EFFECTS OF FEEDING DIETS CONTAINING VARIOUS
DIETARY PROTEIN AND LIPID RATIOS ON THE
GROWTH PERFORMANCE AND THE SENSORY ATTRIBUTES OF
POST-JUVENILE COHO SALMON (*Oncorhynchus kisutch*)
REARED IN SEAWATER

by

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Abstract

Six extruded dry diets formulated to contain one of two levels of digestible protein (37% or 44%) and one of three levels of digestible lipid (16%, 23%, or 30%) on a dry weight basis as well as a seventh commercial diet were used to feed triplicate groups of post-juvenile coho salmon (*Oncorhynchus kisutch*) in sea water. Fish were fed to satiation twice daily for 168 days beginning October, 1997. The performance of the fish was assessed by measuring changes in mean body weight (BW, g), mean specific growth rate (SGR, %, (ln (final mean weight) - ln (initial mean weight)) $\bullet$ 100 $\bullet$ number of experimental days^{-1}), mean feed intake (g $\bullet$ fish^{-1}), mean feed efficiency ratio (FE, weight gained (g) $\bullet$ dry feed intake (g)$^{-1}$), and mean protein efficiency ratio (PER, wet weight gained (g) $\bullet$ protein consumption (g)$^{-1}$) of each replicate group every 28 days. In addition, mean protein deposition (%PD, %, protein gained in fish (g) $\bullet$ 100 $\bullet$ protein consumed (g)$^{-1}$) and mean gross energy utilization (GEU, %, gross energy gained in fish $\bullet$ 100 $\bullet$ gross energy consumed (MJ)$^{-1}$) of each replicate group were determined at the end of the experiment. On the final day, Day 168, of the growth experiment, samples were taken from each replicate group per diet treatment for determinations of whole body and muscle proximate compositions. Fatty acid compositions and astaxanthin concentrations in both the experimental diets and fish flesh were also assessed by gas chromatography (GC) and high performance liquid chromatography (HPLC), respectively. In addition, skinned fillets from pan-sized coho salmon were sampled for the determination of effects of dietary treatments on various sensory attributes. The degree of pigmentation of raw fillets was analyzed using the Roche Color Card (RCC) for Salmonids, SalmoFan (SF), and a Hunter Lab Labscan. The texture of cooked fillets was assessed via a Texture Analyzer using the Texture Profile Analysis (TPA). Intensities of salmon aroma, salmon flavor, off-flavor, texture,

and the overall acceptability of cooked fillet samples were evaluated using quantitative descriptive analysis by a group of 11 trained panelists. Although not significantly different (p > 0.05), results from the growth study showed that as a general trend, the coho salmon fed the diets contained the higher levels of lipid (23 - 30 %) exhibited improved feed efficiency, protein efficiently ratio, percent protein deposition, and percent gross energy utilization. Also, as a general trend (p > 0.05), the diets containing higher protein content supported better growth than those that had lower protein content. However, fish fed the lower protein level (37%) diets generally (p > 0.05) demonstrated higher protein efficiency ratio, % protein deposition, and gross energy utilization. Colorimeter studies showed that fish fed the diets with the high levels of lipid (23% or 30%) generally had higher values for Hunter a, Hunter b, chroma, and astaxanthin content in raw flesh. Color intensities also increased (p < 0.05) with increasing fish size. Texture profile analysis failed to show any significant difference among cooked fillets from salmon given the different dietary treatments; however, the analysis indicated that salmon generally (p > 0.05) become softer with increasing size. Sensory assessments by sensory panel revealed that fillet from fish fed the diet with high protein and high lipid content had significantly (p < 0.05) greater salmon flavor and softer texture than the fillets from those fed the control diet. The intensities of salmon aroma, off-flavor, and overall acceptability of fillets were not affected (p> 0.05) by dietary treatment as were detected by the sensory panel.

Table of Contents

List of Tables

List of Figures

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1. Introduction

Feed accounts for 35%-60% of the operating costs of salmon farms; moreover, the protein sources account for 50%-66% of the cost of the feed. In order to decrease the operating costs of salmon farms, one strategy is to decrease the protein fraction of salmon feeds as much as possible by incorporating more non-protein energy in the diet in the form of lipid and to a much lesser extent carbohydrate (see below). Currently, the Pacific salmon grower diets that are manufactured by EWOS Canada Ltd. contain about 45% protein and 18% lipid. A reduction in the protein content from 45% to 38%-40% by concurrent elevation of dietary lipid content from 18% to 30%-33% could lead to an approximately $50 of saving per tonne of feed.

Presently, the dietary levels of protein and lipid (45% and 18%, respectively) were chosen based on previous nutrition studies conducted on juvenile Pacific salmon, or on salmon that were still at the fresh water stage of their life history (Fowler, 1980; Cho, 1990; Higgs et al., 1995). Recent studies (Anderson et al., 1996; Einen and Roem, 1997) on large (>500g) Atlantic salmon suggest that high energy diets containing 20%-30% lipid result in the most cost efficient production of this salmon species. Wilson and Halver (1986) suggested that the growth of salmonids declined as they increased in body size. Consequently, less protein will likely be required in relation to their dietary energy requirements (NRC, 1993; Higgs et al., 1995). Information on the use of high energy diets for large Pacific salmon (>500g) is lacking, however.

Aside from the growth performance of salmonids, there are also other controversy issues regarding the effect of feeding salmonids with high fat diets. Although the feeding of high energy diets may lead to lipid deposition along the alimentary tract and to a decreased dressed carcass weight in salmonids (Silver et al., 1993; Arzel et al., 1994), Torrissen (1985) reported that deposition of astaxanthin in rainbow trout flesh was positively correlated to levels of fat in the diet. Alternatively, Sheehan et al. (1996) noted that increased dietary lipid

1

levels enhanced the degree of gaping of fillet muscle and resulted in a decrease in the overall appearance of the fillet. Andersen et al. (1997) also observed that fillets from trout fed a high fat diet had higher autolytic protease activity than those from fish ingesting a diet with lower fat content. In addition, they also reported that trout fed the high fat diet had softer flesh texture. Finally, depending on the dietary lipid sources, increased dietary lipid levels may lead to increased levels of polyunsaturated fatty acids (PUFAs) in fish fillets. Fillets containing high levels of PUFAs are more prone to lipid oxidation and thus can result in more intense fishy aroma and flavor (German and Kinsella, 1985; Hsieh and Kinsella, 1986; Fowler et al., 1994).

Hence, careful assessment of the optimal dietary ratios of digestible protein and energy is important for lowering the operating costs of salmon farms, promoting optimal growth performance, and improving the sensory qualities of market-size coho salmon. The objectives of this study were to (1) determine the effects of digestible protein to digestible lipid on weight gain and other performance characteristics of farmed coho salmon in ambient sea water, and (2) evaluate the effect of varying the levels of dietary protein and lipid on the flesh quality of pan-size coho salmon.

2. Literature Review

2.1. Growth of Salmonids

2.1.1. Energy Requirements of Salmonids

Energy is required for sustenance of life processes and maintenance of existing tissues in all living organisms (Cho and Kaushik, 1990). Due to the poikilothermic and ammoniotelic nature of fish and the aqueous environment in which fish live, basal metabolic energy requirements of salmonids have been estimated to be 10 to 30 times lower than those of domestic animals (Brett and Groves, 1979) and to be predominantly affected by water temperature and fish size. The energy requirement of fasting salmonids (genus *Oncorhynchus*) have been estimated to be 10 to 70 kJ $\cdot$ kg body weight$^{-0.82}$ $\cdot$ day^{-1} (Cho and Kaushik, 1990; Kaushik and Gomes, 1988; Kaushik and Médale, 1994) at temperatures ranging from 7.5 to 20.0 °C.

Maintenance energy requirements have been found to be almost two-fold higher than fasting metabolic rates (Kaushik and Gomes, 1988). Further, the maintenance energy requirement of rainbow trout (*Oncorhynchus mykiss*) has been reported to vary between 85 to 110 KJ $\cdot$ kg^{-1} $\cdot$ day^{-1}. Storebakken *et al.* (1991) reported that 60 KJ $\cdot$ kg^{-1} $\cdot$ day^{-1} were required for maintenance of rainbow trout at 15 °C.

A positive energy balance is essential for growth. The dietary energy requirement for growth is, however, in excess of the amount of chemical potential energy that is required to synthesize tissues in the form of protein and lipids. A portion of dietary energy is utilized for the synthetic processes involved in the formation of the compounds making up the new tissue. Storebakken *et al.* (1991) suggested that the daily energy requirements for maximum growth of salmonids range from 270 to 320 kJ $\cdot$ kg^{-1} $\cdot$ day^{-1}. Kim and Kaushik (1992) stated that the digestible energy requirement per unit weight gain of rainbow trout was 17.5 MJ $\cdot$ kg^{-1} weight gain. Cho and Kaushik (1990) suggested that a digestible energy level between 14 and 17 MJ $\cdot$ kg^{-1} feed is required for production diets for salmonids. In addition, it was also

found that young fish require less energy per unit weight gain than older fish. Values of 10 and 18 KJ • kg^{-1} gain were required for fry and large fish (5 kg), respectively (Cho, 1990).

A diet that will support a rapid rate of growth supplies not only sufficient dietary energy, but also a balanced supply of energy from carbohydrate, protein, and lipid sources.

2.1.2. Carbohydrate Requirements of Salmonids

Although diets of salmonids in freshwater can contain reasonable high levels of carbohydrate (Higgs et al., 1995), studies of the stomach contents of salmonids in seawater (Embody and Gordon, 1924) suggested that wild salmonids consumed very little carbohydrate in their natural diets. Their energy needs are met largely by the high levels of protein and lipid in their prey. For the aquaculture industry, it would be more economical to replace a portion of the dietary protein and lipid energy with energy from carbohydrates. The ability of salmonids to use digestible carbohydrate as a major source of dietary energy is, however, somewhat controversial.

2.1.2.1. Digestibility of Carbohydrates

Early studies suggested that salmonids have limited capacity to digest carbohydrates. Digestion coefficients of less than 40% for crude starch were reported by Singh and Nose (1967), Palmer and Ryman , (1972), Bergor and Brèque (1983), ánd Spannhof and Plantikow (1983). However, it appears that the digestibility of carbohydrates depends upon the nature or complexity of the carbohydrate source (Bergot, 1979). Bergot and Brèque (1983) suggested that starch digestibility can be improved through technological treatments such as gelatinization and high temperature extrusion.

A number of studies have been conducted in which gelatinized starch has been incorporated into the diets of juvenile trout (15 to 100 g) held in 15 to 18 °C freshwater (Kaushik and Oliva-Téles, 1985; Kaushik et al., 1989; Kim and Kaushik, 1992). The results

from these experiments showed that weight gain, feed:gain ratio, and daily growth index of fish were not affected by the levels of digestible starch in the diets. In addition, protein and energy retention efficiencies were improved by increasing the levels of gelatinized starch in the diets.

2.1.2.2. Glucose Utilization of Salmonids

Salmonids have high absorptive capacity for glucose, which is the principal product of carbohydrate digestion. Plasma glucose concentrations are known to increase rapidly in response to ingestion of a meal high in glucose (Brauge et al., 1995). Indeed, blood glucose concentrations have been shown to be related directly to the dietary level of digestible carbohydrate (Kim and Kaushik, 1992; Brauge et al., 1994). However, the available evidence indicates that salmonids have limited ability to utilize the absorbed glucose as an energy source.

In order for glucose to be converted to glycogen or be metabolized through the Embden-Meyerhof and pentose-phosphate pathways, glucose must be phosphorylated by hexokinase to form glucose-6-phosphate. Low levels of hexokinase activity in salmonid liver and skeletal tissues are believed to be partly responsible for the poor rate of glucose utilization in salmonids (Knox et al., 1980). Failure to control plasma glucose concentrations leads to prolonged hyperglycemia (Palmer and Ryman, 1972; Bergot, 1979; Mazur et al., 1992). Hilton and Atkinson (1982) reported that plasma glucose concentrations of rainbow trout remained unchanged when diets contained more than 15% of digestible carbohydrates. Enlarged livers have also been observed (Hilton and Dixon,1982; Hilton et al., 1982) due to excessive liver glycogen accumulation. The liver is important for in the detoxification of noxious substances in the body. Hilton and Dixon (1982) and Dixon and Hilton (1985) observed a relationship between the duration of unconsciousness of trout following

administration of anesthetics and liver glycogen concentration when more than 20% of the diets were composed of digestible carbohydrate.

Nevertheless, salmonids are capable of metabolically utilizing a low level of digestible carbohydrate. In this regard, Higgs *et al.* (1995) recommended that the diets of salmonids should contain no more than 15% digestible carbohydrate.

2.1.3. Protein and Amino Acids Requirements of Salmonids

As carnivores, salmonids are well adapted to use protein not only as a source of amino acids for growth, but also as an energy source (Cho and Kaushik, 1990; Luquet and Watanabe 1986). Thus, they require as much as 30 to 60 % protein in their diet for growth and to provide adequate energy (Kim, 1997). In the wild, where dietary carbohydrate is less abundant, salmonids derive most of their needs for glucose through the process of gluconeogenesis. Alanine, serine, and glycine together with lactate and glycerol are used as substrates for gluconeogenesis and the glucose that is formed is used for red blood cells, nervous tissue, and gonads (Walton 1985). As mentioned above, dietary protein is needed not only for energy, tissue repair and maintenance, but also adequate amounts of protein are essential to support growth and protein synthesis - the optimal goal of the aquacultural industry (Hardy, 1991).

Limited information is available concerning the amount of dietary protein needed to support basal metabolism of salmonids. A range of 1.25 to 2.60 g protein $\cdot$ kg^{-1} body weight $\cdot$ day^{-1} has been established for a number of salmonids species at various stages of their life history (Brett and Zala, 1975; McCallum, 1985; Kaushik and Gomes, 1988). McCallum (1985) and Cowey and Luquet (1983) suggested that the maintenance protein requirements increase with rising water temperature due to elevation of the metabolic rate of salmonids.

The dietary protein requirements of salmonids for maximum growth in freshwater have been extensively studied and summarized by Higgs *et al.* (1995). The requirement is

known to be influenced by a number of factors, including the size of the fish, dietary energy content, and the availability of individual amino acids from the dietary ingredients (Wilson, 1989).

Rainbow trout requires 35 to 45% digestible protein in their diet on a dry weight basis for maximum growth provided that the levels and sources of the other energy yielding nutrients are optimal and the quality of protein is excellent (Cho, 1990). A range of 35 to 47% digestible protein is required in the diets for chinook and coho salmon (*O. tshawytscha and O. kisutch*) (Fowler, 1980; Clarke and Higgs, 1984, as cited by Higgs *et al.*, 1995). Diets for chum salmon (*O. keta*) should contain a range of 38 to 43% digestible protein to promote maximum growth (Akiyama *et al.* (1981), as cited by Higgs *et al.*, 1995).

There is little information on the dietary protein requirements of salmonids in seawater. Ogata and Konno (1985) reported that smolt production of cherry salmon (*O. masou*) improved by 2 to 3 fold when the protein level in the diets was increased from 24 to 41 %. Studies on rainbow trout (Lall and Bishop, 1976; Zeitoun *et al.*, 1973) and post-juvenile chinook salmon (Archdekin *et al.*, 1988) suggested that more dietary protein was required to support osmoregulation in seawater. However, Silver *et al.* (1993) showed that chinook salmon in seawater require 41 to 43.5% digestible protein in their diets for maximum growth. Moreover, they found that the dietary need of chinook salmon in seawater for digestible protein in relation to digestible energy (21 to 23 g • MJ^{-1} digestible energy) was similar to the requirement established for chinook salmon in freshwater (23 to 25 g • MJ^{-1} digestible energy). Silver *et al.* (1993) suggested that it was energy, not protein, that was required in greater amount to support osmoregulation of salmonids in seawater. In general, the dietary protein requirement of fish declines as they increase in size (Wilson and Halver, 1986). Current recommendations from the National Research Council (NRC, 1993) suggest that as much as 50 % protein is needed in the diet to maximize growth during the very early stages in the life of a salmon and subsequently the protein requirement declines to about 40

% at about one year of age and then further declines to about 35 % for salmon older than one year.

The dietary protein requirement of salmonids is strongly influenced by the level of digestible energy in the diet. Akiyama *et al.* (1981), for example, reported that the optimal level of dietary protein for chum salmon fry increased from 38 to 43% when dietary lipid content was decreased from 10.9 to 5.5% regardless of water temperature. In fact, salmonids generally eat to satisfy energy demands (Lee and Putnam 1973, Cho and Kaushik, 1985). Intake of protein is therefore regulated by the energy available in the diet. A proper balance of protein and energy in the diets is therefore essential to support optimal growth of fish. A deficiency of digestible energy in the diet as well as an excess amount of dietary protein can also compromise protein utilization and salmon growth since the amino acids will undergo deaminiation in order to support the energy requirement of salmonids. However, when fish are fed diets containing the same level of available energy but decreased levels of digestible protein, the utilization of dietary protein becomes more efficient (DeSilva *et al.*, 1991; Kim and Kaushik, 1992; Brauge *et al.*, 1994). The amount of digestible protein in the diet must therefore be considered in relation to the level and source of non-protein energy and the overall digestible energy content of the diet. Pike *et al.* (1990) suggested that optimal diets for growth of rainbow trout should contain 45 to 50 % metabolizable energy from protein in starter diets, 35 to 40 % for grower diets, and 40 to 45 % for broodstock diets.

A proper balance of amino acids in salmonid diets is essential for optimal growth of salmonids. Salmonids have the capability of modifying dietary amino acids to produce other amino acids; nevertheless, ten amino acids are known to be essential for maximum growth of salmon. These include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, and all of these amino acids need to be present in the diet in correct amounts and balance and in highly bioavailable forms. Cowey

(1979) stated that the dietary balance between essential and non-essential amino acids is also important to minimize the use of nitrogen from essential amino acids to synthesize non-essential amino acids. Although the dietary essential amino acid requirements of salmonids in fresh water are known for several species (Ogino and Nanri, 1980; Arai, 1981; Cowey, 1988; Kaushik *et al.*, 1988; Wilson 1989), little information is available concerning the essential amino acid needs of salmonids in seawater.

In general, it is believed that the essential amino acid profile in formulated diets should mirror the amino acid composition of fish eggs (Arai, 1981; Ogata *et al.*, 1983), fish muscle (Cowey and Luquet, 1983, as cited by Higgs *et al.*, 1995), and whole body tissue (Arai, 1981; Ketola, 1982; Wilson and Poe, 1985) of the species being studied. A study by Wilson and Cowey (1985) suggested that dietary essential amino acid requirements among fish species should be similar since the amino acid compositions of rainbow trout, Atlantic salmon (*Salmo salar*), coho salmon, cherry salmon, and channel catfish were not observed to vary significantly.

2.1.4. Lipid and Fatty Acids Requirements of Salmonids

Lipids serve as an important energy source in fish diets. The gross energy content per unit weight of lipid is much higher than values for carbohydrate and protein. The gross energy values for carbohydrate, protein, and lipid are 17.2, 23.6, and 39.5 KJ $\cdot$ g^{-1}, respectively (Tacon, 1987; Brett and Groves, 1979). Several studies have shown that the provision of adequate levels of dietary lipid can minimize the use of costly protein as an energy source (Takeuchi *et al.*, 1978; Cowey and Sargent, 1979; Watanabe, 1982). LeGrow and Beamish (1986) stated that an increase in dietary lipid level with a concomitant decrease in dietary protein content in the formulation of feeds could enhance growth efficiency by decreasing the energy expenditure for apparent heat increment. Dietary lipid inclusion levels of 15 to 20% of dry matter or 6 to 8 MJ of gross energy of lipid origin have yielded excellent

protein utilization and growth rates for juvenile rainbow trout, chinook salmon, and coho salmon (Lee and Putnam, 1973; Takeuchi *et al.*, 1978; Hilton *et al.*, 1982; Watanabe, 1982; Davies, 1989; Silver *et al.*, 1993). In general, dietary lipid concentration has been found to be positively correlated with values obtained in salmonids for protein efficiency ratio and net protein utilization.

Recent studies on Atlantic salmon in seawater (Pike, 1990; Johnsen *et al.*, 1995; Hillestad and Johnsen, 1994; Hemre *et al.*, 1995; Anderson *et al.*, 1996) showed that the use of diets containing as much as 30% digestible lipid maximized growth and feed efficiency. The growth rates of brown trout (*Salmo trutta*) have also been improved by increasing the dietary lipid level from 21 to 29 % (Arzel *et al.*, 1994). However, diets containing high levels of lipid were not found to be advantageous for rearing chinook salmon in seawater (Silver *et al.*, 1993 Weatherup *et al.* (1997) also reported that growth of rainbow trout (initial mean weight, 170g) was impaired when dietary lipid content was increased from 15 to 20 % even when the dietary protein level was maintained at approximately 44%. The researchers stated that, in some situations, high fat diets can improve feed conversion efficiency without sacrificing growth rate. Since fish in general eat to acquire sufficient energy, increasing dietary lipid in a diet may lead to subsequent decrease of total feed consumption. If the decrease in total feed consumption is too severe, dietary protein intake may be inadequate and lead to poorer growth.

It has been observed that diets containing more than 20% lipid on a dry weight basis have led to excessive increase in visceral fat content that was discarded after processing (Cowey and Sargent, 1979; Davies, 1989; Silver *et al.*, 1993; Arzel *et al.*, 1994). In addition, feeding diets containing over 20% lipid content to Pacific salmon during summer months when water temperature exceed 11°C, and when ration levels were high, have resulted in oil-water emulsions on the surface and sides of net pens. This suggests that some of the fat

10

from the high fat pellets was regurgitated by the fish and not fully digested and thus was discharged into the environment (Higgs et al., 1995).

Although the quantity of lipid in salmon diets is important as a non-protein energy source, the quality of the lipid is also of great importance. Salmonids do not possess the $\Delta 12$ and $\Delta 15$ desaturase enzymes that are required for the production of linoleic acid (C18:2ω6) and linolenic acid (C18:3ω3), the parent acids of the ω6 and ω3 families, respectively (Owen et al., 1975; Henderson and Tocher, 1987). Consequently, fatty acids of the linoleic and linolenic families must be supplied in the diet for normal growth, food conversion, and survival of salmonids (Yu and Sinnhuber, 1979; Watanabe et al., 1974; Watanabe, 1982). With the exception of rainbow trout, Arctic charr, coho salmon in fresh water and juvenile chum salmon in freshwater and seawater, knowledge of the quantitative needs for lipids and essential fatty acids by salmonids is limited (Cowey, 1992; Higgs and Dong, 1999). In addition, there seem to be notable differences between salmonid species in regard to their utilization of nutritional lipids and their needs for essential fatty acids (Takeuchi and Watanabe, 1982; Watanabe, 1982; Arzel et al., 1994).

Takeuchi et al. (1979) fed chum salmon, in a fresh water environment, diets that contained various levels of different fatty acids: C18:2ω6, C18:3ω3, C20:5ω3, and a mixture of C20:5ω3 and C22:6ω3 (ω-3 HUFA). The essential fatty acid (EFA) deficient diets resulted in poor growth, low feed efficiency, high mortality, and swollen, pale livers after two weeks of feeding. The addition of ω-3 fatty acids and / or ω-3 HUFA, to the EFA-deficient diets vastly improved growth and feed efficiency, and the supplemental effect of 0.5% C20:5ω3 and 0.5% ω-HUFA on the growth of chum salmon slightly exceeded that noted for salmon fed the diet containing 1% C18:3ω3 alone. The best weight gain and feed efficiency were obtained with the fish receiving the diet supplemented with both 1% C18:2ω6 and 1% C18:3ω3 or ω-3 HUFA. Similar results were obtained for rainbow trout in freshwater (Watanabe et al., 1974).

In addition, the requirements of chum salmon, held in both seawater and freshwater, for EFA were the same (Takeuchi and Watanabe, 1982). Yu and Sinnhuber (1979) reported that the optimum level of dietary ω-3 fatty acids for coho salmon in freshwater ranged from 1 to 2.5%.

Information on the dietary requirements of salmonids for ω-6 fatty acids is limited. Takeuchi et al. (1979) reported that chum salmon required 1% of $C18:3\omega3$ and 1% of $C18:2\omega6$ in the diet, while absence of ω-6 fatty acids in the diet did not affect growth of chinook salmon (Mugrditchian et al., 1981; Dosanjh et al., 1988), rainbow trout, coho salmon, and masu salmon (Watanabe, 1988). In fact, coho salmon fed diets containing more than 1% ω-6 fatty acids showed depressed growth rate and feed conversion efficiency (Yu and Sinnhuber, 1979).

2.2. Quality Attributes of Salmonids

The consumer's acceptance of fishery products depends on several attributes of food quality . The important attributes are color and appearance, flavor, and texture (Haard, 1992). Consumers also expect the sensory characteristics of cultured fish to be similar to those of free-living or wild fish.

2.2.1. Color and Appearance of Fish

2.2.1.1. Pigmentation of Salmonids

Color and appearance are particularly important to the market acceptability of fishery products. The distinctive pink or red flesh color of salmonids is especially essential for consumer identification and acceptance of the product. In wild salmonids, flesh pigmentation results from the deposition of oxycarotenoid in the tissue of the fish from their natural diet (Hata and Hata, 1975). Since salmonids are incapable of de novo synthesis of carotenoid pigments (Simpson, 1982), carotenoid pigments must be included in the diets of cultured salmon. Work has been done to utilize crustaceans, crustacean by-products and meal, and

pigment extracts (Saito and Regier, 1971; Spinelli et al., 1974; Spinelli and Mahnken, 1978; Arai et al., 1987; Torrissen et al., 1989), red yeast (Johnson et al., 1980; Gentles and Harrd, 1991), synthetic canthaxanthin (Schmidt and Baker, 1969; Bauernfeind, 1976), and synthetic astaxanthin (Torrissen, 1995) as pigmentation sources in salmonid diets.

In salmonids, two oxycarotenoids, astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) and canthaxanthin (β,β-carotene-4,4'-dione) are responsible for the red to orange coloring of the flesh, skin, and fins. Of the two synthetic pigments, astaxanthin is the predominant pigment and represents more than 90% of total carotenoids in the flesh of wild salmon (Khare et al., 1973; Schiedt et al., 1985, 1986; Skrede and Storebakken, 1986a). Astaxanthin is also more efficiently utilized for flesh pigmentation than canthaxanthin (Foss et al., 1984, 1987; Bjerkeng et al., 1990; Choubert and Storebakken, 1989, 1996; Torrissen, 1986; 1989).

Synthetic astaxanthin is the most important commercial source of astaxanthin (Carophyll Pink®, Hoffmann-La Roche, Basel, Switzerland). Synthetic astaxanthin is currently being supplemented in feeds at 35 to 75 mg per kilogram. The addition of astaxanthin to the feed raises its cost by 15 to 25 % depending upon the dosage. However, only up to 15% of the ingested astaxanthin is recovered from the muscle depending on the dose fed and the salmonid species (Torrissen et al., 1989; Storebakken and No, 1992). Work on the bioavailability astaxanthin may result in substantial savings.

Retention of carotenoids in fish tissues depends on absorption, transport, metabolism, and excretion of the carotenoid compounds (Torrissen et al., 1989). Absorption of astaxanthin occurs in the intestine by passive diffusion (Hollander and Ruble, 1978). Choubert et al. (1994) reported a linear response in blood plasma astaxanthin as the dietary concentrations of astaxanthin were increased over a range from 12.5 to 200 mg per kilogram of dry diet. Astaxanthin is carried by chylomicrons and very low density lipoprotein in blood (Choubert et al., 1987). Flesh is the major tissue for storing carotenoids, followed by

skin, gut, and liver (Hata and Hata, 1975; Sivtseva, 1982). Unmodified astaxanthin is retained in the white muscle (Pozo *et al.*, 1988) of sexually immature rainbow trout in its free form (Foss *et al.*, 1984). Henmi *et al.* (1987, 1989) reported that astaxanthin and canthaxanthin are non-specifically bound to actomyosin in the muscle of sockeye salmon by weak, hydrophobic bonds. They also suggested that the one β-ionone ring binds to a hydrophobic binding site on the surface of actomyosin. The hydroxyl and keto groups contribute to further stabilization of the complex by weak hydrogen bonds. Retention of dietary carotenoids in trout is known to range from 3 to 18% for astaxanthin and 2 to 7 % for canthaxanthin (Torrissen and Braekkan, 1979; Foss *et al.*, 1984; Choubert and Storebakken, 1989).

