charr prior to the experiments. n = 3 determinations.

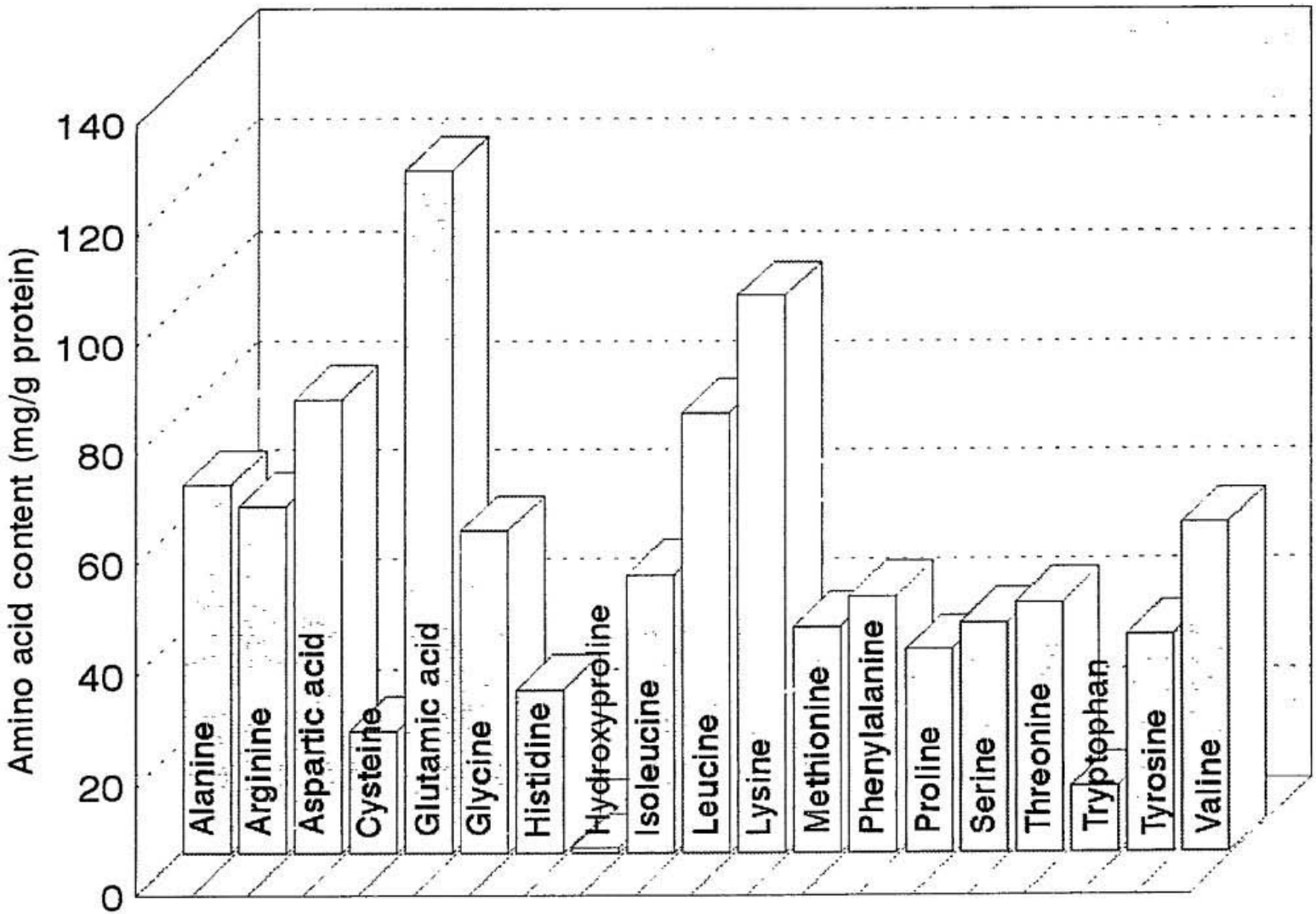


Fig. 5. The contents of total amino acids (mg/g protein) of flesh of Arctic charr reared at a density of 40 kg/m^3 at the end of the experiments. n= 3 determinations.

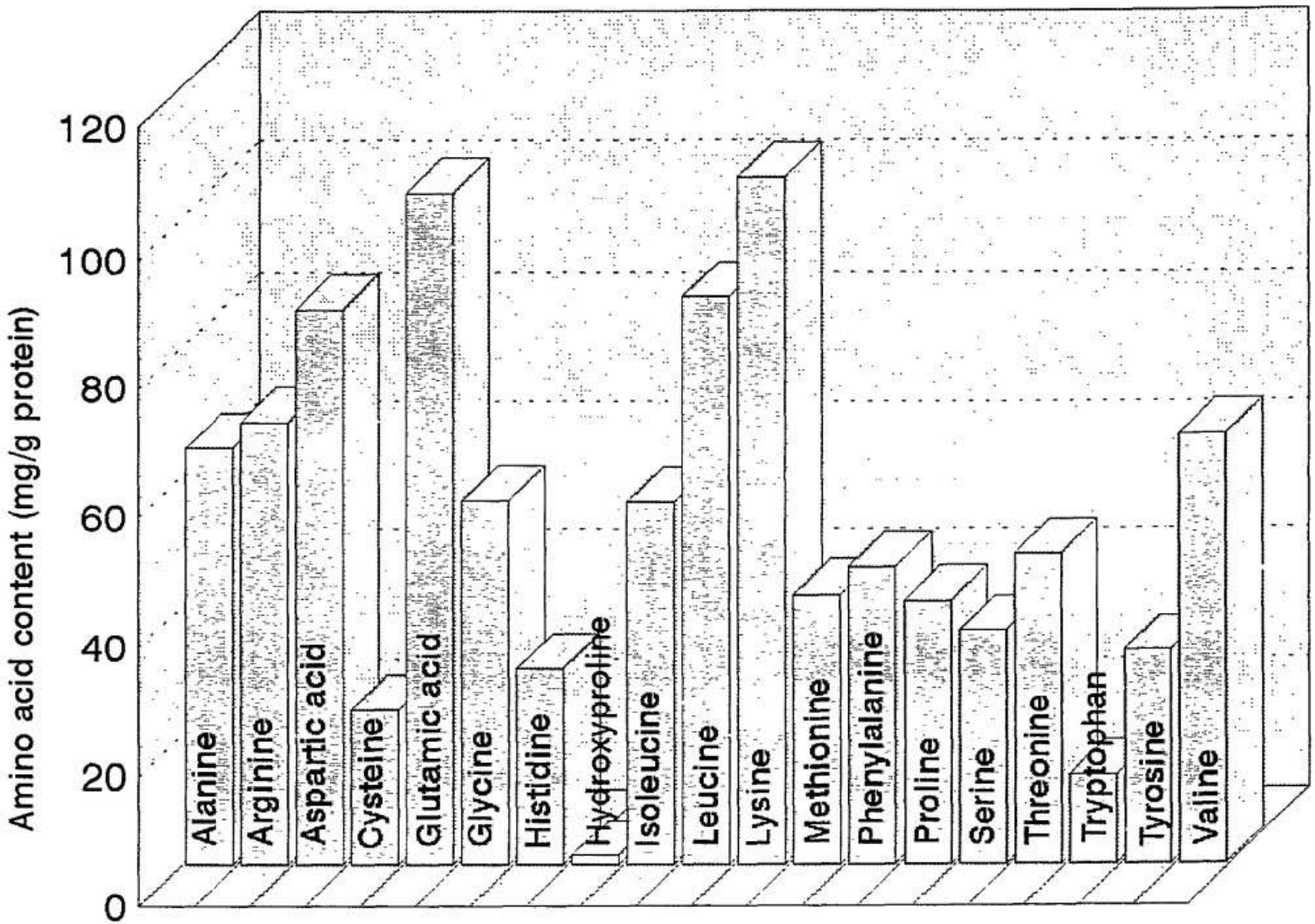


Fig. 6. The contents of total amino acids (mg/g protein) of flesh of Arctic charr reared at a density of 50 kg/m^3 at the end of the experiments. n = 3 determinations.

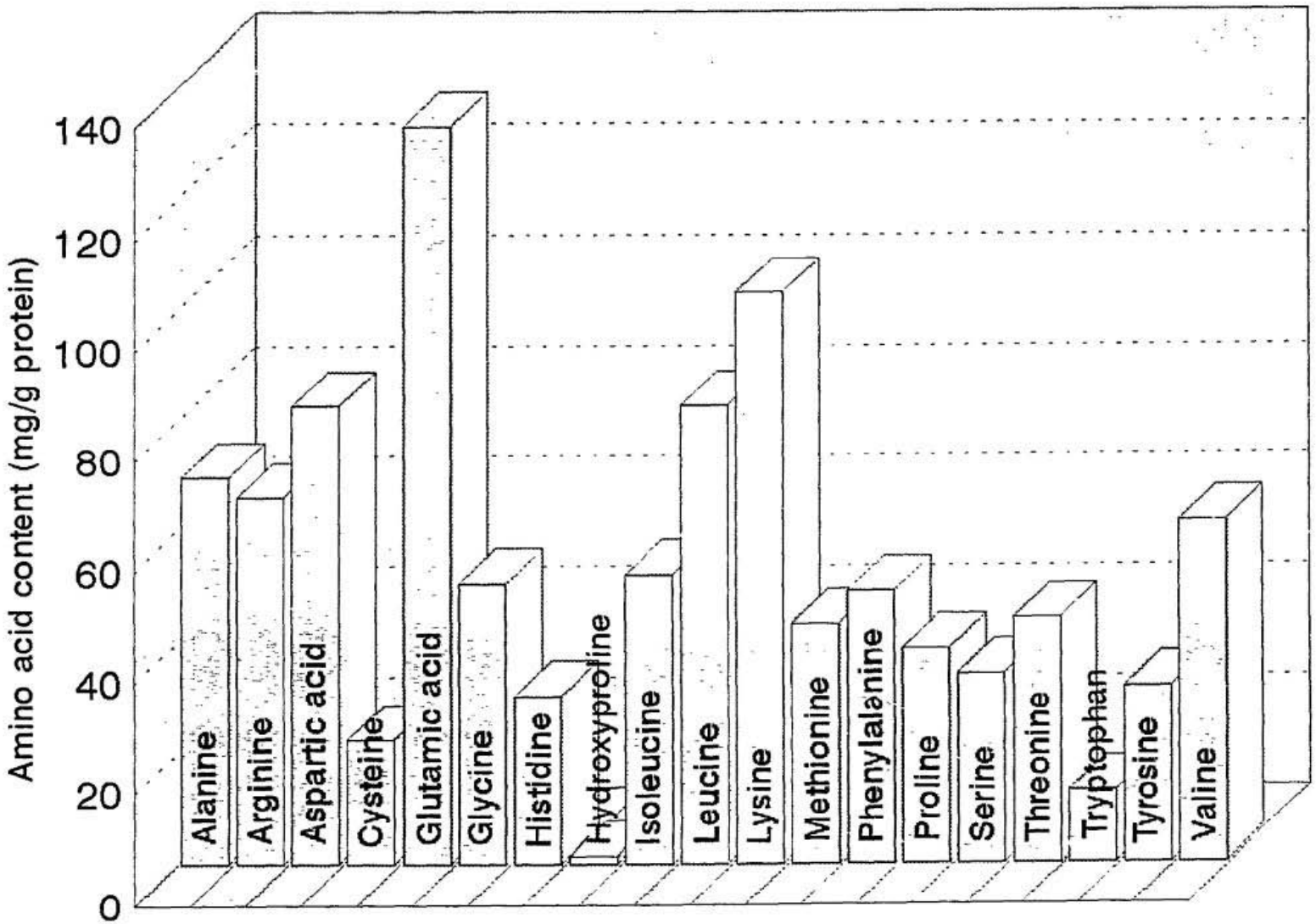


Fig. 7. The contents of total amino acids (mg/g protein) of flesh of Arctic charr reared at a density of 75 kg/m^3 at the end of the experiments. n = 3 determinations.

week-16 as compared to those on week-5, but then increased significantly ($p<0.05$) at the end of the experiment. However, the relative amount of some other amino acids increased but then decreased significantly ($p<0.05$) at the end of the experiments (see Appendix 1-3 for details).

4.1.3.2 Free amino acids

Free amino acid (FAA) content (μg/g wet weight) of muscle tissues of reared Arctic charr is shown in Table 11. The total amount of FAA was 7861.29 ± 389.68, 7952.58 ± 489.03, and 6470.75 ± 309.14 μg/g for fish at densities of 40, 50 and 75 kg/m^3, respectively. These levels were much higher than those of feed and of fish flesh prior to the start of the experiments (Table 12). Statistical analysis (t-test) showed that total FAA contents of fish at densities of 40 and 50 kg/m^3 were not significantly ($p>0.05$) different from one another, however, both were significantly ($p<0.05$) higher than that of fish at a density of 75 kg/m^3 (Fig. 8). Regression analysis also indicated a fairly good inverse relationship between stocking density and FAA contents of fish ($r = -0.9441$). Individual free amino acids also varied significantly among density groups (see Appendix 4-6).

4.1.3.3 Lipid fatty acid composition

The fatty acid compositions of feed and fish flesh prior to the start of the experiments and that of fish during the experiments are given in Table 13. The

Table 11. Total free amino acid contents (μg/g) of flesh of Arctic charr reared at different stocking densities at the end of the experiments.[1]

Free amino acid	Density, kg/m^3		
	40	50	75
Alanine	525.80 ± 72.79^a	549.40 ± 91.54^a	450.36 ± 10.46^b
Anserine	2671.08 ± 347.93^a	1868.40 ± 168.03^b	1946.63 ± 287.90^b
Arginine	201.44 ± 19.54^a	287.82 ± 73.28^b	178.65 ± 14.52^a
Aspartic acid	212.52 ± 28.23^a	244.93 ± 48.99^a	169.33 ± 15.40^b
Carnosine	0^a	5.47 ± 5.47^b	4.25 ± 4.25^b
Cystine	28.32 ± 4.66^a	31.71 ± 1.48^a	31.71 ± 3.68^a
Glutamic acid	327.67 ± 8.55^a	438.35 ± 106.80^b	301.68 ± 8.23^c
Glutamine	119.87 ± 10.21^a	174.66 ± 53.77^b	103.16 ± 6.52^c
Glycine	666.33 ± 92.17^a	831.00 ± 209.36^a	665.12 ± 79.81^a
Histidine	276.16 ± 51.46^a	258.54 ± 2.17^a	230.81 ± 48.77^a
Hydroxyproline	30.93 ± 17.64^{ab}	31.01 ± 1.93^b	27.09 ± 3.13^c
Isoleucine	135.37 ± 7.77^a	174.63 ± 41.98^a	114.51 ± 10.09^b
Leucine	260.69 ± 0.78^a	343.93 ± 95.50^a	210.67 ± 14.40^b
Lysine	355.55 ± 8.23^a	430.71 ± 119.72^a	297.95 ± 39.79^b
Methionine	114.11 ± 1.93^a	143.32 ± 8.39^a	92.19 ± 4.31^b
Phenylalanine	167.42 ± 9.29^a	198.00 ± 42.69^a	138.85 ± 2.21^b
Proline	161.41 ± 3.76^a	192.36 ± 30.82^b	170.53 ± 3.78^b
Serine	225.49 ± 6.32^a	281.55 ± 71.09^a	180.53 ± 24.38^b
Taurine	796.32 ± 42.47^a	770.52 ± 134.96^{ab}	668.67 ± 52.55^b
Threonine	210.92 ± 15.71^a	254.99 ± 61.84^a	187.19 ± 16.84^b
Tryptophan	23.40 ± 2.67^a	31.81 ± 9.71^a	16.16 ± 4.32^b
Tyrosine	125.11 ± 9.21^a	143.96 ± 32.61^a	100.33 ± 1.05^b
Valine	225.41 ± 6.43^a	265.50 ± 62.42^a	184.43 ± 8.45^b
Total FAA	7861.29 ± 389.68^a	7952.58 ± 489.03^a	6470.75 ± 309.14^b

[1]Results are mean values of 4 determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Table 12. Total free amino acid contents (μg/g) of Feed and Arctic charr flesh prior to the experiments.[1]

Free amino acid	Feed	Fish prior to the experiments
Alanine	124.64 ± 6.53	142.28 ± 4.71
Anserine	25.72 ± 4.13	496.93 ± 12.39
Arginine	83.03 ± 5.60	53.82 ± 2.34
Aspartic acid	50.46 ± 9.65	54.08 ± 3.16
Carnosine	0	14.35 ± 1.65
Cystine	39.04 ± 2.87	21.24 ± 2.76
Glutamic acid	100.17 ± 4.60	109.25 ± 3.11
Glutamine	7.78 ± 0.86	32.45 ± 1.87
Glycine	42.20 ± 2.54	295.36 ± 6.63
Histidine	302.99 ± 16.34	65.56 ± 6.10
Hydroxyproline	1.83 ± 0.16	23.54 ± 1.32
Isoleucine	30.25 ± 2.73	35.58 ± 3.08
Leucine	55.89 ± 3.32	67.64 ± 1.99
Lysine	53.60 ± 5.43	74.51 ± 4.63
Methionine	16.83 ± 1.46	33.49 ± 2.58
Phenylalanine	27.20 ± 3.43	42.16 ± 3.64
Proline	33.74 ± 3.62	54.86 ± 2.25
Serine	21.49 ± 3.54	91.01 ± 4.67
Taurine	415.21 ± 14.60	259.45 ± 8.44
Threonine	35.83 ± 4.27	61.03 ± 2.47
Tryptophan	12.91 ± 0.86	7.16 ± 0.44
Tyrosine	16.75 ± 1.84	36.37 ± 0.52
Valine	51.38 ± 3.27	53.94 ± 3.25
Total FAA	1548.91 ± 51.63	2126.05 ± 43.78

[1]Results are mean values of triplicate determinations ± standard deviation.

