DIETARY PROTEIN QUALITY AND QUANTITY FOR ATLANTIC SALMON (SALMO SALAR) REARED IN SEA WATER

by

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ABSTRACT

Two separate studies were conducted with Atlantic salmon (Salmo salar) reared in seawater. The first study investigated various in vitro and in vivo methods for the measurement of the quality of the protein in fish meals. The second study was designed to estimate the optimal dietary ratio of digestible protein (DP) to digestible energy (DE) for Atlantic salmon (0.5 - 1.2 kg body weight). A second objective of the latter study was to investigate the effect of feeding diets varying in levels of digestible protein and lipid on the proximate composition, pigment deposition, and the total fatty acid profiles of whole-fillets of Atlantic salmon.

Chemical (chemical composition, biogenic amines, total volatile basic-nitrogen, anisidine value, peroxide value, thiobarbituric acid value, and iodine value), in vitro (dilute-pepsin and multi-enzyme digestibilities) and in vivo assays using rats and fish as test animals were conducted on various fish meals. The results indicated that dilute-pepsin digestibility was a suitable in vitro assay to evaluate the protein quality of herring meals for Atlantic salmon. However, this assay correlated poorly with the results from in vivo assays when other types of fish meals (menhaden, mackerel, silver hake, groundfish) were included. Apparent metabolizable crude protein values determined using rats as the test animal, was the only in vivo assay that was found to correlate with digestibility values determined using salmon when the entire range of fish meals was assessed.

Based on growth and protein and energy utilization data, the estimated optimal dietary ratio of DP to DE to rear Atlantic salmon over a size range of 0.5 kg to 1.2 kg in seawater was 17.4 g DP·MJ DE. This ratio was supplied by a diet containing 360 g DP (404 g crude protein) and 20.7 MJ DE (24.0 MJ gross energy) $\cdot kg^{-1}$ dry matter. It would

also appear from this study that Atlantic salmon reared in seawater are able to tolerate up to 220 g starch·kg^{-1} dry matter without any detrimental effects on growth, although liver glycogen and hepatosomatic indexes were increased. Work is still needed to determine the optimal ratio of non-protein energy sources to reduce the utilization of protein for energy in salmon diets.

Deposition of canthaxanthin in the fillets of Atlantic salmon was not influenced by changes in the ratio of dietary protein and lipid. The total lipid content in whole-fillets (red and white muscle with the skin removed) from Atlantic salmon was directly and significantly influenced by the dietary lipid level. The fatty acid profile of the fillets was not only affected by the dietary lipid level and the ratio of digestible protein to lipid, but also by the residual lipid content of the fish meal portion of the diet. Although the total n-3 fatty acid content of the fillets was not significantly changed by alterations of the dietary protein or lipid levels, the percentage of 20:5 n-3 did increase in direct relation to the dietary lipid level.

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ADCP-F	-Apparent digestible crude protein determined using fish.
ADCP-R	-Apparent digestible crude protein determined using rats.
ADGE-F	-Apparent digestible gross energy determined using fish.
ADOM-F	-Apparent digestible organic matter determined using fish.
AMCP-R	-Apparent metabolizable crude protein determined using rats.
BV	-Biological value.
CP	-Crude protein.
DE	-Digestible energy.
DM	-Dry matter.
GE	-Gross energy.
GEU	-Gross energy utilization.
HSI	-Hepatosomatic index.
NPU	-Net protein utilization.
PER	-Protein efficiency ratio.
PPV	-Protein productive value.
SGR	-Specific growth rate.
TBA	-Thiobarbituric acid.
TD	-True digestibility.
TVBN	-Total volatile basic-nitrogen.

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CHAPTER ONE - GENERAL INTRODUCTION

In 1992, aquaculture provided nearly 14% (19.3 million tonnes) of global fisheries production (wild and farmed). This accounted for 98.6% and 7.5% respectively, of the total world production of Atlantic and Pacific salmon (FAO, 1994). Fish produced by aquaculture in British Columbia was worth $140 million in 1993 and is now the largest BC agricultural export (Anonymous, 1995). British Columbia produces approximately 20,000 tonnes or 6% of the total world harvest of farmed salmon (Anonymous, 1995). Global and Canadian aquaculture continues to grow, with an increase in volume of existing farmed species and the introduction of new species.

In intensive aquaculture, both for salmonids and other species, feed represents the largest portion (40 to 60%) of the total operating cost (Tacon, 1994; Higgs et al., 1995a). Much of the cost of the feed (again 40 to 60%) is due to the extensive use of fish meal to furnish most of the protein in the feed (Higgs et al., 1995a). The other component that adds a substantial cost (approximately 15%) to the feed is carotenoid pigment (astaxanthin or canthaxanthin). With the increase in global production of farmed fish, there is an increased demand for premium quality fish meal for inclusion in fish feeds. Yet, the total global production of fish meal (6 - 7 million tonnes) has not increased for some time (Smith and Kilpatrick, 1991). If global fish meal production remains static, then fish meal for use in aquaculture feeds must come from a reduction in its use in other animal feed sectors. Presently, only 15% of global fish meal is used in the aquaculture (finfish and shellfish) industry; while, over 50% is used in the poultry industry (New et al., 1995). Any strategy that reduces the overall cost of feed and/or improves its utilization also improves the viability of aquaculture.

There is considerable research in progress to identify and develop economical

alternative sources of protein and energy for inclusion in salmon diets, but these initiatives have not been entirely successful (Åsgård, 1988; Higgs et al., 1988). Also, considerable efforts are being made to improve fish meal quality so that maximum nutritive value can be obtained from this expensive dietary component.

Current research findings suggest that the highest quality fish meals result from the processing of extremely fresh whole fish under low-temperature cooking and drying conditions (McCallum and Higgs, 1989; Clancy, 1992; Anderson et al., 1993). However, there is still uncertainty regarding the extent of reduction in quality of fish meals that result from fluctuations in the composition and freshness of the raw material, the cooking and drying conditions during meal manufacture, and the subsequent meal storage conditions.

As in all animals, nutrient requirements for fish change with the stage of development (NRC, 1993). Salmonids preferentially use protein and lipids for energy purposes, although carbohydrates may, to a certain extent, satisfy their energy needs. Thus the non-protein energy sources spare the use of protein as an energy source (Wilson, 1989a). Optimization of the dietary ratio of protein to energy and optimization of the sources and levels of non-protein energy components need further research.

The purpose of aquaculture is to produce a product for human consumption. As our knowledge of human nutritional requirements and the health benefits of certain nutrients grows, the consumption of fish and fish products may play a very important role in human health. Fish, especially from the marine environment, are an excellent source of polyunsaturated fatty acids (PUFA) (Ackman, 1989). The role of PUFA's, especially eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3), in human health is a controversial issue, but their essentiality in the human diet is being

increasingly demonstrated and recognized (Singh and Chandra, 1988; Weaver and Holob, 1988; Lands, 1989; Innis, 1991; Nelson et al., 1991). The fatty acid composition of fish responds to complex influences, including nutritional status (diet composition and feed intake), water salinity and temperature, stage of development, and spawning migration (Higgs et al., 1995b).

This thesis covers the broad subject area of protein quality and quantity for salmonids, specifically Atlantic salmon (Salmo salar) reared in seawater. The thesis is comprised of seven chapters. The first chapter is this brief introduction. The second chapter is a review of the literature and is meant to give the reader an understanding of the measurement of protein quality for salmonids and the metabolism of protein by salmonids. The third chapter deals with the assessment of the nutritive value of a range of fish meals for Atlantic salmon reared in seawater and a comparison of the effectiveness of various in vitro and in vivo assays for predicting fish meal quality. The fourth chapter deals with the estimation of the optimal dietary ratio of digestible protein to digestible energy ($g \cdot MJ^{-1}$) for Atlantic salmon (0.5 - 1.2 kg) reared in seawater. This chapter also considers the dietary ratio of lipid to carbohydrate for supplying the non-protein energy in diets for Atlantic salmon. The fifth chapter investigates the influence of changing the dietary ratio of protein to lipid on the proximate composition and the total (polar and neutral) fatty acid profiles in fillets from Atlantic salmon reared in seawater. A second objective reported in this chapter was to investigate the influence of the same dietary factors on the extent of canthaxanthin deposition in the fillets of these fish. Chapters six and seven cover, respectively, the general discussion of the results and the general conclusions.

CHAPTER TWO - PROTEIN FOR SALMONID FISH: QUALITY EVALUATION, METABOLISM AND REQUIREMENTS

INTRODUCTION

The increasing production of farmed fish leads to increased demands for premium quality fish meal for inclusion in fish feeds. Since feed represents the largest portion (40 to 60%) of the total operating cost of farmed salmonid production, it is important that efforts be made to reduce feed costs.

Fish meals comprise the main source of protein (30 to 60%) in salmonid diets. Some protein of fish origin can also be included in the raw or ensiled form (Tacon and Jackson, 1985; Tacon, 1994). Other animal protein sources such as blood meal, meat meal, poultry by-product meal, skimmed milk powder, and hydrolysed feather meal are used to a lesser extent (Tacon and Jackson, 1985; Tacon, 1994). This is also the case with plant protein products. Attempts to completely replace the fish meal component of salmonid diets with other ingredients of plant and animal origin have met with little success until recently (Higgs et al., 1988; Prendergast et al., 1994).

Fish meal quality is affected by several factors, including: the characteristics of the raw material (origin, species, season, whole fish or fish scraps, and storage conditions before meal production), the processing methods (cooking and drying temperatures, level and quality of fat and solubles, grinding conditions, and antioxidant addition), and the transportation and storage conditions of the meal (Tarr and Biely, 1973).