Several factors can affect the extent of flesh pigmentation of salmonids. It has been demonstrated that the degree of flesh pigmentation in rainbow trout (Torrissen and Naevdal, 1984, 1988; Gjerde and Gjedrem, 1984), Atlantic salmon (Gjerde and Gjedrem, 1984), coho salmon (Iwamoto *et al.*, 1990), and chinook salmon (McCallum *et al.*, 1987; Withler, 1987) is genetically determined. The time required to reach a carotenoid level of 6 mg per kilogram in the flesh varies notably with fish size and growth rate, but also with dietary carotenoid source (Austreng *et al.*, 1987). Röpke (1988) stated that fish weighing between 0.5 to 1.0 kg reach a satisfactory level of pigmentation if they increase their body weight by 30 - 50 %. March *et al.* (1990) observed that the intensity of flesh pigmentation in coho salmons fed a diet supplemented with astaxanthin was significantly correlated with body weight. A number of studies have shown that salmonids must reach some minimum body weight before pigmentation of their flesh can occur (Yarzhombeck, 1970; Spinelli and Mahnken, 1978; McCallum *et al.*, 1987; Torrissen, 1989). It has been observed that the carotenoid concentration in the flesh of immature trout did not increase further when the dietary pigment concentration exceeded 50 mg • kg^{-1} (Bjerkeng *et al.*, 1990). This was attributed to a decrease in carotenoid pigment digestibility as dietary pigment concentration increased

(Choubert and Storebakken, 1989; Torrissen *et al.*, 1990). Diet compositions also affect pigmentation. Digestibility coefficients for astaxanthin and canthaxanthin and carotenoid deposition in rainbow trout (Torrissen, 1985; Torrissen *et al.*, 1990) and Atlantic salmon (Einen and Roem, 1997) were observed to increase with increasing dietary lipid levels. Carotenoid pigment digestibility was also noted to be associated with the composition of lipid in the diet. Hardy *et al.* (1987), for example, observed that the carotenoid pigment concentration in the flesh of Atlantic salmon was lower when the dietary supplemental lipid source was tallow (high in saturated fatty acids) rather than menhaden oil (high in polyunsaturated fatty acids). In addition, an adverse effect of vitamin A on pigmentation in rainbow trout has been observed (Abdul-Malak *et al.*, 1975, as cited by Storebakken and No, 1992), while vitamin E was found to enhance cathaxanthin deposition in trout flesh (Pozo *et al.*, 1988).

2.2.1.2. Determination of Color and Appearance of Fish

There are two main methods of assessing the degree of pigmentation of salmon flesh: by chemically determining the astaxanthin concentration in the flesh, or by defining the color appearance. Definition of the color appearance can be done by subjective color perception or by characterization of the color by measuring its composition instrumentally (Christiansen *et al.*, 1995).

2.2.1.2.1. Quantification of Astaxanthin in Fish Flesh

Retention of carotenoid pigments in the flesh of salmonids can be determined chemically by acetone extraction (Foss *et al.*, 1984) or chloroform / methanol extraction (Kiessling *et al.*, 1995). Thereafter, identification and quantification of carotenoid pigments can be performed by spectrophotometry and chromatography. A range of molar extinction coefficients, from 1600 to 2500 $cm^{-1} \cdot M^{-1}$, were used to quantify astaxanthin in acetone in

the case of spectrophotometric quantification (Johnson *et al.*, 1976; Torrissen and Naevdal, 1984, 1988). Bjerkeng *et al.* (1997) reported that the molar extinction coefficients for all-*E*-astaxanthin, 13Z, 9Z, and di-Z-isomers are 2100, 1350, 1750, and 1900 $cm^{-1} \cdot M^{-1}$, respectively.

2.2.1.2.2. Determination of Color and Appearance of Fish by Color Cards

Methods used for visual determination of the flesh pigmentation of salmonids have included trained sensory panels and the use of color cards and tiles (Ostrander *et al.*, 1976; Little *et al.*, 1979). In the former, panelists have been asked to rank the color of fish fillet samples using a descriptive scale (Little *et al.*, 1979) or to differentiate the color differences of fish fillet samples using a triangle test (Skede and Storebakken, 1986a).

The first attempt to standardize the evaluation of color in salmon was conducted by Francis and Clydesdale (1975, as cited by Skrede *et al.*, 1990a). Bolton *et al.* (1967) later developed colored tiles to simulate the flesh color of canned salmon. The tiles were designed to be used under standardized conditions along with an opened can of salmon. To standardize the color evaluation for the aquacultural industry, color standards from the Natural Color System (NCS) were selected by a sensory panel to match the color of raw flesh of astaxanthin-fed Atlantic salmon (Skrede *et al.*, 1990a). The color card that was developed is commonly known as the Roche Color Card for Salmonids (Roche Vitamins and Fine Chemicals Division, Hoffman-LaRoche Inc., Basel, Switzerland) scale 11-18. A visual score of 13 or greater is considered to be an intensity of color suitable for marketed fish (Smith *et al.*, 1992).

As mentioned above, the Roche Color Card (RCC) was developed for the assessment of flesh pigmentation of astaxanthin-fed Atlantic salmon and Christiansen *et al.* (1995) reported that the RCC provided a good prediction of the astaxanthin concentration in the flesh of Atlantic salmon fed diets containing various levels of astaxanthin. Smith *et al.*

(1992) also reported that visual assessment of flesh color of pan-size coho salmon using the RCC correlated well with the astaxanthin concentration in the flesh. In 1998, a new set of color standards known as the SalmoFan™ (SF) was developed to assess the degree of flesh pigmentation in salmonid species. The new set of color standard provides a color scale from 20 to 34 and should allow a more accurate assessment of pigmentation.

2.2.1.2.3. Color Assessment by Instrumental Color Analysis

Color is a matter of perception. Several color systems have been developed to express all color within the range of human perception into common numerical codes (Hunt, 1977). The tristimulus R, G, B values are the primary system developed to measure color. It is believed that any color of the visible spectrum can be matched by mixing together three different wavelengths of light in different proportions. The amounts of red, green, and blue lights required to simulate the color of a test sample are recorded as R, G, and B values, respectively. However, negative R, G, and B values are often obtained using the above system. Manipulations of the R, G, and B values have resulted in the X, Y, Z tristiumulus values that could be use to eliminate the negative values.

Transformations of the X, Y, Z tristimulus values result in the L*, a*, and b* values used in the CIE Color Space system and the Hunter L, Hunter a, and Hunter b values used in the Hunter Lab systems. These two systems were introduced to describe color attributes that are independent of brightness (Hunt, 1977) and they have been used to determine the extent of flesh pigmentation of salmonids (Schmidt and Idler, 1958; Saito, 1969; Skrede and Storebakken, 1986b; No and Storebakken, 1991a, b; Smith *et al.*, 1992; Christiansen *et al.*, 1995). In these systems, salmon color may be described by the parameters L, a, and b where L represents lightness, a redness and b yellowness of the sample. The ratio of redness and yellowness ($\tan^{-1}$ (b*/a*) or $\tan^{-1}$ (b/a)) in the fish flesh can be expressed as hue, H°_{ab}; the level of saturation of the color can be represented by chroma, C_{ab} (($a^2+b^2)^{1/2}$).

Using a Gardner automatic colorimeter, Schmidt and Idler (1958) observed that redness (a value) was proportional to the carotenoid content of sockeye salmon before and after canning. Saito (1969) also reported similar results in Atlantic salmon. The a value was found to correlate well with carotenoid concentration in the flesh of rainbow trout (No and Storebakken, 1991a, b) and coho salmon (Smith *et al.*, 1992). In addition, the a value can be use as a predictor of the flesh color of Atlantic salmon (Skrede and Storebakken, 1986a, b; Christiansen *et al.*, 1995). Yellowness (b value) of salmon flesh was found to increase with increasing concentrations of astaxanthin in the flesh (No and Storebakken 1991a, b; Smith *et al.*, 1992; Christiansen *et al.*, 1995). Lightness (L value) of the fish flesh is also affected by concentration of astaxanthin; L values decrease with increasing astaxanthin level (No and Storebakken 1991a, b; Skrede and Storebakken, 1986a; Smith *et al.*, 1992; Christiansen *et al.*, 1995). Finally, Schmidt and Idler (1958) and Skrede and Storebakken (1986a) reported that hue was the best predictor of processed flesh color from raw flesh while Christiansen *et al.* (1995) found a low correlation between hue and the actual astaxanthin content. Instrumentally measured color has also been found to be highly correlated with values obtained from sensory tests (Skrede *et al.*, 1990a) and color cards (Skrede *et al.*, 1990b).

2.2.2. Texture of Fish

The flesh texture of salmonids is an extremely influential determinant of preference of fish (Wesson *et al.*, 1979). Szczesniak (1990) defined texture as reactions of a food structure to a applied force in the mouth on manipulation and mastication.

2.2.2.1. Factors Affecting Texture of Raw Fish

The texture of fish muscle is affected by the species, age and size of the fish within the species, and nutritional state (Hatae *et al.*, 1984, 1990; Ghittino, 1972; Hurling *et al.*, 1996). Treatments after slaughtering also have an important impact on texture and

consumer acceptability of fish meat (Dunajski, 1979; Montero and Borderias, 1990; Nilsson and Ekstrand 1993).

Unlike red meat, connective tissue in fish muscle does not play an important role in the texture of fish (Dunajski, 1979). Consequently, collagen fibrils have been demonstrated to play an important role in defining raw fish meat texture. The presence of collagen maintain toughness and the integrity of the fish muscle (Bremner and Hallett, 1986; Love, 1988; Ando et al., 1991). Sato et al (1991) and Hatae et al. (1986) examined many fresh water and marine fishes and found that the higher the collagen content the less tender the raw fish meat.

Gaping is a textural problem that is difficult to detect before filleting. Gaping in raw fish muscle occurs when links between the muscle fibers and collagen fibrils break and the muscle cells separate (Love, 1988). Gaping of raw blue grenadiers (*Macruronus novaezelandize*) (Bremner and Hallett, 1985), rainbow trout (Dawood et al., 1986; Ando et al., 1991) and Atlantic salmon (Andersen et al., 1994) increase with storage time on ice. Montero and Borderias (1990) found that the solubility of collagen and protease activity in trout (*Salmo irideus*) increased considerably within 72 hours after death. Using a transmission electron microscope, Hallett and Bremner (1988) found that the majority of muscle fibers in blue grenadier were detached after 11 days of storage. Nilsson and Ekstrand (1993) reported that ice storage induced enzyme leakage from muscle tissue of rainbow trout. Negative correlation between protease autolytic activity and texture (Andersen et al., 1997) indicated that an increase in autolytic activity gives a softer texture. Andersen et al. (1997) also reported that autolytic protease activity was higher in the muscle of fish fed higher fat diets than those fed diets with lower fat content.

The extend of gaping has also been found to be related to the content of glycogen in muscle. Love (1988) reported that high content of glycogen in cod (*Gadus morhua*) muscle resulted in low post-mortem pH and increased gaping. In salmonids, gaping correlated

positively to fat content in fish fillet (Stefanusen, 1986; Thorsen, 1989; as cited by Andersen *et al.*, 1997). Muscle from Atlantic salmon (Andersen *et al.*, 1994) and rainbow trout (Andersen *et al.*, 1997) fed diets with higher fat content demonstrated less resistance against compression when tested by texture measurements, indicating a softer consistency. Andersen *et al.* (1994) also reported that fillets without gaping had significantly higher protein content than fillets with gaping.

2.2.2.2. Factors Affecting Texture of Cooked Fish

Collagen of fish is thermally denatured during cooking and as a result generally has very little influence on cooked fish texture. Hatae *et al.* (1990) reported that there was no relation between collagen content and tenderness in cooked fish meat. The texture of muscle after cooking is more a consequence of the state of the myofibrilllar protein (Dunajski, 1979).

Kanoh *et al.*,(1988), Hatae *et al.* (1984, 1990), and Hurling *et al.* (1996) reported an inverse correlation between fiber diameter and sensory firmness. Hurling *et al.* (1996) suggested that this relationship is valid for all fish species. Hatae *et al.* (1990) also observed that species with firm texture had considerably higher levels of coagulated protein between the muscle fibers. When fish muscle is cooked, sarcoplasmic protein is released from the contracting muscle fiber and is coagulated in the interstitial spaces. When the cooked tissue is compressed, the heat-coagulating protein obstructs displacement of the fibers, resulting in firmer texture.

Little work has been conducted to examine the effects of dietary composition on the texture of processed fish. Ghittino (1972) suggested that the composition of the diet fed to trout influences the sensory qualities of the fish flesh, with dry diets producing firm flesh with an acceptable flavor and the traditional wet diets yielding softer flesh with a higher fat content. Studies have shown that the lipid content in fish flesh increases with increased

dietary lipid content. Dunajski (1979) stated that water and lipid decreased the structural factors of muscle tissue, lowering its mechanical strength. Thus, the tenderness of muscles should be greater in fatty fish species containing more fat, while in lean species tenderness increases with water content. Ghittino (1972, as cited by Seurman et al., 1979) reported that trout fed wet diets had higher muscle fat content and that this yielded softer flesh. Orban et al. (1996) also observed that cultured sea bream (Sparus aurata) had higher lipid concentration and slightly softer flesh texture than their wild counterparts. When rainbow trout were fed diets containing different levels of fat (9% and 12 %), lipid content and texture of the cooked fillets remained unaffected (Seurman et al. 1979). On the other hand, Sheehan et al. (1996) reported that smoked fillets from fish fed diets containing higher fat content had significantly firmer texture. They also found that smoked fish fillets from fish fed diets with medium (25%) and high (30%) fat content showed a two-fold increase in gaping over that observed in the fillets from fish fed the diet with the lowest fat (20%) content.

The composition of fatty acids accumulated in muscle lipid was also found to affect the texture of fish meat. Lipids of vegetable origin were noted to diminish the textual quality of brown trout (Arzel et al., 1993, as cited by Andersen et al., 1997) and of Atlantic salmon (Thomassen and Røsjø, 1989). However, no differences were observed when rainbow trout (Boggio et al., 1985), brook charr (Guillou et al., 1995), channel catfish (Morris et al., 1995), and Atlantic salmon (Hardy et al., 1987; Koshio et al., 1994) were fed diets containing lipids from various sources.

2.2.2.3. Measurements of Fish Texture

The texture of a raw fish fillet is commonly examined in the industry by the finger test (Botta, 1991), while sensory panels are often employed to assess texture of cooked fish muscle (Borderías et al., 1983; Sawyer et al., 1988; Hatae et al., 1996; Sigholt et al., 1997). Instrumental analysis is suitable to assess the texture of both raw fish (Botta, 1991; Andersen

et al., 1994, 1997) and cooked fish (Johnson *et al.*, 1980; Bordeías *et al.*, 1983; Dunrance and Collins, 1991; Reid and Durance, 1992).

Regardless of which method is chosen to conduct a textural assessment, it is important to obtain muscle samples from the same location on different fillets. Collagen and fat are distributed unevenly along a fish fillet (Aursand, 1994) and the texture of a fish varies at dissimilar locations of the fish muscle. Limited information is available in the literature on differences in texture measurements taken at different locations on fillets (Botta, 1991). The emphasis is that the sample be representative of the whole fish fillet. In this regard, Azam *et al.* (1989) and Botta (1991) suggested that measurements of fish texture should be made at three locations along the fillet and then the mean from the three measurements should be used.

2.2.2.3.1.   Determination of Fish Texture by Finger Test

The texture of raw fish fillet is commonly examined by the "finger test" under commercial situations (Botta, 1991). The examiner will press a finger on the skin of the fillet and the firmness and hardness of the fillets is then evaluated. The examiner will also observe the mark or hole left in the fillet after pressing. Although this method is rapid and non-destructive, it depends largely on the skill and experience of the person performing the test.

2.2.2.3.2. Determination of Fish Texture by Instrumental Analysis

Textural properties of a food are considered to be the mechanical characteristics resulting from pressure exerted on the teeth, tongue, and roof of the mouth during transportation and preparation of the food in the mouth (Szczesniak, 1963). A number of devices have been developed to assess the mechanical properties of a food sample. The main techniques applied for fish may be divided into compressing (Bourne, 1978; Johnson *et*

22

al., 1980; Botta, 1991), shearing (Kramer et al., 1951; Möller, 1980-1981), and pulling (Weinberg, 1983; Weinberg and Angel, 1984). Mechanical properties including shear resistance, hardness, firmness and tensile resistance can be measured from the information extracted from the force-deformation curves when the food is under stress (Barroso et al., 1998).

Another technique commonly used to measure the texture properties of fish is the Texture Profile Analysis or TPA. TPA compresses a fish sample twice in a back-and-forth movement which imitates the movement of the jaw during mastication. The force-deformation curve is analyzed to determine a number or texture parameters. These include maximum force at first and second compression cycles considered as hardness, and ratio of the force areas under the first and second compression considered as cohesiveness (Peleg, 1976; Bourne, 1978). Firmness can be measured as the maximum slope of the first peak (Durance and Collins, 1991).

Results from these tests vary depending on the locations of the fish sample and on the segmentation and orientation of the fillet structure during the examination (Borderías et al., 1983). Reproducibility of texture measurements can be improved if minced fish is used (Borderías et al., 1983; Botta, 1991; Reid and Durance, 1992) because of increased homogeneity of the sample. Nevertheless, Barroso et al. (1998) stated that unless information about the mince is requested, texture analysis should be performed on a fillet or a part of a fillet that represents the entire fish. In addition, if a cooked fish fillet is analyzed, thermal denaturation of collagen brings about a total loss of binding properties of the connective tissue and the myotome layers can slip apart very easily when compressed in the texturometer (Dunajski, 1979; Borderías et al., 1983; Hatae et al., 1990). As a result, the resistance of the fibers to mechanical disintegration should be measured. Barroso et al. (1998) suggested that single flakes should be used to analyze texture of cooked fish.

2.2.3   Aroma and Flavor of Fish

According to Rutledge and Husdon (1990), flavoring compounds can be segregated into three major categories, namely aromatics compounds that are released from food in the mouth, soluble substances that stimulate the taste buds, and lastly other chemical feeling factors. A study of red sea bream (Konosu and Watanabe, 1979) revealed that the taste active compounds in fish included free amino acids, peptides, organic acids, quaternary ammonium bases, and minerals. The concentration of these components were also found to be higher in wild fish compared to cultured fish. Wild yellowtail (Endo *et al.*, 1974), sea bream (Konosu and Watanabe, 1979), and red drums (Jahncke *et al.*, 1988) were found to have stronger flavor than their cultured counterparts. Hatae *et al.* (1989) noted that cultured red sea bream, flounder, and yellowtail had inferior taste and aroma to the respective wild fish in preference tests. Ostrander *et al.*, (1976) also noted that significant differences in aroma and flavor were detected among salmon and trout obtained from different pen-rearing environments.

Characteristic aroma compounds in fresh fish are derived from polyunsaturated fatty acids by enzyme catalyzed reactions (Josephson *et al.*, 1984). Actions of 12- and 15-lipoxygenases on specific fatty acids were responsible for the development of fresh fish aroma (Josephson *et al.*, 1984). However, lipid oxidation has also been found to contribute to a rapid deterioration of quality, producing fishy, rancid, or other undesirable sensory characteristics (German and Kinsella, 1985; Hsieh and Kinsella, 1986; Fowler *et al.*, 1994).

Since the fatty acid composition of fish tissue generally reflects dietary fatty acid composition (Dosanjh *et al.*, 1984; Yu and Sinnhuber, 1979; Hardy *et al.*, 1987; Bakir *et al.*, 1993), flavor and aroma of a fish can be influenced by the dietary lipid sources (Waagbø *et al.*, 1993). Lovell (1988) reported that fishy flavor has been noted in catfish fed diets containing high levels of menhaden oil. However, Dupree *et al.* (1979) and Morris *et al.* (1995) did not find that addition of menhaden oil into catfish diets had any adverse effect on

the sensory parameters related to the flavor and aroma of the fish. White amur fed diets containing higher levels of monounsaturated fatty acids had less desirable flavor (Bakir *et al.*, 1993).

Several studies have examined the effects of dietary lipid source on sensory characteristics of salmonids. Lipids of vegetable origin were found to diminish the sensory qualities of brown trout (Arzel *et al.*, 1994) and Atlantic salmon (Thomassen and Røsjø, 1989). However, such differences could not be detected in other studies involving rainbow trout (Boggio *et al.*, 1985) and Atlantic salmon (Hardy *et al.*, 1987; Koshio *et al.*, 1994). In a study conducted by Skonberg *et al.* (1993), a significant number of panelists were able to differentiate between fillets from the dietary treatments based on aroma. Fillets from trout and coho salmon fed a diet containing herring oil had a fishier aroma than those from fish fed a diet containing sunflower oil. Because of the high monounsaturated fatty acid content of sunflower oil, sunflower oil was less prone to lipid oxidation and the panelists also preferred fillets from fish fed the sunflower oil treatment compared to fillets from fish fed the herring oil containing diets.

2.2.3.1. Determination of Sensory Attributes by Sensory Panel

Sensory assessment of a fish sample involves panelists use judging attributes such as appearance, flavor, odor, and texture (Botta, 1995). A number of tests including the triangle test (Skonberg *et al.*, 1993; Sigholt *et al.*, 1997) and the ranking test (Sheehan *et al.*, 1996) have been used to assess the intensities of texture, oiliness, and color of salmon fed diets with various fat contents.

Descriptive scales have been used by Ostrander *et al.* (1976), Reid and Durance (1992), Morris *et al.* (1995), and Sigholt *et al.* (1997) for scoring cooked fish samples for a variety of attributes. A number of descriptive terms have been used including tough, firm, flaky, soft, and mushy for texture (Ostrander *et al.*, 1976; Simeonidou *et al.* 1997), salmon

odor, seaweed-like, stale, sour, strong ammonia, and fecal for odor (Ostrander *et al.*, 1976; Simeonidou *et al.*, 1995), putrid, astringent, fishy, boiled chicken, and earthy for off-flavor (Morris *et al.*, 1995), and sweet, meaty, neutral, rancid, bitter, and woody for the taste of fish (Ostrander *et al.*, 1976; Simeonidou *et al.*, 1997). These terms have been equally and evenly anchored along the scales. Each judge records an evaluation by making a vertical line across the horizontal line at the point that best describes her/his perception of the magnitude of the attributes. On an unstructured interval scale, words describe only the two different extremes of the attribute being assessed and are placed or anchored at or near opposite ends of a horizontal line. The words reflecting the most intense aspect of the attribute should be placed at of near the right end of the line. Unstructured scales, with verbal anchors at the ends only, eliminate the problem of unequal intervals that are associated with structured scales (Stone *et al.*, 1974; Larmond, 1977).

3.   Materials and Methods

3.1.   Experiment Facility

On May 9, 1997, approximately 2000 coho salmon (*Oncorhynchus kisutch*) were transferred from Target Marine, Sunshine Coast, B.C., to the West Vancouver Laboratory, Fisheries and Oceans Canada ($49^0$15'N, $123^0$10'W). All growth experiments were then conducted in the latter location. The fish were transferred to salt water on May 22, 1997.

A total of 945 coho salmon that had been selected for uniform size (40 to 60 g) were randomly and equally distributed into twenty one 4000 L circular outdoor fiberglass tanks at the West Vancouver Laboratory. Each tank was supplied with flowing (25 to 40 L • min^{-1}), aerated (DO, 7.1 to 9.2 mg • L^{-1}), ambient temperature (7.8 to 10.9 °C), and filtered seawater (salinity, 30 to 35 %). The water was delivered to circulate around in each tank so that the fish were forced to swim against a current (surface water velocity, 10 cm • sec^{-1}) to minimize their aggressive behavior. Supplemental aeration provided by compressed air passed through a diffuser stone was also used in each tank.

Fish were allowed to acclimate to the tanks for 61 days before the beginning of the experiment. During the acclimation period, the fish were fed by hand twice daily with the "Vextra" control diet (EWOS Canada Ltd., Surrey, B.C.). Starting from October, 1997, triplicate groups of 45 coho salmon (range in initial mean weight of the groups, 93.7 to 124.4 g) were each given one of seven dietary treatments using a randomized block design.

3.2.   Diet Formulations

Six of the extruded dry diets were formulated to contain one of two levels of digestible protein (37% or 44%) and one of three levels of digestible lipid (16%, 23%, or 30%) on a dry weight basis (Table 1). In each experimental diet, equal portions premium quality fish meal from two sources were used as the main source of protein. The fish meal protein was 92% digestible. Menhaden oil was used as the main lipid source and this was

Table 1. *Ingredient composition of diets (g • kg^{-1} DM) used to evaluate the optimal dietary ratio of digestible protein to digestible lipid for rearing coho salmon in seawater.*

Ingredients	Diets					
	1	2	3	4	5	6
High Quality Fish Meal Source I	265.51	265.51	265.51	315.75	315.75	315.75
High Quality Fish Meal Source II	266.21	266.21	266.21	316.57	316.57	316.57
Whole Wheat	73.65	73.65	73.65	89.58	89.58	89.58
Pregelatinized Wheat Starch	260.08	182.30	104.53	154.46	76.68	0.00
Vitamin Supplement[1]	5.40	5.40	5.40	5.40	5.40	5.40
Mineral Supplement[1]	4.10	4.10	4.10	4.10	4.10	4.10
Menhaden Oil	119.72	197.50	275.27	108.81	186.59	263.27
Choline Chloride (60%)	3.33	3.33	3.33	3.33	3.33	3.33
Astaxanthin Premix (2%)	2.00	2.00	2.00	2.00	2.00	2.00

[1] The vitamin and mineral supplements ensured that all diets met the known vitamin and mineral needs of salmonids.

28

90% digestible. The fish meal sources and the whole wheat were adjusted proportionately between the diets containing 37 % or 44% protein to maintain equivalent essential amino acid balance. Moreover, levels of pregelatinized wheat starch were varied to minimize differences between diets in digestible energy content. Equal levels of vitamin and mineral supplements were included in all experimental diets to ensure that the vitamin and mineral needs of the salmon were met. Choline chloride and an astaxanthin premix were added as a source of choline and carotenoid pigment.

3.3. Feeding and Sampling

All groups were fed their prescribed diets by hand twice daily to satiation. The numbers of waste pellets were counted manually in order to accurately estimate daily feed intake. Fish were sampled every 28 days for a total of 168 days. Twenty four hours before each sampling, feed was withheld from each group. The weights and fork lengths of all fish in each group were measured individually following the dual anesthetic procedure of Krieberg and Powell (1991). In this regard, 0.25 ppm of Marinil® were used to sedate the fish followed by 60 ppm of MS222® for complete anesthesia. Accurate records of actual rations ingested, mortality, and water quality parameters were kept daily. Percent mortality between Day 0 and 168 was 11.5% of the total number of fish at the start of the study. Most mortality was ascribed to a low incidence of bacterial kidney disease and the pattern of mortality was random and uninfluenced by diet treatments.

3.4. Chemical Analyses

3.4.1. Sample Collection and Preparation

On the initial day of the experiment, ten fish common to all groups were randomly taken for determination of whole body proximate composition. Right fillets of ten other fish were also sampled for determination of muscle proximate composition. Both whole body and

muscle proximate compositions were analyzed in five composite samples of two fish each.

On the final day, Day 168, of the growth experiment, six to seven fish from each replicate group per diet treatment were sampled for determination of whole body proximate composition. In addition, skinned right fillets from another eight to ten fish were collected for determination of muscle proximate composition. Individual fish samples were used to determine the whole body proximate contents and composite samples of two fish each were used to determine the muscle proximate content. The skinned left fillets of the latter fish were also taken for astaxanthin determination.

Whole fish, right fillets, and left fillets were stored on ice immediately after killing. Within 2 hours of killing, the samples were separately vacuum packed in air impermeable bags (O_2 transmission; 2.3 cc • m^{-2} • 24 $hour^{-1}$, H_2O transmission; 7.8 g • m^{-2} • 24 $hour^{-1}$) and kept at -40 ^0C prior to chemical analyses. At the time of analysis, the samples were partly thawed, chopped into pieces, and thoroughly homogenized in a food processor (Braun$^®$K1000, type 3210). Samples of the homogenate were then analyzed in duplicate.

3.4.2. Ash and Moisture Determinations

Proximate analyses of whole fish and fillets were conducted as described by Higgs *et al.* (1979). Homogenized fish and diet samples were weighted in pre-weighed, labeled crucibles. Samples were then dried in an oven (Isotemp Oven, Fisher Scientific, Pittsburgh, PA) at 100^{0}C for 16 hours. Differences in weight before and after air-drying was considered to be the moisture content of the sample.

To determine the ash content of the samples, previously air-dry samples were ignited in a muffle furnace (Isotemp Muffle Furnace, Fisher Scientific, Pittsburgh, PA) at 600^{0}C for 2 hours. The weight of the remaining sample was considered to be the ash content of the sample.

3.4.3. Protein Determination

Protein content was assessed by the Kjeldahl method with the use of an autoanalyzer (Technicon AutoAnalyzer II, Technicon Industrial Systems, Tarrytown, NY). Approximately 0.5 g of whole fish, fish muscle or 0.2 g of feed samples were added to labeled digestion tubes followed by anti-bumping granules (BHD) and Kjeltab® (catalyst of 89.70 % potassium sulfate and 10.30 % cupric sulfate in tablet of about 3.9 g). In a fumehood, 10.0 mL of concentrated sulfuric acid (Anachemia, 95.0 to 98.0 %) were then added followed by 3 to 5 mL of concentrated hydrogen peroxide (Anachemia, 99.0%) until the solution turned light blue. Subsequently, the digesting tubes were placed on a heated, 410 to 430 °C, digestor (Technicon BD-40 Heating Unit and Technicon BD-20/40 Control Unit) and the samples were allowed to undergo digestion for 35 min. Thereafter, the digested samples were cooled and distilled water was added to make up to a volume of 50.0 mL. Diluted samples were then injected into an autoanalyzer. The level of nitrogen in each sample was obtained. A factor of 6.25 was used to convert % nitrogen levels to the protein content in each sample.