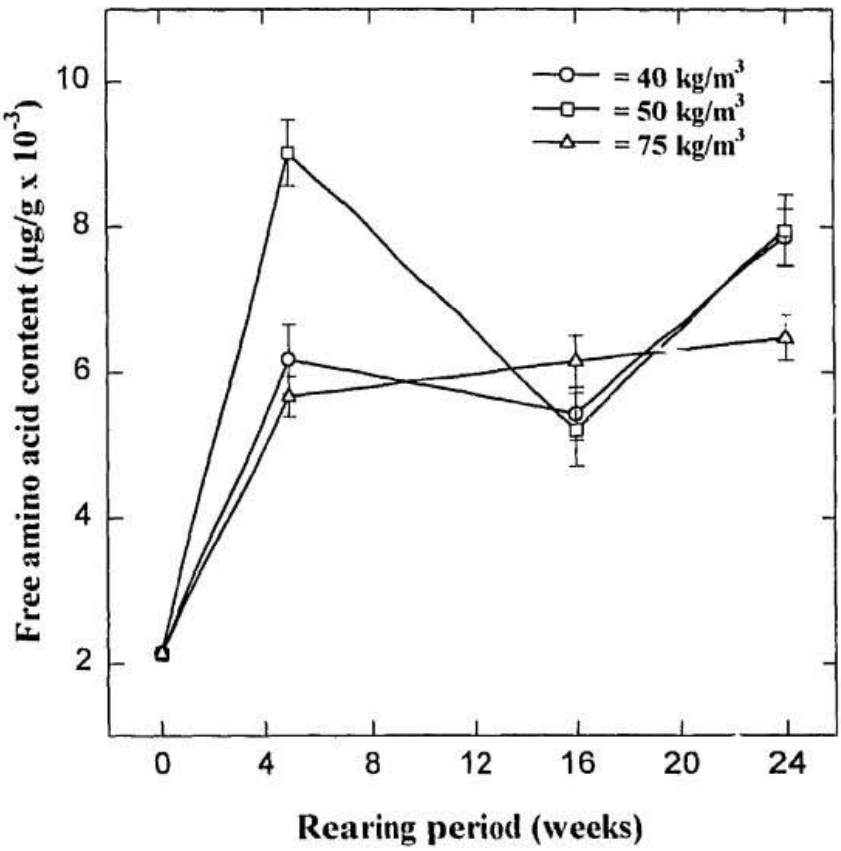


Fig. 8. Total free amino acid contents (µg/g tissue) of flesh of Arctic charr fed on a canthaxanthin-pigmented diet over a 24-week period. n = 4 determinations per period. Vertical bars = standard deviations.

Table 13. Fatty acid composition (%) of total lipid of feed, fish prior to and after 24 weeks of feeding.

Fatty acid	Feed	Fish prior to the experiments	Density, kg/m³		
			40	50	75
14:0	8.07 ± 0.01[a]	3.76 ± 0.40[b]	5.75 ± 0.13[c]	5.56 ± 0.01[c]	5.51 ± 0.04[c]
16:0	22.58 ± 0.04[a]	14.87 ± 0.30[b]	15.91 ± 0.38[c]	15.65 ± 0.01[c]	15.27 ± 0.02[d]
18:0	4.25 ± 0.04[a]	4.25 ± 0.27[a]	3.49 ± 0.02[b]	3.57 ± 0.15[b]	3.46 ± 0.05[b]
20:0	0.34 ± 0.00[a]	-	0.57 ± 0.01[b]	0.58 ± 0.01[b]	0.56 ± 0.01[b]
Σ SFA	**35.23 ± 0.01[a]**	**22.88 ± 0.01[b]**	**25.72 ± 0.53[c]**	**25.35 ± 0.17[c]**	**24.80 ± 0.08[c]**
14:1ω-5	0.29 ± 0.00[a]	-	0.27 ± 0.01[a]	0.26 ± 0.00[a]	0.26 ± 0.01[a]
16:1ω-7	10.93 ± 0.03[a]	7.34 ± 0.34[b]	12.50 ± 0.23[c]	11.94 ± 0.03[d]	11.77 ± 0.07[d]
18:1ω-9	12.38 ± 0.01[a]	19.48 ± 0.35[b]	18.10 ± 0.41[c]	17.42 ± 0.12[d]	16.20 ± 0.06[e]
18:1ω-7	3.43 ± 0.00[a]	-	3.86 ± 0.09[b]	3.95 ± 0.03[b]	3.73 ± 0.03[c]
20:1ω-9	2.76 ± 0.01[a]	4.56 ± 0.25[b]	3.72 ± 0.15[c]	3.79 ± 0.08[c]	3.68 ± 0.07[c]
22:1ω-11	1.81 ± 0.02[a]	2.80 ± 0.28[b]	2.00 ± 0.55[ab]	2.65 ± 0.06[b]	2.69 ± 0.06[b]
24:1ω-9	0.43 ± 0.01[a]	-	0.32 ± 0.01[a]	0.34 ± 0.01[a]	0.39 ± 0.01[a]
ΣMUFA	**32.02 ± 0.02[a]**	**34.17 ± 0.22[b]**	**40.76 ± 0.33[c]**	**40.34 ± 0.06[c]**	**38.71 ± 0.16[d]**
18:2ω-6	4.04 ± 0.02[a]	4.23 ± 0.11[a]	4.59 ± 0.08[b]	4.64 ± 0.01[b]	5.36 ± 0.58[c]
18:3ω-3	1.41 ± 0.01[a]	0.95 ± 0.15[b]	2.30 ± 0.00[c]	2.34 ± 0.00[c]	2.41 ± 0.02[c]
18:4ω-3	2.49 ± 0.01[a]	1.08 ± 0.15[b]	1.70 ± 0.04[c]	1.61 ± 0.01[c]	1.84 ± 0.03[c]
20:2ω-6	0.18 ± 0.01[a]	-	0.28 ± 0.00[a]	0.32 ± 0.01[a]	0.29 ± 0.01[a]
20:3ω-6	0.26 ± 0.00[a]	-	0.34 ± 0.00[a]	0.36 ± 0.01[a]	0.35 ± 0.00[a]
20:4ω-6	1.17 ± 0.01[a]	1.46 ± 0.13[b]	1.07 ± 0.02[c]	1.12 ± 0.02[c]	1.18 ± 0.02[a]
20:5ω-3	12.10 ± 0.01[a]	10.56 ± 0.53[b]	9.62 ± 0.29[c]	9.34 ± 0.05[c]	10.39 ± 0.37[b]
22:5ω-3	2.46 ± 0.02[a]	2.82 ± 0.12[b]	2.43 ± 0.07[a]	2.43 ± 0.03[a]	2.62 ± 0.06[c]
22:6ω-3	8.66 ± 0.00[a]	21.88 ± 0.51[b]	11.19 ± 0.49[c]	12.15 ± 0.04[d]	12.67 ± 0.32[d]
Σ PUFA	**32.76 ± 0.01[a]**	**42.96 ± 0.22[b]**	**33.50 ± 0.82[ac]**	**34.34 ± 0.06[c]**	**37.11 ± 0.53[d]**
U/S	**1.84 ± 0.00[a]**	**3.37 ± 0.00[b]**	**2.90 ± 0.09[c]**	**2.95 ± 0.03[c]**	**3.06 ± 0.04[c]**
Σω-6	**5.65 ± 0.02[a]**	**5.69 ± 0.08[a]**	**6.27 ± 0.06[b]**	**6.43 ± 0.01[c]**	**7.16 ± 0.56[d]**
Σω-3	**27.11 ± 0.01[a]**	**37.28 ± 0.15[b]**	**27.23 ± 0.88[a]**	**27.92 ± 0.06[a]**	**29.93 ± 0.06[c]**
ω-6/ω-3	**0.21 ± 0.00[a]**	**0.15 ± 0.00[b]**	**0.23 ± 0.01[a]**	**0.23 ± 0.00[a]**	**0.24 ± 0.02[a]**

[1]Results are mean values of triplicate determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another. SFA, MUFA and PUFA are saturated, monounsaturated and polyunsaturated fatty acids, respectively. U/S, ratio of unsaturated to saturated fatty acids.

amount of saturated fat in fish, both before (22.88 ± 0.01%) and after 24 weeks of rearing (25.72 ± 0.53%, 25.35 ± 0.17% and 24.80 ± 0.08%, respectively) for fish at densities of 40, 50 and 75 kg/m^3, respectively, were significantly ($p<0.05$) lower than that of the feed (35.23 ± 0.01%). In contrast, the contents of mono- and polyunsaturated fatty acids were significantly ($p<0.05$) higher in Arctic charr flesh as compared to that of the feed. Unsaturated fatty acids constituted up to 74.23 to 75.82% of the total lipids in the experimental fish, 77.13% in fish before the start of the experiment and 64.78% in the feed. After 24 weeks of rearing period, the content of monounsaturated fatty acids (MUFA) in fish flesh increased significantly ($p<0.05$) from an initial amount of 34.17 ± 0.22% to a maximum of 40.76 ± 0.33% at the end of the experiments. On the other hand, polyunsaturated fatty acids (PUFA) decreased significantly ($p<0.05$) from an initial amount of 42.96 ± 0.22% to a minimum of 33.50 ± 0.82%. There was also a tendency for the content of MUFA to decrease with increasing stocking density, whereas that of PUFA increased with increasing stocking density (Fig. 9 and 10).

The ratio of the unsaturated to saturated (U/S) fatty acids increased with increasing stocking density. The U/S values were 2.90 ± 0.09, 2.95 ± 0.03 and 3.06 ± 0.04 for fish at densities of 40, 50 and 75 kg/m^3, respectively. However, these values were slightly, but significantly ($p<0.05$), lower than that of fish before the experiment (3.37 ± 0.00), and significantly ($p<0.05$) higher as

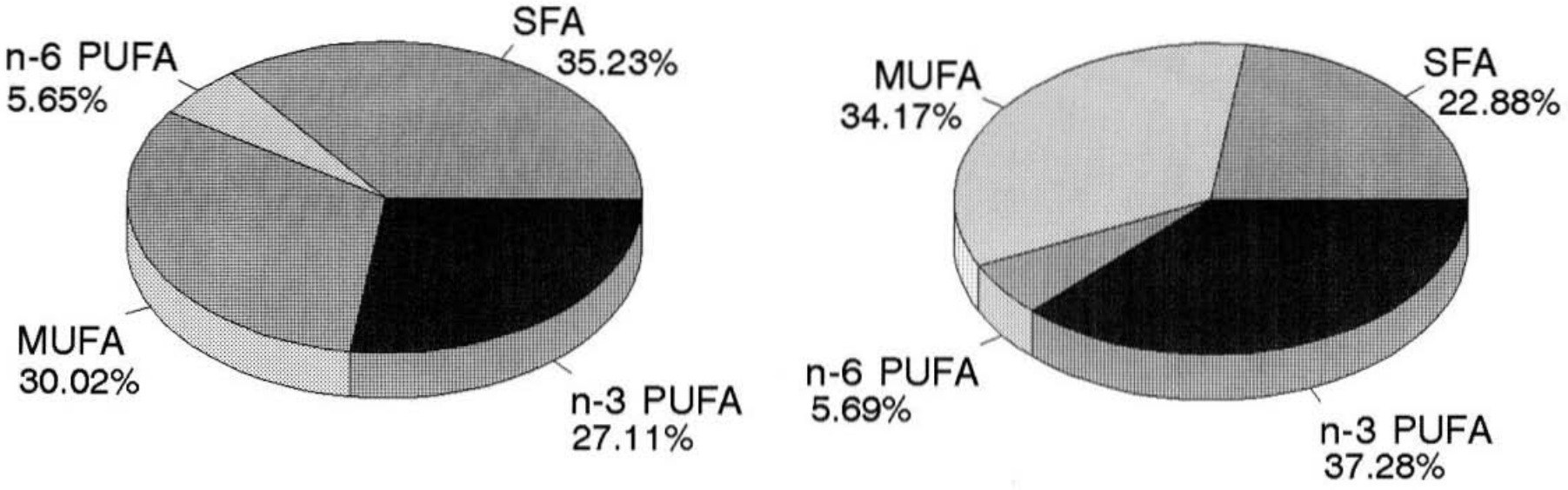
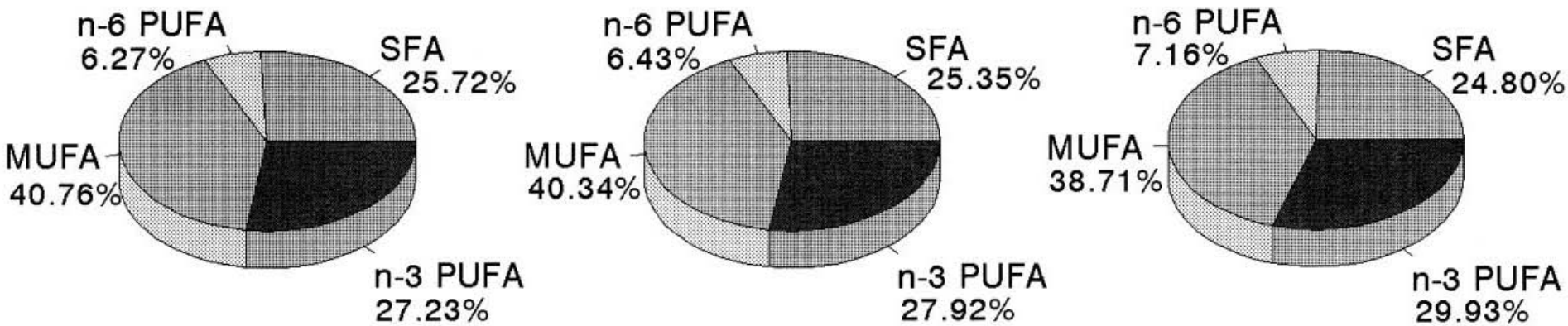


Fig. 9. Fatty acid composition (%) of total lipids of feed and Arctic charr prior to the experiments. n = 3 determinations.



40 kg/m^3 50 kg/m^3 75 kg/m^3

Fig. 10. Fatty acid composition (%) of total lipids of Arctic charr reared at different stocking densities at the end of the experiments. n = 3 determinations.

compared to that of the feed (1.84 ± 0.00). The ratio of ω-6 to ω-3 fatty acids of experimental fish was similar to that of the feed, but both were significantly ($p<0.05$) higher than that of fish at the beginning of the experiment. The omega-6 PUFA accounted for up to 7.16 ± 0.56% of total lipids, whereas ω-3 PUFA accounted for up to 29.93 ± 0.06% of it. Both ω-6 and ω-3 PUFA increased with increasing stocking density. In feed and fish flesh prior to the experiments, ω-6 fatty acids constituted 5.65 ± 0.02% and 5.69 ± 0.08%, respectively, of the total lipids, whereas ω-3 fatty acids accounted for 27.11 ± 0.01% and 37.28 ± 0.15%, respectively. Within density groups, there was a slight change in the proportion of fatty acids in fish flesh among sampling dates, especially for PUFA. However, their total saturated and MUFA, however remained relatively constant. The amount of PUFA decreased from 36.01 ± 0.45% to 33.50 ± 0.82% and from 36.49 ± 0.25% to 34.34 ± 0.06% in flesh of fish at densities of 40 and 50 kg/m^3, respectively. On the other hand, fish at a density of 75 kg/m^3 showed an increase in their PUFA from 33.98 ± 1.37% on week-16 to 37.11 ± 0.53% at the end of the experiment. The ratio of unsaturated to saturated lipids also decreased for fish at densities of 40 and 50 kg/m^3, whereas that of fish at 75 kg/m^3 increased (see Appendices 7-9 for details).

Results of regression analysis further indicated that saturated and monounsaturated fatty acids correlated inversely with stocking density ($r = -0.9914$ and $r = -0.9963$, respectively). On the other hand, polyunsaturated, ω-6

and ω-3 fatty acids were directly correlated with stocking density ($r = 0.9984$, $r = 0.9938$, and $r = 0.9995$ for polyunsaturated, ω-6 and ω-3 fatty acids, respectively). A similar relationship was observed between U/S ratio and stocking density ($r = 0.9995$).

4.1.4 Pigmentation

4.1.4.1 Colour parameters

The Hunter L^*, a^*, b^* values of belly skin, fillet and homogenized tissues of reared Arctic charr are given in Table 14. The Hunter L^*, a^*, b^* values varied significantly ($p<0.05$) among density groups. For belly skin of fish, these colour parameters decreased marginally, but significantly ($p<0.05$) with increasing stocking density. The fillet and homogenized tissue of fish at a density of 75 kg/m^3 were found to have the highest Hunter L^* value, followed by fish at densities of 40 and 50 kg/m^3, respectively. However, fish at a density of 50 kg/m^3 exhibited the highest Hunter a^* and b^* values of their fillet and homogenized tissue, followed by fish at densities of 40 and 75 kg/m^3, respectively (see Appendix 10, 11, and 12 for more details).

Regression analysis demonstrated that there was no relationship between the Hunter L^*, a^*, b^* values of fish belly and the concentration of carotenoids in the fish skin ($r = -0.2450$, -0.1429, -0.2039 for L^*; $r = 0.2092$, -0.6738, -0.8504 for a^*; $r = 0.8386$, 0.4820, and -0.1479 for b^* for fish at densities of 40,

Table 14. The Hunter L^*, a^*, b^* values of Arctic charr reared at different stocking densities at the end of the experiments.[1]

Hunter value	Stocking density, kg/m^3		
	40	50	75
L^*: B	79.44 ± 0.14^a	78.66 ± 0.94^b	77.70 ± 0.60^c
F	48.52 ± 1.66^a	45.65 ± 2.64^b	48.73 ± 2.21^a
H	69.34 ± 0.47^a	65.76 ± 3.34^b	71.55 ± 0.66^c
a^*: B	-1.26 ± 0.07^a	-1.29 ± 0.40^{ab}	-1.36 ± 0.01^b
F	14.51 ± 1.23^a	15.08 ± 0.16^a	12.40 ± 1.13^b
H	5.78 ± 0.03^a	6.55 ± 0.68^b	4.58 ± 0.40^c
b^*: B	6.02 ± 3.22^a	5.97 ± 1.19^a	4.46 ± 0.58^b
F	21.78 ± 1.64^a	23.17 ± 0.50^a	20.87 ± 1.05^a
H	18.77 ± 0.43^a	19.33 ± 0.73^a	17.02 ± 0.63^b

[1]Results are mean values of 30 determinations ± standard deviation. B, belly; F, fillet; H, homogenized tissues. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

50 and 75 kg/m^3, respectively). In contrast, fillets and homogenized tissues of fish from all density groups showed an inverse correlation between their Hunter L^* values and the content of flesh carotenoids ($r = -0.9844$, -0.9480 and -0.9245 for fish at densities of 40, 50 and 75 kg/m^3, respectively). Their a^* and b^* values, however, exhibited a direct correlation with their carotenoid contents ($r = 0.9514$, 0.9824, 0.9040 for a^*, and $r = 0.9924$, 0.9527 and 0.9558 for b^* for fish at densities of 40, 50 and 75 kg/m^3, respectively) (Fig. 11-13).