Fish, in common with other animals, do not have a requirement for protein per se, but rather a requirement for a well-balanced mixture of essential and non-essential

amino acids (Wilson and Halver, 1986). It has been demonstrated that fish require ten essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Cowey and Sargent, 1972). Non-essential amino acids can be synthesized from non-specific precursor carbon and nitrogen. If one assumes a sufficient and an appropriate supply of dietary energy, an inadequate protein intake or a lack of one or more of the essential amino acids in the diet results in a reduction or cessation of growth. This reduction or cessation is due to the withdrawal of protein from less vital tissues to maintain the function of tissues most critical for the survival of the organism. Alternatively, if excess protein is supplied in the diet, only part of it will be used to synthesize new proteins. The remainder will be catabolized to supply energy or will be converted to fat and deposited in fat stores (Walton, 1985).

The use of feedstuffs that are highly digestible is a major goal for sustainable aquaculture. Such use would greatly reduce environmental pollution. Although the use of high quality fish meal in salmonid feeds would help to attain this goal, there are other biological, nutritional, and economical considerations to take into account (Cho et al., 1994). From an economic standpoint, the inclusion of fish meal in formulated salmonid diets should be restricted to that level needed to provide the amino acids required for optimal growth and health. The energy needs of the fish should be met with the less expensive non-protein energy sources, i.e. lipids and carbohydrates. Also, the balance of nutrients and energy in the diet should be adjusted according to life cycle requirements.

This chapter covers the various methods used for evaluating the quality and utilization of protein in fish meal and reviews knowledge regarding the protein

requirements of salmonids (Salmo spp., Oncorhynchus spp., and Salvelinus spp.).

PROTEIN QUALITY EVALUATION OF FISH MEALS

The quality of a protein depends on its amino acid profile and the availability of the amino acids to a particular animal. A protein source of excellent quality is one that provides all of the essential amino acids in quantities that meet the needs of the animal and in ratios that closely mimic the requirements of the particular animal (Woodham, 1969). Only one amino acid needs to be limiting for the protein to be classified as being of poor quality (Haque and Njaa, 1988; Haque et al.,1989).

As mentioned previously, various fish meals comprise the main protein component in salmonid diets. This is because the amino acid composition of fish meals closely corresponds to the fish's requirements, and, thus, excellent growth rates are supported. Fish meals are also highly palatable and generally are good sources of minerals and of n-3 polyunsaturated fatty acids.

To understand how the quality of fish meal can be affected through processing, one must have some understanding of the manufacturing process. The bulk of fish meal is produced from fish caught specifically for that purpose (FAO, 1975). However, in some regions of the world, fish meals are by-products of the roe industry. The major species used for fish meal production are herring, mackerel, anchovy, sardines, and capelin. Another source of material for fish meal production is the cuttings and offal from the processing of a wide range of species, including groundfish and salmon. In regions where fish are specifically caught for fish meal production, the freshness of the raw material is of great importance. This is especially true when large amounts of fish are caught in a short period, because the inevitable delays before processing can result

in varying degrees of enzymatic and bacterial breakdown. This breakdown leads to the generation of non-volatile and volatile amines that can reduce nutritive value. The bacterial degradation of protein and release of ammonia may result in a significant loss of protein and the production of malodorous compounds (Farber, 1965). These compounds may survive processing and affect the quality of the final product.

The processing of raw fish into fish meal involves separation of the solid (protein and ash) and liquid (water and lipid) phases (FAO, 1975; Stansby, 1990). The principal method of processing is wet reduction (FAO, 1975; Stansby, 1990). In this process, the raw material is first cooked to coagulate the protein and to liberate bound water and oil. Thereafter, partial removal of water and oil is accomplished by pressing the coagulate to yield a solid phase (presscake) that contains 60 to 80% of the oil-free dry matter (protein and bones), and an oil and liquid phase (press water) that contains the rest of the solids (dissolved and suspended protein, vitamins, and soluble minerals). The main part of the solids in the press water is removed by centrifugation. The oil is subsequently removed by further centrifugation. The water phase from the centrifuges (called stickwater) is concentrated by evaporation and thoroughly mixed into the presscake, which is then dehydrated to produce a whole meal (presscake plus solubles). Drying of the meal can be accomplished by flame-drying and/or indirect steam-drying. Flame-drying involves direct contact of the meal being dried with a current of flue gases diluted with secondary air (FAO, 1975). With indirect steam-drying, the meal is fed continuously past steam-heated elements (tubes, discs, coils) (FAO, 1975), and a countercurrent of air is blown through the drier to facilitate removal of water vapour. The dried material is milled and then an antioxidant (usually ethoxyquin) is added; the fish meal is stored in bags or in bulk bins (FAO, 1975;

Stansby, 1990).

If excessive heat is applied during the drying process, amino acids present in the fish protein may be rendered nutritionally unavailable, thus lowering the protein quality of the meal (Bender, 1972; Tarr and Biely, 1973). Proteins present in other animal by-products can also be affected adversely during their processing into meals, whereas the quality of plant protein sources is influenced not only by the processing conditions, but also by the presence of antinutritional factors both common (e.g. fibre, phenolic compounds, and phytic acid) and unique (e.g. protease inhibitors in soybeans or glucosinulates in canola) (Bell, 1993; Liener, 1994). Because these alternate protein sources presently contribute only a small portion of the protein in the diets of salmonids (see Tacon and Jackson, 1985; Åsgård, 1988; Tacon, 1994), they will not be discussed further.

Methods for evaluating protein quality

Protein quality can be assessed by chemical assays, microbiological assays, and/or animal growth studies. Growth studies are costly and time consuming. Consequently, more rapid and less expensive assays are generally used. These assays include: assessment of in vitro digestibility, measurement of available lysine, measurement of sulphydryl and disulphide bonds, and measurement of bacterial growth.

In vitro assays

In vitro protein digestibility tests are rapid and relatively inexpensive. The tests may involve the use of individual proteolytic enzymes (usually pepsin) (Olley and Pirie,

1966; Satterlee et al., 1977; Williams, 1984), a combination of enzymes (Gauthier et al., 1982; Brule and Savoie, 1988), or a combination of enzymes together with the measurement of pH response (Hsu et al., 1977; Pedersen and Eggum, 1983; Jecsai et al., 1987; Dimes and Haard, 1994).

March et al. (1966) found that pepsin digestibility values for fish meals (after chloroform-methanol extraction) were significantly correlated with biological estimates of their protein quality in poultry. It was later shown that pepsin concentration and digestion temperature significantly influenced the sensitivity of the test (March and Hickling, 1982).

Hsu et al. (1977) used a multienzyme (trypsin, chymotrypsin, and peptidase) technique for estimating the protein digestibility of a variety of food products. They measured the fall in pH associated with the release of carboxyl groups after addition of the enzyme mixture. There was a good correlation ($r = 0.90$) between the pH drop after ten minutes of incubation and the in vivo digestion of 23 different food products by rats. Pedersen and Eggum (1983) modified the multienzyme technique to work under a constant pH of 7.98 (pH-stat), the pH being maintained by the addition of sodium hydroxide (NaOH) (0.10 N). The amount of NaOH added was used to calculate the digestibility of the feedstuff. The method gave a more accurate prediction of the digestibility of both animal and plant products by rats than the method of Hsu et al. (1977).

In vitro methods of assessing protein digestibility depend on the estimation of the extent of proteolysis after the addition of enzymes. Generally, these methods employ a closed system. The reaction is arrested either by heating the digestion mixture or by precipitating the protein with a strong acid, followed by filtration or centrifugation. The

assessment of protein digestibility by these methods is usually determined by nitrogen or amino acid analyses of the different digestion fractions. A shortcoming of the closed system is that there is the possibility of inhibition due to the accumulation of digestion products or the drop in pH that occurs following the release of carboxyl groups from proteolysis. Because of these shortcomings, several workers have tried to simulate digestion in the animal (an open system) by utilizing a dialysis bag to remove the digestion products (Mauron et al., 1955; Steinhart and Kirchgessner, 1973; Gauthier et al., 1982). To date, such methods have met with only limited success.

Until recently, evaluation of the quality of protein for fish has been conducted in systems utilizing mammalian digestive enzymes (March et al., 1985; Anderson et al., 1993). In an attempt to develop an in vitro assay applicable to the evaluation of components destined for inclusion in fish diets, several workers have used fish enzymes in in vitro studies (Grabner, 1985; Eid and Matty, 1989a; Dimes and Haard, 1994; Dimes et al., 1994a, b). Dimes and co-workers (Dimes and Haard, 1994; Dimes et al., 1994a, b) have utilized crude enzymatic extracts from fish pyloric caeca. However, fish age and strain and the previous dietary history may influence the activity of these crude enzymatic extracts, and thereby affect the results obtained (Dimes and Haard, 1994). Further complications have been the long reaction times needed due to the low incubation temperatures employed when using fish enzymes, and the lack of commercially available digestive enzymes from fish. At present, only one fish enzyme is available commercially - trypsin (Type XX-S from Atlantic cod (Gadus morhua), Sigma Chemical Company, St. Louis, MO, 1994 Catalogue).

It has been shown that the heating of protein to temperatures above 115°C causes destruction of several amino acids, including lysine and cysteine (Ledward,

1979). Since the level of lysine is nutritionally marginal in many proteins, the influence of processing conditions on its availability has been studied extensively (Carpenter, 1973). Reducing sugars react with the ϵ-NH_2 of lysine, reducing availability (Maillard reaction), but this is not a major problem with fish meals because of the lack of reducing sugars in the meals. However, the ϵ-NH_2 group of lysine may react with asparagine, glutamine, or breakdown products of cyst(e)ine to form secondary intramolecular covalent bonds, rendering products that are resistant to digestion (Bjarnason and Carpenter, 1970). Also, carbonyl compounds arising from lipid oxidation (Gardner, 1979) react with the ϵ-NH_2 of lysine. The reactions occur slowly at ambient temperatures (20 - 25°C) and the rate of reaction increases with temperature. A high correlation has often been found between levels of available lysine in fish meal and their ranking for quality in feeding tests; this has been especially true when the fish meals have been produced using extremely high temperature treatments. However, in other studies involving fish meals produced under commercial and laboratory conditions, no correlation has been found between the level of available lysine and growth or other biological responses of rats or fish (Carpenter, 1973; McCallum and Higgs, 1989; Anderson et al., 1993).