3.4.4. Crude Lipid Determination

Crude lipid levels were determined using a modified method of Bligh and Dyer (1959) and Folch *et al.* (1957). A 4 g blended fish sample was first washed using 10 mL of chloroform (Anachemia, >99.8%) and 20 mL of methanol (Anachemia, >99.8%). The mixture was mixed (Sorvall® Omni-mixer, Ivan Sorvall, Inc, Norwalk, US) at ~10,000 RPM for 120 seconds followed by an addition of 10mL of chloroform to create a biphasic solution. The mixture was mixed again for 30 seconds. Finally, 8mL of distilled water were added and the mixture was mixed for another 30 seconds.

The mixture was filtered (Whatman No. 1 Filter Paper) under vacuum to remove all solid residue and then the filtrate was allowed to separate into two phases. In a 50 mL glass

31

graduated cylinder, the volume of the lower chloroform layer were recorded. The upper methanol phase was then suctioned off. Five milliliters of the chloroform layer were pipetted into a pre-weighed, pre-heated aluminum weigh boat. The weigh boat was heated in a fume hood to evaporate the chloroform leaving a thin lipid coating from the sample in the weigh boat. The boat was then transferred to a drying oven (Isotemp Oven, Fisher Scientific, Pittsburgh, PA) at 100^0C for an hour to remove residual chloroform. After cooling, the weight gained by the boat was recorded and this was considered to be the amount of lipid in the 5.00 mL of the sample solution.

3.4.5. Fatty Acid Profile Determination

The fatty acid profiles of fish muscle and feed were determined by methylating (Christie , 1973) the fatty acids in the samples and then the levels (% of total fatty acids) of fatty acid methyl esters (FAME) were quantified by gas chromatography (GC).

3.4.5.1. Sample Extraction

After lipid extraction as described in Section 3.4.4, the chloroform layer from each sample was collected and stored in sealed bottles at -40 ^{0}C (Wheaton, U.S.A.) prior to methyl esterification. The chloroform layer was brought back to room temperature just before methyl esterification. A volume of the chloroform layer containing at least 60 mg of fatty acids was pipetted into a glass test tube. In a 30 ^{0}C water bath, the sample was then concentrated to ~ 1 mL by blowing medical grade nitrogen gas (Praxair) on top of the solution. To methylate the fatty acids in the sample, 1.00 mL of benzene (Anachemia, distilled) and 200 μL of sodium methoxide (Aldrich Chemicals, 0.5 % sodium metal in methanol) were added and the solution was heated in a 50 ^{0}C water bath for 10 minutes. After methylation, the non-polar FAME were separated from other lipid constituents by the addition of 5 mL of hexane (Anachemia, > 99.9 %), 5.0 mL of distilled water, and 0.10 mL of

acetic acid (Anachemia, > 95.0 %). The sample was then separated into two layers. The top hexane layer was pipetted to a new test tube to which 5 mL of hexane were added. A small amount (tip of a spatula) of anhydrous sodium sulfate (BHD) was added to remove any residual water. The solution was then pipetted into a new test tube and concentrated to < 2 mL by blowing nitrogen gas on top of the solution in a 30 °C water bath. Thereafter, the sample was transferred to a GC vial (Varian, U.S.A.) and stored at -18 °C prior to GC analysis.

3.4.5.2. Gas Chromatography

The levels (% of total fatty acids) of fatty acid methyl esters (FAME) were determined by Gas Chromatography (Varian 3400). A volume of 0.1 μL of sample was injected into a 0.25 mm by 30 m column (Supelco Inc., SP-2300). The injection temperature was 220 °C and the final temperature of the column was 195 °C. The temperature at the detector was 250 °C. The flow rate of air, helium, hydrogen and oxygen gases was set at 0.75 mL per minute. In each case, the sample was detected by a flame ionization detector and area under each peak was integrated (Varian 4290). Peak identifications were performed by injecting custom prepared standard mixtures (Supelco Inc.) of FAME. The retention times of peaks from the standard FAME were used to identify fatty acids in the samples.

3.4.6. Astaxanthin Determination

Astaxanthin concentrations of the left fillets were determined individually according to the method described by Kiessling *et al.* (1995) using high performance liquid chromatography (HPLC). Astaxanthin levels in the fish feeds were also assessed in a similar manner. Whole left fillets were individually packed in air-impermeable bags and stored in -40 °C prior to astaxanthin extraction. Fish feeds were stored at -20 °C.

3.4.6.1. Sample Extraction

On the day of astaxanthin extraction, fillets were partly thawed, chopped, and homogenized (Braun®K1000, type 3210) until a paste of even consistency was produced. Astaxanthin was extracted from 1 g of fish homogenate using 20 mL of methanol, 20 mL of chloroform, and 8 mL of distilled water. Each sample was extracted three times and the chloroform layers from each extraction were pooled and the volume was recorded. From the pooled solution, a 20 mL aliquot was pipetted to a round bottom flask for rotary evaporation on a 50 ^{0}C water bath to evaporate off the chloroform. Depending on the concentration of the sample, the sample was then dissolved in 1 to 5 mL of n-hexane (Anachemia, HPLC grade, > 99.9 %) to ensure that the level of astaxanthin of the sample fell within the detection range of the HPLC. From the diluted solution, 1.0 mL of the sample was pipetted to a HPLC vial (Wheaton, U.S.A.) for HPLC analysis. In the case when the sample could not be analyzed on the same day, the sample in the vial would be wrapped in foil and stored at -40 ^{0}C overnight.

Astaxanthin levels in the fish feeds were quantified in a similar manner. The fish feed was first ground (Braun Coffee Bean Grinder, Type 4014), and 1 g of the ground feed was extracted following the method of lipid extraction by Bligh and Dyer (1959). A 1.00 mL aliquot of the chloroform layer was transferred to a round bottom flask. Chloroform was evaporated and the sample was prepared in the same manner as described above for HPLC analysis.

The above procedures were performed under minimum lighting and the samples were covered by foil at all time.

3.4.6.2. High Performance Liquid Chromatography

Quantification of astaxanthin in a sample was performed by HPLC (Waters, LC Module I). The machine was previously calibrated with various concentrations of pure

astaxanthin (Hoffman-LaRoche, Basel, Switzerland). A volume of 50 μL of the sample in hexane was automatically injected (Water 715 Ultra WISP™) and the flow rate of the system was controlled by a Waters 600E PowerLine™ Controller. The sample was then pumped through a 3.9 mm by 150 mm column (μPorasil™, Waters) with a pore size of 125Å. The mobile phase was 82 % hexane (Anachemia, HPLC grade, > 99.9 %) and 18 % acetone (Anachemia, HPLC grade, > 99.9 %). Presence of astaxanthin in the sample was then detected by a UV visible detector (Waters 486 Tunable Absorbance Detector) at 472 nm.

Due to the unavailability of different astaxanthin isomer standards, only the presence of all-*E*-astaxanthin isomer was identified and quantified in the samples.

3.5. Sensory Parameters of the Fish Fillets

On the last day of the growth experiment, eight pan-size (>300 g) fish from each replicate group were taken. No anesthetic was used to kill these fish; they were individually killed by a sharp blow to the head. Fish were filleted and skinned immediately after killing and the fillets were stored on ice prior to being vacuum packaged in air barrier bags. Packaged fillets were stored at -40 ^{o}C pending sensory evaluations, including flesh color of the skinned raw fillets, texture profile of cooked fillet, and aroma, flavor, uncharacteristic flavor, texture and overall acceptability of the cooked fillet.

3.5.1. Quantification of Raw Fillet Color

Raw skinned muscle from the dorsal region of the fillet was used for color determination of the fish fillet. Salmon fillets were partly thawed at -4 ^{0}C overnight before color assessment.

3.5.1.1. Quantification of Raw Fillet Color by Color Cards

Raw skinned fillet color was assessed visually by the Roche Color Card for

Salmonids (RCC) and the SalmoFan for Salmonids (SF) (Hoffman LaRoche, Basel, Switzerland). Salmon fillets were partly thawed at -4 ^{0}C overnight before assessment. Thawed salmon fillets were placed against a white background and a fluorescent light (Spectratlite™ F40T12 fluorescent lamp, Philips Lighting, U.S.A.) was used to create a natural lighting condition (Color temperature at 5900 ^{o}Kelvin, color rendering index or Ra at 90 @5900 ^{o}K). Dual lamps were used to eliminate any shadowing effect. The color of each raw, thawed fillet was then compared with the color cards. A scale of 11-18 was used using the RCC whereas a scale of 20-35 was employed using the SF. All color assessments were performed by a single judge.

3.5.1.2. Quantification of Raw Fillet Color by Colourimetric Measurements

The same fillet portion that was used in the color card assessments was employed for colorimetric measurements by using the Hunterlab Color Difference Reflectance Spectrophotometer (Hunter Lab LabScan) with a 1.27 cm aperture and a standard illuminant D_{65} to simulate daylight at a correlated color temperature ~6500 ^{o}K). Each fillet was placed flesh-side down on a glass plate and positioned over the viewing area so that the reading was taken just anterior to the dorsal fin, and equidistant between the dorsal ridge and the vertebral ridge. Each fillet was rotated 90^0 to the right, read four times, and the resulting values averaged. All readings, including zeroing and standardizing the instrument, were made through a plate of glass 2 mm thick. A black tile was used to zero the colorimeter, and a white tile (No. C2-14178) was used to standardize the instrument.

Averaged Hunter L, a, and b scores were recorded on one fillet from each fish. Hue, H^0_{ab}, was calculated as tan^{-1} (b/a) and chroma, C_{ab}, was measured as $(a^2+b^2)^{1/2}$.

3.5.2. Determinations of Cooked Fillet Texture

The same portion of fillet that was used for color assessment was cooked for texture

analysis using the method described by Bourne (1978). The texture profile analysis (TPA) was followed using the Texture Analyzer (TA. XT2, Stable Micro System, England) to achieve a quantitative measurement of the cooked coho salmon.

3.5.2.1. Preparation of the Cooked Fillet Sample

The fillets were wrapped in two layers of aluminum foil with shiny side in and the foil packages were then placed in a preheated 190 ^{0}C oven and baked for 10 minutes. A preliminary study had shown that the above cooking time was enough to cook the fish muscle so that it was no longer translucent and these conditions also prevented overcooking the fish samples. Once the samples were cooked and cooled to room temperature, four consecutive fish flake layers were separated and stacked on top of each other on the platform of the texture analyzer for texture profile analysis (TPA).

3.5.2.2. Conditions of the Texture Analyzer

Prior to the beginning of analyses, the texture analyzer was calibrated using a 5.0 kg load. As determined by a preliminary study, a No. 4 flat ended probe was used to perform the TPA test on the fish flakes at a speed of 0.5 mm • sec^{-1} and the penetration depth was set to be 3.0 mm. Hardness, firmness, and cohesiveness of the fillet sample were determined. Hardness, maximum force (N) required to compress the sample to the pre-set depth, was measured as the maximum height (g) of the peak multiplied by a factor of 9.8 m • sec^{-2}. Hardness of both the first and the second bites were determined. Firmness as described by Durance and Collins (1991), was measured as the maximum slope of the first peak. Finally, cohesiveness was considered to the ratio of the force areas under the first and second bites (Bourne, 1978).

3.5.3. Sensory Parameters Analysis by Sensory Panel

Aroma, flavor, uncharacteristic flavor, texture, and the overall acceptability of cooked fish fillet samples were assessed by a trained sensory panel to obtain quantitative descriptive data on the sensory parameters of the fish samples.

3.5.3.1. Selection of Descriptive Terms

Aroma, flavor, and texture were initially used to obtain data regarding these three attributes. Preliminary studies showed that "non-characteristic flavor" should be included to differentiate the difference between "characteristic flavor" and "non-characteristic flavor". In addition, the term "overall acceptability" was also included to assess individual preference of the panelist.

3.5.3.2. Construction of the Evaluation Ballot

The evaluation ballot consisted of five unstructured scales anchored with a term at both ends for each of the attributes being assessed (Figure. 1). The terms used for each sensory attribute were as follows: "very weak salmon aroma" and "very strong salmon aroma" for aroma; "very weak salmon flavor" and "very strong salmon flavor" for flavor; "not perceptible" and "very strong un-characteristic flavor" for non-characteristic flavor; "very tender" and "very tough" for texture; and "dislike very much" and "like very much" for overall acceptability.

The panelists were asked to indicate their score by placing a vertical line through the scale at the appropriate spot. A numerical score was then generated by measuring, with a metric ruler, from the left side of the scale to the point where the vertical line crossed the line. For example, a score of zero suggested that the sample had no salmon aroma, no salmon flavor, no non-characteristic flavor, was very tender, or was not acceptable. On the other hand, a score of 13 suggested that the sample had very strong salmon aroma, very strong salmon flavor, very strong non-characteristic flavor, was very tough, or was very acceptable.

Figure 1. *Sensory ballot used to evaluate sensory attributes of cooked fillets of pan-sized coho salmon. The coho were reared in seawater and fed diets with various ratios of digestible protein and lipid for 168 days*

Note that the actual ballot was printed on legal size paper to provide more spacing at the bottom for comments from panelists.

Sensory Quality of Coho Salmon Fed Grower Diets Containing Varying Levels of Lipid and Protein

Name:_____   Date:_____

Please evaluate the aroma, flavour, off-flavour and texture of each sample of cooked salmon that you are provided with.

Please indicate the codes of the samples: ____, ____, ____, ____, ____, ____, ____.

1. **Salmon Aroma** - make vertical lines on the horizontal line to indicate your rating of the salmon aroma of each salmon sample provided. Label each vertical line with the code number of the sample it represents.

————|——|————
       very weak salmon aroma   very strong salmon aroma

2. **Salmon Flavor** - make vertical lines on the horizontal line to indicate your rating of the salmon flavor of each salmon sample provided. Label each vertical line with the code number of the sample it represents.

————|——|————
       very weak salmon flavor   very strong salmon flavor

3. **Uncharacteristic - Flavor** - make vertical lines on the horizontal line to indicate your rating of the uncharacteristic-flavor, if any, of each salmon sample provided. Label each vertical line with the code number of the sample it represents.

————|——|————
       not perceptible   very strong uncharacteristic-flavor

4. **Texture** - make vertical lines on the horizontal line to indicate your rating of the texture of each salmon sample provided. Label each vertical line with the code number of the sample it represents.

————|——|————
       very tender   very tough

5. **Overall Acceptability** - make vertical lines on the horizontal line to indicate your rating of the overall acceptability of each salmon sample provided. Label each vertical line with the code number of the sample it represents.

————|——|————
       dislike very much   like very much

Comments:

39

3.5.3.3. Selection and Training of Sensory Panelists

A group of eleven panelists were recruited from the staff and students of the University of British Columbia. Selected panelists were generally fish eaters and showed interest in the experiment.

A total of three training sessions were conducted. The main purposes of the training sessions were to: (1) acclimate the panelists to the sensory panel environment; (2) determine whether evaluation of seven samples per sensory session would induce fatigue in the panelists; (3) determine the appropriate cooking time, and (4) familiarize the panelists with the descriptive terms used on the evaluation ballot.

During the training sessions, a variety of samples was presented to expose them to a wide range of sensory attributes. Chicken was used as an extreme reference for no characteristic aroma, no characteristic flavor, very strong non-characteristic flavor, very tough texture, and poor overall acceptability. Fresh farmed Atlantic salmon purchased from a local market was considered to be a good reference for the other extreme in each case, i.e., it represented strong characteristic aroma, strong characteristic flavor, no non-characteristic flavor, very tender texture, and high overall acceptability. In addition, fresh farmed trout, overcooked Atlantic salmon, Atlantic salmon that was exposed to two freeze-thaw cycles, and shrimp were all used to represent different degrees of the five attributes mentioned above.

Training sessions were conducted in a round table format as described by Rutledge and Hudson (1990). Each panelist was provided with the samples mentioned above. Some of the samples were provided in duplicate and even triplicate to elevate the repeatability of the panelist. During the training sessions, the panelists were asked to unwrap the foil package, sniff the aroma, and record the score for aroma on the ballot. Subsequently, the panelists were asked to take a bite of the sample and chew the sample around in the mouth to assess its flavor and texture attributes. Finally, the panelists were asked to give a score

on the overall acceptability of the fish sample. Once the evaluation of one sample was completed. Glacial water and unsalted crackers were provided to each of the panelists to rinse their mouths and to clean their palate between samples. The panelists were asked to evaluate the rest of the sample using the same procedures. During the training sessions, open discussions among the panelists were encouraged to minimize the difference of conceptualization of a particular descriptive term.

3.5.3.4. Preparation and Cooking of Fish Samples

Three sensory panel sessions were scheduled for each replicate group of salmon samples from each of the seven dietary treatments. A total of 9 sensory panels were scheduled. On the day before a panel session was to take place, three fillets from each of the seven dietary treatments were selected at random and transferred to an incubator at -4 ^{0}C. This allowed the fillets to partially thaw overnight for easier handling on the day of the sensory panel.

Partially thawed fillet samples were sliced to a thickness of approximately 2 mm by a meat slicer (Hobart Model 410 Slicer, Don Mills, ON). The whole fillet was sliced except for the section posterior to the dorsal fin which was kept for instrumental color and texture analysis. Fillet slices from three fish from the same dietary treatment were combined and were separated into three sections, i.e. anterior, middle, and posterior. Slices from each section were then randomly selected and combined to form a uniform sample of the fillet. Efforts were made to ensure that each panelist would receive a similar sample composed of slices from different locations of the fillets.

The composite samples were then wrapped in two layers of aluminum foil. Samples needed for both the morning and afternoon sessions were prepared in the morning and stored in a 4 ^{0}C cooler until the samples were needed.

Samples were removed from the cooler approximately 30 minutes before the

scheduled sensory panel time. Upon the arrival of the panelists, the samples were placed in a preheated oven and cooked for 5 minutes at 190 °C as determined during the training sessions. Panelists were asked to stay in the sensory panel area to ensure that the cooked samples were served as promptly as possible. Cooked samples were individually placed in a cup that was labeled with random 3-digit code. One sample from each of the seven dietary treatments was presented. Each panelist received all seven samples at the same time on a paper plate covered with foil to maintain the temperature of the samples.

3.5.3.5. Sampling Procedure during a Sensory Session

The sensory tests were conducted in the sensory panel room located in the Food Science Building at the University of British Columbia. Red fluorescent lighting was employed to minimize the effect of the fish flesh color on the assessment of the other sensory attributes. As mentioned above, fish samples for a particular panelist were only cooked upon the arrival of the panelist. Panelists would evaluate the fish samples the same way that they did during the training sessions except that no communication was allowed. Following the sensory session, a small treat was be given to each panelist. In addition, each panelist received a $ 5 honorarium for each sensory session that they attended.

3.6. Statistical Analysis of Data

Minitab Statistical Software, Version 12.0, was used to perform various statistical analyses of the data that were collected during the experiment. Two-way analysis of variance (ANOVA) was performed to examine the effect of protein level, lipid level, and the interaction of the various protein and lipid levels of the 6 diet treatments (Diet 1 to 6). It was shown that dietary protein and lipid levels alone had no effect on the various attributes assessed during the present experiment, while the interactions of the protein and lipid levels demonstrated significant effects in various observations. As a result, the six experimental

diets and the control commercial diet (Diet 7) were considered to be seven different diets using diet as a single factor during data analysis.

Graphs shown in the present report were constructed using Microsoft Excel, Version 5.0.

3.6.1. Statistical Analysis of the Performance Parameters

Fish growth and other aspects of performance were assessed by the following variables: initial and final mean weights (g), specific growth rates (SGR, (ln final weight (g)-ln initial weight (g)) • number of experimental days^{-1} • 100), dry feed intake (DFI, total dry feed intake (g) • fish^{-1}), feed efficiency ratios (FE, wet weight gained (g) • dry feed consumption (g)$^{-1}$), protein efficiency ratios (PER, wet weight gain (g) • protein consumption (g)$^{-1}$), percent protein deposition (%PD, protein gained in fish (g) • total protein consumed (g)$^{-1}$ • 100, and gross energy utilization (GEU, gross energy gained by the fish (MJ) • total gross energy consumed (MJ)$^{-1}$ • 100).

The average initial and final mean weights from each replicate group were used; thus, three observations were obtained per diet treatment. The effect of dietary treatment (dietary digestible protein and lipid levels) on the performance of the fish after each 28-day experimental period was assessed. The effects of diet treatment on the growth performance after every 28 days were treated statistically with an ANOVA test with the main effect of diet treatment. In addition, the time effects on the growth of fish from each dietary treatment were statistically determined with an ANOVA with time as the main effect. When significant effects were found, differences among treatments were further assessed by the Tukey's multiple comparison test. The null-hypothesis was rejected at a level of 5%.

3.6.2. Statistical Analysis of Chemical Compositions

The levels of proximate constituents in the feed, i.e., percentages of dry matter, ash,

protein, and lipid, are presented on a dry weight basis, while those for whole fish and fish muscle are shown and have been statistically assessed on a percent wet weight basis using the General Linear Model of the ANOVA test.

In addition, the whole body proximate composition data were analyzed by analysis of covariance (ANCOVA). The effect of dietary treatment on the log of the absolute amount of a body component (g), with the log of the body weight (g) as the covariant, was performed as recommended by Shearer (1994). Slopes obtained from the regression equations for each replicate group were compared with ANOVA with diet as the main effect.

Percentages of individual fatty acids in fish muscle lipid and astaxanthin content in fish flesh were also statistically assessed using ANOVA with diet as the main effect. When significant effects were found, differences among treatments were further assessed by Tukey's multiple comparison test. The null-hypothesis was rejected at a level of 5%.

3.6.3. Statistical Analysis of Sensory Qualities

3.6.3.1. Statistical Analysis of Color Parameters and Texture Profile

Data obtained from colorimetric analysis of the raw fillet and from texture profile analysis of the cooked fillet were grouped into various weight intervals depending on the whole body of the salmon to remove the effect of dissimilar fish weights on the data. Data were statistically tested using ANOVA with diet as the main effect for each weight interval

A preliminaty statisticaly study of the diet effect on the various color parameters of the flesh pigmentation of the salmon showed that the salmon fed the commercial control diet, which contained no added astaxanthin, were significantly ($p_D < 0.05$) different from those noted for salmon fed the other diets. As a result, statistical analyses on dietary effect of the various color parameters were performed on the data obtained for diets 1 to 6 only.

The effect of fish size on both the color parameters and textural qualities of salmon within the same dietary treatment (data arranged in rows) was assessed using ANOVA with

size as the main effect.

When significant effects were found, differences among treatments were further assessed by Tukey's multiple comparison test. The null-hypothesis was rejected at a level of 5%.

3.6.3.2. Z-Score Transformation of Data Obtained from Sensory Panel

A General Linear Model of ANOVA was used to assess the effect of fish diet, block, and panelist, on the sensory attributes of the cooked fillet samples. It was found that the individual panelists had significant effects on the evaluation of the samples mainly because the different panelists marked differently along the line scales during the sensory trials. Hence, the sensory data were transformed using Equation 1, where x equals the actual scores from a panelist for a sensory characteristic, X and the s.d. are the mean and standard deviation of all the scores for a sensory characteristic from the same panelist, and z is the transformed score.

$$z = \frac{x - X}{s.d.}$$ Equation 1

Score transformation was performed in order to standardize the scores from different panelists (Reid and Durance, 1992). The transformed scores were analyzed statistically using dietary treatment as the main effect. Differences among dietary treatments were further assessed by Tukey's multiple comparison test. The null-hypothesis was rejected at a level of 5 %.

4. Results

4.1. Chemical Analyses of the Experimental Diets

4.1.1. Proximate Compositions of the Experimental Diets

Proximate analyses (Table 2) revealed that the actual levels of digestible protein and lipid for diets 1, 4, 5, and 6 were close to the theoretical values; however, diets 2 and 3 contained less lipid than expected which also led to proportional elevations in their estimated percentages of digestible protein. The actual ratios of digestible protein to digestible lipid (DE:DP) in the seven diets used were 38:16, 39:18, 41:24, 45:16, 45:22, 45:28, and 43:24, respectively. Using caloric equivalents for digestible proteins, digestible lipid, and crude carbohydrate, the digestible energy contents of the experimental diets were calculated. As expected, the diets containing the medium and high levels of lipid had greater estimated digestible energy content than those containing less digestible lipid. Also, the diets containing the higher levels of protein had greater digestible energy content. Digestible energy (DE) of the diets ranged from 19.2 to 21.7 MJ $\cdot$ kg^{-1} of dry feed. Ratios of digestible protein to digestible energy (DP:DE) fell within the range of 19.6 to 23.1 g $\cdot$ MJ^{-1}.

4.1.2. Fatty Acid Compositions of the Experimental Diets

The contents of twenty fatty acids (% of total lipid, Table 3) in the experimental diets were determined using gas chromatography (GC). The major fatty acids found in the diets were C16:0, C16:1ω7, C18:1ω9, C18:2ω6, C18:3ω3, C20:1ω9, C22:1ω11, C20:5ω3, C22:5ω3, and C22:6ω3. Although the diets used in the present experiment should have been made with the same supplemental lipid source, the fatty acid composition profiles were different among diets (Table 3), especially diets 3 and 5. Levels of C18:2ω6 in diets 3 and 5 were 39.24 and 38.69 %, respectively, compared to a range of 1.41 to 5.37 % in other diets. Diets 3 and 5 also contained 5.94 and 6.11 % of C18:3ω3 compared to a range of 0.65 to 1.35 % in other diets. Due to the elevated percentages of C18:2ω6 and C18:3ω3 in diets 3 and 5, percentages of

other fatty acids were relatively lower than those of diets 1, 2, 4, 6, and 7. As a result, total ω-6 fatty acids percentages, and ω-6 to ω-3 fatty acid ratios in diets 3 and 5 were higher than noted for other diets, and the total percentages of ω-3 and HUFA's were lower in diets 3 and 5.

4.1.3. Astaxanthin Contents in the Experimental Diets

Astaxanthin contents in the experimental diets were estimated by high performance lipid chromatography (Table 4). All diets contained less astaxanthin than the expected level of 40 ppm. Astaxanthin levels ranged from 26.72 to 31.88 ppm (dry weight basis) in the six experimental diets, while the control diet, diet 7, contained only 1.02 ppm of added astaxanthin.

4.2. Growth Performance of Post-Juvenile Coho Salmon

4.2.1. Weights of Post-Juvenile Coho Salmon

Coho salmon in all groups had statistically equivalent ($p_D > 0.05$) mean weights, ranging from 102.0 to 117.1 g at the beginning of the experiment (Table 5 & Figure 2). Over time, all groups of salmon gained weight ($p_T < 0.05$). Although no significant differences were observed among dietary treatments ($p_D > 0.05$), salmon fed diet 6 (DP:DL, 45:27) had the highest mean body weight throughout the experiment (Figure 2). On Day 168, mean whole body weight of salmon fed diet 6 (DP:DL, 45:27) was significantly greater ($p_D < 0.05$) than that of salmon fed diet 3 (DP:DL, 41:23). As a general trend, coho salmon fed the diets with the higher level of digestible protein (45%, diets 4, 5, and 6), and increased levels of digestible lipid (22 and 27%) had greater gains in whole body weight. For salmon fed the diets with the lower level of digestible protein (38 to 41%, diets 1, 2, and 3), the trend for growth was not what was expected; i.e., the salmon fed the diet with the highest level of lipid (diet 3; DP:DL, 41:23) had the lowest final mean body weight.

4.2.2. Specific Growth Rates of Post-Juvenile Coho Salmon

No significant differences ($p_D > 0.05$) were observed in the specific growth rates (SGR, %, (ln (final mean body weight) - ln (initial mean body weight)) • 100 • number of experimental day^{-1})) of the coho salmon fed the test diets with various ratios of digestible protein and digestible lipid (Table 6 & Figure 3).

During the first 28 days of the experiment, SGRs ranged from 0.82 to 1.06 %; after 168 days, SGRs ranged from 0.53 to 0.71%. Throughout the experiment, SGRs of salmon fed diets 1 to 5 declined significantly ($p_T < 0.05$), while SGRs of salmon fed diets containing high levels of protein and lipid (diets 6 and 7) were not affected ($p_T > 0.05$).

As a general trend, the salmon fed the diets with the higher level of protein (diets 4, 5, and 6), and increased levels of digestible lipid supported the best SGRs throughout the experiment. This trend was not evident when the salmon were fed the diets with the lower level of protein.