4.1.4.2 Total carotenoid pigments

The content of total carotenoids of fish flesh over the 24 weeks of experimentation is given in Table 15. The carotenoid levels in fish flesh increased significantly ($p<0.05$) from an initial amount of 0.47 ± 0.10 mg/kg to 4.80 ± 0.99, 6.08 ± 1.18, and 3.95 ± 0.39 mg/kg for fish at densities of 40, 50 and 75 kg/m^3, respectively, at the end of the experiments. On week-16, fish at densities of 40 and 75 kg/m^3 attained maximum contents of carotenoids of 5.04 ± 0.70 and 4.60 ± 0.88 mg/kg, respectively, whereas that of fish at a density of 50 kg/m^3 continued to increase throughout the experimental period. At the end of the experiment, the pigment content of fish at 40 kg/m^3 stocking density decreased slightly, but not to any significant ($p>0.05$) extent, while that of fish at a density of 75 kg/m^3 exhibited a significant ($p<0.05$) decrease as compared to that on week-16 (Fig. 14A). On week-8 and 16, fish from all density groups

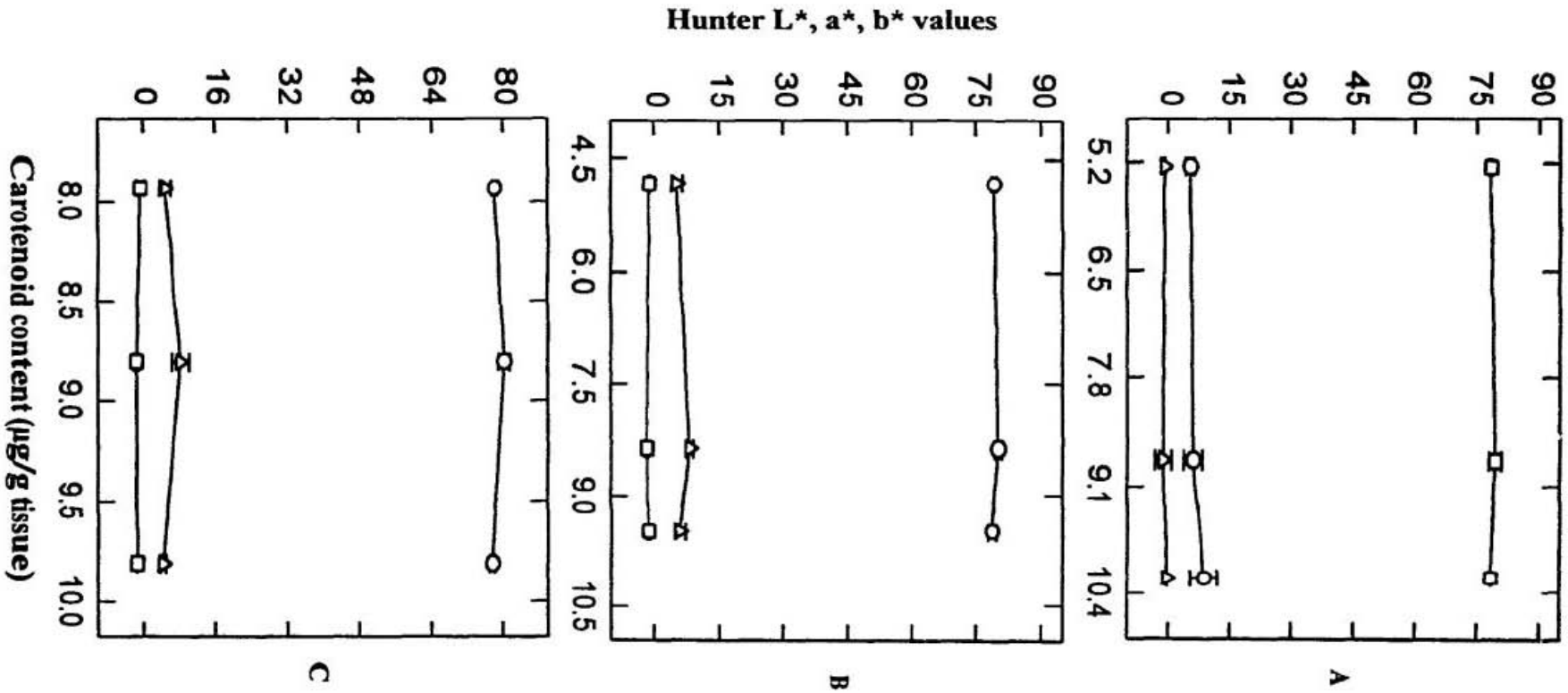


Fig. 11. Hunter L*,a*, b* values of belly skin of Arctic charr at different carotenoid concentrations. (A= 40 kg/m^3; B = 50 kg/m^3; C = 75 kg/m^3; L* = ; a* = ; b* =). n = 30 determinations per period. Vertical bars = standard deviations.

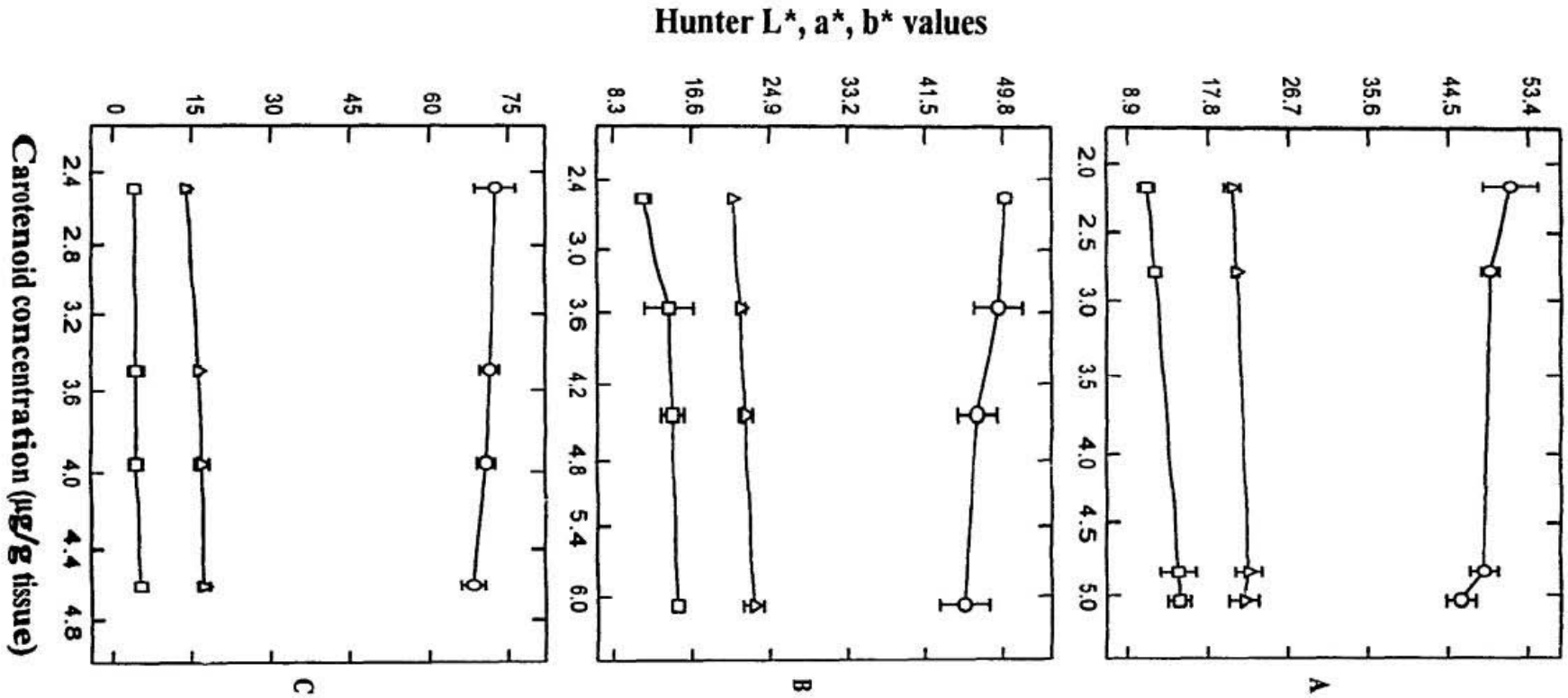


Fig. 12. Hunter L*, a*, b* values of fillet of Arctic charr at different carotenoid concentrations. (A = 40 kg/m^3; B = 50 kg/m^3; C = 75 kg/m^3; L* = -O-; a* = -□-; b* = -△-). n = 30 determinations per period. Vertical bars = standard deviations.

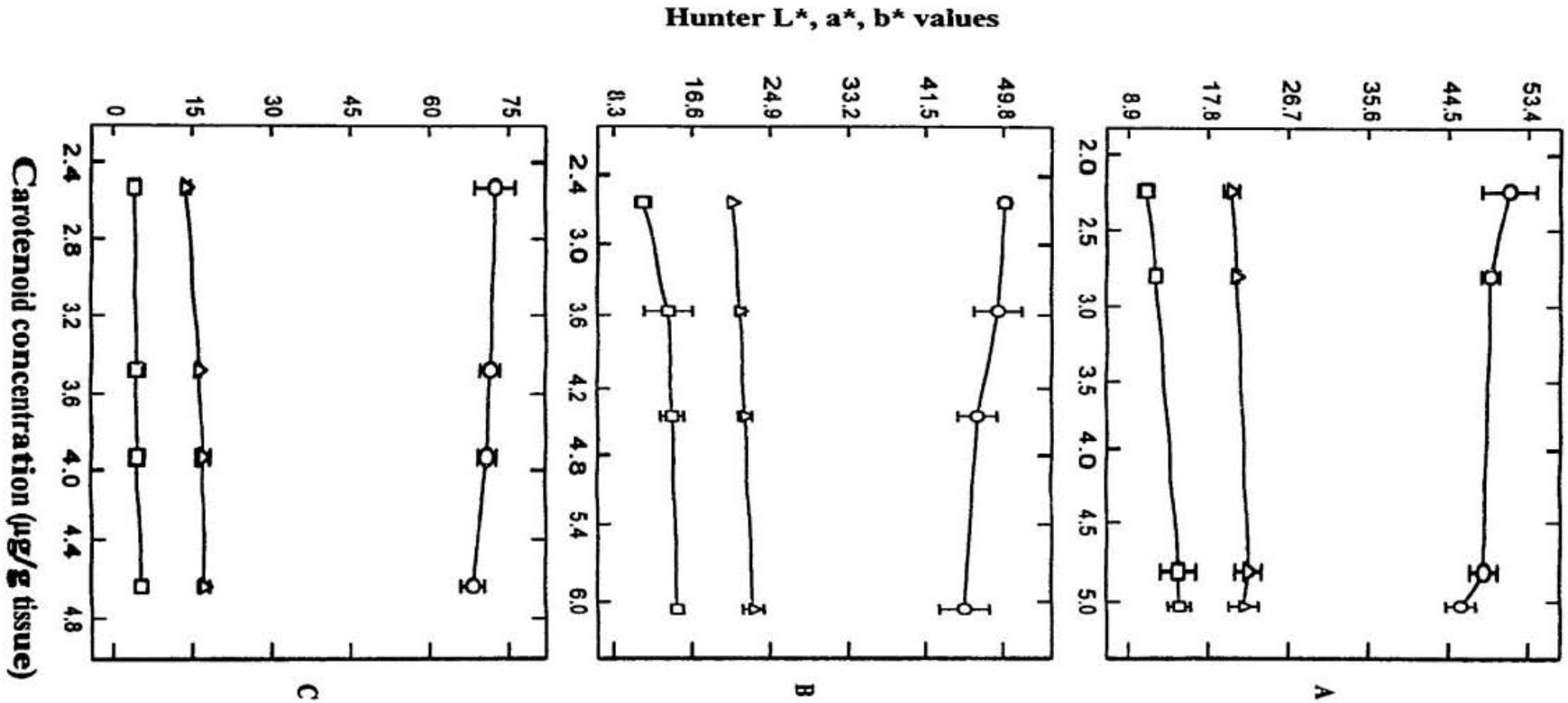


Fig. 13. Hunter L*, a*, b* values of homogenized charr tissue at different carotenoid concentrations. (A = 40 kg/m^3; B = 50 kg/m^3; C = 75 kg/m^3; L* = -o-; a* = -□-; b* = -△-). n = 30 determinations per period. Vertical bars = standard deviations.

Table 15. Total carotenoid contents (mg/kg) of flesh and skin of Arctic charr fed on a canthaxanthin-pigmented feed.

Feeding period, weeks	Stocking density, kg/m^3		
	40	50	75
Flesh[1]:			
8	2.19 ± 0.59^{ax}	2.60 ± 0.51^{ax}	2.50 ± 0.54^{ax}
11	2.77 ± 0.43^{ax}	3.56 ± 0.85^{by}	3.47 ± 0.41^{by}
16	5.04 ± 0.70^{bx}	4.48 ± 0.38^{cx}	4.60 ± 0.88^{cx}
24	4.80 ± 0.99^{bx}	6.08 ± 1.18^{dy}	3.95 ± 0.39^{bz}
Skin[2]:			
8	5.24 ± 0.30^{ax}	4.79 ± 0.08^{ay}	7.93 ± 0.28^{az}
16	10.20 ± 2.86^{bx}	8.42 ± 2.13^{bx}	8.82 ± 1.95^{abx}
24	8.82 ± 0.01^{bx}	9.53 ± 0.84^{bxy}	9.81 ± 0.57^{by}
Initial[3]: Flesh	0.47 ± 0.10		
Skin	1.05 ± 0.05		

[1,2,3]Results are mean values of 18, 6 and 3 determinations ± standard deviation, respectively. Values in each column with the same superscript (a,b,c,d) are not significantly different ($p>0.05$) from one another. Values in each row with the same superscript (x,y,z) are not significantly different ($p>0.05$) from one another.

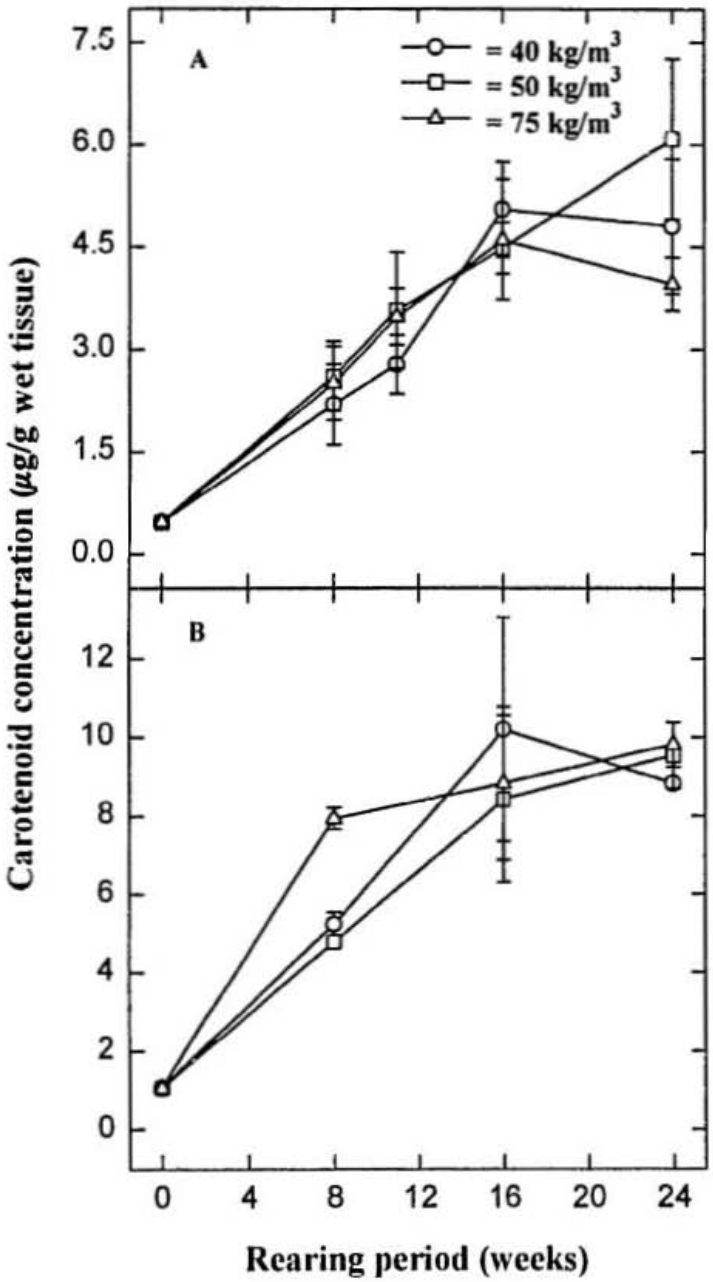


Fig. 14. Total carotenoid content of Arctic charr flesh (A) and skin (B) fed on a canthaxanthin-supplemented diet over a 24-week feeding period. n = 18 and 6 determinations per period for A and B, respectively. Vertical bars = standard deviations.

attained a similar level of flesh carotenoids ($p>0.05$). However, at the end of the experiment, their carotenoid contents varied significantly ($p<0.05$) with fish at a density of 50 kg/m^3 having the highest level followed by fish at densities of 40 and 75 kg/m^3, respectively. These facts suggest that stocking density does not affect deposition of carotenoid pigments in the flesh of Arctic charr. Regression analysis further indicated that there was indeed no relationship between stocking density and carotenoid contents of fish flesh ($r = -0.6034$).

Similarly, the total carotenoid content of fish skin increased during the experiments, except for that of fish at a density of 40 kg/m^3 which decreased at the end of the study (Table 15). The initial content of carotenoids in the skin of Arctic charr was 1.05 ± 0.05 µg/g. On week-8, the carotenoid contents of fish skin were 5.24 ± 0.30, 4.79 ± 0.08 and 7.93 ± 0.28 µg/g for fish at densities of 40, 50 and 75 kg/m^3, respectively. Fish at a density of 75 kg/m^3 deposited considerably higher amounts of carotenoids in their skin at early stages of the experiment as compared to that of other densities. On week-16, skin of fish from all stocking densities had a similar content of carotenoids ($p>0.05$). At the end of the experiment, however, the content of skin carotenoids of fish at a density of 40 kg/m^3 was significantly lower ($p<0.05$) than that of the other densities (Fig. 14B). Regression analysis indicated that there was a moderate relationship between stocking density and the total content of skin carotenoids ($r = 0.8826$).