Sulphydryl groups and disulphide bonds are important for maintaining the structure and function of native proteins, and thus they play important roles in the functional properties of proteinaceous foods (Ledward, 1979). Heat application that decreases the ratio of cysteine to cystine residues has been found to reduce the utilization of the protein in blood meal by rats, chicks, and turkeys (Waibel et al., 1977) and in fish meals by fish (Opstvedt et al., 1984). It has been assumed that disulphide cross-linkages hamper the attack by proteolytic enzymes (Friedman et al., 1982), thus

decreasing protein digestibility.

Opstvedt et al. (1984) showed that -SH (sulphydryl) groups in pollock and mackerel meals were converted to S-S (disulphide) bonds when processing temperatures were increased from 50°C to 115°C. At 115°C cystine plus cysteine were destroyed and lost as hydrogen sulphide (Bjarnason and Carpenter, 1970; Opstvedt et al., 1984), with some 10% of the cystine/cysteine fraction being lost from pollock or mackerel heated in a retort at 115°C for 20 minutes (Opstvedt et al., 1984).

During processing, 6% to 12% of the methionine in fish meal is oxidized. Consequently, the meals contain a blend of methionine and methionine sulphoxide (Slump and Schreuder, 1973; Njaa, 1980; Aksnes and Njaa, 1984; Haaland et al., 1989; Haaland and Njaa, 1990). In diets in which methionine is the first-limiting amino acid, the amount of methionine sulphoxide could prove to be an important indicator of fish meal quality. This assumes however, that the animal does not utilize methionine sulphoxide for tissue formation. This is not the case in rats and chickens (Miller et al., 1970; Aksnes and Njaa, 1984; Haaland et al., 1989). It is not known whether fish are able to utilize methionine sulphoxide for growth, but it is noteworthy that methionine sulphoxide is present in the plasma of fish (Thebault, 1985).

Only when an amino acid is first-limiting does the in vitro evaluation of its availability correlate well with the biological assessment of protein quality. Comparisons of amino acid levels in various fish meals with those in fish egg proteins or fish muscle proteins have revealed that histidine, threonine, or valine may be the first-limiting amino acids in fish meals for growing fish (Hepher, 1988). It is doubtful however, that histidine is indeed limiting for growth. The high level of histidine in fish flesh is due to its high concentration in the non-protein compounds anserine, carnosine, and ophidine (Shirai

et al., 1983; Ogata and Murai, 1994). The presence of these histidine-containing compounds in fish flesh would serve to bias the estimation of histidine as the first limiting amino acid when fish muscle protein is used as the reference standard. The estimated histidine requirement for the optimal growth of salmonids ranges from 1.6% to 1.8% of the dietary protein (NRC, 1993). This requirement is met by the average content of 2.3% to 2.6% histidine in the protein of fish meal (NRC, 1993). This is not to detract from the importance of histidine-containing compounds in fish muscle physiology, and perhaps extra dietary histidine may be required to ensure optimal levels of these compounds in fish muscle, but not for optimal growth.

Microbiological activity and chemical changes during storage of raw fish are responsible for the progressive decline of organoleptic quality. Specifically, enzymatic (natural and bacterial) breakdown of both proteins and the osmoregulatory agent trimethylamine oxide, present in marine species (Love, 1970), results in the production of ammonia, monomethylamine, trimethylamine, and other volatile amines. The test for total volatile basic-nitrogen (TVBN), that measures low molecular weight volatile bases and amine compounds produced by decarboxylation of amino acids, has been commonly used for assessment of the quality of unprocessed fish (Farber, 1965; Woyewoda et al., 1986). However, this measure has little value in predicting the quality of protein in fish meals, since the volatile compounds are lost during the drying phase in fish meal production (Clancy, 1992; Anderson et al., 1993). This test is best used to evaluate the freshness of the raw material before processing (Clancy, 1992; Anderson et al., 1993).

Microbiological assays

The use of proteolytic microorganisms that use a wide array of digestive enzymes offers a significant improvement over the earlier enzymatic methods that have been described. Their use has the unique ability to combine the measurement of amino acid levels and their availability into one test for the evaluation of protein quality.

Streptococcus zymogenes, a proteolytic bacterium capable of digesting intact food proteins, was used in the early microbiological assays (Ford, 1960). The general procedure involves predigesting the protein source with an enzyme (pepsin or papain) and incorporating the digest into a culture medium devoid of protein. Assay tubes are then inoculated with a culture of the bacterium. Growth response is measured either by assessing turbidity, caused by growth of the bacterium, or by determining the extent of reduction of triphenyltetrazolium chloride (Ford, 1960). Turbidity can be assessed photometrically, whereas triphenyltetrazolium chloride is used when the test protein causes the incubation solution to become turbid. Enzymatic reduction of triphenyltetrazolium chloride produces a red compound that can be quantitatively analysed photometrically. A standard protein source, vitamin-free casein, is used as a control. Predigestion of the test protein, using papain, has been found to reduce the lag phase of bacterial growth and has enabled the assay to be completed within 48 hours. Subsequent work has shown that the results obtained are dependent upon the conditions of this predigestion. For instance, an increase in the strength or amount of papain or the use of a sequence of pepsin, trypsin, and erepsin has resulted in higher protein quality values (Ford, 1964; Miller et al., 1965).

Tests of protein quality that involve the use of the protozoan Tetrahymena pyriformis W. are closer to biological testing using mammals than those using bacteria.

This is because, T. pyriformis has a similar pattern of requirement for amino acids to that of the growing rat and it is able to utilize intact proteins (Shorrock, 1976). T. pyriformis has been found to give higher values than Streptococcus zymogenes, but enzymatic predigestion is still required to obtain the best correlation with protein quality evaluations using rats (Shorrock, 1976). The maintenance of cultures of test organisms, especially during times of non-use, is the greatest hindrance to their widespread application.

Biological assays with fish

Several methods have been used in nutritional studies with fish to estimate protein quality. Nose (1962) examined carcass nitrogen retention, whereas other researchers have conducted growth studies using multiple dietary protein levels for each protein source. The two variables that are most used for judging protein quality in growth studies with fish are protein efficiency ratio (PER) and net protein utilization (NPU).

PER is defined as grams of live weight gain per gram of protein consumed. This variable should be measured using several dietary levels of the test protein to avoid penalizing both high and low quality proteins, the maximum PER value is then accepted as being indicative of the nutritive value of the protein source. PER determinations do not make allowances for maintenance and they assume that the live weight gain is of constant composition, which is not necessarily so. Further, PER values are influenced by feed (protein) intake, size and age of the fish, length of the study, and composition (amounts of other essential nutrients and energy) of the experimental diet.

NPU is a more precise index of the amount of the dietary protein used or retained

by fish. NPU combines into a single index both the biological value and the digestibility of a protein. NPU determinations do not require measurement of nitrogenous wastes in the water, yet the procedure still accounts for exogenous and endogenous nitrogen losses. NPU is defined as follows:

$$(B - B_k) / I$$

where B = body nitrogen of animals fed the test protein, B_k = body nitrogen of animals on a nitrogen-free diet, and I = nitrogen intake of animals fed the test protein diet. It is sometimes difficult or impossible to stimulate fish to eat a protein-free diet. An alternative is to feed a diet containing a level of protein near or below maintenance (Cowey et al., 1974). Thus, the formula for NPU becomes:

$$(B - (B_k - I_k)) / I$$

Where, I_k = nitrogen intake of animals fed a low protein diet (Cowey et al., 1974).

Ogino et al. (1980) working with carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss) used the following formula to calculate NPU

$$\frac{\text{Body nitrogen gain of test group (g)} + \left[\frac{(W_1 + W_2)}{2} \times \frac{95}{1000} \times \frac{1}{1000} \times \text{day} \right]}{\text{Protein intake of test group (g)}}$$

where W_1 = initial body weight, W_2 = final body weight of the test group, and day = days of feeding. The endogenous loss of nitrogen was determined by carcass analysis after feeding a protein-free diet during the experimental period. They found that the value for the endogenous loss of nitrogen in rainbow trout was 95 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$ and changed in direct proportion to body weight. Yet, as with PER, NPU values are influenced by feed (protein) intake, size and age of the fish, length of the study, and

composition of the experimental diet, also, endogenous losses of nitrogen are influenced by water temperature and duration of the experimental period.

Although PER is sensitive to dietary concentration of protein, the linear response of body weight gain to graded levels of protein intake, achieved by controlled feeding, provides a useful technique (slope-ratios) for comparing the quality of protein sources. March et al. (1985) used slope-ratios based on the linear responses of body weight gain of rainbow trout to graded levels of protein intake to estimate the protein quality of laboratory-made fish meals . They found that protein level and feed intake could be accounted for with this method. McCallum and Higgs (1989) determined PER, NPU, and slope-ratios for different marine protein sources subjected to various heat treatments. They found that slope-ratios derived from an analysis of covariance of body protein gains of juvenile chinook salmon (Oncorhynchus tshawytscha) against protein intake as the covariate, was a more discriminating method than the slope-ratio assessment of protein quality as used by March et al. (1985). Both methods have the drawback of needing numerous treatments to be effective, with at least three dietary levels needed for each protein source. This type of study requires a considerable number of tanks of fish and it is expensive to conduct.