4.2.3. Dry Feed Intakes of Post-Juvenile Coho Salmon

Feed intakes (g • fish^{-1}) were influenced by dietary treatments as shown in Table 7 and Figure 4. Starting on Day 83 of the experiment, salmon fed diet 6 (DP:DL, 45:27) had the highest feed intake per fish and this was significantly greater different ($p_D < 0.05$) than that of salmon fed diet 3 (DP:DL, 41:23). Two general, but opposite, trends were observed for feed intake. In the case of salmon fed diets with less digestible protein (38 to 41%, diets 1, 2, and 3), feed intake decreased as the level of digestible lipid and digestible energy in the diets increased. On the other hand, in the salmon fed the diets containing the higher level of protein (45%, diets, 4, 5, and 6), salmon fed diets with low (16%) or intermediate (22%) levels of lipid had similar feed intakes. In addition, salmon fed the diet with the highest level (27%) of lipid and digestible energy (21.67 MJ • kg^{-1}) had elevated feed intake.

4.2.4. Feed Efficiency Ratios of Post-Juvenile Coho Salmon Fed the Experimental Diets

Feed efficiency ratios (FE, mean weight gained (g) • dry feed intake^{-1}(g)) were influenced by dietary treatments during parts of the experiment (Table 8 and Figure 5). During the first 28 days of the experiment, FEs ranged from 1.00 to 1.20 and were not significantly different ($p_D > 0.05$) among dietary treatments. In general, the salmon fed the diets with the lower protein content (diets 1, 2, and 3) exhibited improved FEs as the levels of lipid and energy in the diets were increased. This trend was also noted in salmon fed diets with the higher protein level (diets 4, 5, and 6) during the latter part of the study but for the first 112 days, fish fed the diet with the intermediate lipid level (diet 5) had the highest FE ratio.

After 56 days, FE ranged from 0.88 to 1.13 and the aforementioned trends continued. Salmon fed diet 5 had the highest feed efficiency ratio which was significantly different ($p_D < 0.05$) from the FEs observed for salmon fed diets 1, 2, and 4. After 83 days, FEs continued to drop in some groups and ranged from 0.88 to 1.11. The trends continued and FEs for salmon fed diets 3 and 5 were significantly ($p_D < 0.05$) higher than those for salmon fed diets 1 and 2. The same trends continued after 112 days. For salmon fed the diets with the lower protein level, FEs improved as the levels of digestible lipid and energy increased. In fact, the mean FE for salmon fed diet 3 was significantly ($p_D < 0.05$) higher that those for salmon fed diets 1 and 2. With respect to salmon fed the diets with the higher protein level, the same trend existed. However, FE of salmon fed diet 5 was not significantly different from those for salmon fed diets 4 and 6. After 140 days and up to the end of the experiment, the same trends continued except that FE for salmon fed diet 5 fell to an intermediate position relative to those for fish fed diets 4 and 6.

No significant differences ($p_D > 0.05$) were found in overall FE values among fish given the dietary treatments. Over time, the FE values estimated for the fish given the different dietary treatments declined progressively. For fish fed diets 1, 2, 3, and 4, the decline in FEs was significantly ($p_T < 0.05$) affected by time.

4.2.5. Protein Efficiency Ratios of Post-Juvenile Coho Salmon Fed the Experimental Diets

Protein efficiency ratios (PER, mean wet weight gained (g) • protein consumption $(g)^{-1}$) of post-juvenile coho were significantly affected by both dietary treatment ($p_D < 0.05$) and the duration ($p_T < 0.05$) of measurement for most of the dietary treatments (Table 9 and Figure 6). During the first 28 days of the experiment, PERs ranged from 2.15 to 2.63 $g • g^{-1}$. Although no significant differences ($p_D > 0.05$) were observed for PER, a few trends in the data should be mentioned. In general, salmon fed diets containing the lower protein level (38 to 41%) had improved PER values as the levels of digestible lipid and energy were increased. This trend, however, was not observed among salmon fed the diets with the higher level of protein until the latter part of the study. Also, for fish fed the diets containing the same digestible lipid level (16 or 23%), a lower protein level in the diets resulted in improved values for PER.

After Day 56 and Day 83, PER values dropped to ranges of 1.83 to 2.39 $g • g^{-1}$ and 1.90 to 2.51 $g • g^{-1}$, respectively. Similar trends to those described above were found. In this regard, salmon fed diets 3 and 5 had significantly ($p_D < 0.05$) higher PERs than that noted for salmon fed diet 4 after 56 days. Also, salmon fed diet 3 had a significantly ($p_D < 0.05$) higher value for PER than noted for salmon fed diet 4 after 83 days. After 112 days of the experiment, PER continued to decline and the trend trends continued. At this point, the value for PER of salmon fed diet 3 was significantly ($p_D < 0.05$) higher that those for salmon fed diets 1, 2, and 4. After 140 days and until the end of the experiment, the salmon fed the diets containing either of the protein levels, generally had improved values for PER as the levels of digestible lipid and energy in the diets were increased. Throughout the experiment, salmon fed diet 3 (low protein, high lipid and energy) had the highest value for PER among all dietary treatments and after Day 28, the PER value for salmon fed diet 3 were significantly ($p_D < 0.05$) higher than that for salmon fed diet 4 (high protein and low lipid and energy). For all dietary treatments,

PERs declined over time. For salmon fed diets 1 and 4, the declines were significantly ($p_T <$ 0.05) affected by time.

4.2.6. Percent Protein Deposition of Post-Juvenile Coho Salmon

Values for percent protein deposited (%PD, protein gained in the fish (g) • 100 • total protein consumed $(g)^{-1}$) by the coho salmon are shown in Table 10 in relation to diet treatment. As was observed for PER, salmon fed diet 3 (low protein, high lipid) had the highest mean value for %PD; 46.82 % of total dietary protein consumed was converted to body protein. The value obtained for % PD of salmon fed diet 3 was also significantly higher ($p_D < 0.05$) than that of salmon fed diet 4, where only 33.59 % of dietary protein was converted to body protein. As expected, the salmon fed the diets containing the same level of digestible protein with increased levels of digestible lipid and energy exhibited the best conversion of dietary protein to body protein. Also, as a general trend, the salmon fed the diets with the same level of digestible lipid had decreased values for %PD as the dietary protein level was increased.

4.2.7. Gross Energy Utilization of Post-Juvenile Coho Salmon

Gross energy utilization (GEU, %, gross energy gained by the salmon(MJ) • 100 • total gross energy consumed $(MJ)^{-1}$) of the salmon fed the diets with the different ratios of digestible protein and lipid varied between 27.01 and 35.32 % and was not significantly affected ($p_D <$ 0.05) by dietary treatment (Table 10). As observed for the other performance parameters, the salmon fed the diets with the same protein content, but with increased levels of lipid and energy showed improved values for GEU. Also, the salmon fed the diets with the lower (38 to 41%) protein levels showed a trend for higher GEU values than that found for fish fed the diets with the higher (45%) protein level.

4.3. Chemical Analyses of Pan-Sized Coho Salmon

4.3.1. Whole Body Proximate Compositions of Pan-Sized Coho Salmon

Whole body ash and protein contents of coho salmon fed the diets with the various levels of DP and DL were not significantly affected by dietary treatments after 168 days (Table 11). The ash contents of salmon fed the different diets decreased from 2.28% (wet weight basis) on day 0 to a narrow range of 1.70 to 1.85% on day 168; protein content increased from 15.63% on day 0 to a narrow range of 18.03 to 18.34% on day 168. Moisture and lipid percentages in the whole body were significantly ($p_D < 0.05$) influenced by dietary treatment. Salmon fed the commercial control diet, diet 7, generally had lower lipid and higher moisture contents than those fed the other diets. As a general trend, the lipid content in the salmon body increased as the dietary lipid level was raised at each dietary protein level. Also, salmon fed the diets with the lower level of protein generally had greater whole body lipid content than noted in salmon consuming the diets with higher protein content.

Whole body proximate compositions of salmon from different replicate groups were examined using analysis of covariance (ANCOVA) to remove the effect of size during comparison. Using this method, the log weight of each proximate component was regressed against log fish weight. These log-log plots produced linear relationships. Slopes of the equations reflected the effect of body weight on the changes of the proximate composition of the fish body. Statistical analysis of the slopes of the regression equations suggested that dietary treatment had no effect ($p > 0.05$) on whole body proximate compositions (Table 12). Instead, the changes in whole body proximate composition were dependent upon whole fish weight.

4.3.2. Muscle Proximate Compositions of Pan-Sized Coho Salmon

As was observed for whole body proximate composition, the ash and protein contents in the muscle of post-juvenile coho salmon were not affected ($p > 0.05$) by dietary treatment (Table 13). The ash content in muscle increased from 1.65 % (wet weight basis) on day 0 to a

narrow range of 1.95 to 2.12 % on day 168. Protein content also increased slightly from 19.82 % to a range of 20.11 to 20.78%. Muscle lipid content was, however, significantly ($p < 0.05$) affected by dietary treatment. Muscle lipid percentages in salmon fed diets 2 and 6, 4.99 and 4.67%, respectively, were significantly ($p < 0.05$) higher than those noted in muscle from salmon fed diets 1 and 4, 3.55 and 3.27%, respectively. For salmon fed diets containing the higher protein level (45%), increased levels of lipid in the diets also led to progressively higher muscle lipid content. Moisture levels in the salmon muscle were found to be inversely related to lipid levels and were not affected significantly ($p > 0.05$) by dietary treatment.

4.3.3. Fatty Acid Compositions of Fillets of Pan-Sized Coho Salmon

Percentages of fatty acids in the muscle lipid of the coho salmon given the different diet treatments are shown in Tables 14 to 17. Due to the large differences in fatty acid composition that originally existed in the fish diets (Table 3), the fatty acid compositions of the muscle lipid of the salmon fed the different diets were significantly ($p < 0.05$) different from each other. However, percentages of $C18:1\omega9$, $C22:1\omega9$, and $C22:4\omega6$ in the salmon muscle lipid were not affected ($p > 0.05$) by dietary treatment. Percentages of these fatty acids ranged respectively from 17.0 - 19.2%, 0.38 - 1.63%, and 0.46 - 1.20% in salmon muscle lipid whereas the ranges for the levels of these fatty acids in the diets were greater.

Percentages of total saturated fatty acids, unsaturated fatty acids, total ω-3 fatty acids, total ω-6 fatty acids, sum of $C20:5\omega3$ and $C22:6\omega3$ ($\omega3$-HUFA), and ratios of total $\omega6$ to total $\omega3$ fatty acids were significantly different ($p < 0.05$) in muscle obtained from salmon fed the different dietary treatments (Table 18). In general, the fatty acid contents in the salmon muscle lipids mirrored the fatty acid compositions of their respective diets

4.4. Sensory Qualities of Pan-Sized Coho Salmon

4.4.1. Pigmentation in Raw Salmon Fillets

4.4.1.1. Astaxanthin Content in Raw Salmon Fillets

Levels of astaxanthin in the flesh of the salmon fed the diets containing the various ratios of dietary protein and dietary lipid appeared to be significantly affected by both dietary treatment (p_D < 0.05) and body size (p_S < 0.05) (Table 19). Salmon fed diet 7 achieved low levels of flesh pigmentation as a consequence of the absence of astaxanthin in the diet (Table 4). The dietary effect on the flesh pigmentation of the salmon fed diets 1 to 6 was evident (p_D < 0.05) when they weighed less than 200.0 g. At this stage, the salmon fed the diets containing the higher levels of lipid achieved higher levels of flesh pigmentation. However, this trend was less apparent as the salmon increased in size, especially when the dietary protein content was high. In salmon weighing more that 500.0 g, the astaxanthin concentration in their flesh reached a plateau at around 7.5 - 8.0 ppm under the conditions of this study.

If one excludes the results for fish fed diet 7, pattern of astaxanthin concentration in the salmon muscle was similar to that for muscle lipid concentration in the salmon fed the different diets; for example, salmon fed diet 2 had the highest levels of lipid and astaxanthin in their muscle for most size ranges. Also, among the salmon fed the diets with the higher protein level (diets 4, 5, and 6), both muscle lipid level and astaxanthin concentration increased as the lipid concentration in the diets increased. Regression analysis (Figure 9) showed that the astaxanthin level in salmon muscle was positively correlated (r^2 = 0.642) with the muscle lipid content.

4.4.1.2. Color Determinations of Raw Salmon Fillets

Results from visual determinations using the Roche Color Card (RCC) and SalmonFan (SF) were similar (Tables 20 and 21, respectively). As was mentioned previously, diet 7 contained very low level of astaxanthin as compared to the other diets. As a result, the salmon fed diet 7 achieved low RCC and SF scores. However, results obtained from both scales showed that the extent of pigmentation of the fillets from salmon fed the experimental diets

(diets 1 to 6) was not influenced ($p_D > 0.05$) by dietary treatment. In general, larger salmon received higher RCC and SF scores than smaller ones. The size effect was significant ($p_S < 0.05$) for the pigmentation of fillets from salmon fed diets 4 and 6 on the RCC scale and for fillets from salmon fed diets 2, 4, and 6 on the SF scale.

When considering both the flesh astaxanthin content and the RCC and SF scores for salmon in the same weight ranges (data in columns), it appeared that in some cases the fillets containing the highest astaxanthin content (Table 19) had relatively lower RCC and SF scores (Table 20 and 21, receptively).

Color scores of the fillets determined by the Hunter Lab Labscan are shown in Tables 22 to 26. Hunter L scores of the fillets were not affected by dietary treatment ($p_D > 0.05$) or by the size of the salmon ($p_S > 0.05$). With a few exceptions, fillets containing higher levels of astaxanthin generally had lower Hunter L scores.

Results from Hunter a, Hunter b, and Chroma (Tables 23, 24, and 25, respectively) determinations showed that the dietary effect on the flesh pigmentation of the fillets of the salmon fed diets 1 to 6 were significantly influenced ($p_D < 0.05$) for salmon weighing less than 500.0 g. Again, in all cases, fillets from salmon fed diet 7 had lower scores. With a few exceptions, higher Hunter a, Hunter b, and Chroma scores were observed for fillets from larger salmon and for fillets containing higher flesh astaxanthin concentrations. Hue scores (Table 26) of raw salmon fillets, in general, decreased as flesh astaxanthin increased. Fillets from salmon fed diet 7 had significantly ($p_D < 0.05$) higher Hue scores than fillets from salmon fed the other diets. A size effect was also observed ($p_S < 0.05$) for fillets from salmon fed diets 2, 3, 4, 5, and 7.

4.4.2. Texture Profiles of Cooked Salmon Fillets

Hardness (Tables 27 and 28) of the cooked fillets from the salmon fed the diets containing the various ratios of protein and lipid were not significantly affected by dietary

treatment ($p_D > 0.05$). However, it appeared that the texture of the cooked fillets from the salmon fed diets 1, 5, 6, and 7 were significantly affected by fish size ($p_S < 0.05$); the fillets from the larger salmon appeared to be less hard than fillets from the smaller salmon.

By contrast, firmness (Table 29) of the cooked fillets from the salmon fed the different diets was not significantly affected by dietary treatment ($p_D > 0.05$) nor by fish size in most cases ($p_S > 0.05$). However, as a general trend, the fillets from the larger salmon appeared to be less firm than those from smaller salmon.

Results from the present showed that cohesiveness (Table 30) of the cooked fillets from the salmon fed the different diets was not affected by dietary treatment ($p_D > 0.05$). In addition, cohesiveness of the cooked fillets did not show any trend with fish size ($p_S > 0.05$).

4.4.3.   Sensory Attributes of Cooked Salmon Fillets Determined by Sensory Panel

Results from the sensory evaluations are shown in Table 31. Dietary treatments were not found to have any effect ($p > 0.05$) on the intensity of salmon aroma and the level of off-flavor in the cooked samples. Fillets from salmon fed diet 6, however, had significantly ($p < 0.05$) more intense salmon flavor than those from salmon fed diets 1, 3, 5, and 7. Moreover, the fillets from salmon fed diet 6 were judged to be the most tender and were significantly ($p < 0.05$) softer than those from salmon fed diet 7. Scores relating to the overall acceptability of the fillets were highest for salmon fed diets 2 and 6 and they were significantly ($p < 0.05$) higher than the mean score obtained for fillets from salmon fed diet 7.

Table 2. Levels of proximate constituents (% dry matter), digestible protein (DP), digestible lipid (DL), digestible energy (DE, MJ · kg⁻¹ dry feed), and digestible protein to digestible energy ratios (DP:DE, g · MJ⁻¹) in the experimental diets used to evaluate the optimal dietary ratio of digestible protein to digestible lipid for rearing coho salmon in seawater.

Diet	Dry Matter	Ash	Protein	Lipid	DP[2]	DL[2]	DE[3]	DP:DE
1 (37:16)[1]	96.3	9.47	41.3	17.9	38.0	16.1	19.2	19.8
2 (37:23)	95.1	9.78	42.6	20.3	39.2	18.3	19.5	20.1
3 (37:30)	96.5	10.1	44.1	26.1	40.6	23.5	20.7	19.6
4 (44:16)	97.3	11.1	49.1	18.1	45.3	16.3	19.7	23.1
5 (44:23)	97.0	10.8	48.4	24.2	44.5	21.8	20.6	21.6
6 (44:30)	98.1	11.3	49.0	30.5	44.5	27.4	21.7	20.5
Control	93.5	11.1	47.7	27.1	42.9	24.3	20.2	21.3

[1] Values in parentheses denote the theoretical dietary levels (%) of digestible protein and lipid, respectively

[2] Digestibility coefficients for protein and lipid in diets 1 to 6 were assumed to be 92% and 90%, respectively based on the individual digestibility coefficients established for the dietary protein sources. In the case of the control diet, protein and lipid were assumed to be 90 % digestible.

[3] Gross energy values of digestible protein, digestible lipid, and crude carbohydrates were 23.6, 36.4 and 15.9 kJ · g⁻¹, respectively.

Table 3. *Fatty acid composition (% of total fatty acids) in the lipid fraction of the experimental diets determined by gas chromatography. The experimental diets were used to rear coho salmon in seawater.*

Fatty Acids	Diet 1 (38:16)[1]	2 (39:18)	3 (41:23)	4 (45:16)	5 (45:22)	6 (45:27)	7 (43:24)
14:0	5.28	6.82	2.08	6.09	1.20	7.97	8.69
15:0	0.28	0.30	0.09	0.33	0.05	0.44	0.31
16:0	13.90	14.54	11.90	15.83	10.55	14.85	16.03
16:1ω7	5.98	6.78	1.51	5.80	1.01	4.59	8.96
18:0	2.68	2.01	3.93	3.16	3.87	1.45	3.32
18:1ω9	7.02	10.43	18.92	6.20	19.20	12.61	15.47
18:1ω7	1.40	1.72	0.01	1.73	0.01	0.02	1.91
18:2ω6	4.74	2.02	39.24	5.37	38.69	1.41	3.23
18:3ω6	0.07	0.11	0.01	0.12	0.01	0.06	0.24
18:3ω3	1.35	1.04	5.94	1.34	6.11	1.23	0.65
20:1ω9	2.98	12.09	2.80	3.68	0.03	14.40	1.11
18:4ω3	0.70	0.08	0.01	0.21	0.03	0.06	0.21
20:3ω6	0.72	0.26	0.03	1.05	0.02	0.48	0.79
20:4ω6	0.87	1.00	0.49	2.25	3.50	0.74	0.43
22:1ω9	0.00	0.00	0.15	0.01	2.10	0.00	0.01
22:1ω11	15.15	12.80	2.98	4.10	2.79	19.20	8.22
20:5ω3	19.44	11.74	3.12	24.50	4.21	9.39	16.41
22:4ω6	0.52	0.50	0.05	0.62	0.08	0.05	0.06
22:5ω3	7.17	5.16	1.92	5.53	3.11	1.45	7.31
22:6ω3	9.78	10.60	4.79	12.09	3.35	9.59	6.66
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Total Sat.	22.14	23.67	18.01	25.41	15.67	24.73	28.34
Total Unsat.	77.86	76.33	81.99	74.59	84.33	75.27	71.66
Total ω3	38.44	28.62	15.78	43.67	16.81	21.71	31.24
Total ω6	6.92	3.89	39.82	9.41	42.30	2.74	4.75
Total HUFA ω3	29.22	22.34	7.91	36.59	7.56	18.98	23.07
ω6:ω3	0.18	0.14	2.52	0.22	2.52	0.13	0.15

[1] Values in parentheses denote the estimated levels of digestible protein and lipid on a dry weight basis.

Table 4. *Astaxanthin (ppm, dry weight basis) levels in experimental diets determined by high performance liquid chromatography (HPLC). The diets were formulated to contain 40ppm of added astaxanthin.*

Diet	Astaxanthin (ppm)
1 (38:16)[1]	26.72
2 (39:18)	29.91
3 (41:23)	30.76
4 (45:16)	31.88
5 (45:22)	27.66
6 (45:27)	31.18
7 (43:24)	1.02

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

Table 5. *Mean whole body weights (g, n=3) of post-juvenile coho salmon fed diets with different levels of digestible protein and lipid at 28-day intervals. The salmon were reared in seawater for a total of 168 days.*

Diet[1]	Day 0	Day 28	Day 56	Day 83	Day 112	Day 140	Day 168	F_T[5]	P_T[5]
1 (38:16)	107.1 a[2] u[3] (7.7)[3]	140.0 a uv (9.8)	168.1 a vw (12.8)	198.3 a vw (18.6)	223.0 a xy (17.7)	258.6 a yz (19.2)	283.0 ab z	51.21	0.000
2 (39:18)	113.4 a v (3.3)	145.0 a w (6.6)	164.8 a wx (10.8)	189.6 a xy (9.6)	214.6 a y (11.4)	256.6 a z (7.2)	279.1 ab z (21.2)	83.66	0.000
3 (41:23)	102.0 a v (7.4)	130.6 a vw (13.9)	154.2 a vwx (22.5)	183.3 a wxy (20.4)	209.7 a xyz (29.5)	235.3 a yz (29.1)	246.6 a z (22.3)	18.16	0.000
4 (45:16)	106.3 a w (6.4)	141.7 a wx (12.7)	165.0 a wxy (15.6)	198.5 a xyz (27.1)	222.7 a yz (37.6)	238.4 a z (31.0)	266.8 ab z (34.3)	14.29	0.000
5 (45:22)	103.6 a x (9.3)	139.0 a xy (22.7)	172.0 a xyz (27.6)	209.2 a xyz (35.9)	235.6 a xyz (47.0)	257.4 a yz (74.8)	287.5 ab z (81.8)	5.33	0.000
6 (45:27)	117.1 a v (6.4)	158.0 a vw (18.8)	200.7 a vwx (30.5)	243.9 a wxy (42.9)	284.6 a xyz (48.3)	339.5 a yz (68.0)	386.6 b z (62.2)	14.12	0.000
7 (43:24)	114.2 a w (6.0)	143.7 a w (9.1)	172.7 a wx (18.2)	209.4 a wxy (27.4)	242.2 a xyz (38.5)	290.8 a yz (49.9)	331.9 ab z (57.0)	15.48	0.000
F_D[4]	2.13	0.99	1.43	1.50	1.54	1.85	2.86		
P_D[4]	0.115	0.467	0.272	0.248	0.238	0.160	0.049		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a and b) are significantly different ($p<0.05$).

[2] Means within a row that do not have or share a common postscript letter (u, v, w, x, y, and z) are significantly different ($p<0.05$).

[3] Standard deviation.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
   P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_T = Ratio of the time groups mean square to the population's error mean square.
   P_T = Probability of committing a Type I error, i.e. probability that there is no difference over time.

Mean Whole Body Weight (g)

0 50 100 150 200 250 300 350 400 450

Day 0 Day 28 Day 56 Day 83 Day 112 Day 140 Day 168

Experiment Days

☐ Diet 1 (38:16)
■ Diet 2 (39:18)
▦ Diet 3 (41:23)
☐ Deit 4 (45:16)
▨ Diet 5 (45:22)
▨ Diet 6 (45:27)
▨ Diet 7 (43:24)

Figure 2. Mean whole body weights (g, n=3) of post-juvenile coho salmon fed diets with different levels of digestible protein and lipid (values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis) at 28-day intervals. The salmon were reared in seawater for a total of 168 days.

Table 6. *Mean specific growth rates (% body weight • day^{-1}, (In final mean weight – In initial mean weight) • 100 • number of experimental days^{-1}, n=3) of post-juvenile coho salmon fed diets with different levels of digestible protein and lipid after each 28-day interval. The salmon were reared in seawater for 168 days.*

Diet[1]	28 Days[2]	56 Days	83 Days	112 Days	140 Days	168 Days	F_T[5]	P_T[5]
1 (38:16)	0.96 a w (0.05)[3]	0.81 a x (0.01)	0.74 a xy (0.06)	0.66 a yz (0.06)	0.63 a yz (0.05)	0.58 a z (0.05)	23.26	0.000
2 (39:18)	0.88 a y (0.16)	0.67 a yz (0.10)	0.62 a z (0.08)	0.57 a z (0.07)	0.58 a z (0.04)	0.54 a z (0.06)	5.42	0.008
3 (41:23)	0.87 a y (0.13)	0.73 a yz (0.14)	0.70 a yz (0.05)	0.64 a yz (0.07)	0.59 a z (0.05)	0.53 a z (0.02)	5.91	0.006
4 (45:16)	1.02 a y (0.18)	0.78 a yz (0.08)	0.75 a yz (0.12)	0.65 a z (0.12)	0.57 a z (0.06)	0.55 a z (0.05)	7.88	0.002
5 (45:22)	1.03 a y (0.26)	0.906 a yz (0.13)	0.84 a yz (0.10)	0.72 a yz (0.10)	0.63 a yz (0.15)	0.59 a z (0.11)	3.68	0.030
6 (45:27)	1.06 a z (0.22)	0.95 a z (0.17)	0.87 a z (0.15)	0.79 a z (0.11)	0.75 a z (0.12)	0.71 a z (0.07)	2.44	0.096
7 (43:24)	0.82 a z (0.11)	0.74 a z (0.15)	0.73 a z (0.13)	0.67 a z (0.12)	0.66 a z (0.10)	0.63 a z (0.09)	1.07	0.426
F_D[4]	0.88	2.07	2.05	1.54	1.45	2.50		
P_D	0.536	0.124	0.127	0.235	0.265	0.074		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a) are significantly different ($p < 0.05$).
Means within a row that do not have or share a common postscript letter (w, x, y, and z) are significantly different ($p < 0.05$).

[3] Standard deviation.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_T = Ratio of the time groups mean square to the population's error mean square.
P_T = Probability of committing a Type I error, i.e. probability that there is no difference over time.

Figure 3. *Mean specific growth rates (% body weight • day $^{-1}$, ((ln final mean weight – ln initial mean weight) • 100 • number of experimental days $^{-1}$, n=3) of post-juvenile coho salmon fed diets with different levels of digestible protein and lipid after each 28-day interval. The salmon were reared in seawater for 168 days. Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.*

Table 7. *Mean dry feed intakes (g • fish^{-1}, n=3) of post-juvenile coho salmon fed diets with different levels of digestible protein and lipid at 28-day intervals. The salmon were reared for 168 days in seawater*

Diet[1]	28 Days[2]	56 Days	83 Days	112 Days	140 Days	168 Days	F_T[5]	P_T[5]
1 (38:16)	33.1 a u (3.5)[3]	69.3 a v (8.1)	104.3 ab w (8.7)	144.4 ab x (10.0)	185.5 ab y (10.6)	228.5 ab z (9.8)	208.78	0.000
2 (39:18)	29.6 a u (4.1)	57.2 a v (3.8)	85.9 ab w (4.2)	121.5 ab x (7.3)	158.7 ab y (7.9)	190.8 ab z (14.5)	179.91	0.000
3 (41:23)	24.5 a w (4.5)	49.0 a wx (12.0)	74.0 a wxy (15.4)	99.7 a xyz (20.3)	124.1 a yz (24.7)	152.2 a z (28.0)	18.47	0.000
4 (45:16)	32.2 a v (5.5)	65.0 a vw (7.7)	97.9 ab wx (15.4)	133.2 ab xy (25.6)	168.1 ab yz (32.6)	208.6 ab z (36.8)	22.83	0.000
5 (45:22)	29.3 a w (9.5)	61.2 a wx (18.1)	94.9 ab wxy (23.3)	131.6 ab xyz (27.1)	163.7 ab yz (36.8)	201.4 ab z (42.6)	15.37	0.000
6 (45:27)	36.4 a v (5.6)	79.6 a vw (14.5)	123.2 b wx (18.3)	173.2 b xy (19.7)	223.4 b y (24.3)	278.0 b z (25.7)	66.28	0.000
7 (43:24)	29.2 a w (6.0)	63.7 a wx (15.2)	100.6 ab wxy (24.6)	141.4 ab xyz (36.3)	183.0 ab yz (45.3)	230.6 ab z (57.0)	13.64	0.000
F_D[4]	1.26	1.83	2.40	2.90	3.29	3.92		
P_D	0.335	0.164	0.083	0.047	0.031	0.017		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.
[2] Means within a column that do not have or share a common postscript letter (a and b) are significantly different (p<0.05).
Means within a row that do not have or share a common postscript letter (u, v, w, x, y, and z) are significantly different (p<0.05).
[3] Standard deviation.
[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.
[5] F_T = Ratio of the time groups mean square to the population's error mean square.
P_T = Probability of committing a Type I error, i.e. probability that there is no difference over time.