4.1.4.3 Individual carotenoids

The concentration of individual carotenoid pigments in flesh of Arctic charr fed on a canthaxanthin-supplemented diet over the 24 weeks of experiment is presented in Table 16. The composition of carotenoid pigments in feed used in this study is given in Table 17. In fish flesh, only four individual carotenoids were identified, namely echinenone, canthaxanthin, 4′-hydroxyechinenone, and lutein (Figs. 15-17). The amounts of echinenone, canthaxanthin and lutein in fish flesh increased significantly ($p<0.05$) from 0.28 ± 0.02, 1.51 ± 0.13 and 0.15 ± 0.01 mg/kg tissue on week-8 to 0.66 ± .07, 3.69 ± 0.39 and 0.23 ± 0.03 mg/kg on week-16, respectively, for fish at a density of 40 kg/m^3, whereas those of fish at 75 kg/m^3 stocking density increased from 0.32 ± 0.03, 1.73 ± 0.15 and 0.17 ± 0.01 mg/kg on week-8 to 0.60 ± 0.04, 3.37 ± 0.25 and 0.21 ± 0.02 ± 0.02 mg/kg on week-16, respectively. The amounts of these carotenoids decreased significantly ($p<0.05$) at the end of the experiment. The content of 4′-hydroxyechinenone, however, increased significantly ($p<0.05$) in both density groups to the end of the experimental periods. In contrast, fish at a density of 50 kg/m^3 exhibited a significant ($p<0.05$) increase in all fractions of their flesh carotenoids over the entire rearing periods (Fig. 18A,B,C). In flesh of fish from all density groups, canthaxanthin accounted for approximately 71.58 ± 1.02% of total carotenoids. Lutein constituted about 3.82 ± 0.93%, and the rest was divided equally between echinenone and 4′-hydroxyechinenone (12.33 ± 0.40%

Table 16. Concentrations (mg/kg wet tissue) of major carotenoids of Arctic charr fed on a carotenoid-containing feed for 24 weeks.[1]

Density/ Fraction Number	R_f	λ_{max}	Feeding period on a canthaxanthin-containing diet, weeks			
			8	11	16	24
40 kg/m^3:						
I	0.84	456	0.28 ± 0.02^{aw}	0.36 ± 0.01^{ax}	0.66 ± 0.07^{av}	0.59 ± 0.09^{az}
II	0.75	466	1.51 ± 0.13^{ax}	2.00 ± 0.07^{ay}	3.69 ± 0.39^{az}	3.43 ± 0.53^{az}
III	0.41	454	0.25 ± 0.02^{ax}	0.27 ± 0.01^{ax}	0.46 ± 0.05^{av}	0.58 ± 0.09^{az}
IV	0.28	422,444,472	0.15 ± 0.01^{ax}	0.14 ± 0.01^{ax}	0.23 ± 0.03^{av}	0.18 ± 0.03^{az}
50 kg/m^3:						
I	0.84	456	0.33 ± 0.04^{bw}	0.46 ± 0.06^{bx}	0.58 ± 0.02^{bv}	0.69 ± 0.08^{bz}
II	0.75	466	1.80 ± 0.23^{bw}	2.57 ± 0.34^{bx}	3.28 ± 0.13^{bv}	3.99 ± 0.45^{bz}
III	0.41	454	0.30 ± 0.04^{bx}	0.34 ± 0.06^{bv}	0.41 ± 0.02^{bz}	0.68 ± 0.08^{bz}
IV	0.28	422,444,472	0.17 ± 0.02^{bx}	0.18 ± 0.02^{bx}	0.21 ± 0.01^{bz}	0.21 ± 0.02^{bz}
75 kg/m^3:						
I	0.84	456	0.32 ± 0.03^{bw}	0.45 ± 0.02^{bx}	0.60 ± 0.04^{bv}	0.49 ± 0.01^{cz}
II	0.75	466	1.73 ± 0.15^{bw}	2.51 ± 0.12^{bx}	3.37 ± 0.25^{bv}	2.83 ± 0.04^{cz}
III	0.41	454	0.29 ± 0.02^{bw}	0.34 ± 0.02^{bx}	0.42 ± 0.03^{bv}	0.48 ± 0.01^{cz}
IV	0.28	422,444,472	0.17 ± 0.01^{bx}	0.18 ± 0.01^{bx}	0.21 ± 0.02^{bv}	0.15 ± 0.00^{cz}

[1]Results are mean values of 18 determinations ± standard deviation. Values in each row with the same superscript (w,x,y,z) are not significantly different ($p>0.05$) from one another. Values in each column for the same fraction with the same superscript (a,b) are not significantly different ($p>0.05$) from one another.

Table 17. Composition of carotenoid pigments in Arctic charr feed.[1]

Carotenoid	mg/kg feed	% of total
Echinenone	1.69 ± 0.19	2.28
Canthaxanthin	66.87 ± 1.25	90.15
Lutein	5.61 ± 0.33	7.57
Total	74.19 ± 0.60	100.00

[1]Values are mean ± standard deviation of triplicate determinations.

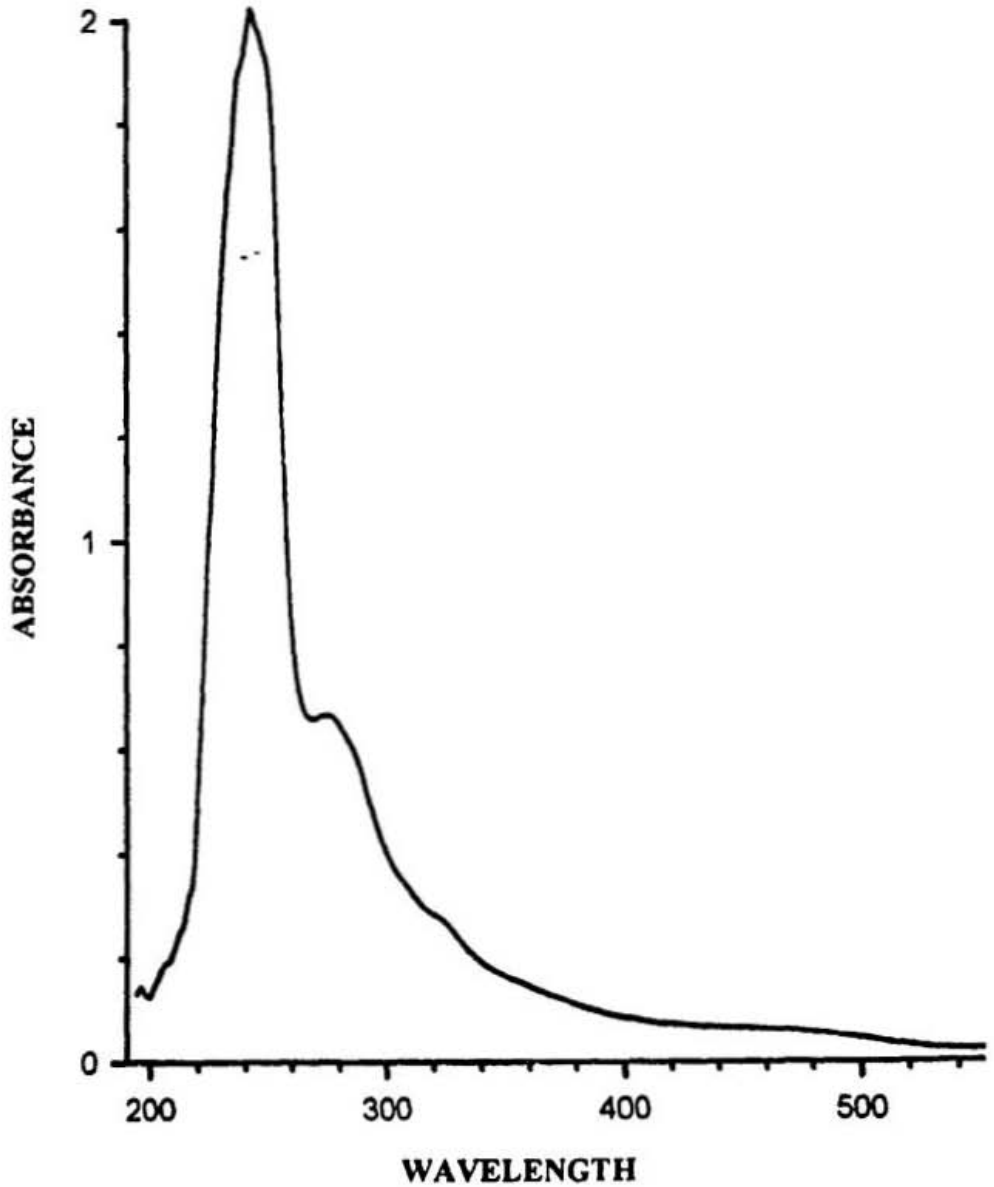


Fig. 15. UV spectra of the non-carotenoid pigments from Arctic charr flesh.

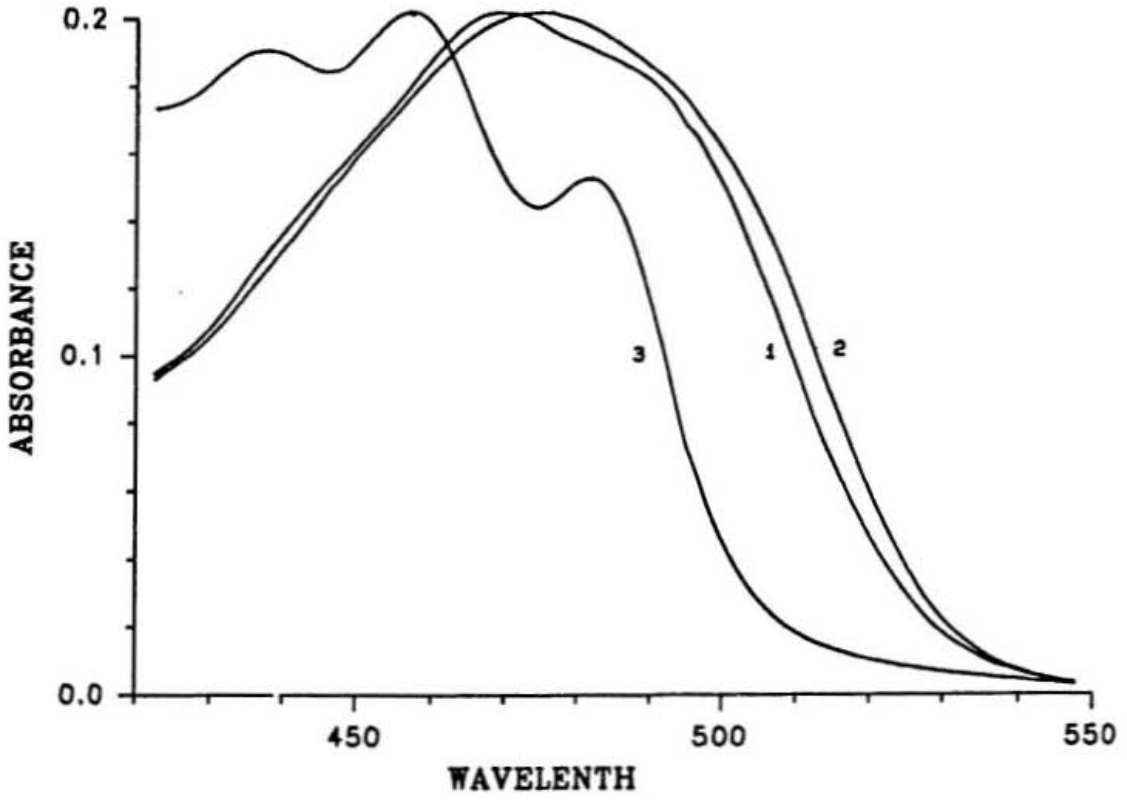


Fig. 16. UV spectra in the visible range for echinenone (1), canthaxanthin (2) and lutein (3) from Arctic charr flesh.

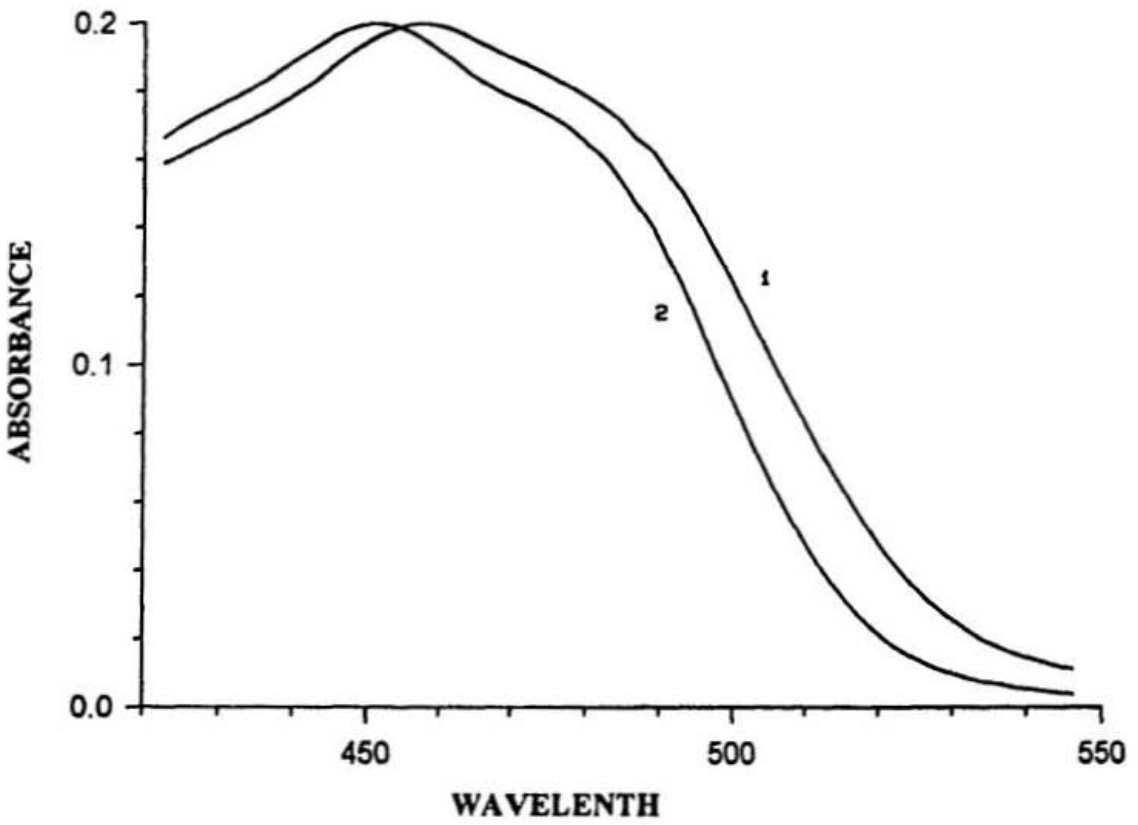


Fig. 17. UV spectra of echinenone (1) and 4'-hydroxyechinenone (2) from Arctic charr flesh.

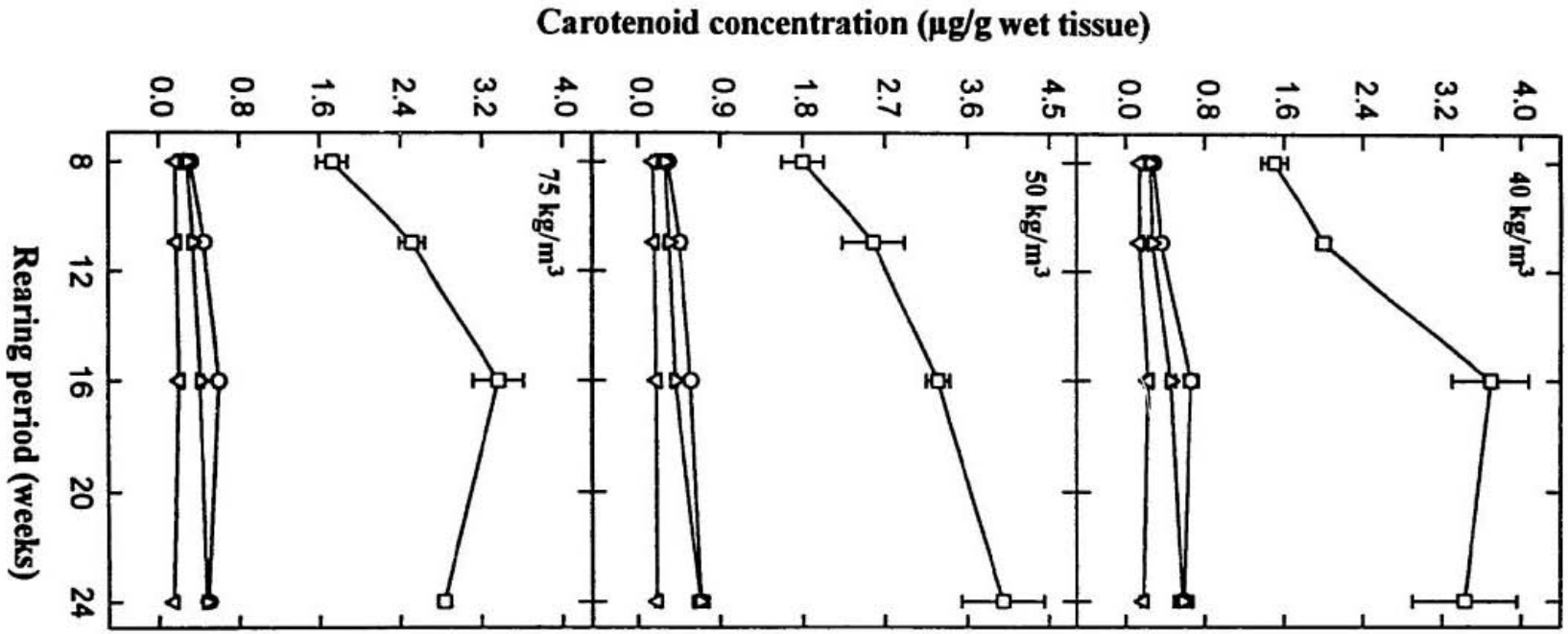


Fig. 18. The contents of echinenone (-o-), 4'-hydroxyechinenoe (-◇-), canthaxanthin (-□-) and lutein (-▽-) of Arctic charr flesh fed on a canthaxanthin-supplemented diet over a 24-week period. n = 18 determinations per period. Vertical bars = standard deviations.

and 12.27 ± 1.05%, respectively) (Table 18).