Comparisons of in vitro and in vivo studies

Studies that have compared the results of in vitro assays with those from in vivo measurements have typically used the rat as the test animal (Hsu et al., 1977; Pedersen and Eggum, 1983; Jecsai et al., 1987; Samman and Farias, 1993). There have been several studies that have compared different in vitro results with the growth or digestibility values obtained using of fish (Opstvedt et al., 1984; Eid and Matty,

1989a; Mundheim and Opstvedt, 1989; Anderson et al., 1993; Dimes and Haard, 1994; Dimes et al., 1994b) and other studies that have used the growth rate of salmonids (Nose, 1962; Ogino and Nanri, 1980; March et al., 1985; McCallum and Higgs, 1989; Anderson et al., 1993) and the nitrogen excretion rates of carp to evaluate the quality of various protein sources (Eid and Matty, 1989b).

In one study, regression equations based on in vitro results using fish enzymes and in vivo digestibility coefficients were developed to predict protein quality (Dimes and Haard, 1994). In this regard, Dimes and Haard (1994), working with nine feedstuffs of plant and animal origin, obtained an r^2 of 0.82 for the relationship between degree of hydrolysis (a pH-stat method) and the apparent digestibility coefficients of the protein in the feedstuffs for rainbow trout. Other regression equations have been based on in vitro results using mammalian enzymes and in vivo digestibility results with rats. A problem with many methods has been the need to develop separate regression equations for animal and plant proteins. The applicability of the mammalian findings to fish is questionable (Haard, 1995).

Chicken growth

Miller and Kifer (1970a) showed that the evaluation of the quality of the protein in fish meal, when the meal was used as the sole source of dietary protein for chicks, was influenced by an excess level of lysine and by various anionic components in the mineral mixture. Antacids were found to be beneficial supplements for some fish meals. Decreasing the dietary contents of chloride (Cl^-) and sulphate (SO_4^{2-}) and simultaneously increasing the bicarbonate (HCO_3^-) content improved the growth responses of chicks by 9 to 40% when they were given various fish meals as the sole

source of dietary protein (Miller, 1970; Miller and Kifer, 1970b). The situation in fish is less clear. Wilson et al. (1985) found no significant effect of dietary electrolyte balance (Na^+, K^+, Cl^-) (-90 to +638 meq·kg^{-1} diet) on growth or acid-base balance in rainbow trout reared in fresh water, although slight acidosis did occur with the highest dietary electrolyte balance (+638 meq·kg^{-1}) . In other work, Chiu et al. (1987) found that high dietary levels of K^+ (362 meq·kg^{-1}), Na^+ (192 meq·kg^{-1}), and Cl^- (349 meq·kg^{-1}), irrespective of overall cation-anion balance, depressed growth rate and feed utilization of rainbow trout fingerlings reared in fresh water.

Gizzard erosion has been observed in chicks fed diets containing poor quality fish meals; gizzerosine, a product of the thermal combination of histamine and lysine (Okazaki et al., 1983) has been identified as the cause of the problem. Because chicks are known to be sensitive to dietary inclusion of poor quality fish meal, they have become the animal of choice for evaluation of fish meal quality. Two studies have assessed the effects of dietary gizzerosine on fish (Watanabe et al., 1987; Fairgrieve et al., 1994). In the latter study, no correlation was found between gizzerosine levels in fish meals and estimates of their nutritional value for rainbow trout.

Digestibility evaluation using mink

In recent years mink (Mustela vison) have been used as a test animal for evaluation of feedstuff quality for fish. This is most likely because mink and carnivorous fish have a similar gastrointestinal tract morphology. Mundheim and Opstvedt (1989) evaluated the quality of 16 different capelin meals that were processed according to protocols established for the production of NorSeaMink® and Norse-LT94® fish meals (see Pike et al. 1990 for the quality standards). The coefficients of simple determination

(r^2) between apparent protein digestibility by mink and apparent protein digestibility by Atlantic salmon and between the former and apparent protein digestibility by rainbow trout were 0.630 and 0.605, respectively. These relationships are rather weak. Also, they are applicable only to fish meals within this limited range of quality.

Digestibility evaluation using fish

As in all studies involving the use of live animals, facilities must be available for the proper housing and care of the test fish species. The aquatic environment can make it difficult to obtain accurate determinations of feedstuff digestibility because of nutrient leaching from faeces.

Faeces have been collected by dissection, anal suction, or manual stripping (Austreng, 1978; Windell et al., 1978a; Spyridakis et al., 1989; Hajen et al., 1993a, b), continuous filtration (Choubert et al., 1982), netting or pipetting (Windell et al., 1978a; Spyridakis et al., 1989), sedimentation (Cho et al., 1975; Rychly and Spannhof, 1979; Satoh et al., 1992; Hajen et al., 1993a, b), and from specially designed metabolic chambers (Post et al., 1965; Smith, 1971; Smith et al., 1980). The advantages and disadvantages of each of these methods have been reviewed (Cho et al., 1982; Hajen et al., 1993a). These different methods of faecal collection may influence the accuracy of the measurement of the amount of nutrients not absorbed from the intestinal tract because of contamination or leaching of nutrients from the faeces. Such error will result in artificially inflated or depressed values for nutrient digestibilities (Windell et al., 1978a; Choubert et al., 1979). The most reliable procedures currently in use include manual stripping or intestinal dissection, the Choubert system (continuous filtration) and the "Guelph" system (sedimentation).

The apparent digestibility coefficients determined using salmonids (mostly rainbow trout) for the protein fraction from various feedstuffs average 80.1 ± 10.6% (mean ± sd, n=19 with a range of 60% to 90%) for plant sources and 73.7 ± 22.1% (mean ± sd, n=21 with a range of 30% to 97%) for animal protein sources (NRC, 1993). The digestibility of the protein from fish meals for salmonids has an average value of 87.7 ± 3.4% (mean ± sd, n=6 with a range of 83% to 92%). Much work has been done on the evaluation of the digestibility of many common feedstuffs for fish (for a comprehensive summary see NRC, 1993).

PROTEIN METABOLISM

Digestion

Fish vary widely with respect to digestive tract morphology and endogenous enzymatic secretions. It is beyond the scope of this review to cover this topic and the reader should refer to articles by Kapoor et al. (1975), Ash (1985), Smith (1989), and Wilson (1989b). In salmonids, pepsin is produced in the stomach and trypsin, chymotrypsin, carboxypeptidases, collagenase, and elastase are synthesized in the pancreas for secretion into the small intestine to aid in protein digestion (Fänge and Grove, 1979; Ash, 1985).

Protein synthesis and degradation

Proteins are the major organic components in fish tissue, making up approximately 65 to 75% of the dry-matter (Wilson, 1989a). Protein accretion is under the control of two processes: protein synthesis and protein catabolism (Fauconneau, 1985; Houlihan et al., 1986). The methods used to measure rates of protein synthesis

in mammals have recently been adapted for use with fish. The methods include the constant infusion technique (Garlick and Marshall, 1972), and the use of a single high-dose injection of labelled amino acid to flood the intracellular pools and stabilize precursor-specific activity over the course of the measurement (Garlick et al., 1980). Protein degradation rates have generally been estimated from the difference between the amount of protein synthesized and the amount deposited. As protein deposition is measured over a period of weeks and protein synthesis over a period of minutes or hours, this is not an especially satisfactory procedure. Currently, there is no satisfactory precise procedure for this measurement.

In fish white muscle, protein turnover represents only a small part of protein synthesis, whereas in mammals it may reach over 60% of protein synthesis (Reeds and Lobley, 1980). Mechanisms of muscular growth associated either with an increase or a decrease in protein turnover rate are similar in fish and in mammals. Yet turnover rates of resident proteins in fish muscle are low when compared with those of other fish tissues, like gill and liver, and when compared to muscle protein synthesis rates (Houlihan et al., 1988).

In man and other mammals, level of protein intake has the most important influence on protein deposition. Increased protein intake seems to stimulate both protein synthesis and protein degradation. The intake of non-protein energy, while affecting nitrogen retention, appears to act in a manner different from that of dietary protein. These two dietary factors appear to have a summative rather than an interactive effect on protein turnover, and may stimulate protein accretion by different mechanisms (Reeds and Fuller, 1983). Decreases in muscle-protein synthesis in response to low-protein and/or low-energy content in the diet have been observed in

mammals (Millward et al., 1976) and fish (Houlihan et al., 1988). Increased rates of protein synthesis in fish have been accompanied by elevated efficiencies in the retention of synthesized protein (Houlihan et al., 1988). Houlihan et al. (1988) estimated that the energy required for protein synthesis accounted for 24% of the total oxygen consumption of Atlantic cod exhibiting no growth and 42% in growing cod (2% BW·day^{-1}). In general, one would assume that the energy cost of protein synthesis in fish should be the same as that in mammals, since the mechanisms are the same. The lower proportional energy cost of protein synthesis in growing mammals (15 to 22%) (Garlick et al., 1976; Nicholas et al., 1977; Reeds and Lobley, 1980) can be explained by the lower basal energy requirements of fish compared to mammals.

Fractional rates of protein synthesis and degradation in all tissues of rainbow trout are inversely related to body size (Houlihan et al., 1986). Comparisons of these measurements in tissues sampled from different species should therefore be undertaken only when the dissimilar species are of a similar size. Fractional rates of protein synthesis in visceral tissues of mammals are appreciably greater than those in skeletal muscle (Reeds and Lobley, 1980). Values obtained from fish indicate a similar relationship, with synthesis rates in gill, liver, and visceral tissues approximately 20 times greater than in skeletal muscle. In fish white muscle, 76% of synthesized protein is retained as growth compared with, for example, 4% in gill, a metabolically-active tissue with a fractional rate of protein synthesis of approximately 9%·day^{-1} (Smith, 1981; Houlihan et al., 1986).

Protein catabolism

Protein is degraded by two separate systems within the cell. One system is the

ubiquitin-mediated pathway that requires direct energy input, and the other is lysosomal degradation that requires no direct energy input (Glaumann and Ballard, 1987; Ciechanover and Schwartz, 1994). These systems yield free amino acids that then may be recycled into new proteins or catabolized.