Figure 4. *Mean dry feed intakes (g • fish⁻¹, n=3) of post-juvenile coho salmon fed diets with different levels of digestible protein and lipid at 28-day intervals. The salmon were reared in seawater for 168 days. Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.*

99

Table 8. *Mean feed efficiency ratios (mean weight gained (g) • dry feed intake^{-1} (g), n=3) of post-juvenile coho salmon fed diets with different levels of digestible protein and lipid at 28-day intervals. The salmon were reared in seawater for 168 days.*

Diet[1]	28 Days	56 Days	83 Days	112 Days	140 Days	168 Days	F_T[5]	P_T[5]
1 (38:16)	1.00 a y (0.05)[3]	0.88 a yz (0.03)	0.88 a yz (0.10)	0.80 ab z (0.08)	0.82 a yz (0.06)	0.77 a z (0.05)	4.37	0.017
2 (39:8)	1.06 a y (0.08)	0.90 a yz (0.13)	0.88 a yz (0.09)	0.83 b z (0.07)	0.90 a yz (0.02)	0.87 a yz (0.06)	2.91	0.060
3 (41:23)	1.16 a y (0.06)	1.05 ab yz (0.06)	1.11 b yz (0.06)	1.08 c yz (0.06)	1.08 a yz (0.09)	0.96 a z (0.10)	2.52	0.088
4 (45:16)	1.10 a y (0.06)	0.90 a z (0.07)	0.93 ab yz (0.09)	0.86 abc z (0.09)	0.79 a z (0.05)	0.77 a z (0.05)	8.72	0.001
5 (45:22)	1.20 a z (0.13)	1.13 b z (0.08)	1.11 b z (0.02)	0.99 bc z (0.09)	0.91 a z (0.19)	0.89 a z (0.16)	3.08	0.051
6 (45:27)	1.11 a z (0.16)	1.04 ab z (0.11)	1.02 ab z (0.15)	0.96 abc z (0.14)	0.98 a z (0.18)	0.96 a z (0.12)	0.47	0.793
7 (43:24)	1.02 a z (0.05)	0.92 ab z (0.02)	0.95 ab z (0.04)	0.90 abc z (0.02)	0.97 a z (0.09)	0.94 a z (0.04)	2.10	0.136
F_D[4]	1.90	4.47	3.82	4.12	2.29	2.40		
P_D[4]	0.152	0.010	0.018	0.014	0.095	0.083		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.
[2] Means within a column that do not have or share a common postscript letter (a, b, and c) are significantly different ($p < 0.05$).
[3] Means within a row that do not have or share a common postscript letter (y and z) are significantly different ($p < 0.05$).
[3] Standard deviation.
[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
  P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.
[5] F_T = Ratio of the time groups mean square to the population's error mean square.
  P_T = Probability of committing a Type I error, i.e. probability that there is no difference over time.

Feed Efficiency Ratio (FE, Wet Weight Gain (g) * Dry Feed Intake-1 (g-1)

Experiment Days

Day 28 Day 56 Day 83 Day 112 Day 140 Day 168

□ Diet 1 (38:16)
■ Diet 2 (39:18)
⊞ Diet 3 (41:23)
□ Diet 4 (45:16)
▨ Diet 5 (45:22)
▩ Diet 6 (45:27)
▨ Diet 7 (43:24)

Figure 5. *Mean feed efficiency ratios (mean weight gained (g) • dry feed intake^{-1} (g), n=3) of post-juvenile coho salmon fed diets with different levels of digestible protein and lipid at 28-day intervals. The salmon were reared in seawater for 168 days. Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.*

Table 9. *Mean protein efficiency ratios (PER, mean wet weight gained (g) • protein consumption $(g)^{-1}$) of post-juvenile coho salmon fed diets with different levels of digestible protein and digestible lipid after each 28-day interval. The salmon were reared in seawater for 168 days.*

Diet[1]	28 Days	56 Days	83 Days	112 Days	140 Days	168 Days	F_T[5]	P_T[5]
1 (38:16)	2.41 a y[2] (0.13)[3]	2.14 ab yz (0.07)	2.12 ab yz (0.25)	1.94 b z (0.19)	1.98 ab z (0.14)	1.86 ab z (0.12)	4.38	0.017
2 (39:18)	2.49 a z (0.18)	2.10 ab z (0.31)	2.08 ab z (0.21)	1.95 b z (0.16)	2.12 ab z (0.05)	2.03 a z (0.14)	2.89	0.060
3 (41:23)	2.63 a y (0.13)	2.39 a yz (0.13)	2.51 a yz (0.14)	2.45 a yz (0.13)	2.45 a yz (0.20)	2.18 a z (0.22)	2.53	0.088
4 (45:16)	2.23 a y (0.13)	1.83 b z (0.14)	1.90 b yz (0.18)	1.76 b z (0.18)	1.60 b z (0.09)	1.57 b z (0.10)	8.68	0.001
5 (45:22)	2.49 a z (0.27)	2.33 a z (0.16)	2.30 ab z (0.03)	2.05 ab z (0.18)	1.88 ab z (0.40)	1.84 ab z (0.34)	3.08	0.051
6 (45:27)	2.26 a z (0.32)	2.12 ab z (0.22)	2.07 ab z (0.30)	1.95 ab z (0.28)	2.01 ab z (0.37)	1.96 ab z (0.25)	0.47	0.794
7 (43:24)	2.15 a z (0.11)	1.92 ab z (0.04)	1.99 ab z (0.08)	1.89 b z (0.05)	2.02 ab z (0.20)	1.98 ab z (0.09)	2.10	0.136
F_D[7]	2.38	3.93	3.43	4.42	3.37	3.48		
P_D[4]	0.085	0.016	0.026	0.010	0.029	0.026		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a and b) are significantly different (p<0.05). Means within a row that do not have or share a common postscript letter (y and z) are significantly different (p<0.05).

[3] Standard deviation.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
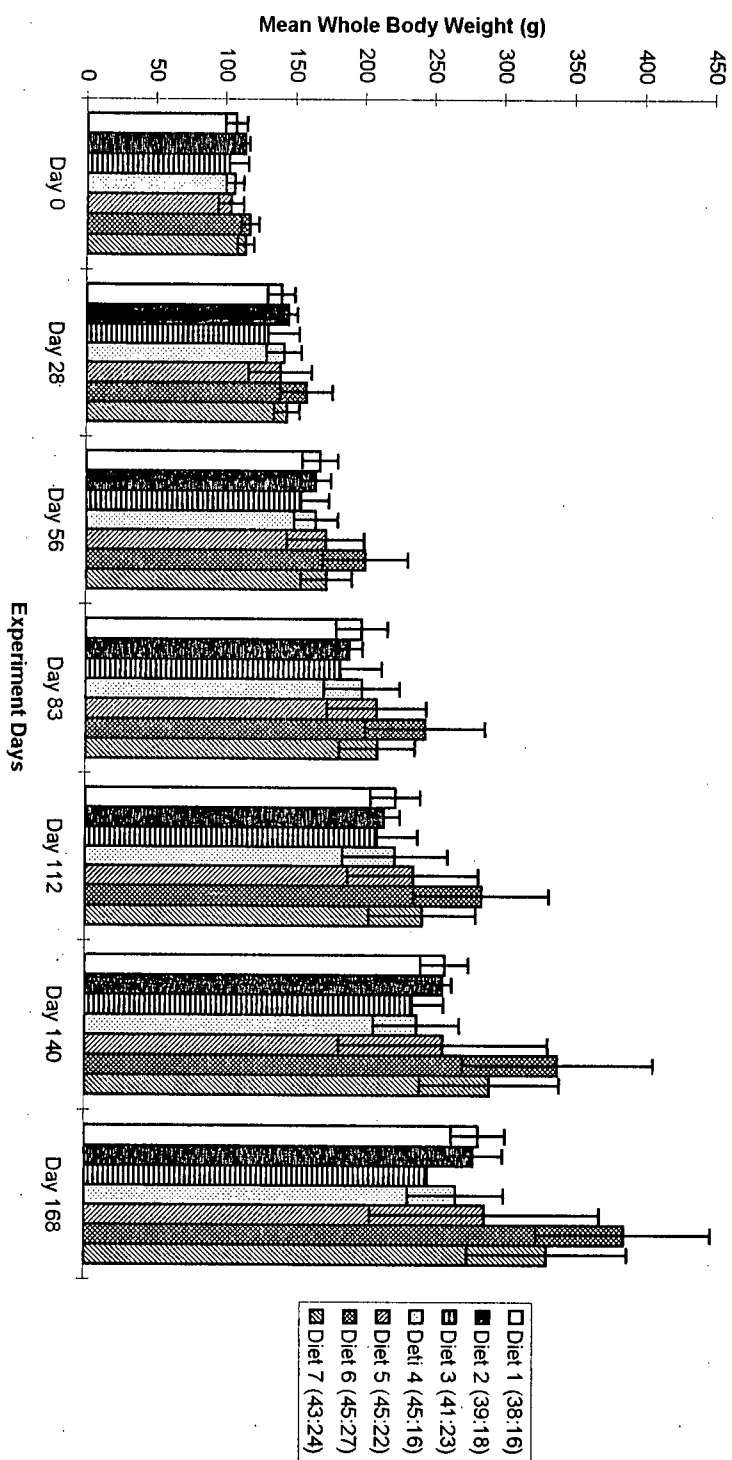P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_T = Ratio of the time groups mean square to the population's error mean square.
P_T = Probability of committing a Type I error, i.e. probability that there is no difference over time.

Figure 6. *Mean protein efficiency ratios (PER, mean wet weight gained (g) • protein consumption (g)$^{-1}$) of post-juvenile coho salmon fed with diets containing different levels of digestible protein and lipid after each 28-day interval. The salmon were reared in seawater for 168 days. Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.*

Table 10. *Percent protein deposition (%PD, protein gained in the salmon (g) • 100 • total protein consumed (g)$^{-1}$) and gross energy utilization (GEU, gross energy gained in the salmon (MJ) • 100 • total gross energy consumed (MJ^{-1})) of coho salmon over the experimental period of 168 days. The coho salmon, reared in seawater, were fed diets containing different levels of digestible protein and lipid.*

Diet	% PD	GEU
1 (38:16)[1]	40.37 ab (2.40)	29.15 a (2.69)
2 (39:18)	43.78 ab (2.68)	34.36 a (3.31)
3 (41:23)	46.82 a (5.47)	35.32 a (2.08)
4 (45:16)	33.59 b (1.75)	27.01 a (1.56)
5 (45:22)	39.77 ab (6.63)	30.45 a (8.33)
6 (45:27)	42.02 ab (4.15)	32.88 a (5.16)
7 (43:24)	43.47 ab (1.09)	31.95 a (1.92)
F^4	3.38	1.45
P^5	0.028	0.265

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.
[2] Standard deviation.
[3] Means within a column that do not have or share a common postscript letter are significantly different (p<0.05).
[4] Ratio of the groups mean square to the population's error mean square.
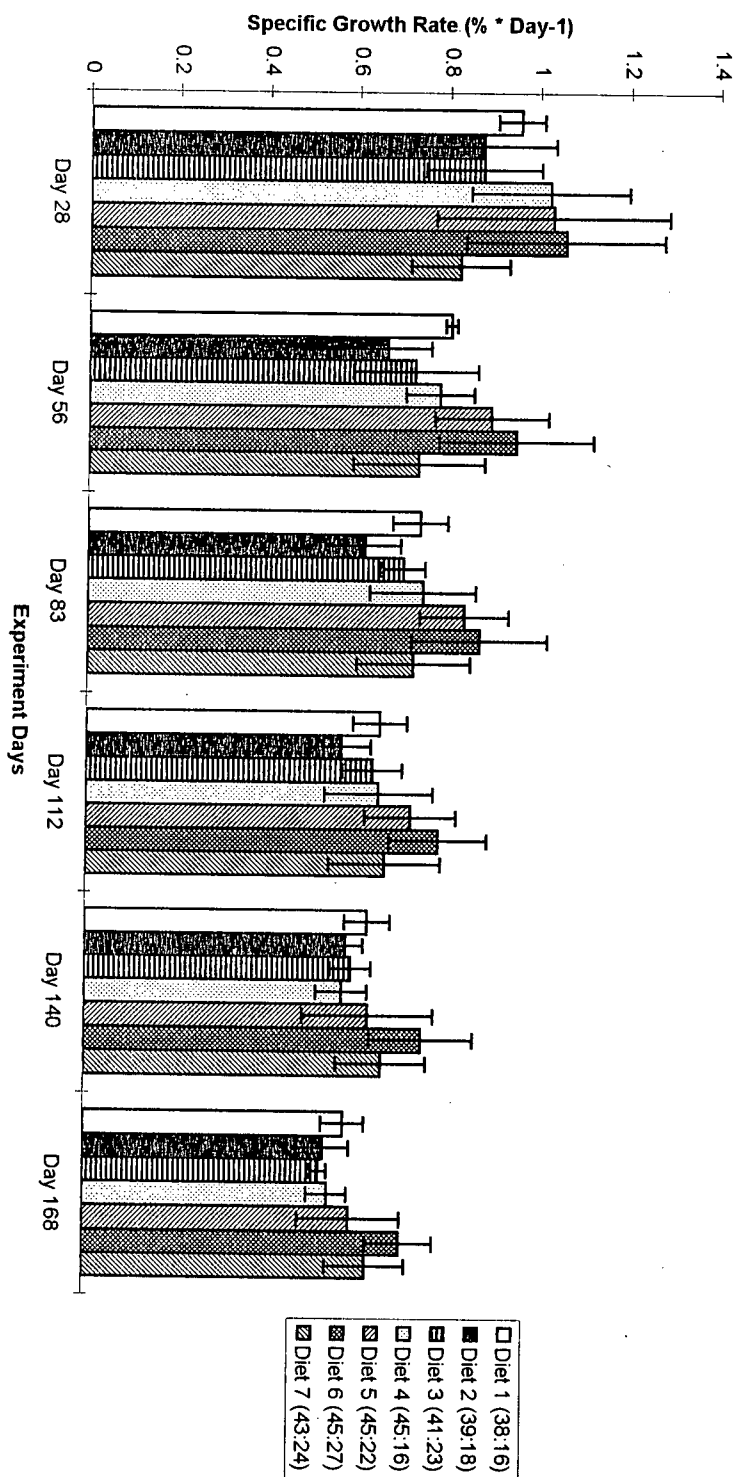[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

70

Table 11. *Whole body proximate compositions (%, wet weight basis) of coho salmon at Day 0 and Day 168 of the experiment. The salmon were reared in seawater and fed diets containing different levels of digestible protein and lipid.*

	n	Moisture	Ash	Protein	Lipid
Day 0	5	72.33 (0.92)[2]	2.28 (0.38)	15.63 (0.93)	8.47 (0.93)
Day 168 Diet 1 (38:16)[1]	20	72.42 ab[3] (2.11)	1.80a (0.23)	18.31a (0.78)	7.63 ab (2.09)
2 (39:18)	18	71.58 a (1.72)	1.75a (0.16)	18.10a (0.89)	8.41 a (2.20)
3 (41:23)	18	71.99 ab (2.09)	1.84a (0.26)	18.03a (0.75)	8.32 a (1.91)
4 (45:16)	20	73.07 ab (1.01)	1.81a (0.22)	18.12a (0.95)	7.07 ab (0.87)
5 (45:22)	18	72.86 ab (2.04)	1.85a (0.27)	18.29a (0.85)	7.16 ab (1.81)
6 (45:27)	18	71.71 ab (1.31)	1.80a (0.33)	18.27a (0.85)	7.93 ab (1.44)
7 (43:24)	19	72.94 b (0.95)	1.70a (0.25)	18.34a (0.78)	6.83 b (1.17)
F[4]		2.62	0.73	0.35	2.60
P[5]		0.022	0.629	0.910	0.023

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Standard deviation.

[3] Means within a column that do not have or share a common postscript letter are significantly different (p<0.05).

[4] Ratio of the groups mean square to the population's error mean square.

[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

Table 12. *Slope, b, of allometric analysis (log Y = a + b • log X, where Y equals absolute body proximate content, and X equals fish weight) of gain in proximate constituents in relation to body weight gain of post-juvenile coho salmon fed diets with various levels of digestible protein and lipid. The salmon were reared in seawater for 168 days.*

Diet	Protein	Lipid	Moisture	Ash
1 (38:16)[1]	1.023 a[3] (0.033)[2]	1.124 a (0.316)	0.988 a (0.027)	0.956 a (0.062)
2 (39:18)	0.977 a (0.026)	1.139 a (0.164)	0.986 a (0.018)	1.033 a (0.068)
3 (41:23)	1.025 a (0.021)	1.105 a (0.052)	0.984 a (0.006)	1.000 a (0.033)
4 (45:16)	1.055 a (0.035)	0.972 a (0.115)	0.992 a (0.003)	0.950 a (0.039)
5 (45:22)	1.035 a (0.032)	1.184 a (0.032)	0.975 a (0.006)	0.858 a (0.051)
6 (45:27)	1.009 a (0.044)	1.121 a (0.080)	0.984 a (0.004)	1.048 a (0.110)
7 (43:24)	1.016 a (0.018)	0.963 a (0.020)	1.000 a (0.002)	0.914 a (0.060)
F[4]	1.80	1.00	1.64	3.17
P[5]	0.171	0.463	0.210	0.053

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.
[2] Standard deviation.
[3] Means within a column that do not have or share a common postscript letter are significantly different ($p < 0.05$).
[4] Ratio of the groups mean square to the population's error mean square.
[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.
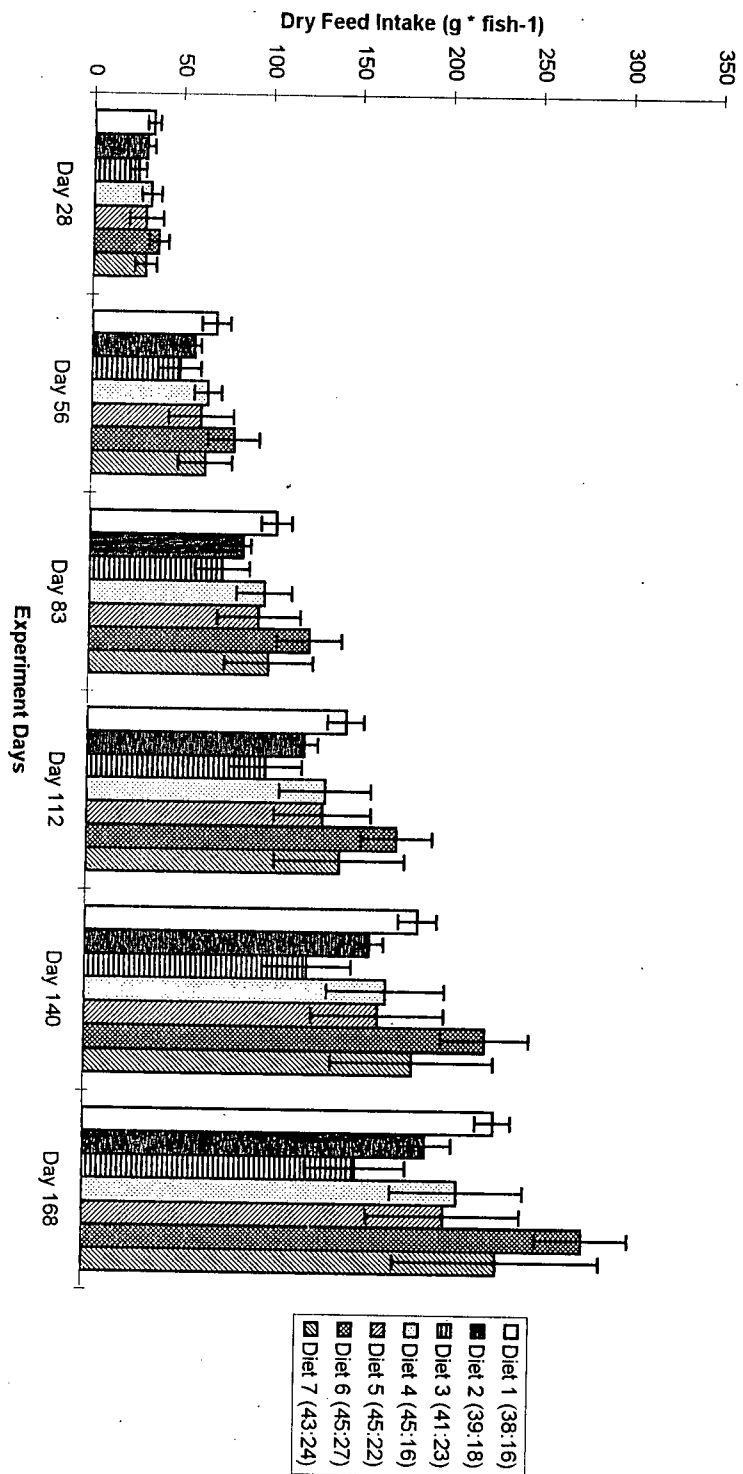
Table 13. *Muscle proximate compositions (%, wet weight basis) of coho salmon at Day 0 and Day 168 of the experiment. The salmon were reared in seawater and fed diets containing different levels of digestible protein and lipid.*

	n	Moisture	Ash	Protein	Lipid
Day 0	5	76.00 $(0.65)^2$	1.65 (0.07)	19.82 (1.01)	2.62 (0.35)
Day 168 Diet 1 $(38:16)^1$	15	74.4 a^3 (0.91)	2.09 a (0.21)	20.75 a (0.64)	3.55 a (0.82)
2 (39:18)	13	73.23 a (1.07)	2.05 a (0.16)	20.54 a (0.66)	4.99 b (0.76)
3 (41:23)	13	74.34 a (1.61)	1.95 a (0.29)	20.11 a (0.77)	3.89 ab (1.07)
4 (45:16)	13	74.53 a (0.79)	2.03 a (0.21)	20.54 a (0.47)	3.27 a (0.59)
5 (45:22)	12	73.81 a (1.60)	2.06 a (0.21)	20.63 a (0.38)	4.16 ab (1.54)
6 (45:27)	13	73.40 a (0.92)	2.12 a (0.25)	20.78 a (0.90)	4.67 b (1.04)
7 (43:24)	12	73.62 a (0.68)	1.98 a (0.14)	20.44 a (0.43)	4.31 ab (0.79)
F^4		2.68	0.83	1.33	5.54
P^5		0.053	0.548	0.258	0.000

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Standard deviation.

[3] Means within a column that do not have or share a common postscript letter are significantly different ($p<0.05$).

[4] Ratio of the groups mean square to the population's error mean square.

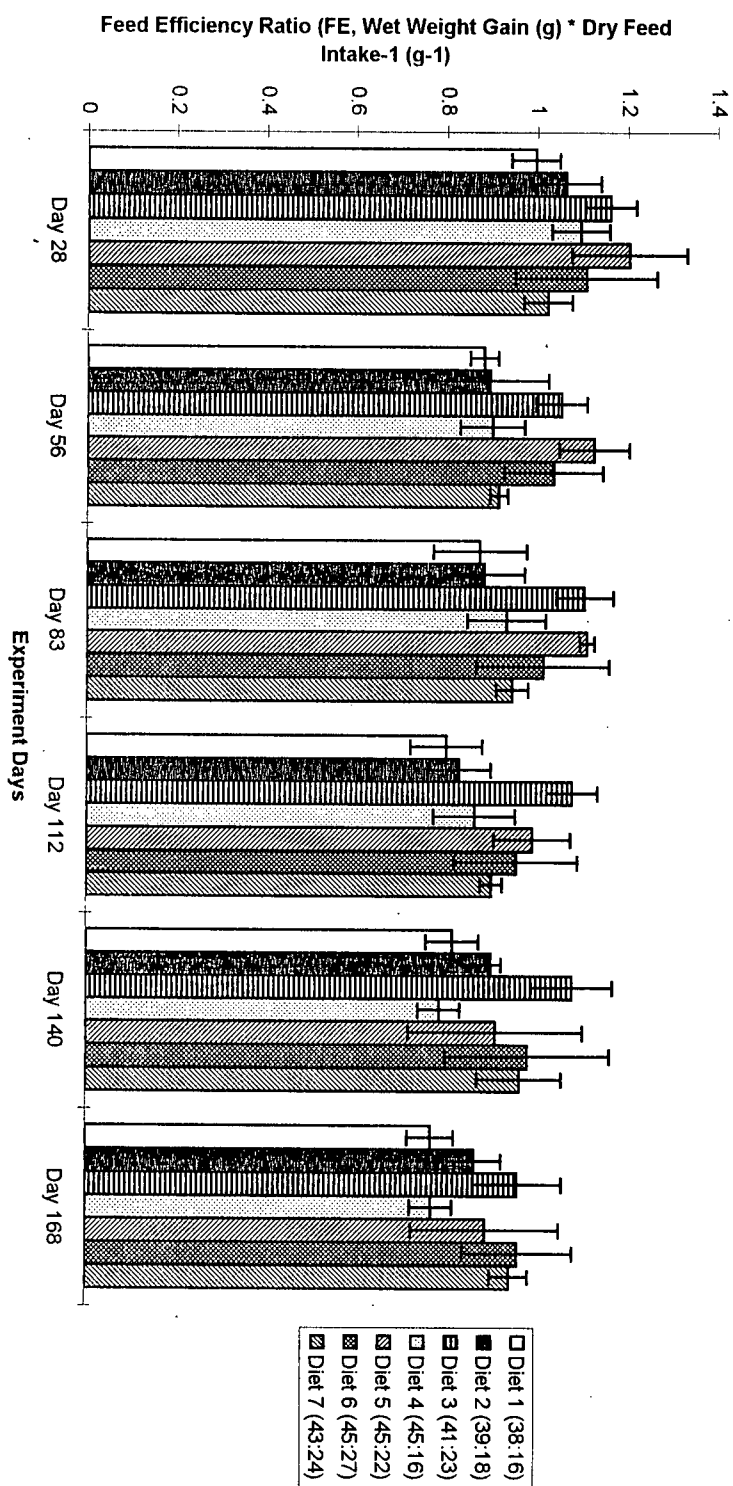[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

Table 14. *Percentages of C14:0, C15:0, C16:0, C16:1ω7, and C18:0 of total fatty acids in the lipid fraction of coho salmon muscle determined by gas chromatography. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	n	14:0	15:0	16:0	16:1ω7	18:0
1 (38:16)	14	3.94 a[3] (0.52)[2]	0.23 a (0.07)	15.07 a (1.73)	6.58 a (0.95)	3.52 ab (0.41)
2 (39:18)	15	4.97 b (0.29)	0.29 b (0.03)	13.78 bc (0.74)	5.16 b (0.21)	2.38 b (0.15)
3 (41:23)	13	2.25 c (0.23)	0.13 c (0.02)	11.99 d (0.87)	2.76 c (0.34)	3.47 ab (0.20)
4 (45:16)	13	4.07 a (0.60)	0.27 ab (0.05)	15.55 e (1.21)	6.52 a (0.91)	3.69 ab (0.55)
5 (45:22)	12	1.50 d (0.17)	0.10 c (0.02)	12.12 d (1.00)	2.28 c (0.67)	5.31 a (4.53)
6 (45:27)	13	4.77 b (0.46)	0.26 ab (0.05)	12.59 bd (1.23)	4.66 b (0.39)	2.17 b (0.29)
7 (43:24)	13	5.53 e (0.67)	0.26 ab (0.03)	14.68 ace (0.96)	9.50 d (1.15)	3.29 b (0.26)
F[4]		134.36	39.44	21.61	148.51	4.93
P[5]		0.000	0.000	0.000	0.000	0.000

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Standard deviation.

[3] Means within a column that do not have or share a common postscript letter are significantly different ($p<0.05$).

[4] Ratio of the groups mean square to the population's error mean square.

[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

Table 15. *Percentages of C18:1ω9, C18:1ω7, C18:2ω6, C18:3ω6, and C18:3ω3 of total fatty acids in the lipid fraction of coho salmon muscle determined by gas chromatography. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	n	18:1ω9	18:1ω7	18:2ω6	18:3ω6	18:3ω3
1 (38:16)	14	17.72 a[3] (1.95)[2]	0.39 ab (0.56)	4.81 a (1.23)	0.31 a (0.07)	1.05 a (0.35)
2 (39:18)	15	17.94 a (0.96)	0.72 bc (0.52)	2.58 a (0.20)	0.09 b (0.04)	0.99 a (0.07)
3 (41:23)	13	19.16 a (2.01)	0.11 a (0.18)	24.19 b (4.50)	0.06 b (0.03)	3.07 b (0.59)
4 (45:16)	13	17.05 a (1.35)	0.67 bc (0.48)	4.27 a (0.56)	0.21 a (0.08)	1.06 a (0.17)
5 (45:22)	12	18.10 a (6.07)	0.10 a (0.15)	30.82 c (4.88)	0.60 c (0.22)	3.82 c (0.69)
6 (45:27)	13	17.16 a (1.35)	0.91 bc (0.31)	2.13 a (0.63)	0.08 b (0.04)	1.07 a (0.11)
7 (43:24)	13	17.89 a (1.74)	1.10 c (0.66)	3.88 a (0.41)	0.23 a (0.04)	0.77 a (0.09)
F[4]		0.91	9.51	285.81	54.71	140.34
p[5]		0.488	0.000	0.000	0.000	0.000

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Standard deviation.