The skin of Arctic charr contained five fractions of individual carotenoids identified as β-carotene, lutein ester, echinenone, isocryptoxanthin, and canthaxanthin. In this tissue, approximately 50% of the total carotenoids were accounted for by β-carotene (48.95 ± 2.36%) followed by echinenone (28.11 ± 2.53%) and lutein esters (10.49 ± 1.02%). The rest was shared about equally between isocryptoxanthin and canthaxanthin (6.25 ± 1.47% and 6.18 ± 0.97%, respectively) (Table 18). In gonads, lutein esters, 4'-hydroxyechinenone, isocryptoxanthin and canthaxanthin were also identified. Lutein esters and 4'-hydroxyechinenone constituted the highest amount of carotenoids in fish gonads (37.66 ± 3.01% and 33.53 ± 2.71%, respectively). Isocryptoxanthin and canthaxanthin made up 10.98 ± 2.54% and 17.81 ± 4.25%, respectively (Table 18). The liver of fish contained only reductive metabolites of canthaxanthin, except lutein esters, namely echinenone and 4'-hydroxyechinenone. Echinenone was the main carotenoid component in fish liver (55.14 ± 1.63%). Lutein esters and 4'-hydroxyechinenone shared the rest, almost equally (20.33 ± 3.43% and 24.41 ± 4.12%, respectively) (Table 18). It is interesting that the liver of fish lacks canthaxanthin, despite the fact that this carotenoid was the major pigment in the diet.

Table 18. Proportion (%) of individual carotenoids present in flesh, skin, gonads, and liver of reared Arctic charr.[1]

Individual carotenoid	Flesh[2]	Skin[3]	Gonads[4]	Liver[4]
β-carotene	-	48.95 ± 2.36	-	-
Lutein	3.82 ± 0.93	-	-	-
Lutein ester	-	10.49 ± 1.02	37.66 ± 3.01	20.33 ± 3.43
Echinenone	12.33 ± 0.40	28.11 ± 2.53	-	55.14 ± 1.63
Hydroxyechinenone	12.27 ± 1.05	-	33.53 ± 2.71	24.41 ± 4.12
Isocryptoxanthin	-	6.25 ± 1.47	10.98 ± 2.54	-
Canthaxanthin	71.58 ± 1.02	6.18 ± 0.97	17.81 ± 4.25	-

[1]Results are mean values of [2]18, [3]6 and [4]3 determinations ± standard deviation.

4.2 Discussion

4.2.1 Performance

It is generally accepted that growth of fish is density-dependent, but numerous studies have shown that growth rate in many fish populations is not well correlated with density **(Ross and Almeida, 1986)**. **Brown *et al* (1991)** have reported a significantly ($p<0.05$) greater growth rate for Arctic charr held at a high stocking density (44 kg/m^3) than that of charr held at low densities of 8.7 and 20 kg/m^3, respectively. Similar observations were made by **Jobling (1985, 1987)**, **Wallace *et al* (1988)** and **Jorgensen *et al* (1993)**, among others. **Jorgensen *et al* (1993)** have shown that the SGR of Arctic charr at different stocking densities were -0.10 to 0.13 (15 kg/m^3), 0.86 (60 kg/m^3) and 1.13 (120 kg/m^3). In contrast, **Carlander and Payne (1977)** found a significant ($p<0.05$) inverse correlation between density and growth rate of walleye *(Stizosterdion vitreum)*. However, only approximately 17% of the variability in growth rate was attributed to changes in stocking density, whereas substantially greater variability (62%) was explained by fluctuations in water level. The present study demonstrated that growth rates decreased as stocking density increased ($r = -0.9522$). These results agree with many other reports regarding the existence of an inverse relationship between growth and stocking density in fish culture **(e.g. Kincaid *et al*, 1976; Backiel and Le Cren, 1978; Vijayan and Leatherland, 1988)**. Since the water level was adjusted in this study to maintain

the original stocking densities, it is unlikely that fluctuation in water level contributed to changes on the growth of Arctic charr. The limiting factors for growth of fish at extremely high stocking densities are probably of physical rather than behavioural nature. For example, elevated ammonia levels, inadequate oxygen supply, or as suggested by **Wedemeyer (1976)**, limited food availability because of restricted freedom of movement might be responsible for this observation. **Holm *et al* (1990)** have demonstrated that continuous availability of feed did not completely compensate for the density-linked growth depression.

The inverse relationship between stocking density and growth observed in this study was in contrast with the findings of **Jobling (1985; 1987)** and **Wallace *et al* (1988)**. Similarly, **Baker and Ayles (1990)** reported that growth performance declined when densities exceeded 40-50 kg/m^3. **Christiansen *et al.* (1991)** also found that fish stocked at high densities of up to 110 kg/m^3 show significantly ($p<0.05$) lower growth performance than fish stocked at low densities, ranging from 23 to 70 kg/m^3. In this experiment, the growth rate (% weight gain/day) of Arctic charr were 0.76, 0.70, and 0.66 at stocking densities of 40, 50, and 75 kg/m^3, respectively. These values are slightly higher than that of Arctic charr reported by **Torrissen and Shearer (1992)** (0.69 for fast growing and 0.52 for slow growing strains), and that of brook charr *(Salvelinus fontinalis)* reported by **Vijayan and Leatherland (1988)** (0.51 to 0.72). In contrast, other

investigators have reported that the SGR of rainbow trout ranged from 1.06 to 2.08 **(Papoutsoglou *et al*, 1987)**, and 0.61 to 1.05 **(Quinton and Blake, 1990)**. According to **Hepher (1988)**, the growth rate of fish is very high during the larval and juvenile stages of development. It can reach 40% or more of the fry weight/day. The growth rate decreases with increasing weight, and fish of 1.0 kg each usually grow less than 1% per day. Densities of 61 kg/m^3 or less have been recommended as a mean of minimizing stress in rainbow trout *(S. gairdneri)* after handling **(Wedemeyer, 1976)**. This latter author also reported that stocking density of 16 kg/m^3 or higher caused posthandling stress in coho salmon *(O. kisutch)*. Changes in the specific growth rate of Arctic charr during the present experiments indicated that increases in fish density and size may impair the growth of fish. The fact that optimal stocking density for Arctic charr appears to be high compared to those reported for other salmonids remains unexplained.

Feed conversion ratio (FCR) was found to decrease with increasing fish density ($r = -0.9696$). This agrees with the findings of **Fagerlund *et al* (1981)** where the FCR values decreased with increasing stocking density. The FCR values of Arctic charr in the present study varied from 1.05 to 2.05 which are not as good as those reported by **Torrissen and Shearer (1992)** for Arctic charr (0.66 and 0.82), but are comparable to those of rainbow trout reported by **Papoutsoglou *et al* (1987)** (1.10 to 1.46) and **Trzebiatowski *et al* (1981)** (1.89

to 2.14). In contrast, **Quinton and Blake (1990)** reported that the FCR of trout was between 0.16 and 0.47. In this study, the FCR of Arctic charr improved from 2.49, 4.01, and 4.94 on week-5 to 1.05, 1.40, and 2.05 at the end of the experiment for fish reared at densities of 40, 50 and 75 kg/m^3, respectively. Stocking density was also found to inversely correlated with the FCR of fish ($r = -0.9980$). Whatever the reasons for changes in feed conversion ratio, the fast pace at which changes occurred indicates that short term feeding trials may give estimates of growth rate and feed conversion which cannot be sustained over a long period of time.

The PER of Arctic charr in this experiment increased from 1.03 to 2.31, 0.64 to 1.86, and from 0.54 to 1.25, respectively, for fish at densities of 40, 50, and 75 kg/m^3. These values are slightly lower than those reported by **Torrissen and Shearer (1992)** for Arctic charr (2.90 for Hammerfest strain and 2.30 for Skogseid strain), but are comparable to those for trout (1.60 to 2.00) **(Papoutsoglou *et al*, 1987)** and for striped bass (1.70 to 2.40) **(Millikin, 1982)**. In a feeding trial with carp *(C. carpio)*, **O'Grady and Spillett (1985)** reported PER values of 0.90-1.80 in laboratory trials and 1.20-1.60 in pond trials. From the data presented, it is evident that PER of fish improves as the weight of fish increases. Regression analysis also indicated that PER values were strongly correlated with stocking density ($r = -0.9886$).

The hepatosomatic index (HSI) of Arctic charr observed in this experiment ranged from 1.37 to 1.43. Neither the stocking density nor the size of fish was found to significantly ($p<0.05$) affect the HSI of fish. **Leatherland and Cho (1985)** observed that the HSI of rainbow trout was between 0.79 and 1.12 while **Millikin (1982)** reported that the HSI of striped bass was between 1.50 and 2.70. Meanwhile, **Hung *et al* (1993)** found the HSI of striped bass ranged from 0.50 to 1.83. However, **Leatherland and Cho (1985)** reported that the HSI of rainbow trout reared at a low density of 130 kg/m^3 was significantly ($p<0.05$) higher than those reared at medium (210 kg/m^3) and high (299 kg/m^3) densities. These researchers argued that the high HSI of fish reared at low densities was caused by a higher total hepatic lipid and glycogen reserves than those in fish reared at higher densities.

4.2.2 Proximate composition

Changes in the body composition of fish were primarily in their contents of moisture and lipid. Similar results were also obtained by **Groves (1970)**. This is because additional energy stored as fat simply replaces body water **(Reinitz, 1983)**. During experimentation, moisture and ash contents of Arctic charr from all density groups decreased, whereas protein and lipid contents increased with increasing fish weight. **Tidwel and Robinette (1990)** have made a similar observation for channel catfish *(I. punctatus)*. In this study, a large

decrease in moisture content coincided with a large increase in the amounts of protein and lipid present. Ash contents, on the other hand, decreased at a very slow rate. The decrease in ash content observed in this experiment was in agreement with findings of **Brown and Murphy (1991)** who reported that the whole body ash fraction decreased with increasing fish weight. Other investigators have reported a relative increase in ash content of fish with declining body lipid **(Savitz, 1971; Niimi, 1972).**

Deposition of protein in fish flesh was increased concurrently with increasing feeding period at about the same magnitude and was independent of changes in moisture and lipid contents. **Reinitz (1983)** has also shown that protein contents in salmonids increase with increasing size and age of fish and that changes in moisture and lipid contents did not adversely affect the deposition of proteins. One possible explanation for the increase in protein levels of fish in this study is that the energy sources in feed are adequate to supply energy demand, and support fish growth. Approximately equal proportions of protein and lipid were deposited during the growth period. However, as the energy content of lipid is much greater than that of protein, almost two-thirds of the energy fixed during growth was in the form of lipid and only a third as protein.

Fish at a density of 75 kg/m^3 contained a higher moisture and a lower lipid content as compared to those at lower densities (40 and 50 kg/m^3). Similar findings have been reported by **Fagerlund *et al* (1981).** An increase in moisture

content was due to an extensive cellular shrinkage with a concurrent increase in extracellular fluids **(Love, 1980)**. An increase in lipid content during the growth of fish was expected as reported by other investigators **(Perera and De Silva, 1978; Vanstone and Markerts, 1978)**. With further weight gain during exponential growth, the lipid content remained relatively constant and the amount of lipid per unit weight decreased. In this study, lipid contents in muscles increased during the first 16 weeks, but decreased afterwards, except for that of fish at a stocking density of 50 kg/m^3 which continued to show an increase in its lipid content. Fatty fish (e.g. Salmonidae) exhibit an inverse relationship between their contents of lipid and moisture, but non-fatty fish (e.g. Gadidae) show an inverse relationship between their protein and moisture contents **(Brown and Murphy, 1991)**. The total lipid contents of salmonid fish also varied substantially: 1.7% in sockeye salmon, 2.3% in chum salmon, 3.2% in female fall chum salmon, 4.4% in male fall chum salmon, and 7.4% in pink salmon **(Takama *et al*, 1994)**. These amounts are much lower than that of Arctic charr (8.16 to 12.38%) observed in this experiment.

The increase in moisture and decrease in protein and lipid contents of fish at the end of the experiments may have been due to the initiation of gonad maturation. **Dygert (1990)** has observed that 55% loss of body protein, 4% of ash, and 5% of lipids was accounted for by an increase in these constituents in gonads during sexual maturation. The loss of these constituents was compensated

by an increase in the moisture content. Similarly, **Love (1980)**, **Dawson and Grimm (1980)** and **Iles (1984)** reported that most of the stored proteins that were lost during sexual maturation were used for gonad development.

4.2.3 Total and free amino acids

The amino acid compositions of flesh of Arctic charr in this study varied significantly ($p<0.05$) with stocking density. Among density groups, fish at a density of 40 kg/m^3 showed the highest content of serine, glycine, and tyrosine, but contained the lowest amount of hydroxyproline, proline, valine, cysteine, methionine, isoleucine, leucine, lysine and arginine. Fish at a density of 50 kg/m^3 attained the highest content of aspartic acid, threonine, proline, valine, cysteine, isoleucine, leucine, lysine and arginine, but contained the lowest amount of glutamic acid, alanine, and phenylalanine compared to those of the other densities. Meanwhile, flesh of fish at a density of 75 kg/m^3 exhibited the highest content of glutamic acid, alanine, methionine, and phenylalanine, but were lowest in the content of threonine, glycine, and tyrosine. However, compared to that of fish prior to the experiment (Table 10), fish from all density groups had lower content of aspartic acid, proline, valine, isoleucine, and tryptophan. The major amino acids in Arctic charr were aspartic acid, glutamic acid, leucine, and lysine, whereas hydroxyproline was the least abundant. Compared to amino acid composition of wild and reared Arctic charr reported by **Shahidi *et al* (1993)**,

the results were very close, but charr in the present study showed a slightly higher content of aspartic acid, glutamic acid, cysteine, methionine, leucine, tryptophan, and lysine, and contained slightly lower amounts of serine, valine, isoleucine, histidine, and arginine. The present results were also higher in the content of cysteine, histidine, isoleucine, leucine, lysine, methionine, tryptophan, and valine, but contained lower amount of aspartic acid, glutamic acid glycine, proline and serine as compared to those of goldfish, golden shiner and fathead minnow reported by **Gatlin (1987)**. Furthermore, results of **Shahidi *et al* (1991)** for cod *(Gadus morhua)* and **Shahidi (1994a,b)** for capelin were close to those of charr in this work. Amino acid content of charr was also similar to that of 20 species of Indian fish reported by **Chandrashekar and Deosthale (1993)**. With respect to essential amino acids (EAA), fish in this experiment exhibited a well-balanced composition of EAA as compared to that of the FAO reference pattern **(FAO, 1979)**. Within density groups, amino acid contents also varied significantly with sampling date. Charr from all stocking densities, in general, showed a significant ($p<0.05$) decrease in their amino acid contents with increasing duration of feeding.

Free amino acids (FAA) are important compounds contributing to the flavour and taste of fish meat **(Kemp and Birch, 1992)**. The FAA of Arctic charr flesh varied significantly ($p<0.05$) among density groups (Table 11). Only three individual FAA had a similar concentration throughout the experimental

period, namely glycine, cysteine, and histidine. The most abundant FAA in Arctic charr were anserine, taurine, glycine, and alanine, whereas the least abundant were carnosine, tryptophan, cystine and hydroxyproline, respectively. Carnosine, however, was not observed in fish at a stocking density of 40 kg/m^3 at the end of the experiments. The importance of carnosine as an antioxidant in muscle foods has been demonstrated by **Chan *et al* (1994a,b)**. **Synowiecki *et al* (1993)** have reported that the major FAA in Arctic charr were alanine, glycine, histidine, taurine and threonine. Similar observations were also reported by **Hata *et al* (1988)** in the ordinary muscles of coho salmon *(O. kisutch)*.

4.2.4 Lipid fatty acid composition

In the present study, fatty acid composition of Arctic charr changed slightly over the course of the experiments. The content of saturated and monounsaturated fatty acids remained relatively constant at approximately 25 and 40% of total amount during this period, respectively, whereas polyunsaturated fatty acids exhibited a more pronounced change (decreased from about 36 to 34% in fish at densities of both 40 and 50 kg/m^3, and increased from about 34 to 37% in fish at a density of 75 kg/m^3). Unsaturated fatty acids, however, accounted for about 75% of total lipids of fish flesh. **Tidwell and Robinette (1990)** reported that during a two-year pond culture of channel catfish *(I. punctatus)*, the content of saturated fatty acids showed a linear decline from 32% at the

beginning to 24% at the time of the harvest. Accompanying this change was a concurrent increase in unsaturated fatty acids, from 68 to 73%. The ratio of unsaturated to saturated fatty acids for charr in the present study (2.90 to 3.10) was slightly higher than that of channel catfish (2.12 to 3.01) reported by **Tidwell and Robinette (1990)**. **Yu et al (1977)** suggested that a mechanism exists in fish that regulates and maintains certain levels of body lipid saturation. It may be that the "set point" of this saturation regulation mechanism changes toward increasing lipids unsaturation with increasing age or size, or both.

The proportional distribution of fatty acids within the polyunsaturated fraction also changes over time. However, these levels differed only slightly from those of the dietary lipids, whereas total saturated and monounsaturated levels and U/S differed substantially. Monoenes were deposited in the fillets at much higher levels than were found in the feed (40% in fillets and 32% in feed). This observation agrees well with those of **Worthington and Lovell (1973)** who found that catfish tend to preferentially deposit palmitoleic (16:1) and oleic (18:1) acids above levels in dietary lipids. **Farkas *et al* (1978)** stated that accumulation of oleic acid in common carp, *C. carpio*, was due to an elevated rate of formation and not to selective deposition. These changes could have important implications for the storage and nutritional qualities of different sizes of fish because monounsaturated fatty acids are not as susceptible to oxidative rancidity as polyunsaturated fatty acids **(Labuza, 1971)**, but have similar

beneficial effects for human health **(Grundy, 1986).**

Diene and triene levels in charr flesh lipids were slightly higher than those in dietary fat (4.23 to 5.65% and 0.95 to 2.76% for diene and triene in fish, respectively, and 4.22 and 0.26% for diene and triene in feed, respectively), largely due to lower levels of linoleic acid (18:2) in the diets. These results are in contrast with those of **Farkas *et al* (1978)** and **Yingst and Stickney (1979)** who suggested that the low levels of linoleic acid may have been due to its use in place of linolenic acid (18:3) in the absence of sufficient dietary levels of the latter. Both dienes and trienes showed significant increases over the grow out period. The trienes (18:3 and 20:3) represent ω-3 and ω-6 fatty acids in this study. Recent studies on Arctic charr have shown that linolenic acid (18:3ω-3) was preferred over linoleic acid as substrate for elongation and desaturation regardless of the diet **(Olsen *et al*, 1991; Olsen and Ringo, 1992).** Similarly, **Satoh *et al* (1989)** reported that catfish are able to modify dietary linolenic acid to eicosapentaenoic acid (20:5ω-3).

The proportion of ω-3 fatty acids in fish flesh was remarkably high in the polyunsaturated fraction. They constituted up to 81.31% of the total polyunsaturated fatty acids. Eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) were dominant (up to 34.71 and 42.33%, respectively) ω-3 fatty acids present. This agrees with findings of **Cowey and Sargent (1977)** who reported that polyunsaturated fatty acids in fish tissues are predominantly of the ω-3 type.