The principal end products of amino acid catabolism are water, ammonium ions (NH_4^+), carbon dioxide (CO_2), and bicarbonate (HCO_3^-). In mammals, the CO_2 and HCO_3^- concentrations are controlled by respiration and urinary excretion. High concentrations of NH_4^+ are toxic. To prevent toxicity, metabolic energy is used to incorporate NH_4^+ into organic compounds (carbamyl phosphate) that can then be further transformed and excreted. The particular compound that is synthesized depends on the life habits of the animal; urea is formed in mammals and uric acid in birds and reptiles. Gill breathing vertebrates are able to dispose of their waste products into the water flowing over the gills and most teleosts are ammonotelic. Gills are the main excretory organs, accounting for 75 to 90% of total nitrogen excretion. Urinary excretion is relatively small, with NH_4^+ as the major nitrogenous catabolite. A small but significant proportion (5 to 15%) of waste nitrogen is also excreted in the urine as urea (Brett and Zala, 1975; Kaushik, 1980) in almost all teleosts. Other minor nitrogenous end products in the urine include uric acid, creatine, and trimethylamine oxide. The major source of urea in teleosts is from purine catabolism (Vellas and Serfaty, 1974).

Most of the NH_4^+ is produced in the liver, with smaller amounts produced in the kidney and muscle. NH_4^+ is formed largely from the catabolism of amino acids. Direct deamination occurs only with a limited number of amino acids, including histidine, serine, glutamine, and glutamate. Other than glutamate dehydrogenase (EC 1.4.1.2), the activities and distribution of these enzymes (histidine deaminase (EC 4.3.1.3),

serine deaminase (EC 4.2.1.13), and glutaminase (EC 3.5.1.2)) in fish tissue are too low for them to be considered as major contributors to NH_4^+ formation (van Waarde, 1983). Glutamate dehydrogenase plays a key role in NH_4^+ formation via the transdeamination pathway, with amino acid specific amino transferases. Glutamate is then oxidatively deaminated to form NH_4^+ and to regenerate alpha-ketoglutarate.

PROTEIN REQUIREMENTS OF SALMONIDS

Protein is required by fish to provide essential amino acids as well as nitrogen for the synthesis of non-essential amino acids. These amino acids are used for maintenance, growth, reproduction, and replacement of depleted tissues. A portion of protein is also utilized for energy, particularly during starvation, migration, and disease.

Maintenance protein requirements

Cells are in a dynamic state. This means that endogenous nutrients are in continual turnover and a portion of these nutrients is lost through normal metabolism. Therefore, for normal protein maintenance, cells require basic amounts of amino acids for synthesis of proteins, nucleic acids, and lesser (in quantity but not importance) nitrogen-containing compounds. Thus, the replacement of this supply of amino acids represents the absolute minimum requirement of the fish for amino acids or the maintenance protein requirement in the diet.

Two methodologies have generally been used to evaluate the excretion of endogenous nitrogen by fish. Direct methods involve measuring the combined faecal, urinary, and branchial nitrogen losses, whereas indirect methods are based on carcass analysis (Ogino et al., 1980).

Although several investigators have studied the retention and the endogenous excretion of nitrogen in a variety of fish, few investigators have determined the maintenance requirements for protein. For sockeye salmon (Oncorhynchus nerka), Brett and Zala (1975) estimated the endogenous protein loss to be 1.38 g protein·kg^{-1} body weight·day^{-1}. The maintenance protein needs of rainbow trout, ranging in weight from 40 g to 200 g, have been estimated to be 1.8 ± 0.3 g protein·kg^{-1} body weight·day^{-1} (Kaushik et al., 1981; Kaushik and Luquet, 1983). Kaushik and Gomes (1988) estimated the maintenance protein requirement for rainbow trout (150g) to be 2.6 g of digestible protein·kg^{-1} body weight·day^{-1}.

Factors affecting gross protein requirements

Fish size and age

Generally, the protein requirement of fish, as a percentage of the diet, decreases with increasing size and age (Hilton and Slinger, 1981; NRC, 1981).

Water temperature

Changes in water temperature have been shown to alter the protein requirements in some species of fish, but not in others. Chinook salmon (5 - 10 g) were found to require 40% protein at 8°C and 55% protein at 15°C by DeLong et al. (1958). However, when rainbow trout were fed practical diets containing 35, 40, or 45% crude protein at temperatures ranging from 9°C to 18°C, no differences in protein requirement could be detected (NRC, 1981). The findings of DeLong et al. (1958) are probably in error because the test diets varied in digestible energy content. Also, the diets contained marginal levels of lipid energy and suboptimal levels of n-3 fatty acids (Higgs

et al., 1995b).

Water temperature affects feed intake, rate of transit of foodstuffs through the digestive tract, metabolic rate, etc. (Brett, 1979). Water temperature appears to affect nitrogen excretion, and consequently overall nitrogen retention, but the response is mediated through its effect on feed intake (Kaushik and Cowey, 1991), and there are no changes in dietary protein needs (NRC, 1993).

Water salinity

Salinity has been shown to affect the protein requirement of the euryhaline rainbow trout. Zeitoun et al. (1973) found that the dietary protein requirement of rainbow trout (6.5 - 20 g) increased from 40 to 45% for fish reared in 10 and 20‰ salt water, respectively. Lall and Bishop (1979) found no difference in the protein requirement of rainbow trout (50 - 100 g) reared in 0 or 32‰ salinity. Zeitoun et al. (1974), working with coho salmon smolts (15 - 20 g), found no difference in the relative protein requirement of fish reared at 10 or 20‰ salinity. The influence of salinity on the relative protein requirement of fish needs further investigation before conclusive statements can be made.

Dietary factors influencing the quantitative protein requirement

Protein is in a constant state of turnover; amino acids must be supplied to the cells in ratios that can be used for synthesis of new proteins. If any one essential amino acid is low or lacking, the excess amino acids are deaminated and the carbon skeletons are used for energy or synthesis of other compounds (Walton and Cowey, 1982). Amino acids, unlike carbohydrates and lipid, are not stored, but when in excess of what

can be used for protein synthesis are deaminated and the carbon skeletons are used for energy purposes or they are converted and stored as fat.

Protein requirements of fish have been extensively investigated, mostly in studies utilizing dose-response curves. The results from such studies have frequently been confounded by differences among diets in digestible energy content, amino acid composition and balance, and digestibility of the dietary proteins.

Dietary energy level

Fish generally eat organoleptically acceptable diets to satisfy their demands for energy (Lee and Putnam, 1973; Kaushik and Luquet, 1983). The protein-sparing action of the non-protein energy sources (lipid and carbohydrate) has been studied especially in rainbow trout (Kaushik et al., 1981; Kaushik and de Oliva Teles, 1985; Cho and Woodward, 1989; Kaushik et al., 1989). It has been suggested that rainbow trout in fresh water need 22 - 25 g of digestible protein per MJ of digestible energy ($DP \cdot MJ^{-1}$ DE) in the diet for optimal growth and protein utilization (Cho and Kaushik, 1990). Other workers have suggested a requirement of 19 - 22 g $DP \cdot MJ^{-1}$ DE for chinook salmon reared in seawater (Silver et al., 1993), which is lower than that estimated for juvenile Pacific salmon (all Oncorhynchus spp.) in fresh water (22 - 25 g $DP \cdot MJ^{-1}$ DE) (Higgs et al., 1995b). Further study is needed in the area for the different life cycle stages of fish, especially the salt versus fresh water life stages.

Dietary energy source

Carbohydrate (Lee and Putnam, 1973; Pieper and Pfeffer, 1979, 1980; Kaushik and de Oliva Teles, 1985; Kim and Kaushik, 1992) and lipid (Lee and Putnam, 1973;

Reinitz et al., 1978; Yu and Sinnhuber, 1981; Beamish and Medland, 1986; Silver et al., 1993) have been shown to spare protein as an energy source in salmonids; lipid being the preferred non-protein energy source (Kaushik et al., 1989).

Carbohydrate is not used efficiently as an energy source by salmonids. Palmer and Ryman (1972) observed this during a test of the oral glucose tolerance of rainbow trout; hyperglycaemia was exhibited 24 hours after administration of glucose. The same effect was seen with juvenile chinook salmon (Mazur et al., 1992). It has been proposed that the poor ability of salmonids to use large amounts of carbohydrate as an energy source may be due to natural selection for low enzyme activities (little need to phosphorylate exogenous glucose) due to the low levels of carbohydrate in their natural diet (Buddington, 1987; Buddington and Hilton, 1987; Buddington et al., 1987), although all enzymes for glycolysis have been identified in fish tissues (Nakai et al., 1970). Newsholme and Start (1973) reported that the ability to phosphorylate glucose is the rate-limiting step in glucose utilization for all animals. In rainbow trout, no glucokinase activity has been demonstrated (Shatton et al., 1971; Nagayama and Ohshima, 1974; Cowey et al., 1977; Nagayama et al., 1980), but work by Borrebaek et al. (1993) has shown the presence of glucokinase in Atlantic salmon (Salmo salar).

There are other physiological factors that may hinder carbohydrate utilization in fish. The role of insulin in carbohydrate metabolism in fish appears to be quite variable between species. In some fish, insulin functions as a major anabolic hormone stimulating glucose uptake by the liver and skeletal muscles (Mommsen and Plisetskaya, 1991). However, these effects are not found in all species of fish. In salmonids, glucose stimulates insulin release from islet cells, but amino acids (especially lysine and arginine) are more potent stimulators of insulin secretion

(Mommsen and Plisetskaya, 1991). Carnivorous fish show a non-insulin dependent diabetes mellitus, rather than what was originally thought to be an insulin-deficiency. It is now thought that carnivorous fish have an impaired post-receptor glucose-transporter system (Gutiérrez et al., 1991; Párrizas et al., 1994). Another difference is that fish have a lower glucose utilization rate than omnivorous mammals (Walton and Cowey, 1982).