[3] Means within a column that do not have or share a common postscript letter are significantly different ($p < 0.05$).

[4] Ratio of the groups mean square to the population's error mean square.

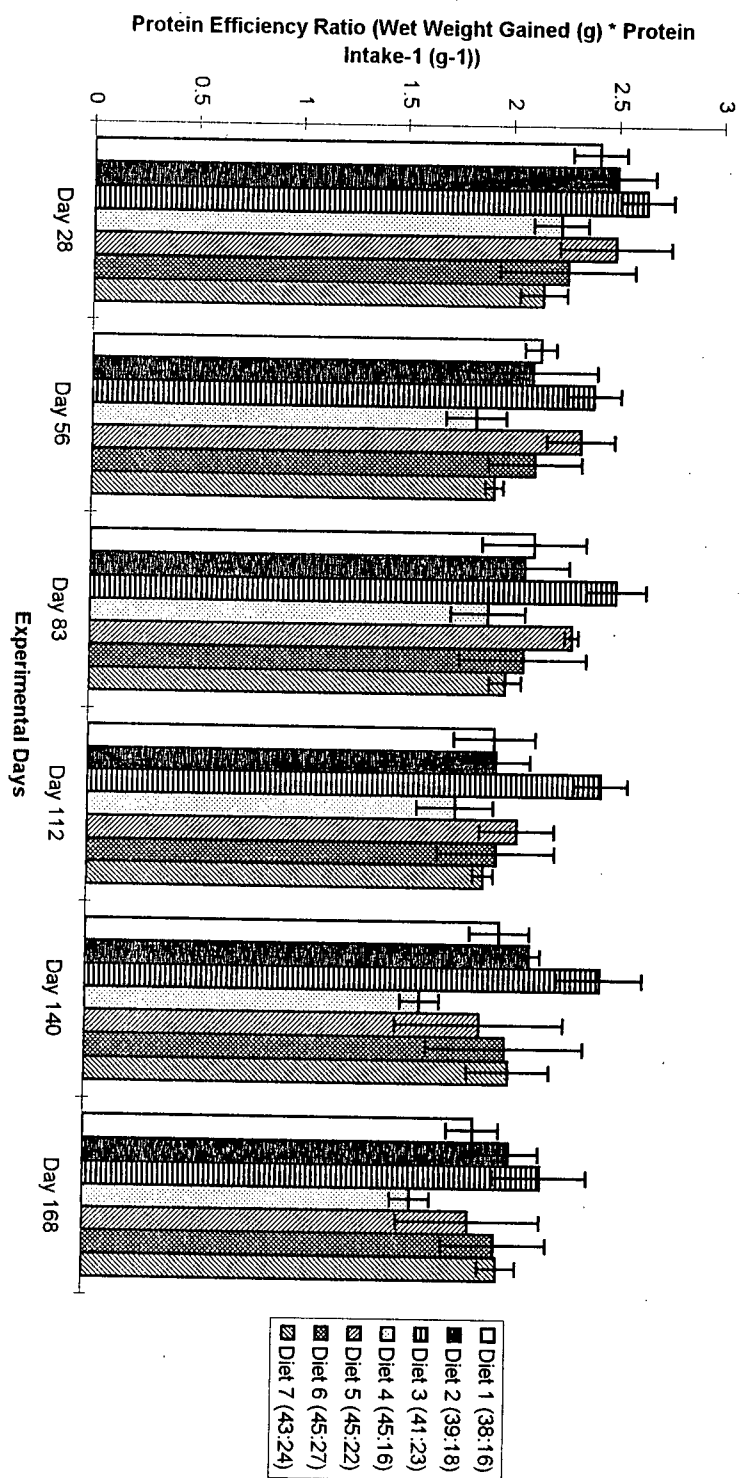[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

75

Table 16. *Percentages of C20:1ω9, C18:4ω3, C20:3ω6, C20:4ω6, and C22:1ω9 of total fatty acids in the lipid fraction of coho salmon muscle determined by gas chromatography. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	n	20:1ω9	18:4ω3	20:3ω6	20:4ω6	22:1ω9
1 (38:16)	14	2.95 a[3] (0.53)[2]	0.27 a (0.17)	0.62 ab (0.350)	1.37 a (0.51)	1.30 a (2.05)
2 (39:18)	15	11.43 b (1.75)	0.13 b (0.06)	0.43 a (0.50)	1.19 a (0.62)	0.83 a (1.13)
3 (41:23)	13	3.63 a (0.44)	0.11 b (0.10)	1.37 b (1.31)	1.49 ab (1.41)	0.38 a (0.82)
4 (45:16)	13	3.49 a (2.49)	0.16 ab (0.10)	0.67 ab (0.84)	2.62 b (1.64)	1.52 a (2.16)
5 (45:22)	12	1.35 c (0.17)	0.12 b (0.08)	0.45 a (0.38)	1.61 ab (1.30)	1.13 a (1.62)
6 (45:27)	13	11.40 b (1.32)	0.11 b (0.08)	0.53 a (0.51)	1.00 a (0.67)	1.18 a (1.60)
7 (43:24)	13	2.32 a (0.47)	0.26 a (0.08)	0.22 a (0.29)	1.15 a (0.34)	1.63 a (1.57)
F[4]		146.76	6.47	3.74	3.64	0.93
P[5]		0.000	0.000	0.002	0.003	0.478

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Standard deviation.

[3] Means within a column that do not have or share a common postscript letter are significantly different ($p < 0.05$).

[4] Ratio of the groups mean square to the population's error mean square.

[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

Table 17. *Percentages of C22:1ω11, C20:5ω3, C22:4ω6, C22:5ω3, and C22:6ω3 of total fatty acis in the lipid fraction of coho salmon muscle determined by gas chromatography. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	n	22:1ω11	20:5ω3	22:4ω6	22:5ω3	22:6ω3
1 (38:16)	14	4.54 a[3] (2.36)[2]	12.54 a (5.64)	1.01 a (0.46)	5.46 a (1.75)	16.35 abc (3.36)
2 (39:18)	15	11.46 b (1.10)	7.66 bc (1.26)	0.71 a (0.70)	3.19 bc (1.03)	14.07 bc (2.02)
3 (41:23)	13	5.08 a (1.79)	4.60 c (1.34)	0.46 a (0.60)	2.86 c (1.69)	12.85 c (2.79)
4 (45:16)	13	3.03 ac (1.51)	9.66 ab (1.50)	1.20 a (1.49)	4.47 ab (0.92)	19.84 a (2.52)
5 (45:22)	12	1.97 c (1.63)	4.74 c (2.99)	0.66 a (1.25)	2.27 b (0.91)	10.96 c (3.34)
6 (45:27)	13	11.40 b (3.52)	7.38 bc (1.85)	0.50 a (0.20)	3.68 bc (1.29)	17.02 ab (3.41)
7 (43:24)	13	2.77 ac (1.16)	11.46 a (2.53)	0.80 a (0.53)	5.08 a (0.82)	17.18 ab (4.29)
F[4]		54.27	16.13	1.33	11.82	11.69
P[5]		0.000	0.000	0.254	0.000	0.000

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.
[2] Standard deviation.
[3] Means within a column that do not have or share a common postscript letter are significantly different ($p<0.05$).
[4] Ratio of the groups mean square to the population's error mean square.
[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

Table 18. *Mean percentages (% of total fatty acids) of different classes and families of fatty acids and ratios of ω6 to ω3 fatty acids in the lipid fraction of coho salmon muscle determined by gas chromatography in relation to diet treatment. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	n	Total Sat.	Total Unsat.	Total ω3	Total ω6	Total HUFA ω3	ω6:ω3
1 (38:16)	14	22.76 a[3] (2.60)[2]	77.24 a (2.60)	35.67 a (5.26)	8.12 a (1.53)	28.88 a (4.83)	0.23 a (0.07)
2 (39:18)	15	21.43 ac (0.91)	78.57 ac (0.91)	26.04 bc (2.48)	5.09 b (1.43)	21.72 b (2.46)	0.20 a (0.09)
3 (41:23)	13	17.84 b (1.22)	82.16 b (1.22)	27.87 bd (4.62)	27.57 c (3.92)	17.44 c (3.43)	0.99 b (0.47)
4 (45:16)	13	23.57 a (2.00)	76.43 a (1.00)	35.19 a (2.79)	8.97 a (1.90)	29.49 a (2.85)	0.25 a (0.08)
5 (45:22)	12	19.03 bc (5.01)	80.98 bc (5.01)	21.91 d (4.81)	34.14 d (4.40)	15.70 c (4.24)	1.56 c (0.72)
6 (45:27)	13	19.79 bc (1.67)	80.21 bc (1.67)	29.26 c (4.70)	4.24 b (0.78)	24.40 b (4.17)	0.14 a (0.04)
7 (43:24)	13	23.77 a (1.36)	76.23 a (1.36)	34.75 a (3.12)	6.28 ab (0.59)	28.63 a (3.92)	0.18 a (0.02)
F[4]		12.36	12.36	34.98	315.15	29.61	76.65
P[5]		0.000	0.000	0.000	0.000	0.000	0.000

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Standard deviation.

[3] Means within a column that do not have or share a common postscript letter are significantly different ($p < 0.05$).

[4] Ratio of the groups mean square to the population's error mean square.

[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

Table 19. Astaxanthin concentrations (ppm, wet weight basis) in the flesh of post-juvenile coho salmon at different weight intervals. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.

Diet[1]	Fish Weight							F_S[5]	P_S[5]
	0.0-100.0	100.1-200.0	200.1-300.0	300.1-400.0	400.1-500.0	500.1-600.0	600.1-700.0		
1 (38:16)	0.31 a x[3,6] (0.27; 8)[2]	1.12 ab xy (0.69; 4)	3.87 a xy (3.19; 4)	3.77 a xy (2.51; 4)	4.28 a yz (2.38; 4)	6.31 a z (0.01; 2)	6.53 a z (0.01; 2)	7.55	0.000
2 (39:18)	0.99 b x (0.36; 6)	1.52 ab xy (0.10; 3)	4.26 a yz (0.95; 2)	6.38 a yz (2.10; 2)	6.44 a yz (1.50; 4)	7.20 a y (0.06; 2)	8.24 a z (0.06; 2)	25.80	0.000
3 (41:23)	1.03 b x (0.36; 8)	1.56 a xy (0.32; 4)	3.93 a y (0.24; 3)	5.40 a z (1.86; 4)	8.72 a z (1.26; 3)	7.55 a z (0.83; 4)	8.05 a z (2.44; 3)	23.71	0.000
4 (45:16)	0.62 ab x (0.12; 5)	0.77 b x (0.37; 8)	3.78 a yz (1.00; 3)	5.69 a yz (1.36; 2)	5.58 a yz (1.45; 5)	7.88 a z (0.11; 2)		44.14	0.000
5 (45:22)	0.66 ab w (0.29; 9)	1.31 ab w (0.20; 3)	4.23 a xy (0.58; 3)	4.93 a y (0.14; 2)	6.06 a y (1.53; 3)	7.97 a z (0.84; 3)	7.43 a yz (0.82; 3)	77.11	0.000
6 (45:27)	0.54 ab x (0.37; 2)	1.93 a x (1.54; 3)	3.84 a xy (0.96; 3)	4.34 a xy (2.81; 2)	7.10 a yz (0.96; 3)	7.29 a yz (1.63; 2)	7.63 a z (1.11; 5)	14.09	0.000
7 (43:24)	0.10 y (0.11; 6)	0.20 yz (0.03; 3)	0.25 z (0.07; 2)	0.39 yz (0.03; 2)	0.29 yz (0.11; 4)	0.40 z (0.15; 6)	0.29 yz (0.13; 2)	5.00	0.004
F_D[4]	7.22	6.20	0.13	0.44	0.50	0.61	0.060		
P_D[5]	0.000	0.001	0.982	0.810	0.773	0.661	0.700		

1 Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

2 Standard deviation; number of observations.

3 Means within a column that do not have or share a common postscript letter (a, b, c, and d) are significantly different ($p < 0.05$).

4 Means within a row that do not have or share a common postscript letter (w, x, y, and z) are significantly different ($p < 0.05$).

5 F_D = Ratio of the diet groups mean square to the population's error mean square.
P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.
F_S = Ratio of the time groups mean square to the population's error mean square.
P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

6 Only the data obtained for diets 1 to 6 were statistically analyzed for the effect of dietary treatment.
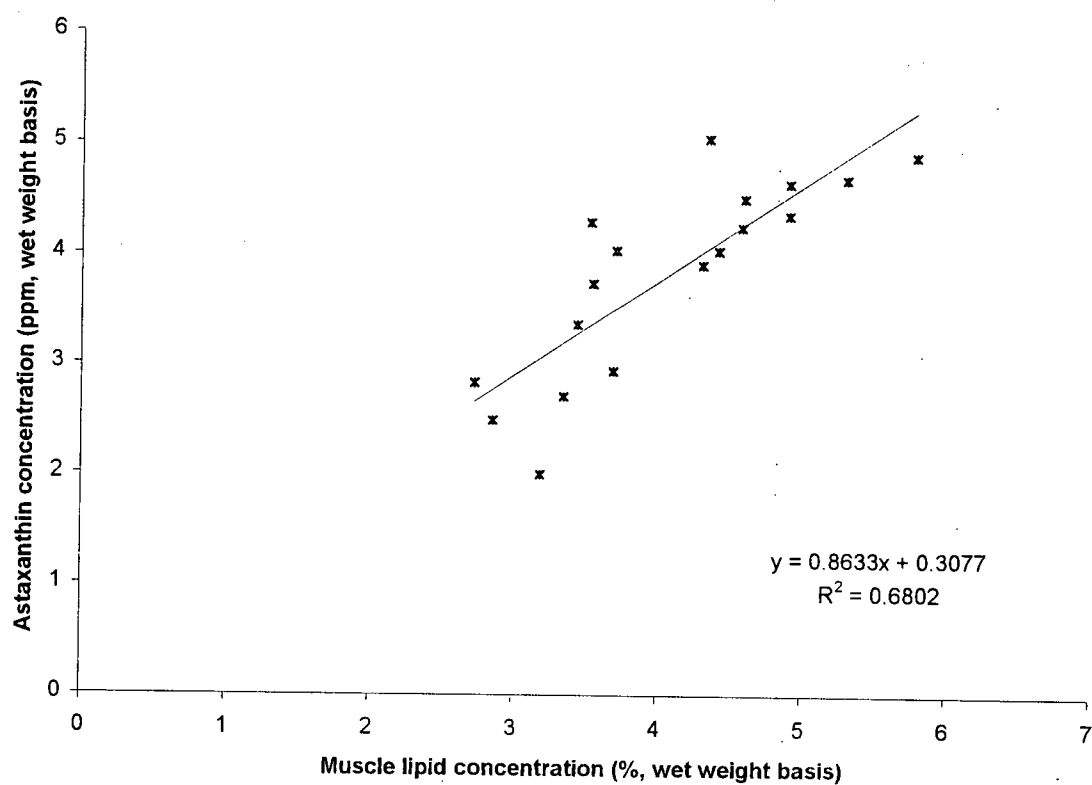
Figure 7. *Correlation between astaxanthin conetent (ppm, wet weight basis) and lipid concentration (%, wet weight basis) in muscle of post-juvenile coho salmon. The salmon were reared in seawater and fed diets with various ratios of digestible protein and lipid for 168 days.*

Table 20. Roche Color Card scores of the flesh of post-juvenile coho salmon at different body weight intervals. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.

Diet[1]	Fish Weight					F_S[5]	P_S[5]
	200.1 -300.0	300.1 -400.0	400.1 -500.0	500.1 -600.0	600.1 -700.0		
1. (38:16)	14 a y[2,6] (1; 4)[3]	15 a yz (1; 6)	14 a yz (1; 7)	15 a yz (0; 3)	16 a z (0; 3)	2.90	0.052
2. (39:18)	13 a z (1; 4)	14 a z (1; 2)	15 a z (1; 10)	15 a z (1; 4)	15 a z (1; 2)	2.92	0.051
3. (41:23)	14 a z (1; 4)	14 a z (1; 10)	14 a z (1; 5)	15 a z (1; 4)	15 a z (1; 3)	2.39	0.085
4. (45:16)	11 a x (1; 2)	13 a y (1; 5)	14 a yz fx(1; 5)	15 a z (1; 2)	16 a z (1; 2)	10.55	0.000
5. (45:22)	13 a z (2; 3)	14 a z (1; 3)	14 a z (1; 7)	15 a z (0; 4)	15 a z (0; 3)	2.77	0.066
6. (45:27)	13 a y (1; 2)	15 a yz (1; 7)		15 a yz (0; 6)	16 a z (1; 4)	3.22	0.040
7. (43:24)	10 z (0; 2)	10 z (0; 4)	11 z (1; 7)	11 z (1; 8)	11 z (1; 5)	1.53	0.230
F_D[4]	2.70	1.12	0.55	0.37	0.85		
P_D[4]	0.069	0.381	0.737	0.861	0.544		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a and b) are significantly different ($p < 0.05$).

Means within a row that do not have or share a common postscript letter (x, y, and z) are significantly different ($p < 0.05$).

[3] Standard deviation; number of observations.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square.

P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_S = Ratio of the time groups mean square to the population's error mean square.

P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

[6] Only the data obtained for diets 1 to 6 were statistically analyzed for the effect of dietary treatment.

Table 21. SalmoFan for Salmonids scores of the flesh of post-juvenile coho salmon at different body weight intervals. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.

Diet[1]	Fish Weight					F_S[5]	P_S[5]
	200.1 -300.0	300.1 -400.0	400.1 -500.0	500.1 -600.0	600.1 -700.0		
1 (38:16)	24 a z[2,6] (2; 4)[3]	25 a z (2; 6)	26 a z (2; 7)	27 a z (2; 3)	28 a z (3; 3)	2.15	0.107
2 (39:18)	23 a z (1; 4)	23 a z (1; 2)	26 a z (3; 10)	27 a z (2; 2)	28 a z (2; 2)	3.22	0.039
3 (41:23)	25 a z (1; 4)	25 a z (2; 10)	24 a z (2; 5)	27 a z (2; 4)	28 a z (1; 3)	2.60	0.067
4 (45:16)	22 a z (4; 2)	23 a y (1; 5)	26 a yz (2; 9)	26 a yz (2; 5)	27 a z (1; 3)	4.80	0.007
5 (45:22)	23 a z (1; 3)	24 a z (1; 3)	25 a z (2; 7)	26 a z (1; 4)	27 a z (2; 3)	2.46	0.082
6 (45:27)	25 a y (1; 2)	26 a z (1; 2)	26 a yz (2; 7)	26 a yz (1; 6)	28 a z (2; 4)	3.68	0.023
7 (43:24)	19 z (0; 2)	19 z (0; 4)	20 z (1; 7)	19 z (1; 8)	19 z (0; 5)	1.17	0.355
F_D[4]	1.71	1.51	0.79	0.34	0.52		
P_D[4]	0.193	0.232	0.560	0.885	0.755		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a and b) are significantly different (p<0.05).
Means within a row that do not have or share a common postscript letter (y and z) are significantly different (p<0.05).

[3] Standard deviation: number of observations.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_S = Ratio of the time groups mean square to the population's error mean square.
P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

[6] Only the data obtained for diets 1 to 6 were statistically analyzed for the effect of dietary treatment.

Table 22. *Hunter L scores of the flesh of post-juvenile coho salmon at different weight intervals. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	Fish Weight 200.1-300.0	300.1-400.0	400.1-500.0	500.1-600.0	600.1-700.0	F_S[5]	P_S[5]
1 (38:16)	30.93 a y z,[2],[6] (2.12; 4)[3]	34.03 a yz (4.02; 6)	32.73 a yz (2.68; 7)	36.30 a z (1.49; 3)	32.59 a yz (9.16; 3)	3.67	0.034
2 (39:18)	37.19 a z (5.14; 4)	35.75 a z (6.37; 2)	35.06 a z (3.02; 10)	34.88 a z (2.62; 4)	34.02 a z (2.78; 2)	0.34	0.884
3 (41:23)	34.98 a z (2.17; 4)	35.00 a z (2.82; 10)	34.92 a z (3.13; 5)	33.26 a z (2.28; 4)	35.73 a z (1.17; 3)	0.50	0.735
4 (45:16)	35.45 a z (6.58; 2)	36.97 a z (4.49; 5)	34.06 a z (4.40; 9)	33.23 a z (1.63; 5)	29.91 a z (2.26; 2)	1.27	0.320
5 (45:22)	33.52 a z (3.39; 3)	35.43 a z (3.06; 4)	35.58 a z (2.41; 7)	34.14 a z (1.25; 4)	33.98 a z (1.27; 3)	0.66	0.626
6 (45:27)	35.67 a z (2.83; 2)	35.80 a z (2.46; 2)	36.34 a z (2.34; 7)	36.46 a z (2.96; 6)	33.00 a z (3.18; 4)	0.68	0.619
7 (43:24)	43.22 z (1.71; 2)	41.74 z (2.98; 4)	39.24 z (2.31; 7)	39.43 z (2.92; 8)	41.66 z (4.77; 5)	1.23	0.325
F_D[4] P_D[4]	1.27 0.336	0.33 0.889	1.42 0.237	1.66 0.186	0.25 0.934		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a and b) are significantly different (p<0.05).

Means within a row that do not have or share a common postscript letter (y and z) are significantly different (p<0.05).

[3] Standard deviation; number of observations.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square.

P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_S = Ratio of the time groups mean square to the population's error mean square.

P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

[6] Only the data obtained for diets 1 to 6 were statistically analyzed for the effect of dietary treatment.

Table 23. *Hunter a scores of the flesh of post-juvenile coho salmon at different weight intervals. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	Fish Weight 200.1-300.0	300.1-400.0	400.1-500.0	500.1-600.0	600.1-700.0	F_S[5]	P_S[5]
1 (38:16)	9.82 a y^{z}[6] (1.67; 4)[3]	13.71 a yz (2.14; 6)	12.05 ab y (1.67; 7)	14.47 a yz (0.46; 3)	16.14 a z (0.46; 3)	4.96	0.007
2 (39:18)	9.77 a wy (0.58; 4)	11.36 a xy (1.06; 2)	13.24 ab y (1.68; 10)	16.03 a z (1.89; 4)	18.80 a z (0.79; 2)	19.74	0.000
3 (41:23)	10.20 a y (0.53; 4)	11.24 a y (1.69; 10)	14.98 a z (1.55; 5)	15.17 a z (2.13; 4)	17.56 a z (2.11; 3)	13.72	0.000
4 (45:16)	4.73 b x (2.15; 2)	11.53 a y (1.18; 5)	12.45 b y (1.53; 9)	14.00 a yz (1.90; 5)	15.94 a z (0.96; 2)	18.81	0.000
5 (45:22)	9.60 a x (1.20; 3)	11.23 a xy (0.17; 3)	12.94 ab xyz (1.45; 7)	14.29 a yz (1.53; 4)	15.28 a z (3.30; 3)	6.30	0.003
6 (45:27)	9.92 a x (0.51; 2)	11.17 a xy (1.62; 2)	14.95 a z (1.55; 7)	14.12 a yz (1.58; 6)	16.13 a z (1.65; 4)	8.74	0.001
7 (43:24)	1.40 z (0.23; 2)	2.40 z (0.83; 4)	2.87 z (1.04; 7)	2.67 z (1.54; 8)	2.28 z (1.38; 5)	0.64	0.640
F_D[4]	3.82	1.85	3.80	1.34	0.89		
P_D[4]	0.018	0.151	0.007	0.290	0.512		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a, b, and c) are significantly different (p<0.05). Means within a row that do not have or share a common postscript letter (w, x, y, and z) are significantly different (p<0.05).

[3] Standard deviation; number of observations.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square. P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_S = Ratio of the time groups mean square to the population's error mean square. P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

[6] Only the data obtained for diets 1 to 6 were statistically analyzed for the effect of dietary treatment.

Table 24. Hunter b scores of the flesh of post-juvenile coho salmon at different weight intervals. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.

Diet[1]	Fish Weight 200.1-300.0	300.1-400.0	400.1-500.0	500.1-600.0	600.1-700.0	F_S[5]	P_S[5]
1 (38:16)	8.96 bc z[2,6] (1.01; 4)[3]	11.48 a z (1.31; 6)	10.64 b z (1.89; 3)	11.12 a z (1.89; 3)	11.54 a z (1.89; 3)	2.38	0.083
2 (39:18)	11.02 a z (0.52; 4)	11.11) a z (1.90; 2)	11.51 ab z (1.17; 10)	11.93 a z (1.44; 4)	12.98 a z (0.54; 2)	1.17	0.377
3 (41:23)	9.70 ab x (0.53; 4)	10.44 a xy (0.53; 10)	12.86 a z (1.52; 5)	11.98 a yz (1.32; 4)	13.56 a z (0.88; 3)	10.35	0.000
4 (45:16)	7.39 c x (0.20; 2)	11.23 a yz (1.64; 5)	10.51 a y (1.22; 9)	11.34 a yz (1.44; 5)	13.29 a z (0.88; 2)	7.50	0.001
5 (45:22)	10.11 ab y (1.35; 3)	11.35 a yz (1.67; 3)	12.06 ab yz (1.20; 7)	12.64 a yz (0.94; 4)	13.00 a z (1.15; 3)	3.14	0.040
6 (45:27)	9.00 abc y (0.35; 2)	10.68 a yz (0.38; 2)	12.29 ab z (1.22; 7)	12.26 a z (1.32; 6)	12.81 a z (1.21; 4)	4.82	0.007
7 (43:24)	8.77 z (0.57; 2)	9.80 z (0.59; 4)	9.47 z (1.22; 7)	9.38 z (1.18; 8)	9.94 z (1.98; 5)	0.39	0.813
F_D[4]	5.74	0.67	4.16	0.59	1.11		
P_D	0.004	0.649	0.004	0.704	0.392		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.
[2] Means within a column that do not have or share a common postscript letter (a, b, and c) are significantly different (p<0.05).
Means within a row that do not have or share a common postscript letter (x, y, and z) are significantly different (p<0.05).
[3] Standard deviation; number of observations.
[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.
[5] F_S = Ratio of the time groups mean square to the population's error mean square.
P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.
[6] Only the data obtained for diets 1 to 6 were statistically analyzed for the effect of dietary treatment.

Table 25. *Chroma scores of the flesh of post-juvenile coho salmon at different weight intervals. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	Fish Weight 200.1-300.0	300.1-400.0	400.1-500.0	500.1-600.0	600.1-700.0	F_S[5]	P_S[5]
1 (38:16)	13.31 a z[2,6] (1.81; 4)[3]	18.07 a z (2.17; 6)	16.57 a z (2.68; 7)	17.43 a z (3.64; 3)	18.24 a z (3.59; 3)	2.26	0.103
2 (39:18)	14.56 a y (0.25; 4)	15.95 ab yz (0.57; 2)	17.68 a yz (1.83; 10)	20.25 a z (2.67; 4)	20.92 a z (2.39; 2)	5.00	0.012
3 (41:23)	14.07 a y (0.67; 4)	15.50 b y (1.38; 10)	19.74 a z (2.16; 5)	19.35 a z (2.39; 4)	21.82 a z (2.69; 3)	12.41	0.000
4 (45:16)	9.38 b x (0.27; 2)	15.35 ab y (0.51; 5)	17.09 a y (2.50; 9)	17.28 a yz (2.16; 5)	20.76 a z (1.16; 2)	11.81	0.000
5 (45:22)	13.56 a x (1.60; 3)	15.70 ab xy (1.24; 3)	17.73 a yz (1.86; 7)	18.38 a yz (1.97; 4)	19.67 a z (2.58; 3)	5.35	0.005
6 (45:27)	13.83 a y (2.01; 2)	15.48 ab yz (0.90; 2)	19.17 a z (1.95; 7)	19.28 a z (2.02; 6)	19.86 a z (2.21; 4)	5.08	0.005
7 (43:24)	9.31 z (0.08; 2)	10.01 z (0.77; 4)	9.69 z (1.50; 7)	10.07 z (1.79; 8)	11.78 z (1.07; 5)	1.97	0.139
F_D[4]	5.17	2.53	1.86	0.96	0.85		
P_D[4]	0.008	0.064	0.125	0.465	0.530		

1 Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.
2 Means within a column that do not have or share a common postscript letter (a, b, and c) are significantly different (p<0.05).
3 Means within a row that do not have or share a common postscript letter (x, y, and z) are significantly different (p<0.05).
4 Standard deviation; number of observations.
F_D = Ratio of the diet groups mean square to the population's error mean square.
P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.
5 F_S = Ratio of the time groups mean square to the population's error mean square.
P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.
6 Only the data obtained for diets 1 to 6 were statistically analyzed for the effect of dietary treatment.

Table 26. Hue scores of the flesh of post-juvenile coho salmon at different weight intervals. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.