The high amount of 20:5 and 22:6 present in the muscles of charr may have been a direct reflection of these fatty acids in the diet (12.10 and 8.66%, respectively). In addition, the ω-3 fatty acids in fish flesh and in the diet were observed to have similar concentrations (27.23 to 29.93% in fish flesh and 27.11% in feed, respectively). **Lovell (1988)** and **Nettleton (1990)** have reported that diet influences the amount of nutritionally important ω-3 fatty acids in the muscles of farmed fish.

The high content of ω-3 PUFA in Arctic charr, therefore, may play an important role in human health since they are known to reduce heart and heart-related diseases **(Barlow and Stansby, 1982; Bradlow, 1986; Kinsella, 1987)**. The long-term consumption of ω-3 polyunsaturated fatty acids has also been associated with alleviation of symptoms of breast cancer, rheumatoid arthritis, multiple sclerosis, and psoriasis **(Goodnight *et al*, 1982; Kinsella, 1988)**. On the other hand, nutritional benefits of consuming ω-3 fatty acids has clearly increased the marketability of salmon **(Carroll, 1986; Herald and Kinsella, 1986; Kinsella, 1986; Lands, 1986; Hearn *et al*, 1987; Stansby, 1990; Holub, 1992; Nettleton, 1992)**.

4.2.5 Pigmentation

4.2.5.1 Colour parameters

Hunter colour parameters (L^*, a^*, b^*) of belly, fillet and homogenized

tissues of individual fish were recorded. It was observed that while Hunter L^* (lightness) values decreased, Hunter a^* (redness) and b^* (yellowness) values increased with increasing carotenoid concentration of fish flesh. The increase in Hunter a^* values indicate a more reddish hue in fish flesh.

At the end of the experiment, fillets and homogenized tissues of fish at stocking densities of 40 and 75 kg/m^3 showed an increase in their Hunter L^* values and a decrease in their Hunter a^* and b^* values as the carotenoid concentration in their muscles decreased. The Hunter L^*, a^* and b^* values depended upon the concentration of flesh carotenoids and generally an increase in the level of feed carotenoids resulted in a more reddish hue in fish flesh. However, no such relationship was observed for colour parameters of the belly skin of the fish. The results of the present study clearly indicate a linear relationship between Hunter L^* and a^* values and carotenoid content of charr flesh. The decrease in L^* and the increase in Hunter a^* values with increasing carotenoid contents are in good agreement with findings of **Saito (1969), Skrede and Storebakken (1986a,b), and Shahidi *et al* (1993)**.

Homogenizing samples prior to colour measurement has been reported to have a conclusive effect on the colour parameters of salmon flesh **(Little and MacKinney, 1969; Schmidt and Choubert, 1969; Choubert, 1982; Skrede and Storebakken, 1986a)**. This effect was demonstrated in the present study when colour parameters of homogenized fish tissues were measured. Compared

with readings on fillets, homogenized samples possessed higher Hunter L^* and lower Hunter a^* and b^* values. According to **Skrede and Storebakken (1986a)**, this effect was likely to be related to the translucency of flesh samples. Translucency causes various amount of light to be trapped within samples unless special precautions are taken such as increasing the exposed relative to the illuminated area.

4.2.5.2 Total carotenoid pigments

At the beginning of the experiments, a low amount (0.47 mg/kg tissue) of carotenoids was present in the muscles of Arctic charr. The concentration of carotenoids was increased steadily during the feeding period until a maximum level of about 6.08 mg/kg was reached at the end of the experiments. **Torrissen *et al* (1989)** have reported that the carotenoid concentrations of about 6 mg/kg in the flesh of trout and 3-4 mg/kg in fillets of farmed salmon as being sufficient to give a satisfactory visual colour impression to fish. The present experiments on Arctic charr fed on a canthaxanthin-containing diet reached these levels after 11-16 weeks of feeding.

Accumulation of carotenoids in the flesh of Arctic charr increased linearly up to week-16 ($r = 0.9827$ and $r = 9948$) for fish at densities of 40 and 75 kg/m^3, respectively; whereas that of fish at a density of 50 kg/m^3 increased linearly over the entire experimental period ($r = 0.9958$). If one assumes that the weight gain

is proportional to feed consumption, the amount of carotenoids ingested should also be proportional to growth. However, this was not the case, at least for fish at a density of 40 or 75 kg/m^3. The decrease in carotenoid content after week-16 of feeding may be due to a saturation effect of pigment in fish flesh. **Torrissen and Torrissen (1984)** and **Torrissen *et al* (1984)** indicated a saturation level of 4 to 5 mg carotenoids per kilogram of tissue of Atlantic salmon *(S. salar)*. However, this saturation level might be influenced by genetic factors **(Torrissen and Naevdal, 1984)**, dietary carotenoid levels **(Torrissen, 1985)**, growth rate and size of fish **(Torrissen, 1986)**. **Torrissen and Naevdal (1984)** reported that rainbow trout accumulated between 5.5 and 6.5 mg carotenoids per kilogram of tissue after 5 to 6 months of feeding on a diet supplemented with 50 mg/kg of canthaxanthin. This agrees well with the results of the present investigation. The decrease in total carotenoid content of fish at densities of 40 and 75 kg/m^3 at the end of the experiment may also have originated from initiation of maturation of fish gonads. Fish undergoing sexual maturation are known to mobilize carotenoids from muscles and transfer them to the gonads and skin **(Crozier, 1970; Tacon, 1981; Storebakken and No, 1992)**.

The skin of charr in this study contained a relatively lower amount of carotenoids (8.82-9.80 mg/kg wet tissue) than those of Arctic charr belly skin reported previously **(Synowiecki *et al*, 1993)**, but were higher than those of rainbow trout reported by **Bjerkeng *et al* (1992)**. However, the final weights of

charr used in the study of Synowiecki *et al* (1993) were much higher (1539 g) as compared with those of charr used in this study (514.31 to 623.43 g). It is known that immature salmonids in a rapid phase of growth deposit carotenoids mainly in their flesh (Torrissen *et al*, 1989).

4.2.5.3 Individual carotenoid pigments

Carotenoid pigments of Arctic charr flesh and skin were separated into five fractions, whereas those of gonads and liver were separated into four and three fractions, respectively. Four fraction of carotenoids in charr flesh exhibited absorption maxima at 456, 466, 454, and 422/444/472 nm, respectively. The electronic spectra of the 5th fraction, eluted from silica gel column with hexane-acetone-methanol (8:1:1, v/v/v), indicated that it was not a carotenoid as it lacked any absorption maxima in the visible range and exhibited only one sharp absorption band at 242 nm. The occurrence of a yellow, non-carotenoid, pigment in the flesh of rainbow trout has also been reported by No and Storebakken (1992). The R_f values of isolated carotenoids were 0.84, 0.75, 0.41, and 0.28. Fractions I-IV, therefore, each contained only one single carotenoid namely echinenone, canthaxanthin, 4'-hydroxyechinenone, and lutein, respectively. The identity of each compound was established using standard canthaxanthin and lutein (Hoffman-La-Roche, Etobicoke, ON) or by comparison of absorption spectra with those reported by Bauernfeind (1981). The R_f value of the main

carotenoid in the flesh of charr (Fraction II) indicated the presence of canthaxanthin. Furthermore, its absorption characteristics were identical to that of canthaxanthin extracted from Carophyll red (R_f=0.75) in fish feed. Arctic charr flesh also contained reductive metabolites of canthaxanthin, namely echinenone (Fraction I) and 4′-hydroxyechinenone (Fraction III). Presence of these carotenoids in fish flesh lends further support to the findings of **Schiedt *et al* (1985)** and **No and Storebakken (1992)** that reductive metabolism of canthaxanthin (β,β-carotene-4,4′-dione) may take place in fish organs. According to **Ghidalia (1985)**, possible intermediates of reductive transformation of canthaxanthin are 4′-hydroxy-echinenone (4′-hydroxy-β,β-carotene-4-one), echinenone (β,β-carotene-4-one), isocrypto-xanthin (β,β-carotene-4-ol), and β-carotene (β,β-carotene), respectively (Fig.19). A portion of echinenone content in the charr flesh might have originated from feed pigments as each gram of feed contained 2.28 µg of echinenone which might have been produced from canthaxanthin. All carotenoids in charr flesh were in the free, unesterified form which are bound to actomyosin via hydrophobic interaction and hydrogen bonding **(Henmi *et al*, 1989)**.

The canthaxanthin content in the flesh of Arctic charr after 24-weeks on diets containing 65 mg/kg Carophyll red reached 3.99 mg/kg sample (approximately 71.58% of the total carotenoids). This was slightly lower than

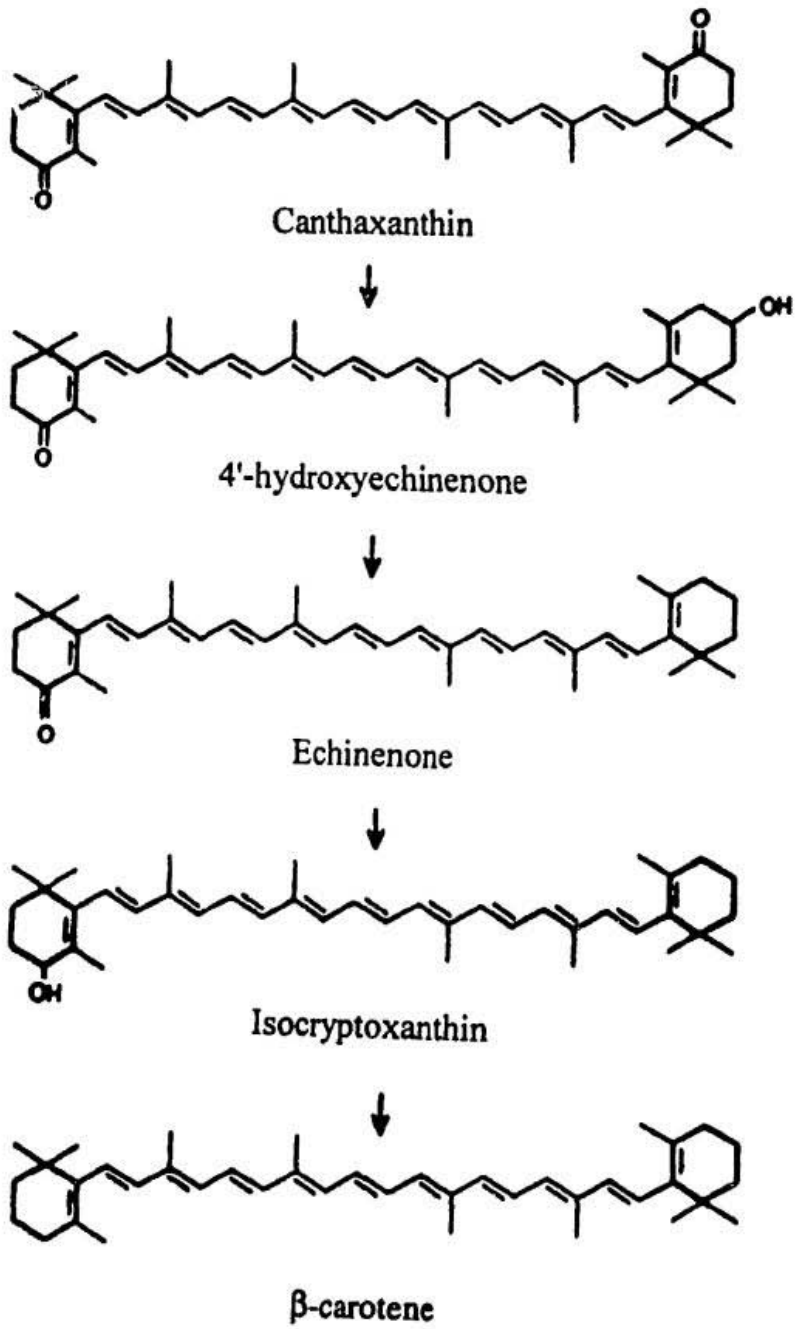


Fig. 19. Possible metabolic pathway of canthaxanthin in Arctic charr organs.

that of charr previously reported by **Shahidi *et al* (1994)** (4.74 mg/kg) and of rainbow trout reported by **No and Storebakken (1992)**. However, these researchers used a much bigger fish (>500 g) as compared with those of charr used in this study (average of 154 g each). It is well known that bigger fish deposit carotenoid pigments more efficiently in their flesh than smaller fish. Existing differences in water temperature, feeding regime, diet formulations and size of fish were responsible for the observed differences. Presence of similar amounts of lutein (0.15-0.23 mg/kg) and smaller amounts of canthaxanthin metabolites (0.48-0.69 mg/kg) in this study as compared to those observed by **Shahidi *et al* (1994)** (0.14-0.23 and 0.56-1.09 mg/kg) could be explained by existing differences in feed composition (i.e. dietary lipids and vitamin E level and lipid quality), stage of sexual maturation and size of fish and also storage of fish before carotenoid determination. According to **Protasowiecka and Kolakowski (1984)**, storage of Antarctic krill meal over a 24 h period at 13°C exhibited considerable changes in its carotenoids composition as its lutein content dropped from 0.19 mg/kg fresh sample to 0.0 mg/kg. In vacuum-packed fillets of Arctic charr and rainbow trout, however, as little as 5% of total carotenoids were lost after storage for up to 6 months at −20°C **(No and Storebakken, 1992; Synowiecki *et al*, 1993)**. The final reduction product of canthaxanthin, β-carotene, was not present in the Arctic charr flesh. Lack of hydroxyl and keto groups in β-carotene makes it to have a very low affinity for actomyosin

(Storebakken and No, 1992). Therefore, unbound β-carotene is transported rapidly from muscle tissues into the skin where it is deposited or is transformed into vitamin A in the intestinal walls as observed by **Simpson *et al* (1981)**. The muscles of Arctic charr contained the same type of carotenoids as those present in the feed used for pigmentation, except for 4'-hydroxyechinenone.

Large amounts of canthaxanthin metabolites were accumulated in charr skins; up to 48.95% of which was β-carotene followed by echinenone (28.11%) and isocryptoxanthin (6.25%), respectively. These values are similar to those reported by **Bjerkeng *et al* (1990)** for the skin of canthaxanthin-fed rainbow trout which contained 46% β-carotene and 29% echinenone. The skin of charr in the present study contained a relatively lower amount of carotenoids (9.38 mg/kg of wet tissue) than those reported previously **(Synowiecki *et al*, 1993)**. It is known that immature salmonids in a rapid phase of growth deposit carotenoids mainly in their flesh, followed by skin, gut and liver **(Torrissen *et al*, 1989)**. However, the fish undergoing sexual maturation mobilize carotenoids from the flesh and transfer them to the skin and gonads **(Crozier, 1970; Storebakken and No, 1992)**.

The total content of carotenoids in the gonads of sexually immature Arctic charr was 2.16 mg/kg tissue. During sexual maturation of salmonid fish a considerable amount of carotenoids are transferred from the fish muscles or gastrointestinal tract to ovaries **(Ando and Hatano, 1986)**. Individual

carotenoids in Arctic charr gonads included some of the canthaxanthin metabolites. Considerably lower amounts of carotenoids (5.23 mg/kg) were found in Arctic charr liver as compared with those in the flesh and skin. The main compounds present in the liver were echinenone (55.14%) and 4'-hydroxy-echinenone (24.41%). Presence of canthaxanthin metabolites in charr liver lends support to the findings of **Hardy *et al* (1990)** who reported that salmonid fish liver is the major metabolic organ for carotenoids.

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The present study demonstrated that there were no differences in the final mean weight gain of fish reared at stocking densities of 40, 50 and 75 kg/m^3. However, there were great variations in individual weight of fish among stocking densities (over 100 g weight difference) as well as within density groups, particularly between densities of 40 and 50 kg/m^3.

The performance parameters of fish, except the hepatosomatic index (HSI), were influenced markedly by stocking density and duration of feeding. The specific growth rate (SGR) of fish decreased linearly with stocking density and duration of feeding. After 24 weeks of feeding, fish at a density of 40 kg/m^3 had the highest SGR value (0.76%/day), followed by fish at densities of 50 and 75 kg/m^3 (0.70 and 0.66%/day, respectively). The feed conversion ratio (FCR) of charr also decreased as stocking density and feeding period increased. As in the case of SGR, fish at a density of 40 kg/m^3 exhibited the best FCR value (1.05), followed by fish at densities of 50 and 75 kg/m^3 (1.40 and 2.05, respectively). Changes in the FCR values of fish at all density groups occurred at a slower pace after 16 weeks of rearing as compared with earlier stages. At the beginning of the experiments, the FCR values of fish, particularly for those at densities of 50 and 75 kg/m^3 were very poor compared to that of fish at a

density of 40 kg/m^3. After 11 weeks of feeding, the FCR of fish at a density of 50 kg/m^3 exhibited a marked improvement while that of fish at densities of 40 and 75 kg/m^3 showed a slower pace. The protein efficiency ratio(PER), on the other hand, increased progressively as rearing was extended, however, it decreased as stocking density increased. As in the SGR and FCR values, fish at 40 kg/m^3 showed the best PER value followed by fish at 50 and 75 kg/m^3 of densities (2.31, 1.86 and 1.25, respectively).

The proximate composition of Arctic charr flesh, except the ash content, changed significantly ($p<0.05$) over the course of the experiments. The moisture, crude protein and total lipid contents varied significantly ($p<0.05$) among and within density groups and among sampling dates. While the moisture content decreased, the crude protein and total lipid contents increased as the fish grew. As the fish underwent sexual maturation, the moisture content increased significantly ($p<0.05$) whereas the total lipid content decreased significantly ($p<0.05$); the decrease in crude protein content was not significant ($p>0.05$). Statistical analysis further indicated that the moisture and ash contents were strongly influenced by the stocking density, but the protein and lipid contents did not.

The contents of total amino acids of charr flesh varied significantly ($p<0.05$) according to stocking density and sampling dates. Aspartic acid, glutamic acid, leucine and lysine were the most abundant amino acids present,

whereas the least abundant one was hydroxyproline. Of the amino acids determined, only cysteine, methionine and histidine showed similar levels in fish flesh before and after the experiments. Within density groups, small, but significant ($p<0.05$) variations in amino acid contents of fish flesh existed among sampling dates. However, the total amino composition of charr was well-balanced with reference to the FAO standards. The total free amino acid contents of charr flesh also varied significantly ($p<0.05$) according to stocking density and sampling dates. The contents of free amino acids in feed and fish prior to experimentation were much lower than those in fish from all density groups at all sampling dates. In feed, the main free amino acids were taurine, glutamic acid, alanine, and histidine, whereas fish flesh contained the same amino acids as well as glycine, lysine and anserine. Among the major amino acids, anserine was one the most abundant. Cysteine and carnosine were least abundant in all fish samples.