The digestibility of cooked starches by rainbow trout is very high (Hilton et al., 1982) as is that of cerelose (D-glucose, 95 to 99% absorption). However, the ingestion of high dietary levels of digestible carbohydrate increases liver size in trout due to excessive accumulation of glycogen and this, in turn, may lead to liver disfunction.

There are marked differences between salmonid species in the estimated maximum tolerable dietary levels of digestible carbohydrate. Indeed estimates range from approximately 14% (Phillips et al., 1948; Hilton and Atkinson, 1982) to 25% (Buhler and Halver, 1961; Luquet, 1971; Bergot, 1979) and even 38% (Kaushik et al., 1989). The differences among these estimates are presumably due to differences in the levels of inclusion of the preferred energy sources (protein and lipid) among the different diets (Hilton et al., 1982). Other factors may involve water temperature, level of dietary intake, and experimental duration, as discussed by Higgs et al. (1995b).

Amino acid composition

The estimation of nutrient requirements is affected by the nature of the diet, i.e. whether it is purified, semi-purified, or consists of practical ingredients. The available levels and balance of amino acids from the various protein sources are also important factors to consider (Wilson et al., 1981; Anderson et al., 1992, 1995). Thus, direct

comparisons of results among different studies are difficult.

Factors influencing digestibility

Digestion of food depends on three main factors: a) the nature of the ingested food and the extent to which it is susceptible to digestive enzymes; b) the activity of the digestive enzymes; and c) the length of time the food is exposed to the action of the digestive enzymes. The availability of the digested material to the animal is also influenced by the rate of uptake (passive or active) by the entrocytes and any condition that may influence nutrient uptake. Each of these main factors is affected by a multitude of secondary factors. These include those associated with: a) the fish itself, such as species, age, size, and physiological condition; b) the environmental conditions, such as water temperature; and c) the feed, e.g. composition, particle size, and amount ingested.

Fish age

The capacity of fish to digest and transport nutrients can change with age (Buddington et al., 1987; Buddington, 1992). It has been reported that the activities of proteolytic enzymes (trypsin, chymotrypsin, aminopeptidase) in rainbow trout increase with age (Lauff and Hofer, 1984; Dimès et al., 1994a). Moreover, trypsin activity in Atlantic salmon has been shown to change with stage of the life cycle (Torrissen and Torrissen, 1984, 1985).

Water temperature

Fish exposed to an increase in water temperature increase their metabolic rate. Accompanying this is an increase in feed intake and rate of transit of ingesta through

the digestive tract (Fänge and Grove, 1979). Several experiments on rainbow trout have shown that the apparent digestibilities of dry matter, crude protein, lipid, and gross energy in practical diets are not significantly influenced by water temperature (Cho and Slinger, 1979; Cho, 1987; Kaushik, 1981). Yet, an interaction between the melting point of lipid and the prevailing water temperature has been observed in digestibility measurements. As the water temperature decreases (from 15 to 5°C) the digestibility of lipids with high melting points (lard and tallow) decreases in rainbow trout, whereas this is not the case for oils from fish, rapeseed, soybean, or linseed that are comparatively more unsaturated (Cho and Kaushik, 1990). These findings suggest that lard and tallow should be avoided as sources of lipid in diets which are designed for fish culture in cold waters (e.g. below 10°C).

Water salinity

Very little is known about the effect of salinity or other factors related to water composition on feedstuff digestibility. MacLeod (1977) found that the digestibility of dry matter, energy, and protein by rainbow trout held in water varying in salinity (0, 7.5, 15, or 32.5‰) for at least 100 days decreased linearly with increasing salinity. Also, Lall and Bishop (1979) observed a decrease in protein digestibility in rainbow trout held in seawater as compared to fresh water. By contrast, Dabrowski et al. (1986) found that rainbow trout (300 - 600 g) fed in both fresh and seawater (20 hours of exposure to seawater), had similar protein digestion and nutrient absorption in the alimentary tract.

In the a seawater environment, fish drink seawater (Smith, 1930; Evans, 1979) absorbing the water and monovalent ions, but not a significant portion of the divalent ions (Smith, 1930, 1983, 1989); therefore, the ash and thus the dry matter content of the faeces is significantly increased. Thus, in digestibility studies conducted in seawater,

the organic matter digestibility rather than dry matter digestibility should be determined. This approach is valid, as the organic matter and marker in the faeces are diluted equally by the added inorganic matter (ash) originating from the seawater.

Feed intake level and feeding frequency

Windell et al. (1978b) were unable to detect any change in the digestibility of protein and lipid by rainbow trout as the level of dietary intake was increased from 4 to 16 $g \cdot kg^{-1}$ body $weight \cdot day^{-1}$. At the highest feeding level, carbohydrate digestibility decreased; this may have been due to the fact that the diet contained 440 g $carbohydrate \cdot kg^{-1}$, an exceptionally high level for trout. Similar results have been obtained with rainbow trout maintained on rations of 6, 9, 12, or 15 $g \cdot kg^{-1}$ body $weight \cdot day^{-1}$ (Cho and Kaushik, 1990). In this latter study, there were no apparent differences due to ration level on the apparent digestibility of dry matter, crude protein, lipid, or energy.

Feeding frequency also does not appear to have any effect on digestibility. Hudon and de la Noüe (1985), for example, were unable to obtain any difference in apparent digestibility of dietary dry matter, protein, and energy in rainbow trout when feeding frequency was increased from two to six times per day.

Processing of dietary components

Processing of feedstuffs affects digestibility. Milling (particle size reduction) increases protein and carbohydrate digestibility for common carp and channel catfish (Ictalurus punctatus) (Mann, 1948; Lovell, 1984). Cooking, especially extrusion, (Smith, 1971; Bergot and Breque, 1983; Vens-Cappell, 1984) has been shown to increase the digestibility of the carbohydrate fraction in various feedstuffs for salmonids, and the protein and carbohydrate fraction for channel catfish (Lovell, 1984). In the case of plant

protein sources, heat treatment is known to reduce the activity of several antinutrients (Bender, 1972; Bell, 1993; Liener, 1994) and therefore to improve digestibility.

Statistical methods used to determine protein requirements

Most of the reported estimates for protein requirements of salmonids have been based on dose-response curves; replicate groups of fish have been fed diets containing graded levels of protein until a measurable difference has appeared in the growth of the test fish. A linear increase in growth rate is normally observed with an increase in the dietary protein level, up to a specific maximum at which the growth rate plateaus.

Various methods have been used to estimate the breakpoint corresponding to the nutrient requirement. Some workers have estimated the requirement by using a growth response curve without the aid of any statistical analysis, or they have used analysis of variance and multiple comparison procedures. Others have used regression analysis to generate the growth response curve (Robbins et al., 1979; Akiyama et al., 1985). With the availability of computers and statistical programs that easily perform the mathematical calculations associated with complicated designs, there is no reason for not using statistics to analyse the growth data. Yet, the misuse of statistics is still quite prevalent. This is especially true for the multiple comparison procedures (Petersen, 1977; Little, 1978, 1981; Dawkins, 1983). The use of regression analysis for dose-response data is the appropriate statistical method, but there is still some debate as to which form of regression is the best for describing the dose-response.

Zeitoun et al. (1976) claim that the advantage of the quadratic regression is "that it provides a better empirical fit to the growth responses of living organisms that do not exhibit the abrupt change from linearity" assumed by the broken-line analysis. Zeitoun

et al. (1976) also present an example where both the broken-line and quadratic regression models are fitted to the same data. The mean square error was noted to be less when quadratic regression was applied to the data. This finding was used to support the use of the quadratic regression model. Work conducted by Santiago (1985) on the quantitative requirements of young Nile tilapia (Tilapia nilotica) for the ten essential amino acids used both models for estimating the amino acid requirements. The method that provided the lower mean square error was chosen as the appropriate statistical method. In four out of ten cases the broken-line model was chosen whereas the quadratic model was selected in the other six.

The review by Baker (1986) provides a good comparison of the quadratic regression and the broken-line model. One problem with quadratic regression is that it does not identify a breakpoint. Therefore, one is forced to select an optimal response at the maximum point on the curve or to arbitrarily select 95% of the maximum point. By contrast, the broken-line model selects an objective breakpoint and it provides an estimate representing the average animal in the population. The estimated requirement set by the maximum response of the quadratic regression line represents the requirement indicative of all the animals in the test population. Thus, the broken-line analysis will predict a requirement that is lower than that predicted by quadratic regression. The fact that there is no universal definition of the requirement makes it difficult to make choices regarding the preferred method to estimate the requirement.

Other non-linear models for describing nutrient intake-response relationships have been developed (Ware et al., 1980; Phillips, 1981, 1982; Mercer, 1982; Mercer et al., 1986); these are based on saturation kinetics. Little work (Cho et al., 1992) has been done to evaluate their usefulness in fish growth studies.

A review of 20 intake-response studies considering the effect of protein level or protein to energy ratio on weight gain of salmonids found that three used Almquist plots (DeLong et al., 1958; Halver et al., 1964; Takeuchi et al., 1978); 11 used multiple comparison procedures (Satia, 1974; Cho et al., 1976; Lall and Bishop,1977, 1979; Beamish and Medland, 1986; LeGrow and Beamish, 1986; Reinitz, 1987; Jayaram and Beamish, 1992; Kim and Kaushik, 1992; Silver et al., 1993; Arzel et al., 1995); three used broken-line analysis (Zeitoun et al., 1973, 1974; Kim et al., 1991); one used regression analysis (Jobling and Wandsvik, 1983); one used orthogonal contrasts (Gulbrandsen and Utne, 1977); and one used factor analysis (Garcia-Riera et al., 1993) to estimate protein requirement. Thus, 15 out of the 20 studies used inappropriate statistics to evaluate the data.