Diet[1]	Fish Weight					F_S[5]	P_S[5]
	200.1 -300.0	300.1 -400.0	400.1 -500.0	500.1 -600.0	600.1 -700.0		
1 (38:16)	0.744 a z[2,6] (0.060; 4)[3]	0.711 a z (0.071; 6)	0.728 a z (0.044; 7)	0.654 ab z (0.009; 3)	0.685 a z (0.096; 3)	1.33	0.290
2 (39:18)	0.852 ab y (0.051; 4)	0.772 a yz (0.132; 2)	0.710 a z (0.058; 10)	0.640 a z (0.037; 4)	0.627 a z (0.010; 2)	8.51	0.001
3 (41:23)	0.760 a z (0.024; 4)	0.742 a z (0.066; 10)	0.706 a z (0.005; 5)	0.670 ab z (0.042; 4)	0.674 a z (0.047; 3)	2.90	0.048
4 (45:16)	1.014 b y (0.217; 2)	0.757 a z (0.057; 5)	0.695 a z (0.037; 9)	0.706 ab z (0.041; 5)	0.681 a z (0.006; 2)	11.85	0.000
5 (45:22)	0.853 ab y (0.106; 3)	0.767 a yz (0.078; 3)	0.745 a yz (0.039; 7)	0.724 b yz (0.025; 4)	0.687 a z (0.069; 3)	3.76	0.022
6 (45:27)	0.843 ab z (0.024; 2)	0.766 a z (0.090; 2)	0.688 a z (0.035; 7)	0.708 b z (0.024; 6)	0.696 a z (0.037; 4)	0.63	0.645
7 (43:24)	1.406 y (0.043; 2)	1.322 yz (0.076; 4)	1.284 z (0.071; 7)	1.310 z (0.133; 8)	1.346 z (0.118; 5)	7.61	0.001
F_D[4]	3.46	0.39	1.93	4.07	0.39		
P_D[4]	0.033	0.847	0.110	0.010	0.850		

1 Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

2 Means within a column that do not have or share a common postscript letter (a, b, and c) are significantly different (p<0.05).

Means within a row that do not have or share a common postscript letter (y and z) are significantly different (p<0.05).

3 Standard deviation; number of observations.

4 F_D = Ratio of the diet groups mean square to the population's error mean square.

P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

5 F_S = Ratio of the time groups mean square to the population's error mean square.

P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

6 Only the data obtained for diets 1 to 6 were statistically analyzed for the effect of dietary treatment.

Table 27. Hardness I (Newton) of cooked coho salmon fillets determined after the first compression by a Texture Analyzer. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.

Diet[1]	Fish Weight 200.1 -300.0	300.1 -400.0	400.1 -500.0	500.1 -600.0	600.1 -700.0	F_S[5]	P_S[5]
1 (38:16)	254.0 a z[2] (76.1; 4)[3]	253.6 a z (35.8; 6)	227.1 a z (29.8; 10)	177.1 a z (60.6; 3)	177.2 a z (33.1; 6)	3.49	0.023
2 (39:18)	243.8 a z (43.8; 4)	230.6 a z (9.7; 2)	187.6 a z (62.5; 11)	185.7 a z (21.8; 7)	134.9 a z (23.2; 2)	0.22	0.101
3 (41:23)	244.0 a z (54.9; 4)	223.1 a z (76.9; 10)	213.9 a z (55.4; 4)	194.0 a z (38.2; 5)	158.9 a z (17.2; 3)	0.98	0.439
4 (45:16)	277.4 a z (65.2; 4)	252.1 a z (58.8; 4)	222.3 a z (58.3; 10)	171.7 a z (21.2; 4)	199.4 a z (80.7; 4)	1.94	0.143
5 (45:22)	288.2 a y (38.3; 4)	201.4 a yz (36.8; 4)	221.6 a yz (34.4; 7)	194.4 a yz (54.8; 6)	164.3 a z (76.3; 6)	3.63	0.018
6 (45:27)	274.4 a y (13.7; 2)	252.2 a yz (18.7; 2)	216.6 a yz (55.0; 8)	212.5 a yz (49.1; 4)	157.3 a z (33.7; 5)	3.12	0.045
7 (43:24)	293.9 a y (21.8; 2)	213.3 a yz (24.6; 6)	214.7 a yz (45.6; 10)	191.8 a z (41.3; 8)	141.6 a z (59.8; 4)	4.81	0.005
F_D[4]	0.49	0.61	0.69	0.41	0.49		
P_D[4]	0.806	0.724	0.655	0.868	0.811		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a) are significantly different ($p < 0.05$).

Means within a row that do not have or share a common postscript letter (y and z) are significantly different ($p < 0.05$).

[3] Standard deviation; number of observations.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square.

P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_S = Ratio of the time groups mean square to the population's error mean square.

P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

Table 28. *Hardness II (Newton) of cooked coho salmon fillets determined after the second compression by a Texture Analyzer. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	Fish Weight 200.1 -300.0	300.1 -400.0	400.1 -500.0	500.1 -600.0	600.1 -700.0	F_S[5]	P_S[5]
1 (38:16)	192.6 a yz[2] (27.8; 4)[3]	219.8 a y (59.7; 6)	190.5 a yz (20.5; 10)	160.8 a yz (54.8; 3)	150.5 a z (29.8; 6)	2.91	0.043
2 (39:18)	206.9 a z (23.1; 4)	196.5 a z (5.7; 2)	163.1 a z (50.8; 11)	159.5 a z (21.9; 7)	123.2 a z (20.9; 2)	1.87	0.153
3 (41:23)	201.1 a z (80.1; 4)	214.1 a z (74.9; 10)	168.7 a z (51.7; 4)	164.2 a z (34.0; 5)	149.9 a z (23.2; 3)	1.04	0.407
4 (45:16)	226.5 a z (17.1; 4)	216.0 a z (42.3; 4)	193.6 a z (50.2; 10)	152.4 a z (18.5; 4)	153.0 a z (45.9; 4)	2.40	0.083
5 (45:22)	259.7 a y (42.9; 4)	179.3 a yz (35.7; 4)	197.1 a yz (30.5; 7)	178.9 a z (54.6; 6)	163.1 a z (39.9; 6)	3.66	0.020
6 (45:27)	252.1 a y (21.6; 2)	237.4 a yz (30.0; 2)	199.9 a yz (40.8; 8)	174.9 a yz (50.6; 4)	155.5 a z (41.6; 5)	3.43	0.027
7 (43:24)	227.6 a z (56.3; 2)	174.7 a z (23.4; 6)	198.1 a z (37.6; 10)	177.9 a z (39.4; 8)	144.6 a z (63.3; 4)	1.98	0.127
F_D[4]	0.96	0.66	1.24	0.33	0.26		
P_D[4]	0.482	0.679	0.301	0.914	0.953		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a) are significantly different (p<0.05).

[3] Means within a row that do not have or share a common postscript letter (y and z) are significantly different (p<0.05).

[3] Standard deviation; number of observations.

[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
 P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

[5] F_S = Ratio of the time groups mean square to the population's error mean square.
 P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

Table 29. *Firmness (Newton · sec⁻¹) of cooked coho salmon fillets determined by a Texture Analyzer. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	Fish Weight 200.1-300.0	300.1-400.0	400.1-500.0	500.1-600.0	600.1-700.0	F_S[5]	P_S[5]
1 (38:16)	50.44 a z[2] (11.15; 4)[3]	45.54 a z (10.52; 6)	48.71 a z (15.02; 10)	36.31 a z (15.02; 3)	31.27 a z (16.06; 6)	2.35	0.084
2 (39:18)	47.83 a z (5.88; 4)	43.56 a z (3.50; 2)	37.38 a z (11.68; 11)	34.32 a z (3.06; 2)		1.89	0.147
3 (41:23)	46.94 a z (20.52; 4)	44.97 a z (11.89; 10)	36.48 a z (6.39; 4)	36.49 a z (6.39; 5)	31.94 a z (22.08; 3)	1.16	0.352
4 (45:16)	48.47 a z (7.98; 4)	48.75 a z (21.92; 4)	32.00 a z (3.26; 10)	32.00 a z (3.26; 4)	34.37 a z (4.41; 4)	1.58	0.219
5 (45:22)	55.31 a z (17.13; 4)	43.72 a z (8.70; 4)	39.01 a z (11.69; 7)	39.01 a z (11.69; 6)	39.72 a z (6.11; 6)	1.89	0.150
6 (45:27)	41.95 a z (5.20; 2)	35.50 a z (5.91; 2)	39.35 a z (11.25; 8)	39.35 a z (11.25; 4)	33.12 a z (8.98; 5)	1.45	0.264
7 (43:24)	40.35 a z (3.12; 2)	37.09 a z (6.06; 6)	38.92 a z (6.88; 10)	38.92 a z (6.88; 8)	29.13 a z (10.10; 4)	1.89	0.146
F_D[4]	0.66	0.57	1.32	0.39	1.08		
P_D[4]	0.682	0.751	0.262	0.880	0.400		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Means within a column that do not have or share a common postscript letter (a) are significantly different (p<0.05).

[3] Means within a row that do not have or share a common postscript letter (z) are significantly different (p<0.05).

[4] Standard deviation; number of observations.

[5] F_D = Ratio of the diet groups mean square to the population's error mean square.
P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.
F_S = Ratio of the time groups mean square to the population's error mean square.
P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

16

Table 30. Cohesiveness of cooked coho salmon fillets determined by a Texture Analyzer. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.

Diet[1]	Fish Weight 200.1 -300.0	300.1 -400.0	400.1 -500.0	500.1 -600.0	600.1 -700.0	F_S[5]	P_S[5]
1 (38:16)	0.4553 a z[2] (0.0149; 4)[3]	0.4802 a z (0.0714; 6)	0.4482 a z (0.0527; 10)	0.4561 a z (0.0232; 3)	0.4570 a z (0.0228; 6)	0.40	0.803
2 (39:18)	0.4540 a z (0.0286; 4)	0.4802 a z (0.0718; 2)	0.4531 a z (0.0521; 11)	0.4624 a z (0.0492; 7)	0.4833 a z (0.0395; 2)	0.39	0.817
3 (41:23)	0.4601 a z (0.0329; 4)	0.4751 a z (0.0443; 10)	0.4704 a z (0.0299; 4)	0.4698 a z (0.0233; 5)	0.5185 a z (0.0059; 3)	0.95	0.450
4 (45:16)	0.4614 a z (0.0105; 4)	0.4964 a z (0.0588; 4)	0.4888 a z (0.0513; 10)	0.4790 a z (0.0233; 4)	0.4825 a z (0.0120; 4)	0.26	0.901
5 (45:22)	0.4790 a z (0.0435; 4)	0.4631 a z (0.0200; 4)	0.4973 a z (0.0300; 7)	0.4488 a z (0.0232; 4)	0.4718 a z (0.0691; 6)	0.86	0.504
6 (45:27)	0.4523 a z (0.0090; 2)	0.4101 a z (0.0234; 2)	0.4689 a z (0.0330; 8)	0.5252 a z (0.1014; 4)	0.4736 a z (0.0271; 5)	1.97	0.146
7 (43:24)	0.4977 a z (0.0004; 2)	0.4885 a z (0.1765; 6)	0.4484 a z (0.0719; 10)	0.4804 a z (0.0251; 8)	0.4590 a z (0.0423; 4)	0.33	0.853
F_D[4]	0.98	0.37	1.29	1.08	0.79		
P_D[4]	0.473	0.894	0.279	0.398	0.588		

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.
[2] Means within a column that do not have or share a common postscript letter (a) are significantly different (p<0.05). Means within a row that do not have or share a common postscript letter (z) are significantly different (p<0.05).
[3] Standard deviation; number of observations.
[4] F_D = Ratio of the diet groups mean square to the population's error mean square.
P_D = Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.
[5] F_S = Ratio of the time groups mean square to the population's error mean square.
P_S = Probability of committing a Type I error, i.e. probability that there is no difference among fish of different sizes.

Table 31. *Sensory attributes, aroma, flavor, off-flavor, texture, and overall acceptability, of cooked coho salmon fillets assessed by 11 panelists at 9 sittings. The salmon were reared in seawater and fed diets containing various levels of digestible protein and lipid for 168 days.*

Diet[1]	Aroma	Flavor	Off-Flavor	Texture	Overall
1 (38:16)	7.16 a[3] (3.63)[2]	7.63 a (3.04)	1.70 a (2.85)	5.82 ab (2.79)	7.50 ab (2.84)
2 (39:18)	8.00 a (3.17)	8.85 ab (2.67)	1.35 a (2.27)	5.54 ab (2.59)	8.32 a (2.54)
3 (41:23)	7.53 a (3.23)	8.94 a (1.08)	1.03 a (1.76)	5.43 ab (2.60)	7.76 ab (2.59)
4 (45:16)	8.05 a (3.23)	8.52 ab (3.09)	1.51 a (2.25)	5.67 ab (2.66)	7.92 ab (2.89)
5 (45:22)	7.09 a (3.41)	7.82 a (2.94)	1.74 a (2.46)	5.59 ab (2.60)	7.50 ab (2.60)
6 (45:27)	7.76 a (3.25)	9.25 b (2.53)	1.43 a (2.23)	4.98 a (2.54)	8.41 a (2.71)
7 (43:24)	7.93 a (3.26)	7.89 a (3.16)	1.82 a (2.88)	6.15 b (2.64)	7.28 b (2.97)
F[4]	1.65	3.78	1.79	3.20	2.43
P[5]	0.130	0.001	0.098	0.004	0.025

[1] Values in parentheses denote the estimated digestible levels (%) of protein and lipid on a dry weight basis.

[2] Standard deviation.

[3] Means within a column that do not have or share a common postscript letter are significantly different (p<0.05).

[4] Ratio of the groups mean square to the population's error mean square.

[5] Probability of committing a Type I error, i.e. probability that there is no difference among dietary treatments.

92

5.   Discussion

5.1.   Chemical Compositions of the Diets

5.1.1.   Proximate Compositions

Proximate analysis (Table 2) revealed that all extruded diets used in the present study contained adequate levels of protein and lipid to support growth of salmonids as recommended by NRC (1993). However, the digestible lipid content in diets 2 and 3 were found to be less than expected. This consequently led to elevated levels of digestible protein in these two diets.

Nevertheless, all diets contained at least 19.22 MJ of digestible energy per kg of dry feed and the digestible protein to digestible energy ratios (DP:DE) ranged from 19.6 g $\cdot$ MJ^{-1} to 23.1 g $\cdot$ MJ^{-1}. Wide ranges of optimal DE content and DP:DE ratios have been recommended by different researchers for different species of salmonids. Cho and Kaushik (1990) and Cowey (1992) suggested that diets for rainbow trout should contain DE levels of 15 to 17 MJ $\cdot$ kg^{-1} and DP:DE ratios ranging from 22 to 24 g $\cdot$ MJ^{-1}. For Atlantic salmon, Hillestrad and Johnsen (1994) reported that a DP:DE ratio of 15 g $\cdot$ MJ^{-1} provided better growth than DP:DE ratios of 17 to 19 g $\cdot$ MJ^{-1} when fish size varied between 0.1 and 0.6 kg. Anderson et al. (1996) also reported that a DP:DE ratio of 17.4 g $\cdot$ MJ^{-1} supported the best growth performance of Atlantic salmon grown from 0.5 to 1.2 kg. Einen and Roem (1997) reported that the optimal dietary DP:DE ratios for maximum growth response were 19 g $\cdot$ MJ^{-1} for Atlantic salmon between 1.0 to 2.5 kg and 16 to 17 g $\cdot$ MJ^{-1} for Atlantic salmon between 2.5 and 5 kg.

Based on the preceding values obtained for Atlantic salmon, it is possible that the dietary DP:DE ratios tested in the present study on coho salmon exceeded the optimal levels required to support growth of this species in seawater. In this regard, the diets may have provided excess protein per unit energy. However, the present findings suport those of Silver et al. (1993) for chinook salmon in seawater over a similar size range.

5.1.2. Fatty Acid Profile

Although all of the experimental diets were formulated to contain the same source of supplemental marine lipid, namely, menhaden oil, the fatty acid analyses of the lipid fraction of the diets showed that several of the experimental diets had different fatty acid compositions form those that were anticipated. In this regard, of the fatty acid profiles of diets 1, 2, 4, and 6 were similar and resembled the composition of menhaden fish oil. By contrast, the fatty acid profile compositions of diets 3 and 5 resembled each other and were clearly different from the other diets. This strongly suggests that a different supplemental oil source was used to produce diets 3 and 5. Fatty acid analyses showed that diets 3 and 5 contained high levels of $\omega 6$ fatty acids (39.8 % and 42.3 % of total lipid in diets 3 and 5, respectively) and elevated by not excessive levels of $C18:1\omega 9$ and $C 18:3\omega 3$. These findings suggested that diets 3 and 5 were supplemented with a vegetable oil, probably corn oil. According to the manufacture record, four of the six experimental diets were processed on the same day and the other two diets were manufactured on a second day. The dietary fatty acid profiles as well as the fillet fatty composition data clearly suggest that a mistake was made on the second day of diet manufacture.

The elevated levels of ω-6 fatty acids in diets 3 and 5 led to concerns about fish growth and health in the present study. A previous study on juvenile coho salmon in freshwater by Yu and Sinnhuber (1979) found that the growth rate and feed conversion efficiencies of the fish were depressed when the diets contained more than 1% of ω-6 fatty acids. Interestingly, diets 3 and 5 in this study contained much higher levels of ω-6 fatty acids then those used by Yu and Sinnhuber (1979), yet the growth and survuval (Appendix A) of the post-juvenile coho salmons were not significantly depressed relative to fish given the other diet treatments at each protein level. This point, however, requires further investigation and confirmation.

5.1.3. Astaxanthin Content

According to the diet formulations (Table 1), all diets should have been supplemented with 40 ppm (dry weight basis) of astaxanthin. HPLC analysis of astaxanthin content in the diets showed that only 26.7 to 31.9 ppm of astaxanthin were present in the extruded experimental diets (Table 4). Several factors may have contributed to the low levels of astaxanthin found in the diets.

The lower than expected levels of astaxanthin in the diets could have resulted from thermal degradation of the pigment during the high temperature extrusion process. Information on the stability of astaxanthin during extrusion is unavailable. Nevertheless, Gadient and Fenster (1994) reported that stabilities of vitamins A, B_2, E and coated vitamin C in extruded trout feed at 150 °C ranged from 24 to 100 %. It would be reasonable to expect some loss of astaxanthin during thermal extrusion.

In addition, the extraction method used in the present study may not have totally extracted all of the astaxanthin in the diets using Carophyll® Pink as the pigment source leading to underestimation of the actual levels of astaxanthin in the diets. To obtain more accurate results, Schierle & Hardi (1994) suggested that Maxatase® should be used during astaxanthin extraction from diets supplemented with Carophyll® Pink. The enzyme would aid in breaking down the protein matrix surrounding the astaxanthin in the Carophyll® Pink beadlet that is used as a pigmentation source.

Finally, due to the unavailability of astaxanthin isomer standards, only the concentration of all-E-astaxanthin isomer was quantified by the HPLC system used in the present study. It has been reported that Carophyll® Pink consists of a range of 75 to 85 % all-E-astaxanthin (Bjerkeng et al., 1997), and the rest is composed of Z-isomers. The lack of Z-isomer standards prevented quantification of these isomers with the HPLC system used in

this study. At present, it is better to consider that the values shown in Table 4 represent only the all-*E*-astaxanthin available in the diets.

Optimal astaxanthin concentrations in salmonid diets have not been fully elucidated but concentrations used in industry generally range from 35 to 75 ppm. Studies on rainbow trout (Choubert and Storebakken, 1989; Bjerkeng *et al.*, 1990) have shown that the astaxanthin concentration in the flesh of trout does not increase when the dietary pigment concentration is increased above 50 ppm. However, other studies have shown that the apparent digestibility of carotenoid increases to compensate for low carotenoid concentrations in the diet (Choubert and Storebakken, 1989; Torrissen *et al.*, 1990). The retention coefficient of astaxanthin in rainbow trout after six weeks of feeding was noted to be the highest when the dietary astaxanthin concentration was 25.0 ppm (Choubert and Storebakken, 1989). In addition, different salmonids species require different pigmentation strategies at different life stages (Torrissen and Naevdal, 1984, 1988; Gjerde and Gjedrem, 1984; Withler, 1987). For the purposes of the present study, the astaxanthin concentrations in the diets of coho salmon used in the present study should have been adequate to produce acceptable flesh pigmentation (Bjerkeng *et al.*, 1990; March *et al.*, 1990).

5.2.   Growth Performances of Coho Salmon

5.2.1. Growth of Coho Salmon

The findings from the present study showed that the diets containing different ratios of digestible protein and digestible lipid had significant effects ($p_D < 0.05$) on the growth performance of post-juvenile coho salmon reared in seawater. Salmon fed diet 6 (DP:DL, 45:27; DP:DE, 20.5 g $\bullet$ MJ^{-1}) had the highest average weight gain (Table 5), although this was only significantly different from the gain observed for salmon fed diet 3.

Dry feed intakes (g per fish) were influenced by dietary treatments (Table 7). Salmon fed diet 6 containing high protein and high lipid levels had significantly ($p_D < 0.05$) higher

feed intake (278.0 g • fish^{-1}) than the salmon fed the diet (diet 3) containing low dietary protein and high lipid levels (152.2 g • fish^{-1}) after 168 days of the experiment. With the exception of the salmon fed diet 6, the overall feed intakes of the fish fed the test diets were inversely related to dietary lipid level. This trend agrees with the findings of Beamish and Medland (1986), Kaushik and Oliva-Téles (1986), and Kaushik and Médale (1994) for rainbow trout. These investigators suggested that voluntary feed consumption of salmonids is inversely related to dietary DE levels. Salmonids in general consume organoleptically acceptable diets to satisfy energy demands. Hillestad and Johnsen (1994) also found similar results when Atlantic salmon were fed diets containing various ratios of protein and energy. In contrast, Alsted and Jokumsen (1991, as cited by Hillestad et al., 1998) observed that salmon consumed the same amount of food and achieved that same weight gain regardless of the dietary energy content. Hillestad et al. (1998) also reported that Atlantic salmon (initial body weight, 300g) consumed more feed when they were fed a diet containing 30% lipid instead of one that contained 22% lipid.

In the present study, the best growth response was obtained when coho salmon were fed diet 6 (contained 45 % protein and 27 % lipid). When these fish were fed to satiation, their average mean weight from 117.1 g to 386.6 g after six months in seawater. This growth rate was slower than results reported by Hillestad and Johnsen (1994). They reported that for Atlantic salmon held held similar conditions, but were fed isonitrogenous diets that contained different protein and lipid ratios. The fish grew from 100 to 605 g after 7 months in seawater. The best growth response was obtained when the salmon were fed a diet with 37% protein and 26% lipid. Besides the difference in species, extent of genetical selection, and perhaps thermal input, the depressed growth rate in the coho salmon of the present study may have, in part, resulted from a low incidence of bacterial kidney disease (BKD, Renibacterium salmoninarum). In addition, midway through the experiment, it was observed that some of the coho salmon were growing slowly and that these fish had visible

parr marks. This suggests that some of the coho salmon did not undergo complete smoltification. Indeed, they remained or reverted back to the parr stage of their life history and they did not respond to the dietary treatments as expected. Consequently, the average weight of the salmon from each replicate group were lower than expected.

If the results from feed intake and body weight gain are considered together, it appears those salmon that consumed more feed had higher weight gains. Salmon fed diet 6, for example, consumed the most feed (278.0 g) and had the highest weight gain (from 117.1 g to 386.6 g) over the experimental period. Other researchers have also reported that salmonid growth has been directly related to the amount of energy supplied in the feed when the diets were isonitrogenous (Atlantic salmon, Hillestad and Johnsen, 1994) and the fish were fed ad libitum (rainbow trout, Johnsen et al., 1995; Atlantic salmon, Hillestad et al., 1998).

The specific growth rates (SGRs, Table 6) of the coho salmon were not significantly ($p_D > 0.05$) influenced by dietary treatment and the ranged from 0.824 % • day^{-1} to 1.058 % • day^{-1} during the first interfal of the experiment and from 0.525 % • day $^{-1}$ to 0.706 % • day^{-1} over the 168-day study. Except for salmon fed diets 6 and 7, SGRs declined significantly ($p_T < 0.05$) over time. The relatively slower specific growth rates during the later months of the experiment may be related to changes in water temperature and photoperiod as well as increased fish size. Lower water temperature and shorter day lengths in the winter months would be expected to depress growth responses. Brett and Groves (1979) stated that with declining temperature, meal size and digestion rate decrease in salmon at roughly the same rate. Weatherup et al. (1997) also reported that feed intake and growth rates of rainbow trout were suppressed by falling water temperatures. In addition, some researchers observed that digestibilities of protein and energy in diets of rainbow trout are depressed by low water temperature (Choubert et al., 1982; Olive-Téles and Rodrigues, 1993). The reduction in growth rate would be relatively more rapid due to both a poorer feed efficiency and a lower

amount of feed intake per meal. The 3 mm feed particle size may also have depressed growth at the end of the experimental period as fish grew to larger size. Salmon would be required to expend more energy to capture enough feed pellets to support their energy requirement.

In the present study, the decline in specific growth rates were less affected by cultural conditions when salmon were fed diets 6 and 7. Perhaps the high levels of digestible protein and lipid in those diets (DP:DL ratios were 45:27 and 43:24 for diets 6 and 7, respectively) were more favorable for growth during the winter months. The growth rates of coho salmon fed the diets containing high levels of protein and lipid were therefore not affected as much as those for fish fed diets containing less protein and lipid by the cold water in the latter stages of the experiment.

The dissimilar lipid sources in the diets did not seem to affect the growth rate of the coho salmon in the present study. Although diets 3 and 5 contained high levels of ω-6 fatty acids, the growth performances of coho salmon fed these diets were not depressed significantly. Indeed, results similar to the $\omega6$ and $\omega3$ imbalance study on juvenile coho salmon described by Yu and Sinnbuher (1976) were not observed in the present study. The findings agreed with those from previous studies in which a variety of different supplemental lipid sources have been incorporated into salmonids feeds by partial replacement of marine lipid. Greene and Selivonchick (1990) reported that different groups of rainbow trout fed diets containing vegetable, animal, or marine lipid did not show any difference in growth responses. Also, Polvi and Ackman (1992) and Bell et al. (1989) reported that post-smolt Atlantic salmon fed diets with 10% lipid originating from different sources did not show different growth responses to the dietary treatments. Further, studies on Pacific salmon (Dosanjh et al., 1984; Mugrditchian et al., 1981) have reported that fish, fed diets supplemented with linseed oil, canola oil, animal fat, or marine oil as 8% of the diet, did not show any difference in growth rate. Moreover, recent studies (Dosanjh et al., 1998; Arzel et

al., 1994) demonstrated that when as much as 47% to 63% of the lipid content in the diets of Atlantic salmon and brown trout have been replaced with canola oil, no adverse effect on the growth of the fish resulted. However, it is important to emphasize that the use of alternate lipid is possible if the essential fatty acid needs of the fish have been met by appropriate levels and sources of $\omega 3$ fatty acids in the diet. This requirement seems to have been fulfilled in this study since once again the fish fed diets 3 and 5 did not show significant depression of growth.

5.2.2. Feed Protein and Energy Utilization

Results from the present study show that salmon fed diets containing higher levels of lipid had improved values for feed efficiency ratio (FE, Table 8), protein efficiency ratio (PER, Table 9), percent protein deposited (%PD, Table 10), and gross energy utilization (GEU, Table 10). The better efficiency of protein utilization in coho salmon fed high-lipid diets (diets 3 and 6) compared to salmon fed lower lipid diets (diets 1 and 4), suggests that the high lipid contents in the diets exerted a protein-sparing effect. Such an effect has been well demonstrated in rainbow trout (Takeuchi *et al.*, 1978; Kaushik and Oliva-Téles, 1985; Kim and Kaushik, 1992; Weatherup *et al.*, 1997), brown trout (Arzel *et al.*, 1994), red tilapia (De Silva *et al.*, 1991), and yellowtail (Shimeno and Kajiyama, 1980). Recent studies on Atlantic salmon reared in seawater also have found similar results (Hillestad and Johnsen, 1994; Einen and Roem, 1997; Hillestad *et al.*, 1998). The improvement in protein utilization is due to an increasing contribution of non-protein energy mainly in the form of lipid to overall energy expenditure (Cho and Kaushik, 1985, 1990; Kaushik and Cowey, 1991). LeGrow and Beamish (1986) reported that the lowest apparent heat increment was obtained in rainbow trout when they were fed a diet containing a low level of protein (34%) and a high level of lipid (23%). In this situation, less energy was consumed for the deamination of ingested

protein. Improved protein (nitrogen) retention in fish also leads to reduced nitrogen discharge from fish farms (Higgs et al., 1995).

In the present study, feed efficiency ratios of coho salmon improved as the levels of lipid were increased in the diets regardless of their protein content. Overall, FE values ranged from 0.769 g • g^{-1} to 0.961 g • g^{-1} for fish fed the low protein diets (diets 1, 2, and 3) whereas they ranged from 0.770 g • g^{-1} to 0.962 g • g^{-1} among those fed the high protein diets (diets 4, 5, and 6). FE values were not affected by protein content in the diets. This finding suggests that post-juvenile coho salmon were capable of utilizing up to 27% lipid in the diet as a source of energy and essential fatty acids and thereby spared dietary protein for elaboration of new tissue.