The composition of charr lipids was dominated by unsaturated fatty acids. The predominant fatty acids were monounsaturated fatty followed by polyunsaturated constituents. Omega-3 fatty acids made up approximately 80% of the total polyunsaturated fatty acids and only 20% were of the ω-6 type. Among density groups, saturated lipids remained relatively unchanged, but the proportions of mono- and polyunsaturated fatty acids did change. The contents of monounsaturated fatty acids decreased as stocking density increased and the

relative amounts of polyunsaturated decreased. Omega-3 and ω-6 polyunsaturated fatty acids were also increased with increasing stocking density. As a result, the ratio of unsaturated to saturated fatty acids also increased. Within density groups, the contents of saturated and monounsaturated fatty acids increased slightly with prolonged duration of feeding, whereas the contents of polyunsaturated fatty acids decreased.

Hunter $\mathbf{L}^*$, $\mathbf{a}^*$, $\mathbf{b}^*$ values of flesh of Arctic charr were strongly influenced by the contents of carotenoid pigments. While carotenoid contents were inversely correlated with Hunter $\mathbf{L}^*$ (lightness) values of fish flesh, they were directly correlated with Hunter $\mathbf{a}^*$ (redness) and $\mathbf{b}^*$ (yellowness) values. In contrast, colour parameters of belly skin of fish were not influenced by the amounts of deposited carotenoids.

The content of carotenoid pigments in flesh of Arctic charr increased as duration of feeding on pigmented diets was prolonged. However, stocking density and the amount of feed consumed did not affect the deposition of carotenoid pigments in charr flesh. After 11 to 16 weeks of feeding on a diet supplemented with 65 mg/kg of canthaxanthin, fish flesh at all density groups attained the required level of carotenoids considered sufficient (3-4 mg/kg wet tissue) for providing a satisfactory colour impression. The decrease in flesh carotenoid concentration at the end of the experiments may have been due to the saturation effect of carotenoid pigments in fish flesh or the initiation of gonad

maturation or both. In fish flesh, lutein, canthaxanthin, echinenone, and 4′-hydroxyechinenone were identified. Canthaxanthin, as expected, was the main carotenoid (71.58%) in flesh of charr since it was the main pigment (90.15%) in fish feed.

The contents of carotenoid pigments in skin of Arctic charr were also increased with increasing duration of feeding on pigmented diets. As in flesh, the contents of carotenoids in fish skin were not influenced by the stocking density or the amounts of feed consumed. In skin, however, β-carotene (48.95%) and echinenone (28.11%) were the major carotenoid pigments. Lutein, isocryptoxanthin and canthaxanthin were also identified in smaller amounts. In fish gonads, lutein esters and 4′-hydroxyechinenone were the main fractions of carotenoids present. Isocryptoxanthin and canthaxanthin were also present in smaller amounts in the gonads. Lutein esters, echinenone and 4′-hydroxyechinenone were the pigments present in fish liver. Echinenone was the main component of carotenoid pigments in liver, whereas canthaxanthin was absent in this organ. Presence of reductive metabolites of canthaxanthin, namely echinenone, 4″-hydroxyechinenone, isocryptoxanthin and β-carotene, strongly suggested that canthaxanthin was metabolized in Arctic charr organs. In addition, feed used during the experiments contained no β-carotene, 4′-hydroxyechinenone and isocryptoxanthin and very small amounts of echinenone (2.28%). Furthermore, the amount of canthaxanthin in the skin of fish was very

low (6.18% of the total carotenoids), and was totally absent from the liver of fish. Therefore, stocking density and stage of maturation may influence charr flesh quality in aquaculture operations.

5.2 Recommendations

Based on the results of the present study, it is suggested that:

a. To culture Arctic charr with more uniform size and to stock them at a relatively high density. Stocking density of 50 kg/m^3 or less will most likely result in greater variability of fish at the time of harvest. In addition, employing a stocking density higher than 50 kg/m^3 would also reduce production costs. However, if efficiency of feed utilization is the main goal, stocking fish at a lower density would be more beneficial.

b. For pigmentation of sexually immature Arctic charr with average weight of 150 to 200 g each, using 65 mg/kg canthaxanthin in dry feed, 11 to 16 weeks feeding prior to harvesting is required for production of fish with acceptable flesh colouration. This period may be reduced by increasing the amounts of carotenoid supplementation or using larger size fish.

c. It is necessary to investigate the effects of lipid content and quality as well as lipid fatty acid composition of Arctic charr feed in order to understand more about the deposition and metabolism of dietary carotenoids in charr.

d. To produce Arctic charr with high contents of ω-3 fatty acids, it is necessary

to provide sufficient amounts of these fatty acids in fish diet. Inclusion of up to 25% marine lipids in feed is considered sufficient to produce products with enriched ω-3 fatty acids.

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Appendix 1. The contents of total amino acids (mg/g protein) of flesh of Arctic charr reared at a density of 40 kg/m^3 at different sampling dates.[1]

Amino acid	Rearing period, weeks		
	5	16	24
Alanine	68.67 ± 0.56^a	64.70 ± 0.30^b	67.11 ± 0.18^a
Arginine	65.77 ± 0.27^a	61.27 ± 0.23^b	63.16 ± 0.35^c
Aspartic acid	87.46 ± 0.41^a	107.30 ± 0.20^b	82.85 ± 0.50^c
Cysteine	20.93 ± 0.23^a	21.08 ± 2.49^a	21.53 ± 2.95^a
Glutamic acid	110.07 ± 1.03^a	115.12 ± 0.53^b	124.12 ± 3.00^c
Glycine	59.60 ± 0.40^a	54.54 ± 0.24^b	58.68 ± 0.11^a
Histidine	29.49 ± 0.14^a	27.57 ± 0.14^b	30.02 ± 0.11^a
Hydroxyproline	1.06 ± 0.08^a	1.07 ± 0.06^a	1.01 ± 0.05^a
Isoleucine	55.94 ± 0.59^a	53.50 ± 0.20^b	50.70 ± 0.27^c
Leucine	80.44 ± 0.96^a	84.55 ± 0.41^b	80.38 ± 0.40^a
Lysine	103.18 ± 0.54^a	96.33 ± 0.16^b	101.77 ± 0.28^c
Methionine	41.37 ± 0.31^a	38.80 ± 0.25^b	41.50 ± 0.30^a
Phenylalanine	48.40 ± 0.23^a	45.38 ± 0.20^b	47.00 ± 0.11^a
Proline	40.33 ± 0.50^a	36.65 ± 0.35^b	37.32 ± 0.10^b
Serine	42.07 ± 0.26^a	39.75 ± 0.20^b	42.34 ± 0.15^a
Threonine	44.59 ± 0.42^a	50.96 ± 0.15^b	45.84 ± 0.22^a
Tryptophan	13.22 ± 0.11^a	12.64 ± 0.16^{ab}	12.33 ± 0.14^b
Tyrosine	38.97 ± 0.50^a	39.25 ± 0.10^a	39.98 ± 0.18^a
Valine	55.96 ± 0.60^a	58.14 ± 0.17^b	59.98 ± 0.40^b

[1]Results are mean values of triplicate determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Appendix 2. The contents of total amino acids (mg/g protein) of flesh of Arctic charr reared at a density of 50 kg/m^3 at different sampling dates.[1]

Amino acid	Rearing period, weeks		
	5	16	24
Alanine	65.63 ± 0.71^a	67.10 ± 0.15^b	64.60 ± 0.78^a
Arginine	61.17 ± 0.42^a	66.31 ± 0.30^b	68.53 ± 0.67^c
Aspartic acid	81.72 ± 0.66^a	88.28 ± 0.52^b	85.98 ± 3.40^c
Cysteine	23.07 ± 0.65^a	21.86 ± 2.43^a	23.98 ± 3.61^a
Glutamic acid	103.66 ± 0.61^a	120.47 ± 0.85^b	103.69 ± 1.20^a
Glycine	59.52 ± 0.61^a	54.89 ± 0.18^b	56.19 ± 0.27^c
Histidine	31.19 ± 0.10^a	29.08 ± 0.19^b	30.36 ± 1.96^{ab}
Hydroxyproline	1.09 ± 0.05^a	1.92 ± 0.13^b	1.31 ± 0.10^c
Isoleucine	51.08 ± 0.70^a	55.20 ± 0.40^b	55.92 ± 0.33^b
Leucine	89.56 ± 1.40^a	82.15 ± 0.46^b	87.94 ± 0.63^a
Lysine	103.43 ± 0.31^a	101.63 ± 0.90^b	105.94 ± 0.42^a
Methionine	40.87 ± 0.45^{ab}	38.96 ± 0.78^a	41.81 ± 0.26^b
Phenylalanine	47.98 ± 0.44^a	42.08 ± 0.29^b	45.81 ± 0.58^c
Proline	43.00 ± 0.19^a	37.68 ± 0.22^b	40.80 ± 0.95^a
Serine	43.19 ± 0.15^a	40.66 ± 0.17^b	36.23 ± 0.38^a
Threonine	48.13 ± 0.33^a	52.51 ± 0.32^b	47.73 ± 0.46^a
Tryptophan	14.56 ± 0.14^a	13.11 ± 0.13^b	13.68 ± 0.15^b
Tyrosine	34.66 ± 0.37^a	31.38 ± 0.15^b	33.06 ± 1.16^{ab}
Valine	65.53 ± 1.08^a	61.99 ± 0.40^b	66.58 ± 0.87^a

[1]Results are mean ± standard deviation of triplicate determinations. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Appendix 3. The contents of total amino acids (mg/g protein) of flesh of Arctic charr reared at a density of 75 kg/m^3 at different sampling dates.[1]

Amino acid	Rearing period, weeks		
	5	16	24
Alanine	64.54 ± 0.15^a	63.23 ± 0.20^a	69.75 ± 0.53^b
Arginine	62.56 ± 0.18^a	68.53 ± 0.34^b	66.03 ± 0.16^c
Aspartic acid	89.89 ± 0.35^a	86.41 ± 1.02^b	82.63 ± 0.36^c
Cysteine	22.73 ± 0.43^a	23.85 ± 2.77^a	22.36 ± 2.97^a
Glutamic acid	110.81 ± 0.55^a	118.57 ± 1.04^b	132.88 ± 1.43^c
Glycine	55.94 ± 0.25^a	54.76 ± 0.16^a	50.54 ± 0.52^b
Histidine	30.64 ± 0.15^a	32.23 ± 0.25^b	30.19 ± 0.17^a
Hydroxyproline	2.03 ± 0.10^a	1.47 ± 0.12^b	1.24 ± 0.12^b
Isoleucine	50.69 ± 0.16^a	54.37 ± 0.35^b	52.06 ± 0.18^c
Leucine	88.77 ± 0.23^a	84.27 ± 0.51^b	82.74 ± 0.36^b
Lysine	102.15 ± 0.41^a	106.16 ± 0.91^b	103.11 ± 0.22^b
Methionine	42.86 ± 0.40^a	40.10 ± 0.46^b	43.05 ± 0.83^a
Phenylalanine	53.20 ± 0.20^a	50.21 ± 0.29^b	48.99 ± 0.24^c
Proline	43.09 ± 0.15^a	42.48 ± 0.17^b	38.53 ± 0.23^a
Serine	37.06 ± 0.11^a	35.97 ± 0.21^a	34.18 ± 0.49^b
Threonine	49.43 ± 0.12^a	47.47 ± 0.25^b	44.14 ± 0.66^c
Tryptophan	14.35 ± 0.25^a	14.31 ± 0.14^a	12.79 ± 0.10^b
Tyrosine	34.88 ± 0.13^a	32.87 ± 0.32^b	31.76 ± 0.13^c
Valine	57.48 ± 0.17^a	54.56 ± 0.70^b	61.52 ± 0.30^c

[1]Results are mean values of triplicate determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Appendix 4. Total free amino acid contents (μg/g) of flesh of Arctic charr reared at a density of 40 kg/m^3 at different sampling dates.[1]

Free amino acid	Rearing period, weeks		
	5	16	24
Alanine	462.38 ± 65.93^{ab}	400.32 ± 20.63^{a}	525.80 ± 72.79^{b}
Anserine	1541.22 ± 350.32^{a}	1958.13 ± 224.71^{b}	2671.08 ± 447.93^{c}
Arginine	215.21 ± 6.42^{a}	71.10 ± 9.59^{b}	201.44 ± 19.54^{a}
Aspartic acid	180.93 ± 5.63^{a}	51.11 ± 11.73^{b}	212.52 ± 28.23^{c}
Carnosine	25.23 ± 15.14^{a}	17.02 ± 1.94^{a}	0^{b}
Cystine	51.34 ± 7.28^{a}	34.30 ± 5.62^{b}	28.32 ± 2.12^{a}
Glutamic acid	313.65 ± 23.17^{a}	186.09 ± 9.65^{b}	327.67 ± 8.55^{a}
Glutamine	113.03 ± 1.15^{a}	34.13 ± 7.32^{b}	119.87 ± 10.21^{a}
Glycine	616.45 ± 188.30^{a}	754.92 ± 86.36^{a}	666.33 ± 92.17^{a}
Histidine	229.33 ± 52.34	177.38 ± 67.17^{b}	276.16 ± 51.46^{c}
Hydroxyproline	38.74 ± 14.68^{a}	65.12 ± 4.65^{b}	30.93 ± 17.64^{a}
Isoleucine	120.25 ± 0.84^{a}	50.55 ± 7.92^{b}	135.37 ± 7.77^{c}
Leucine	249.52 ± 10.05^{a}	109.00 ± 16.83^{b}	260.69 ± 0.78^{c}
Lysine	292.30 ± 7.37^{a}	253.05 ± 27.27^{b}	355.55 ± 8.23^{c}
Methionine	117.72 ± 4.54^{a}	44.85 ± 9.36^{b}	114.11 ± 1.83^{a}
Phenylalanine	162.08 ± 9.73^{a}	81.39 ± 13.86^{b}	167.42 ± 9.29^{a}
Proline	170.53 ± 40.49^{a}	110.92 ± 4.38^{b}	161.41 ± 3.76^{a}
Serine	219.79 ± 33.92^{a}	115.82 ± 12.02^{b}	225.49 ± 6.32^{a}
Taurine	526.21 ± 118.04^{a}	584.01 ± 26.77^{a}	796.32 ± 42.47^{b}
Threonine	197.80 ± 37.27	162.41 ± 2.29^{b}	210.92 ± 15.71^{c}
Tryptophan	19.62 ± 0.94^{a}	8.06 ± 4.34^{b}	23.40 ± 2.67^{c}
Tyrosine	123.90 ± 2.89^{a}	63.28 ± 12.35^{b}	125.11 ± 9.21^{a}
Valine	192.46 ± 7.57^{a}	103.47 ± 14.68^{b}	225.41 ± 6.43^{c}
Total FAA	6179.68 ± 478.80^{a}	5433.43 ± 355.27^{b}	7861.29 ± 389.68^{c}

[1]Results are mean values of 4 determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Appendix 5. Total free amino acid contents (μg/g) of flesh of Arctic charr reared at a density of 50 kg/m^3 at different sampling dates.[1]

Free amino acid	Rearing period, weeks		
	5	16	24
Alanine	690.13 ± 8.07^a	393.95 ± 34.05^b	549.40 ± 91.54^c
Anserine	1921.46 ± 161.82^a	2062.15 ± 245.76^a	1868.40 ± 168.03^a
Arginine	382.49 ± 29.24^a	57.73 ± 24.43^b	287.82 ± 73.28^c
Aspartic acid	326.58 ± 21.13^a	56.52 ± 27.58^b	244.93 ± 48.99^c
Carnosine	37.78 ± 37.78^a	49.84 ± 30.79^a	5.47 ± 5.47^b
Cystine	78.99 ± 5.16^a	26.09 ± 2.67^b	31.71 ± 1.48^c
Glutamic acid	489.61 ± 22.31^a	169.09 ± 50.04^b	438.35 ± 106.80^a
Glutamine	206.68 ± 23.41^a	36.93 ± 14.42^b	174.66 ± 53.77^a
Glycine	906.69 ± 30.33^a	587.92 ± 54.49^b	831.00 ± 209.36^a
Histidine	280.70 ± 29.48^a	214.58 ± 98.30^a	258.54 ± 2.17^a
Hydroxyproline	50.40 ± 6.70^a	46.56 ± 3.05^a	31.01 ± 1.93^b
Isoleucine	191.97 ± 3.12^a	47.01 ± 15.18^b	174.63 ± 41.98^a
Leucine	445.29 ± 77.57^a	107.60 ± 41.47^b	343.93 ± 95.50^a
Lysine	509.53 ± 45.53^a	190.14 ± 40.43^b	430.71 ± 119.72^a
Methionine	195.13 ± 27.75^a	39.91 ± 12.69^b	143.32 ± 28.39^c
Phenylalanine	264.72 ± 34.22^a	68.81 ± 17.52^b	198.00 ± 42.69^c
Proline	237.82 ± 13.77^a	103.57 ± 27.68^b	192.36 ± 30.82^c
Serine	339.07 ± 0.48^a	110.99 ± 17.73^b	281.55 ± 71.09^a
Taurine	631.72 ± 5.53^a	502.10 ± 155.72^a	770.52 ± 134.96^b
Threonine	267.68 ± 1.84^a	162.17 ± 21.07^b	254.99 ± 61.84^a
Tryptophan	42.37 ± 7.32^a	9.32 ± 7.30^b	31.81 ± 9.71^a
Tyrosine	218.54 ± 30.41^a	65.05 ± 25.12^b	143.96 ± 32.61^c
Valine	308.41 ± 15.13^a	97.95 ± 32.86^b	265.50 ± 62.42^a
Total FAA	9021.27 ± 448.87^a	5206.04 ± 501.92^b	7952.58 ± 489.03^c

[1]Results are mean values of 4 determinations± standard deviation. Values in each row with the same superscript are not significantly different (p>0.05) from one another.