Published requirements

A review of the literature was conducted to find research papers that dealt with the protein requirements of salmonids. The papers were selected only if they were designed to estimate the dietary protein requirement or protein to energy requirement and if the response plateaued or commenced to decline. That is to say that there was at least one diet (protein level) on either side of the maximal growth response.

The data from the 20 studies are provided in Table 1. The estimated protein requirements ranged from 32.9% to 55% of the dietary dry matter. The ingredients used to make the diets in the studies ranged from purified to commercially available ingredients. The digestible energy levels of the diets ranged from 16.0 to 23.1 $MJ \cdot kg^{-1}$ DM. The fish weights ranged from 1.2 g to 500 g and growth rates varied between 0.37 and 2.84 $\% \cdot day^{-1}$ in the different studies.

If one considers the effect of daily protein intake expressed as $g \cdot kg^{-1}$ body weight$\cdot day^{-1}$, on the growth rate of salmonids on the a dose dependent response (Figure 1) is revealed. As protein intake increases, so does the growth rate. A similar relationship can be seen between digestible energy intake (kJ DE$\cdot kg^{-1}$ body weight$\cdot day^{-1}$) and growth rate of salmonids (Figure 2). From the regressions performed here, the intercepts for zero growth (maintenance) corresponded to protein and DE intakes of 1.0 g and 17.3 kJ$\cdot kg^{-1}$ body weight$\cdot day^{-1}$, respectively. These values are close to the literature values of 1.8 ± 0.3 g protein and 19 to 66 kJ DE$\cdot kg^{-1} \cdot day^{-1}$ reported by Cho et al. (1982) and Kaushik and Gomes (1988) for trout. Since these former values were derived from a compilation of studies, the error associated with the intercepts is quite large. This would be expected as the different studies were carried out on different species, strains, and ages of fish held under different husbandry conditions (water temperature, salinity, and feeding level) . Moreover, the diets contained different protein sources.

Table 1. Estimated dietary protein requirements for maximal growth of salmonids.

Species	Experimental weight range (g)	SGR[1] (%·day^{-1})	Water salinity (‰)	Water temperature (°C)	Protein source[2]	Feeding level[3]	Estimated crude protein requirement (g·kg^{-1} DM)	Dietary energy (MJ·kg^{-1}) GE[4]	DE[5]	Digestible protein to energy ratio (g·MJ^{-1} DE)[6]	Reference
Arctic charr (Salvelinus alpinus)	18 - 58	2.03	0	10	Fish meal, wheat, oats, maize, and yeast	100	400	19.1*	17.5	20.6	Jobling and Wandsvik (1983)
Atlantic salmon (Salmo salar)	57 - 207	1.53	32	15 - 16	Casein and gelatin	100	450	22	20.2	21.4	Lall and Bishop (1977)
Brown trout (Salmo trutta)	1.15 - 5.1	1.24	0	11.4 - 15.4	Casein, fish meal, and fish protein concentrate	100	530	24	22.1	21.6	Arzel et al. (1995)
Chinook salmon (Oncorhynchus tshawytscha)	109 - 300	0.97	27	7-16	Fish meal, canola meal, feather meal, wheat mill run	100	460	21	19.9	20.8	Silver et al. (1993)
	5.5 - 7.5	0.44	0	8	Casein, gelatin, and amino acids	100	400	----†	----	----	DeLong et al. (1958)
	5.5 - 10	0.85	0	14.4	Casein, gelatin, and amino acids	100	550	----	----	----	DeLong et al. (1958)
Coho salmon (Oncorhynchus kisutch)	14.5 - 21.8	0.58	10	6.5-10.5	Casein and gelatin	100	400	20	18.2	20.9	Zeitoun et al. (1974)
	14.5 - 20.7	0.51	20	6.5-10.5	Casein and gelatin	100	400	20	18.2	20.9	Zeitoun et al. (1974)
Lake trout (Salvelinus namaycush)	216	----	0	13	Fish meal, soybean meal , wheat middlings	0.75% BW·d^{-1}	400	22.0*	18.3*	20.8*	Jayaram and Beamish (1992)
Sockeye salmon (Oncorhynchus nerka)	1.2 - ?	----	0	10	Casein, gelatin, and amino acids	100	450	----	----	----	Halver et al. (1964)

Table 1 (Con't). Estimated dietary protein requirements for maximal growth of salmonids.

Species	Experimental weight range (g)	SGR[1] (%·day^{-1})	Water salinity (‰)	Water temperature (°C)	Protein source[2]	Feeding level[3]	Estimated crude protein requirement (g·kg^{-1} DM)	Dietary energy (MJ·kg^{-1}) GE[4]	DE[5]	Digestible protein to energy ratio (g·MJ^{-1} DE)[6]	Reference
Rainbow trout (Oncorhynchus mykiss)	200 - 290	1.69	0	15	Fish meal	2% BW·d^{-1}	421	21.5	20	18.9	Garcia-Riera et al. (1993)
	3.5 - 25.5	2.84	0	16 - 27	Fish meal	Pair fed 100% of lowest intake	400	20.5	19.1	18.8	Satia (1974)
	6.5 - 19.0	1.53	10	9 - 12	Casein and gelatin	100	400	19.6	18.2	20.9	Zeitoun et al. (1973)
	6.5 - 20.3	1.63	20	9 - 12	Casein and gelatin	100	450	19.9*	18.5	23.1	Zeitoun et al. (1973)
	13 - 160	1.28	0	12	Commercial ingredients	% of BW based on length	370	21.4	19.8	16.8	Reinitz (1987)
	5.8 - 10	1.95	0	13.5	Fish meal	100	398	21.2	19.8	18.7	Gulbrandsen and Utne (1977)
	24 - 44	2.16	0	13.5	Fish meal	100	385	21.5	20	17.3	Gulbrandsen and Utne (1977)
	68.1 - 95.5	1.21	0	13.5	Fish meal	100	370	21.6	20.2	16.5	Gulbrandsen and Utne (1977)
	1.3 - ?	----	0	10	Casein, gelatin, and amino acids	100	450	----	----	----	Halver et al. (1964)
	0.7 - 84.9	2.14	0	15	Commercial ingredients	100	450	21	16.9*	24.4*	Cho et al. (1976)
	398 - 498	0.37	0	----	Fish meal, soybean meal, wheat middlings	100	430	21.3*	16.0*	24.2	Beamish and Medland (1986)

Table 1 (Con't). Estimated dietary protein requirements for maximal growth of salmonids.

Species	Experimental weight range (g)	SGR[1] (%·day^{-1})	Water salinity (‰)	Water temperature (°C)	Protein source[2]	Feeding level[3]	Estimated crude protein requirement (g·kg^{-1} DM)	Dietary energy (MJ·kg^{-1}) GE[4]	DE[5]	Digestible protein to energy ratio (g·MJ^{-1} DE)[6]	Reference
Rainbow trout (Oncorhynchus mykiss)	10 - 15	----	0	15	Fish meal, soybean meal, wheat middlings	2.0 % BW·d^{-1}	340	20.9*	16.3	18.8	LeGrow and Beamish (1986)
	15 - 30	2.31	0	11 - 14	Casein	100	350	22.8	18.8*	17.7	Takeuchi et al. (1978)
	50 - 100	0.83	0	15	Casein and gelatin	100	500	20.2	18.7	25.4*	Lall and Bishop (1979)
	50 - 120	0.83	32	15	Casein and gelatin	100	500	20.2	18.7	24.9*	Lall and Bishop (1979)
	37 - 110	1.95	0	17.5	Herring meal	100	410	20.1*	17.1*	22.2*	Kim and Kaushik (1992)
	37 - 120	2.1	0	17.5	Herring meal	100	506	20.6*	18.4*	25.9*	Kim and Kaushik (1992)
	9.7 - 30	2.69	0	15	Casein and gelatin	100	329	18.3	17.1	18.3	Kim et al. (1991)

[1] Specific growth rate= $\ln(Wt_2/Wt_1)x100/days$; where Wt_1 and Wt_2 are initial and final weight respectively and days is the experimental growth period.

[2] Main protein source(s) used in the experimental diets.

[3] Feeding level expressed as a percentage of satiation, unless otherwise indicated.

[4] Gross energy (calculated by applying values of 23.64, 39.54, and 17.36 kJ/g for protein, lipid, and starch, respectively).

[5] Digestible energy (calculated by applying values of 21.25, 37.7, and 16.7 kJ/g for protein, lipid, and gelatinized starch, respectively).

[6] Calculated by assuming the protein was 95% digestible for casein- and gelatin-based diets and 90% digestible otherwise.

* The values were determined by experimentation and reported in the respective papers.

†Values were not available from the original papers.

Figure 1. Relationship between the intake of crude protein ($g \cdot kg^{-1}$ body weight$\cdot day^{-1}$) and the specific growth rate ($\%\cdot day^{-1}$) of salmonids.