Although FEs of coho salmon were not significantly ($p_D > 0.05$) affected by the dietary treatments over the 168-day experiment, they were significantly ($p_D < 0.05$) affected during the earlier stages of the experiment. In this regard, FE values after 28 days of the experiment ranged from 0.995 g • g^{-1} to 1.203 g • g^{-1}. The depressed FE values observed towards the end of the experiment likely contributed to the slower growth rates of the fish during this time. Reduction in water temperature as the experiment progressed probably can account for some of the temporal decline in FE. Also, as the fish grow larger, the maintenance energy requirement (per unit growth) increases with fish weight. Therefore, FE values decreased as coho salmon grew larger towards the end of the experiment.

Dietary protein concentration also appeared to influence protein and energy utilization. Coho salmon fed the low protein diets had higher values for PER, %PD, and GEU than those fed the high protein diets. This could have resulted from excess protein provided by the high protein diets. DP:DE ratios of diets 4, 5, and 6 were 23.05, 21.60, and 20.54 g • MJ^{-1}, respectively. The ratios of available protein to energy in these diets far exceeded the recommended DP:DE ratios established by other researchers for Atlantic salmon. Studies on Atlantic salmon held in seawater have suggested that DP:DE ratios ranging from 16 to 19

g • MJ^{-1} were optimal for growth of this species (Andersen *et al.*, 1994; Einen and Roem, 1997). Hillestrad and Johnsen (1994) even suggested that a DP:DE ratio of 15 g • MJ^{-1} was optimal for Atlantic salmon grown from 0.1 to 0.6 kg. The present findings suggested that diets 4, 5, and 6 provided excess levels of protein (45%) for optimal protein retention. Such findings can be explained by a number of factors. Possibly, protein digestibility may have been depressed due to an excess supply of dietary protein and hence less was absorbed in the form of amino acids and small peptides. Alternatively the salmon may have absorbed more amino acids from the higher dietary level of protein and hence these may have elevated amino acid deamination (higher heat increment). Also, in general, salmon fed diets 4, 5, and 6 consumed more and grew faster than those salmon fed diets 1, 2, and 3. Consequently, more protein may have been utilized to support the regular protein turnover in the larger fish; maintenance energy was concurrently elevated. Protein and energy retention in salmon would therefore be decreased when salmon were fed diets containing high levels of protein.

The highest %PD and GEU values found in the present study, for coho salmon in seawater, were achieved when the salmon were fed diet 3 which contained 41 % digestible protein and 23 % digestible lipid. Values for % PD and GEU of these coho salmon were 46.8% and 35.32 %, respectively. These values are higher than those found for rainbow trout (Brauge *et al.*, 1994) and chinook salmon (Silver *et al.*, 1993), but are lower than the values obtained for Atlantic salmon (Hillestad and Johnsen, 1994; Hemre *et al.*, 1995; Andersen *et al.*, 1997; Einen and Roem, 1997). The differences in %PD and GEU values between studies can be attributed to the quality of protein used in the studies (McCallum and Higgs, 1989). Chinook salmon in seawater fed diets containing blends of animal and protein sources had %PD and GEU values of 33.1% and 29.5%, respectively (Silver, 1993). In the present study, premium quality fish meals were used as the main sources of protein in the experiment diets. In the study conducted by Hillestad and Johnsen (1994) on Atlantic

salmon, %PD and GEU values of 51% and 47% were obtained when the fish were maintained on a restricted ration protocol. In addition, the differences possibly can be attributed to the differences in size of the fish. Einen and Roem (1997) suggested that when fish are fed high-energy diets, the dietary protein:energy ratio should be decreased as the fish increase in weight.

5.3. Body Composition of Coho Salmon

5.3.1. Proximate Composition

Whole body proximate analyses showed that the lipid content in the coho salmon was influenced by dietary lipid level (Table 11). Protein and ash content of the salmon were not affected by dietary treatments. Protein content increased from 15.63 % (wet weight basis) at the beginning of the experiment to a narrow range of 18.03 to 18.34 % after 168 days. Ash content also decreased slightly from 2.28% at the start of the experiment to a narrow range or 1.70 to 1.85 %. Generally whole body lipid content increased when coho salmon were fed diets containing higher levels of lipid. In addition, whole body lipid content in salmon fed diets with the lower protein level (diets 1, 2, and 3) was higher (7.63, 8.41, and 8.32%, respectively) than that noted for salmon fed diets 4, 5, and 6 with higher protein (lipid contents were 7.07, 7.16, and 7.93%, respectively). Results from the present study support those reported previously by Andersen et al. (1997) and Einen and Roem (1997) who showed that lipid content in Atlantic salmon increased when the fish were fed higher lipid content diets.

The lipid content of coho salmon in the present study was lower than the values reported by other researchers. Andersen et al. (1997) observed that whole body lipid contents of Atlantic salmon increased from 7.9 % to a range of 10.9 to 14.1 % after the fish were fed diets containing 20 to 29 % of dietary lipid for 112 days. Einen and Roem (1997) also reported that whole body lipid levels of Atlantic salmon, fed diets with 25.6 to 38.9 %

lipid, ranged from 15.1 to 17.2%. The differences in findings between studies may be due to differences in fish size. Coho salmon in the present study grew to a smaller size as compared to the sizes of the Atlantic salmon in the latter studies. Different salmonid species also metabolize and deposit dietary lipid differently.

Whole body lipid content in salmon fed the control diet (diet 7) was significantly ($p <$ 0.05) lower that the whole body lipid contents found in salmon fed diets 2 and 3 despite the fact that diet 7 contained about 24 % digestible lipid. This may have resulted from differences between the diets in ratios of digestible protein to lipid or to differences between the groups in size.

As was stated by Shearer (1994), the levels of whole body proximate constituents such as protein and lipid are influenced strongly by fish size. The effects of dietary treatment on whole body proximate compositions should only be evaluated after the effect of dissimilar fish size has been removed using analysis of covariance (ANCOVA). The results (Table 12) of the present study showed that whole body proximate compositions of the coho salmon were size dependent and that the dietary ratios of digestible protein and digestible lipid had no significant ($p > 0.05$) effects on the levels of the proximate constituents. This finding suggests that the high whole body lipid contents observed in salmon fed the diet with the higher lipid contents likely resulted from the rates of growth supported by these diets. When adequate dietary ratios of digestible protein to lipid were provided, the salmon were able to grow larger and whole body proximate contents were therefore altered as the salmon increased in size.

Similar trends were also observed for the levels of proximate constituents in the muscle of coho salmon (Table 13). Ash and protein content were not affected by dietary treatment while muscle lipid content increased as the dietary lipid content was increased except in the salmon fed diet 3. A range of 3.27 to 4.99 % of lipid was found in the muscle. Studies using larger Atlantic salmon (Hillestrad and Johnsen,1994; Hillestrad et al., 1998;

104

Einen and Roem, 1997; Einen and Skrede, 1998) showed that up to 18.9% of fat was found in the fillets of Atlantic salmon. Again, the differences can be ascribed to dissimilar salmon and fish size between the studies.

Less lipid was found in the fish muscle as compared to the whole body lipid. This is because a portion of the dietary fat and energy is stored in the viscera and under the skin. Silver et al. (1993)and Arzel et al. (1994) also observed increased amounts of visceral fat in fish fed high energy diets. The excess energy stored as visceral fat in the fish would be discarded during the dressing of the salmon for market. More assessment of the lipid content in different parts of the salmon body would allow improved understandings of how dietary lipid is utilized and deposited in the body. This should be a focus in future studies on coho salmon fed diets containing varying ratios of digestible protein and lipid.

Although coho salmon in the present study were unintentionally given different sources of supplemental lipid in their diet, the proximate compositions of the fish fillets fell within the expected ranges. This finding agrees with the work conducted by Waagbø et al. (1993) on the effects of dietary lipid sources on the growth of salmonids. Further, the different dietary lipid sources had no effect on the proximate composition of the salmon in their study.

5.3.2. Fatty Acid Profile in Muscle Lipid

The fatty acid compositions of the salmon fillets reflected to a large extent the dietary lipid compositions. For instance, fatty acids compositions observed in the muscle lipid of salmon fed diets 3 and 5 (Tables 14 to 18) were similar. The results from the present study support those of other researchers (Dosanjh et al., 1984, 1988; Hardy et al., 1987; Kiessling et al., 1989; Thomassen and Røsjø, 1989; Kiessling and Kiessling, 1993) who also reported that fatty acid deposition in fish lipids was markedly influenced by the dietary lipid composition.

The fatty acid profile of muscle lipid did not simply reflect that of dietary lipid, since some fatty acids appeared to be maintained within defined ranges. Even though percentages of $C18:1\omega9$ were higher in diets 3 and 5, the levels of $C18:1\omega9$ in fish muscle was maintained within a narrow range between 17.05 to 19.16%. In addition, although diets 3 and 5 contained a high level of $C18:2\omega6$, the levels other ω-6 fatty acids in the muscle lipid were maintained at similar levels to those of salmon fed the other diets. Similar results have also been reported by Léger *et al.* (1981) for rainbow trout, Donsajh *et al.* (1988) for chinook salmon, and Arzel *et al.* (1994) for brown trout. In this study, little or no desaturation and elongation of $C18:2\omega6$ to longer chain ω-6 fatty acids occurred even when substantial amounts of $C18:2\omega6$ were present in the tissue. Henderson and Tocher (1987) and Bell *et al.* (1989) suggested that bioconversion of $C18:2\omega6$ to highly unsaturated derivatives was effective only in the case of ω-3 HUFA deficiency. In this situation, Hardy *et al.* (1987) observed a significant increase in the dead-end product $C20:2\omega6$ when the level of this fatty acid was compared to those in fish fed diets containing only fish oil.

Although diets 3 and 5 contained high levels of ω-6 fatty acids and relatively lower levels of ω-3 HUFA, the levels of $C20:5\omega3$ and $C22:6\omega3$ fatty acids in the muscle lipid did not appear to be affected. It has been suggested (Arzel *et al.*, 1994) that the desaturases and elongases involved in the bioconversion of fatty acids in salmonids show preferences for the conversion of $C18:3\omega3$ to ω-3 HUFA which are precursors of many physiologically essential compounds.

5.4.   Sensory Attributes of Pan-Sized Coho Salmon

5.4.1. Pigmentation in Raw Salmon Fillets

5.4.1.1. Astaxanthin Content in Raw Salmon Fillets

Astaxanthin concentrations in the flesh of post-juvenile coho salmon appeared to be significantly affected by both dietary treatment ($p_D < 0.05$) and body weight ($p_S < 0.05$) (Table 19). The effect of dietary lipid level was clear even when salmon weighed less than 100.0 g; however, when coho salmon grew above 500.0 g, astaxanthin concentration in the flesh appeared to reach a plateau level at around 7.5 - 8.0 ppm.

The size effect observed in the present study supports the work conducted by Torrissen (1985) and Smith et al. (1992). They reported that flesh pigment levels in coho salmon increased with body weight. When pan-size coho salmon were fed a diet containing 30 ppm of astaxathin for 196 days, Smith et al. (1992) found that muscle astaxanthin levels varied from 2.76 to 8.26 ppm for salmon over a size range of 50 to 400 g. Although earlier studies (Spinelli and Mahnken, 1978; McCallum et al., 1987) suggested that flesh pigmentation only occurred in salmonids after they have achieved a minimum size, results from the present study show that dietary astaxanthin is deposited in the flesh of coho salmon weighing less than 100.0 g. March et al. (1990) also demonstrated that coho salmon weighing less than 40 g was capable of absorbing astaxanthin. Recent work by Thomas (1999) found that up to 2.0 ppm of astaxanthin was deposited in the flesh of chinook salmon weighing less than 100 g.

In the present study, muscle lipid content appeared to be affected by the dietary treatments as shown in Table 13. In addition, astaxanthin concentrations in the flesh were correlated ($r^2 = 0.6802$) positively to the muscle lipid content. This suggests that increased dietary lipid levels led to increased muscle lipid content which, in turn, improved the degree of flesh pigmentation. However, Choubert and Luquet (1983) and Henmi et al. (1987 and 1989) observed that increased lipid content in the diet of salmonids did not enhance carotenoid pigment deposition. The differences in findings between these studies may have resulted from dissimilar sizes of the salmon, experimental duration, and dietary lipid contents. In the study by Choubert and Luquet (1983), dietary lipid level did not exceed 18%

and rainbow trout were only fed over a size range of 100 g to 190 g. When larger salmonids were studied using dietary lipid levels up to 40 %, flesh pigmentation was found to be positively correlated with the lipid level in the diets for rainbow trout (Torrissen 1985, Torrissen et al., 1990; Choubert et al., 1991) and Atlantic salmon (Torrissen et al., 1995; Einen and Roem, 1997, Hillestad et al., 1998).

An earlier study conducted by Windell et al. (1982) showed that an increase in the dietary lipid level decreased the gastric evacuation rate and led to improved digestibility of other dietary components. Consequently, it is possible that the digestibility of astaxanthin could be improved due to increased lipid levels in the diets. Absorbed astaxanthin would then bind to the lipid protein in the serum (Schiedt et al., 1985). Increased astaxanthin and canthaxanthin levels in plasma have been observed by March et al. (1990) and Torrissen (1986) when salmonids were fed a single oral dose of carotenoids. The high lipid diets used in the present study may have facilitated better absorption and transportation of astaxanthin to the muscle.

Salmon flesh pigmentation could be further enhanced by the protein sparing effect exerted by the high lipid diets. After the astaxanthin was transported to the muscle tissue, Henmi et al. (1987, 1989) proposed that astaxanthin was bound to the actomyosin of the muscle of coho salmon. Increasing the turnover rate of muscle protein would likewise increase the turnover rate of deposited astaxanthin as was shown in a study by March et al. (1990). When salmon were fed diets containing higher dietary energy, less muscle protein would be utilized for energy production purpose, and the turnover rates for both muscle protein and the anchored astaxanthin would be decreased. More stable muscle pigmentation in salmon flesh could be achieved using higher lipid diets. As was observed in the present study, salmon fed diets 2 and 3 (DP:DL ratios, 39:18 and 41:23, respectively) had the highest values for percent protein deposition (%PD) and gross energy utilization (GEU)

(Table 10) and the highest degree of muscle pigmentation among salmon fed the seven experimental diets.

The high astaxanthin level (8.05 ppm, diet 3) found in the largest group of fish in this study was close to the plateau levels noted for astaxanthin in the muscle of Atlantic salmon (7.5 to 8.0 ppm) (Torrissen et al., 1995; Einen and Roem, 1997). The plateau level indicated that when the muscle of salmon is close to a saturation level of astaxanthin, the effects of body size and dietary treatments may disappear.

5.4.1.2. Color Determinations of Raw Salmon Fillets

In the present study, visual color evaluation of salmon fillets using the Roche Color Card and SalmoFan for salmonids also indicated that the pigmentation of the fillets was influenced by dietary treatment (Tables 20-21). Fillets from salmon fed diet 6 had the highest RCC and SF scores of 15 and 27, respectively. Fillets from salmon fed the other diets, except diet 7, had RCC and SF scores ranging from 14 to 15 and 24 to 26, respectively. Although visual evaluation of color is a subjective quality evaluation and is affected by viewing conditions (Bolton et al., 1967; Skrede et al., 1990) and by the color perception of the judge (Little, 1979; Skrede et al., 1990), good correlations between astaxanthin concentration and the visual score have been reported for rainbow trout (Foss, 1984, 1987) and Atlantic salmon (Christiansen et al., 1995). Smith et al. (1992) also observed a linear relationship between the RCC score and the astaxanthin concentration in pan-size coho salmon up to a concentration of 8 mg astaxanthin per kilogram fish flesh. However, as astaxanthin concentrations in the flesh increased, the curve became asymptotic, making it difficult to distinguish visual differences as color intensity increased. Torrissen and Naevdal (1984), Foss et al. (1984; 1987) and Storebakken et al. (1987) were in agreement that visual scores are not linear and that they become less sensitive at high carotenoid levels in salmon flesh. In the present study, because the evaluations of RCC score and astaxanthin level were

conducted using fillets from different salmon, the relationship between RCC card score and astaxanthin concentration could not be directly correlated. However, in general, results from the present study showed that as salmon grew larger in size, levels of muscle astaxanthin increased, and higher RCC and SF scores were achieved. Also, because of the size of coho salmon used, plateau levels of RCC and SF scores were not observed.

When considering fillets from coho salmon in the same size range (data in columns), it appeared that the salmon containing the highest levels of flesh astaxanthin had relatively lower RCC and SF scores. In the present study, salmon were fed high levels of dietary lipid and had increased fat levels in their muscle. The presence of a pale yellow fat strip distributed among the myotomes of the fillets may have lead to lower visual color scores. As a result, for the salmon fillets that contained the same astaxanthin concentration, but were higher in lipid content might have had lower visual scores due to the presence of the fat strip among the myotomes.

In the present study, the Hunter Lab color system (Hunter L, a, and b) was also used to evaluate the degree of pigmentation in salmon flesh. However, the International Commission on Illumination (CIE, L*, a*, and b*) system has also been used in a number of studies. As a result, direct comparisons of the HunterLab values of the present study to those of other studies are not valid. However, the Hunter L, a, and b and L*, a*, and b* values were found to be significantly correlated (Skrede and Storebakken, 1986a) and could be compared in terms of general trends.

As has been observed by other researchers (Skrede and Storebakken, 1986 a, b; Christiansen et al., 1995), Hunter L (Table 22) values decreased when astaxanthin concentrations in the salmon fillets increased. The effect of the fat strip was again observed. Nevertheless, it was generally apparent that both the Hunter L and hue (Table 26) values were not good indicators of the levels of astaxanthin in salmon flesh. The HunterLab a (redness, Table 23), Hunter b (yellowness, Table 24), and chroma (saturation, Table 25)

values obtained in the present experiment increased as the salmon increased in size and they reflected the higher astaxanthin concentrations present in the larger salmon. High correlations between these values and flesh astaxanthin concentrations have been demonstrated in previous studies on Atlantic salmon (Skrede and Storebakken, 1986 a, b; Christiansen *et al.*, 1995), rainbow trout (No and Storebakken, 1992), and coho salmon (Smith *et al.*, 1992). In general, it is apparent that Hunter a values serve as the best predictor of actual flesh pigment concentrations while Hunter b and chroma values are also useful. Again, when examining the fillets from salmon in the same weight ranges, it appeared that the salmon fillets containing the highest level of flesh astaxanthin (Table 19) had lower Hunter a, b, and chroma scores. Once, again, this can be explained by the high level of fat deposited between the myotomes of the salmon fillets.

Instrumental measurements of the color of an object were dependent upon the penetration of light into the sample (Schmidt and Cuthbert, 1969). Translucence of salmon fillets will cause light to be trapped within the sample (Little, 1964), possibly affecting the measurements made directly on the fillet. In addition to the fat strip within the fillet layers, blood stains left on the fillet would also influence color measurements.

5.4.2. Texture Profiles of Cooked Salmon Fillets

Texture profile analysis (TPA) revealed that hardness I and II (Tables 27 and 28), firmness (Table 29), and cohesiveness (Table 30) of cooked salmon fillets were not significantly ($p_D < 0.05$)affected by dietary treatment. However, when considering the lipid content in the fish muscle, it appeared that fish containing higher levels of muscle lipid (salmon fed diets 2, 5, and 6) seemed to exert more resistance to applied force during the first and the second compression cycles (hardness I and hardness II, respectively). In contrast, fillets from salmon containing higher muscle lipid levels appeared to be less firm than those from salmon with a lower muscle lipid content. According to Bourne (1978),

hardness can be measured as the peak forces during the two TPA compression cycles when the probe is inserted through the sample to a fixed depth. However, Durance and Collins (1991) suggested that firmness, which measures the maximum slope of the first compression cycle, allows the measurement of the overall resistance of the fish muscle before structural deformation due to compression. Andersen *et al.* (1997) also reported that texture measurements should be obtained from the linear portion of the TPA curve.

Little information is available on the effect of feeding salmon diets containing different ratios of dietary protein and lipid on the texture of salmon fillets. Recent studies (Andersen *et al.*, 1994, 1997) revealed a higher autolytic protease activity and a softer consistency in raw fillets from salmon fed diets with high fat content compared to those fed diets with lower fat content after the fillet had been stored on ice for 11 days. However, the actual mechanisms of the effect of dietary lipid content on fillet texture were not determined. In the present study, fillets containing higher muscle lipid content seemed to have lower resistance against compression before structural deformation. Fat strips distributed between myotome layers may have contributed to the softer texture in the fillets containing higher lipid content.

Results from the present study also indicated that as the fish increased in size, the texture of the cooked fillets became less hard and less firm. Growth of salmon involves an increase in the size of muscle fibers. Hatae *et al.* (1984, 1989, and 1990) showed that fish species with smaller diameter muscle fibers had firmer texture. In the present study, larger sized salmon may have had larger diameter muscle fibers leading to softer texture.

Results from the sensory panel (Table 27) showed that the fillets from salmon fed diet 6 were significantly (p < 0.05) more tender than those from salmon fed the other diets. Again, limited information exists regarding dietary effects on the texture of cooked salmon. In contrast to the finding of the present study, Sheedan *et al.* (1996) reported that smoked salmon from fish fed a 30% fat diet were significantly firmer than those from salmon fed diets containing 20 to 25 % lipid.

In the present study, the dietary treatments appeared to have greater effects on salmon texture when the assessments were conducted using a sensory panel rather than objectively with the Texture Analyzer. The differences in findings may be due to preparation of the samples. Samples used for the sensory analysis were made from slices of fish from different locations along the fillet, while texture analysis was only performed at one location on the fillet due to the limited availability of sample. Therefore, the composite samples used in the sensory panel would provide a better representation of the entire fillet. In addition, Szczesniak (1963) stated that the textural properties of a food represent the combined results from pressure exerted on the oral cavity during the transportation and preparation of the food in the mouth. As a result, fat in fish fillets may have dispersed in the mouth and acted as a lubricant during mastication. Thus, fillet samples may become more palatable and contribute to the tenderness perceived in the mouth of the panelists.

5.4.3. Aroma and Flavor of Cooked Salmon Fillets

Sensory evaluations of the flesh of post-juvenile coho salmon showed that dietary lipid level and sources did not significantly ($p > 0.05$) affect the aroma and off-flavor of the cooked fillets (Table 27). Flavor, texture, and overall acceptability were significantly ($p < 0.05$) affected by the diets fed to the coho salmon. Flesh from salmon fed diet 6 appeared to be the most flavorful, the most tender, and had the highest overall acceptability. Oil in the fillet muscle from salmon fed diet 6 may have served as a carrier for the aromatic compounds which contributed to the high intensities of salmon aroma and flavor that were detected by sensory panelists.

Again, very little information exists regarding the effects of dietary lipid level on sensory qualities of cooked salmon. A study done by Sheehan et al. (1996) showed that supplementing up to 30% of fat in the diets did not affect the flavor of smoked Atlantic salmon. The development of fish aroma, off-flavor and other undesirable sensory

characteristics are believed to be linked to the level of lipid oxidation (Hsieh and Kinsella, 1986) and this depends on the specific fatty acids that are present as well as the specific lipoxygenase involved in hydroperoxide formation (Ackman, 1967; German and Kinsella, 1985; Josephsen *et al.*, 1984). As a result, a number of studies have examined the effects of dietary lipid source on the sensory attributes of salmonids (Boggio *et al.*, 1985; Hardy *et al.*, 1987; Thomassen and Røsjø, 1989). However, the results have been inconclusive.

Hardy *et al.* (1987) reported that the replacement of herring oil with menhaden oil, soybean oil, or tallow in the diets of Atlantic salmon raised in marine net-pens had no effect on the flavor and texture attributes of the fillets. On the other hand, Thomassen and Røsjø(1989) found the intensity of salmon taste was lower when Atlantic salmon were fed diets with capelin oil, soybean oil, and rapeseed oil instead of fish oil alone. In addition, Waagbø *et al.* (1993) reported that rancid flavor was significantly higher in Atlantic salmon, raised on diets with high levels of ω-3 fatty acids, after the fish were frozen at -18 $^{\circ}$C for up to 5 weeks. Skonberg *et al.* (1993) also reported that fishy aroma in coho salmon fed diets with herring oil was stronger than noted for salmon fed diets containing sunflower oil. The authors suggested that a high monounsaturated fatty acid content diet could decrease oxidative rancidity and improve the sensory characteristics of the fillets. In the present study, the salmon fillets were vacuum packed in oxygen barrier plastic bags and kept at -35 $^{\circ}$C in the dark. As a result, any potential for lipid oxidation of the polyunsaturated fatty acids was minimized.

The sensory scores for coho salmon fillets from salmon fed the control diet (diet 7) were lower than those for fillets from salmon fed diets 1 to 6 and thus may reflect differences in the of protein sources that were used to manufacture the diets. Premium grade fish meals were the sole sources of protein in the experimental diets (diets 1 to 6, Table 1), while only 60% of the protein in the control diet was made with fish meal. The other 40% of the dietary

protein in diet 7 originated from alternative protein sources such as soybean meal, poultry-by-product meal, and wheat meal.

6. Conclusions

Results from the present study indicate that diet 6 which contained 45 % digestible protein and 27 % digestible lipid supports the best growth in post-juvenile coho salmon reared in seawater over a size range of 0.1 to 0.4 kg. However, the highest rate of protein and energy utilization were achieved when coho salmon were fed diet 3 which contained 41 % digestible protein and 23 % digestible lipid. DP:DE ratios of these two diets are 20.5 and 19.6 g • MJ^{-1} respectively. These values are higher than the values reported for Atlantic salmon. In this regard, Hillestrad and Johnsen (1994), Anderson et al. (1996), and Einen and Roem (1998) reported that DP:DE ratios ranging from 15 to 19 g • MJ^{-1} provided the best growth for fish over a size range of 0.1 to 5.0 kg.

Data from the present study indicate salmon larger in size achieve higher levels of flesh pigmentation; satisfactory levels of flesh pigmentation can be achieved for coho salmon weighing between 0.4 to 0.5 kg when 30 ppm (dry weight basis) of astaxathin is added to a diet containing 41 % digestible protein and 23 % digestible lipid. Data in the present study also show that with increasing dietary lipid levels there appears to be an increase in flesh astaxanthin concentration for salmon in the same weight ranges. This finding is supported by Torrissen (1985), Torrissen et al. (1990), and Choubert et al. (1991) for rainbow trout and by Torrissen et al. (1995), Einen and Roem (1997), and Hillestad et al. (1998) for Atlantic salmon. However, the weight effects on flesh pigmentation were not addressed in these reports. Nevertheless, the actual mechanisms of how dietary lipid facilitates flesh pigmentation in salmonids remain unclear.

Although instrumental analysis of cooked salmon fillet texture is not significantly affected by dietary treatment, fillets from salmon fed high levels of dietary lipid appeared to be slightly less firm than fillets from salmon fed the other diets. In contrast, Sheehan et al. (1996) reported that smoked salmon from fish fed a 30 % fat diet were firmer than smoked salmon from fish fed lower levels of dietary fat. Results from the present study relating to the

sensory panel, however, showed that cooked salmon fillets from salmon fed a high lipid diet (diet 6) were significantly ($p < 0.05$) more tender than fillets from salmon fed the other diets. The roles of lipid in fish muscle texture are undetermined.

Sensory evaluation of flesh of post-juvenile coho salmon show that ratios digestible protein and digestible lipid did not significantly ($p > 0.05$) affect the aroma and off-flavor of the cooked fillets; flavor, texture, and overall acceptability were significantly ($p < 0.05$) affected by the diets fed to the coho salmon. Cooked fillets from salmon fed a high protein and high energy diet (diet 6) appeared to be the most flavorful, the most tender, and had the highest overall acceptability.

Additional research is required to establish optimal dietary ratios of digestible protein and digestible lipid in coho salmon at different stages of their life history. It is conceivable that a further reduction in the protein content by increasing dietary lipid content may be economical and feasible for optimizing growth of coho salmon, especially at larger sizes than those used in the present study. Biochemical and physiological studies on how dietary lipid facilitates the absorption, transportation, and deposition of astaxanthin in coho salmon at various market weights will allow a reduction in the cost of incorporating astaxanthin into salmonid diets. Further studies on the deposition of dietary lipid in th salmon body will also allow better understandings on how dietary lipid levels affect salmon flesh texture.

7.   References

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Appendix A. *Number of post-juvenile coho salmon that were infected with bacterial kidney disease (BKD) over a period of 168 days. The coho were fed diets containing various levels of digestible protein and lipid (as shown in parentheses) and were reared in seawater.*

Diet	Number of salmon with BKD
1 (38:16)	8 ± 2
2 (39:18)	8 ± 3
3 (41:23)	11 ± 2
4 (45:16)	12 ± 6
5 (45:22)	9 ± 2
6 (45:27)	8 ± 5
7 (43:24)	10 ± 2
F	2.75
P	0.165