Appendix 6. Total free amino acid contents (μg/g) of flesh of Arctic charr flesh reared at a density of 75 kg/m^3 at different sampling dates.[1]

Free amino acid	Rearing period, weeks		
	5	16	24
Alanine	475.21 ± 22.41^a	457.21 ± 132.16^a	450.36 ± 10.46^a
Anserine	1229.23 ± 114.43^a	2275.80 ± 62.43^b	1946.63 ± 287.90^b
Arginine	231.75 ± 0.76^a	100.06 ± 48.78^b	178.65 ± 14.52^c
Aspartic acid	189.95 ± 11.11^a	64.38 ± 29.45^b	169.33 ± 15.40^c
Carnosine	9.92 ± 9.92^{ab}	19.45 ± 0.42^a	4.25 ± 4.25^b
Cystine	47.08 ± 2.19^a	36.86 ± 6.95^b	31.71 ± 6.89^a
Glutamic acid	306.86 ± 5.02^a	218.14 ± 77.24^b	301.68 ± 8.23^a
Glutamine	122.17 ± 1.33^a	49.63 ± 22.17^b	103.16 ± 6.52^c
Glycine	593.74 ± 79.27^a	781.36 ± 124.11^b	665.12 ± 79.81^{ab}
Histidine	154.97 ± 4.91^a	236.25 ± 61.12^b	230.81 ± 48.77^b
Hydroxyproline	38.42 ± 0.33^a	66.32 ± 2.31^b	27.09 ± 3.13^c
Isoleucine	117.37 ± 9.30^a	64.73 ± 25.99^b	114.51 ± 10.09^a
Leucine	265.88 ± 10.14^a	136.15 ± 43.74^b	210.67 ± 14.40^c
Lysine	318.91 ± 14.58^a	216.87 ± 91.08^b	297.95 ± 39.79^{ab}
Methionine	116.47 ± 3.58^a	56.47 ± 20.31^b	92.19 ± 4.31^c
Phenylalanine	157.75 ± 6.19^a	102.88 ± 33.59^b	138.85 ± 2.21^c
Proline	149.82 ± 4.08^a	131.37 ± 28.85^a	170.53 ± 3.78^b
Serine	217.68 ± 6.30^a	148.04 ± 49.05^b	180.53 ± 24.38^b
Taurine	417.28 ± 18.65^a	593.47 ± 3.75^b	668.67 ± 52.55^c
Threonine	173.55 ± 7.69^a	162.47 ± 15.32^a	187.19 ± 16.84^a
Tryptophan	18.89 ± 2.59^a	15.35 ± 1.12^b	16.16 ± 4.32^{ab}
Tyrosine	126.23 ± 5.04^a	79.37 ± 24.79^b	100.33 ± 1.05^b
Valine	192.38 ± 1.69^a	127.98 ± 41.66^b	184.43 ± 8.45^a
Total FAA	5671.38 ± 275.71^a	6145.09 ± 348.15^b	6470.75 ± 309.14^b

[1]Results are mean values of 4 determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Appendix 7. Fatty acid composition (%) of total lipid of Arctic charr reared at a density of 40 kg/m^3 at different sampling dates.[1]

Fatty acid	Rearing period, weeks		
	8	16	24
14:0	5.58 ± 0.04^a	5.73 ± 0.36^a	5.75 ± 0.13^a
16:0	15.06 ± 0.07^a	15.20 ± 0.10^a	15.91 ± 0.38^b
18:0	3.19 ± 0.10^a	3.39 ± 0.11^{ab}	3.49 ± 0.02^a
20:0	0.57 ± 0.01^a	0.57 ± 0.00^a	0.57 ± 0.01^a
Σ SFA	**24.40 ± 0.03^a**	**24.89 ± 0.15^b**	**25.72 ± 0.53^b**
14:1ω-5	0.25 ± 0.00^a	0.25 ± 0.00^a	0.27 ± 0.01^a
16:1ω-7	11.74 ± 0.02^a	11.85 ± 0.01^a	12.50 ± 0.23^b
18:1ω-9	17.30 ± 0.13^a	17.44 ± 0.12^a	18.10 ± 0.41^b
18:1ω-7	3.81 ± 0.02^{ab}	3.69 ± 0.03^a	3.86 ± 0.09^b
20:1ω-9	3.94 ± 0.01^a	3.51 ± 0.10^b	3.72 ± 0.15^{ab}
22:1ω-11	2.86 ± 0.02^a	2.44 ± 0.05^{ab}	2.00 ± 0.55^b
24:1ω-9	0.34 ± 0.01^a	0.31 ± 0.00^a	0.32 ± 0.01^a
Σ MUFA	**40.23 ± 0.13^a**	**39.48 ± 0.26^b**	**40.76 ± 0.33^a**
18:2ω-6	4.76 ± 0.03^a	4.50 ± 0.01^b	4.59 ± 0.08^b
18:3ω-3	2.41 ± 0.01^a	2.33 ± 0.02^a	2.30 ± 0.00^a
18:4ω-3	1.74 ± 0.00^a	1.76 ± 0.03^a	1.70 ± 0.04^a
20:2ω-6	0.31 ± 0.00^a	0.27 ± 0.01^a	0.28 ± 0.00^a
20:3ω-6	0.35 ± 0.01^a	0.35 ± 0.01^a	0.34 ± 0.00^a
20:4ω 6	1.07 ± 0.00^a	1.13 ± 0.01^a	1.07 ± 0.02^a
20:5ω-3	9.94 ± 0.04^a	10.62 ± 0.21^b	9.62 ± 0.29^a
22:5ω-3	2.78 ± 0.02^a	2.61 ± 0.05^b	2.43 ± 0.07^c
22:6ω-3	12.05 ± 0.15^a	12.46 ± 0.16^a	11.19 ± 0.49^b
Σ PUFA	**35.39 ± 0.16^a**	**36.01 ± 0.45^a**	**33.50 ± 0.82^b**
U/S	**3.10 ± 0.01^a**	**3.03 ± 0.01^b**	**2.90 ± 0.09^b**
Σω-6	**6.49 ± 0.03^a**	**6.24 ± 0.01^b**	**6.27 ± 0.06^b**
Σω-3	**28.91 ± 0.19^{ab}**	**29.77 ± 0.46^a**	**27.23 ± 0.88^b**
ω-6/ω-3	**0.23 ± 0.01^a**	**0.21 ± 0.00^a**	**0.23 ± 0.01^a**

[1]Results are mean values of triplicate determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another. SFA, MUFA and PUFA are saturated, monounsaturated and polyunsaturated fatty acids, respectively. U/S, ratio of unsaturated to saturated fatty acids.

Appendix 8. Fatty acid composition (%) of total lipid of Arctic charr reared at a density of 50 kg/m^3 at different sampling dates.[1]

Fatty acid	Rearing period, weeks		
	8	16	24
14:0	5.52 ± 0.22^a	5.64 ± 0.03^a	5.56 ± 0.01^a
16:0	15.74 ± 0.47^a	15.95 ± 0.02^a	15.64 ± 0.01^a
18:0	3.31 ± 0.05^a	3.37 ± 0.04^a	3.57 ± 0.15^a
20:0	0.55 ± 0.01^a	0.58 ± 0.01^a	0.58 ± 0.01^a
Σ SFA	**25.12 ± 0.63^a**	**25.54 ± 0.62^a**	**25.35 ± 0.17^a**
14:1ω-5	0.25 ± 0.02^a	0.26 ± 0.00^a	0.26 ± 0.00^a
16:1ω-7	11.59 ± 0.35^a	12.17 ± 0.04^b	11.94 ± 0.03^{ab}
18:1ω-9	16.88 ± 0.41^a	17.52 ± 0.02^b	17.42 ± 0.12^{ab}
18:1ω-7	3.69 ± 0.10^a	3.80 ± 0.00^a	3.95 ± 0.03^b
20:1ω-9	4.01 ± 0.06^a	3.63 ± 0.04^b	3.79 ± 0.08^c
22:1ω-11	3.01 ± 0.05^a	2.53 ± 0.03^b	2.65 ± 0.06^c
24:1ω-9	0.40 ± 0.02^a	0.33 ± 0.01^a	0.34 ± 0.01^a
Σ MUFA	**39.81 ± 0.99^a**	**40.24 ± 0.06^a**	**40.34 ± 0.25^a**
18:2ω-6	4.57 ± 0.01^a	4.46 ± 0.01^a	4.64 ± 0.01^a
18:3ω-3	3.64 ± 1.36^a	2.32 ± 0.01^a	2.34 ± 0.00^a
18:4ω-3	1.78 ± 0.07^a	1.66 ± 0.01^{ab}	1.61 ± 0.01^b
20:2ω-6	0.43 ± 0.15^a	0.29 ± 0.00^a	0.32 ± 0.01^a
20:3ω-6	0.31 ± 0.02^a	0.34 ± 0.01^a	0.36 ± 0.01^a
20:4ω-6	1.15 ± 0.03^a	1.09 ± 0.03^a	1.12 ± 0.02^a
20:5ω-3	9.78 ± 0.54^a	9.98 ± 0.06^a	9.34 ± 0.05^a
22:5ω-3	2.30 ± 0.13^a	2.44 ± 0.01^a	2.43 ± 0.03^a
22:6ω-3	12.55 ± 1.05^a	11.69 ± 0.07^a	12.15 ± 0.04^a
Σ PUFA	**36.49 ± 0.25^a**	**34.24 ± 0.05^b**	**34.34 ± 0.06^b**
U/S	**3.04 ± 0.05^a**	**2.92 ± 0.01^b**	**2.95 ± 0.03^{ab}**
Σω-6	**6.45 ± 0.17^a**	**6.19 ± 0.01^a**	**6.43 ± 0.01^a**
Σω-3	**30.04 ± 0.42^a**	**28.05 ± 0.04^b**	**27.92 ± 0.06^b**
ω-6/ω-3	**0.22 ± 0.01^a**	**0.22 ± 0.00^a**	**0.23 ± 0.00^a**

[1]Results are mean values of triplicate determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another. SFA, MUFA and PUFA are saturated, monounsaturated and polyunsaturated fatty acids, respectively. U/S, ratio of unsaturated to saturated fatty acids.

Appendix 9. Fatty acid composition (%) of total lipid of Arctic charr reared at a density of 75 kg/m^3 at different sampling dates.[1]

Fatty acid	Rearing period, weeks		
	8	16	24
14:0	5.84 ± 0.09^a	5.48 ± 0.22^{ab}	5.51 ± 0.04^b
16:0	15.66 ± 0.23^a	15.82 ± 0.51^a	15.27 ± 0.02^a
18:0	3.15 ± 0.16^a	3.35 ± 0.11^a	3.46 ± 0.05^a
20:0	0.55 ± 0.00^a	0.54 ± 0.01^a	0.56 ± 0.01^a
Σ SFA	**25.20 ± 0.16^a**	**25.19 ± 0.62^a**	**24.80 ± 0.08^a**
14:1ω-5	0.25 ± 0.00^a	0.26 ± 0.02^a	0.26 ± 0.01^a
16:1ω-7	11.24 ± 0.13^a	12.27 ± 0.39^b	11.77 ± 0.07^b
18:1ω-9	14.78 ± 0.12^a	18.61 ± 0.54^b	16.20 ± 0.06^c
18:1ω-7	3.72 ± 0.06^a	3.69 ± 0.10^a	3.73 ± 0.03^a
20:1ω-9	4.45 ± 0.04^a	3.57 ± 0.08^b	3.68 ± 0.07^{ab}
22:1ω-11	3.53 ± 0.02^a	2.56 ± 0.06^b	2.69 ± 0.06^{ab}
24:1ω-9	0.42 ± 0.01^a	0.33 ± 0.02^a	0.39 ± 0.01^a
Σ MUFA	**38.38 ± 0.37^a**	**41.28 ± 1.20^b**	**38.71 ± 0.16^a**
18:2ω-6	4.75 ± 0.03^{ab}	4.42 ± 0.06^a	5.36 ± 0.58^b
18:3ω-3	2.28 ± 0.03^a	2.25 ± 0.00^a	2.41 ± 0.02^a
18:4ω-3	1.83 ± 0.02^a	1.71 ± 0.04^a	1.84 ± 0.03^a
20:2ω-6	0.31 ± 0.01^a	0.26 ± 0.01^a	0.29 ± 0.01^a
20:3ω-6	0.32 ± 0.00^a	0.33 ± 0.00^a	0.35 ± 0.00^a
20:4ω-6	1.10 ± 0.01^a	1.04 ± 0.03^a	1.18 ± 0.02^a
20:5ω-3	10.11 ± 0.15^a	9.82 ± 0.38^a	10.39 ± 0.37^a
22:5ω-3	2.20 ± 0.06^a	2.40 ± 0.10^{ab}	2.62 ± 0.06^b
22:6ω-3	13.45 ± 0.30^a	11.76 ± 0.89^b	12.67 ± 0.32^{ab}
Σ PUFA	**36.45 ± 0.53^a**	**33.98 ± 1.37^b**	**37.11 ± 0.53^a**
U/S	**2.97 ± 0.03^a**	**2.99 ± 0.08^a**	**3.06 ± 0.04^a**
Σω-6	**6.47 ± 0.02^a**	**6.04 ± 0.04^b**	**7.16 ± 0.56^c**
Σω-3	**30.25 ± 0.29^a**	**27.94 ± 1.41^b**	**29.93 ± 0.06^{ab}**
ω-6/ω-3	**0.22 ± 0.01^a**	**0.22 ± 0.01^a**	**0.24 ± 0.02^a**

[1]Results are mean values of triplicate determinations ± standard deviation. Values in each row with the same superscript are not significantly different (p>0.05) from one another. SFA, MUFA and PUFA are saturated, monounsaturated and polyunsaturated fatty acids, respectively. U/S, ratio of unsaturated to saturated fatty acids.

Appendix 10. Hunter L^*, a^*, b^* values of belly skin of Arctic charr reared at different stocking densities over a 24-week period.[1]

Feeding period, weeks	Density, kg/m^3		
	40	50	75
L^*: 5	71.67 ± 2.74^a	71.50 ± 2.79^a	72.94 ± 1.13^a
8	78.33 ± 0.39^a	79.32 ± 0.59^b	78.22 ± 0.72^a
11	77.13 ± 0.98^a	77.13 ± 0.25^a	79.50 ± 1.47^b
16	78.41 ± 1.51^a	80.11 ± 0.77^b	80.35 ± 1.23^b
24	79.44 ± 0.14^a	78.66 ± 0.94^b	77.70 ± 0.60^c
a^*: 5	-0.93 ± 1.15^a	-1.07 ± 1.43^a	-1.35 ± 0.37^a
8	-0.77 ± 0.04^{ab}	-1.04 ± 0.25^a	-0.60 ± 0.80^b
11	-1.36 ± 0.29^a	-1.56 ± 0.71^{ab}	-1.79 ± 0.29^b
16	-0.37 ± 1.96^a	-1.74 ± 0.34^b	-1.41 ± 0.61^b
24	-1.26 ± 0.07^a	-1.29 ± 0.40^a	-1.36 ± 0.01^a
b^*: 5	11.76 ± 4.22^a	10.66 ± 2.80^a	11.30 ± 0.58^a
8	5.34 ± 1.02^a	5.37 ± 1.25^a	4.97 ± 1.26^a
11	7.74 ± 1.07^a	5.02 ± 2.82^b	5.93 ± 0.41^b
16	8.65 ± 2.22^a	8.22 ± 0.78^a	8.38 ± 1.88^a
24	6.02 ± 3.22^a	5.97 ± 1.19^a	4.46 ± 0.58^b

[1]Results are mean values of 30 determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Appendix 11. Hunter L*, a*, b* values of fillet of Arctic charr reared at different stocking densities.[1]

Feeding period, weeks		Density, kg/m³		
		40	50	75
L*:	5	53.43 ± 1.96[a]	51.59 ± 2.25[b]	51.94 ± 1.86[b]
	8	51.40 ± 3.05[a]	50.01 ± 0.65[a]	50.60 ± 3.85[a]
	11	49.21 ± 0.99[a]	49.25 ± 2.60[a]	50.55 ± 1.81[b]
	16	45.91 ± 1.56[a]	47.03 ± 2.08[a]	46.61 ± 1.72[a]
	24	48.52 ± 1.66[a]	45.65 ± 2.64[b]	48.73 ± 2.21[a]
a*:	5	9.09 ± 1.87[a]	9.22 ± 1.71[a]	8.50 ± 1.75[a]
	8	10.95 ± 0.88[a]	11.47 ± 0.80[a]	11.27 ± 1.19[a]
	11	11.97 ± 0.17[a]	14.14 ± 2.58[b]	13.57 ± 1.54[b]
	16	14.61 ± 1.96[a]	14.58 ± 1.24[a]	13.88 ± 1.34[a]
	24	14.51 ± 1.23[a]	15.08 ± 0.16[a]	12.40 ± 1.18[b]
b*:	5	19.55 ± 0.08[a]	20.52 ± 1.39[a]	19.87 ± 0.94[a]
	8	20.45 ± 0.88[a]	21.11 ± 0.65[a]	20.82 ± 0.87[a]
	11	20.97 ± 0.25[a]	21.28 ± 0.50[a]	21.06 ± 0.34[a]
	16	22.31 ± 1.45[a]	21.39 ± 0.76[a]	21.23 ± 1.33[a]
	24	21.78 ± 1.64[a]	23.17 ± 1.10[a]	20.87 ± 1.05[a]

[1] Results are mean values of 30 determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.

Appendix 12. Hunter L*, a*, b* values of homogenized tissues of Arctic charr reared at different stocking densities.[1]

Feeding period, weeks	Density, kg/m³		
	40	50	75
L*: 5	74.60 ± 1.16[a]	73.57 ± 2.37[ab]	72.71 ± 1.06[b]
8	71.23 ± 1.48[a]	71.56 ± 1.66[ab]	72.56 ± 2.06[b]
11	71.04 ± 0.40[c]	70.07 ± 0.07[b]	71.55 ± 0.66[c]
16	68.66 ± 0.99[a]	70.06 ± 1.81[b]	68.32 ± 1.04[a]
24	69.34 ± 0.47[a]	65.76 ± 3.34[b]	70.86 ± 1.01[c]
a*: 5	1.90 ± 0.41[ab]	2.51 ± 2.02[a]	1.45 ± 0.68[b]
8	3.86 ± 0.44[a]	3.69 ± 0.36[a]	4.15 ± 0.15[b]
11	5.02 ± 0.48[a]	4.89 ± 1.19[ab]	4.50 ± 0.41[b]
16	6.35 ± 0.70[a]	5.72 ± 0.50[b]	5.37 ± 0.65[b]
24	5.78 ± 0.03[a]	6.55 ± 0.68[b]	4.58 ± 0.40[c]
b*: 5	13.24 ± 1.04[ab]	14.17 ± 3.01[a]	12.56 ± 0.99[b]
8	16.18 ± 1.47[a]	15.48 ± 0.16[a]	13.92 ± 3.69[b]
11	16.37 ± 0.59[a]	16.20 ± 1.00[a]	16.35 ± 1.18[a]
16	19.11 ± 0.72[a]	18.46 ± 0.55[b]	17.29 ± 0.98[c]
24	18.77 ± 0.43[a]	19.33 ± 0.73[b]	17.02 ± 0.63[c]

[1]Results are mean values of 30 determinations ± standard deviation. Values in each row with the same superscript are not significantly different ($p>0.05$) from one another.