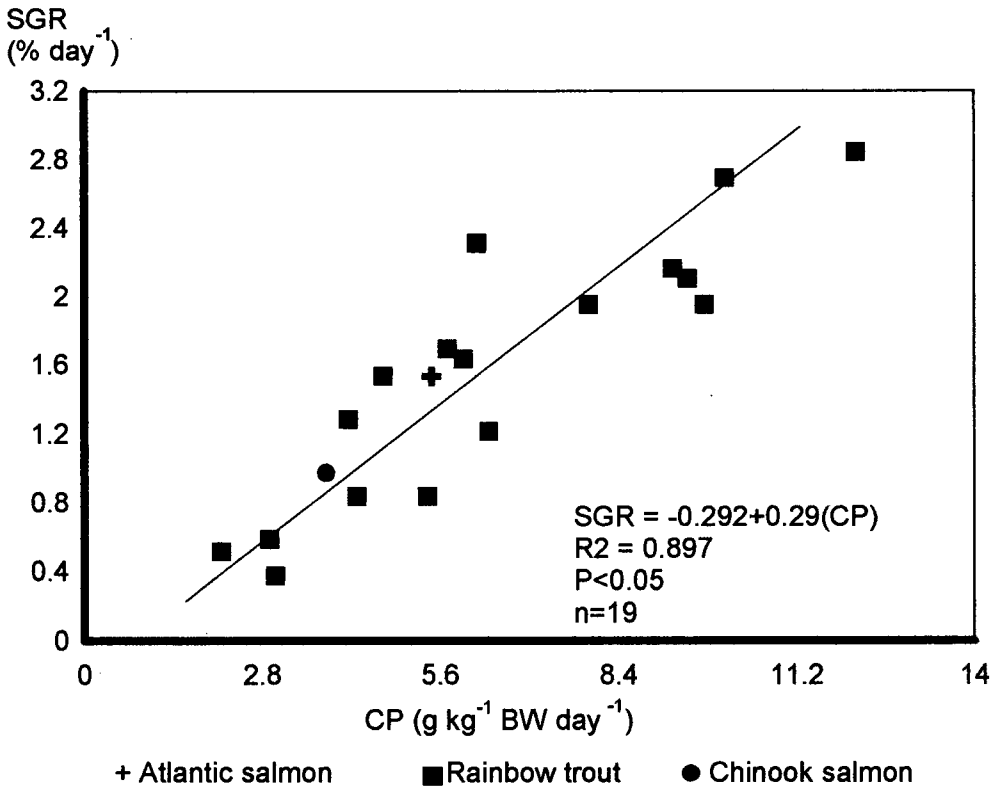
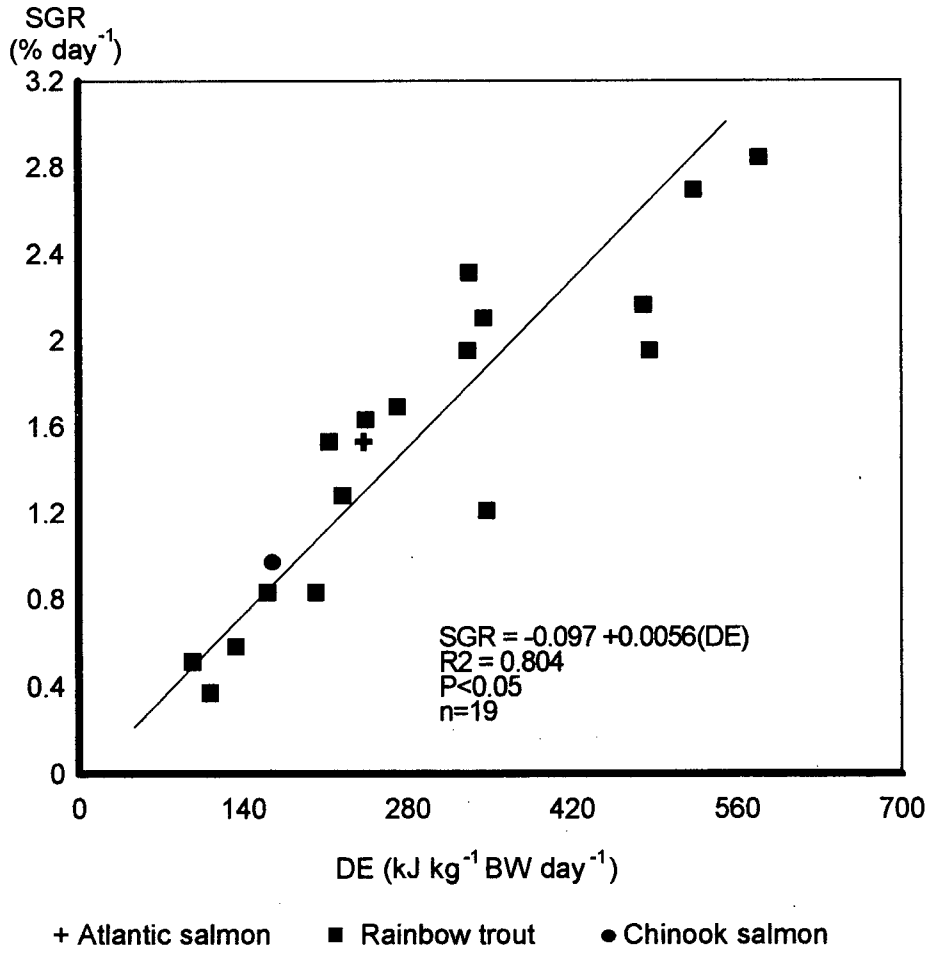


Figure 2. Relationship between digestible energy intake (kJ·kg^{-1} body weight·day^{-1}) and the specific growth rate (%·day^{-1}) of salmonids.



CONCLUSIONS AND RECOMMENDATIONS

Although there are numerous in vitro methods that can be used for the assessing the quality of protein in dietary components for fish, there is still much information that is needed on how well the in vitro measures of quality correlate with the in vivo estimates of quality that have been derived from digestibility and growth studies on fish. It appears at this time that the dilute pepsin (Olley and Pirie, 1966) and the multienzyme pH-stat methods (Pedersen and Eggum, 1983) may give the best estimates of the quality of fish meal protein (Anderson et al., 1993). The pH-stat method using an enzyme fraction from fish pyloric caeca (Dimes and Haard, 1994) has potential as an accurate way to estimate the digestibility and biological value of alternate protein sources for salmonids. Until more purified digestive enzymes of fish origin become commercially available, mammalian enzymes will have to be utilized for such tests. Further evaluation of the usefulness of homoeothermic animal models for predicting protein quality for fish is needed due to the limited number of studies in which direct comparisons have been made.

Although the metabolism of nitrogen in fish is similar in some respects to that in mammalian species, it differs considerably in other respects. The most striking difference is the ability of fish to excrete ammonia directly to the aquatic environment.

From this review, it would appear that the absolute protein requirements ($g \cdot kg^{-1} \cdot day^{-1}$) for salmonids do not differ appreciably among species. Apparent differences in the published literature would appear to be due to variations in experimental conditions. These differences in experimental conditions seem to affect the growth rate of the fish and thus would dictate protein and energy requirements. Standardisation of experimental conditions and the use of a common experimental diet

for requirement studies would help to reduce such variation in the published requirement values. Yet, conditions that mimic the situations encountered during commercial production are also required. In studies that have evaluated the optimal dietary protein to energy ratio for growth, the determination of digestibility coefficients for dietary protein and energy is highly recommended as digestible energy values may be significantly influenced by the source(s) and level of carbohydrate in the diets.

Much work is still needed on the best way to replace protein as a source of energy for salmonids. One way to accomplish this is by enhancing the extent to which non-protein energy sources are used to meet energy demands. One major problem in this regard is the poor digestibility and utilization of high dietary levels of carbohydrates by carnivorous fish. The ratio of digestible carbohydrate to lipid that optimizes the sparing of protein for growth needs to be investigated more closely for each period of the fish's life cycle. With practical feeding studies and an increased understanding of the metabolic utilization of the different components in the diets, a diet that is utilized at maximum efficiency for growth can be formulated and produced.

One step in achieving these goals is to be able to measure the quality of the protein from various fish meals for the use in salmon diets. Reliable, quick, and inexpensive tests for measuring protein quality that can be performed at the feed manufacturing plant are needed. Information on the quality of a fish meal can be used to help optimize diet formulation and this combined with optimum feeding strategies will help achieve maximum utilization of dietary nutrients for growth (one of the most important variables for farmers).

CHAPTER THREE - FISH MEAL QUALITY ASSESSMENT FOR ATLANTIC SALMON REARED IN SEAWATER

INTRODUCTION

Feed accounts for 40 - 60% of the operating expenses of salmon farms. Much of this cost is due to the extensive use of fish meal to furnish most of the protein in the feed (Tacon and Jackson, 1985; Tacon, 1994; Higgs et al., 1995a). There is considerable research in progress however, to identify and develop economical alternative sources of protein and energy for inclusion in salmon diets, but these initiatives have not been entirely successful (Åsgård, 1988; Higgs et al., 1988). Also, considerable efforts are being made to improve fish meal quality so that maximum nutritive value can be obtained from this expensive dietary component. Presently, fish meal quality varies extensively, both among sources and among batches from the same source. The reasons for this variation include: 1) dissimilar composition and degree of freshness of the raw material before conversion into fish meal; 2) variable proportions of whole fish, offal, and filleting residues; 3) differences in processing conditions (cooking and especially drying temperatures employed during meal manufacturing); 4) variation in the ratio of presscake to solubles, which are added to the presscake to make whole fish meals; 5) possible alterations in the general quality of the fish meal due to the addition of poor quality fish solubles; 6) inappropriate levels of antioxidants and moisture in the meals; and 7) suboptimal meal storage and transportation conditions (Higgs et al., 1995a).

Current research findings suggest that the highest quality fish meals result from the processing of extremely fresh whole fish under low-temperature cooking and drying

conditions (McCallum and Higgs, 1989; Anderson et al., 1993). Meals produced under these conditions are designated as low-temperature (LT) type meals. Even within this category there can be variation in quality standards and in the adherence to these standards. However, there is still uncertainty regarding the extent of reduction in the quality of fish meals that result from fluctuations in the composition and freshness of the raw material, the cooking and drying conditions during meal manufacture, and the subsequent meal storage conditions.

The goals of this study were: 1) to assess the nutritive value of a range of fish meals for Atlantic salmon (Salmo salar) reared in seawater; and 2) to compare the effectiveness of various in vitro assays and in vivo assays using rats for predicting fish meal quality for Atlantic salmon reared in seawater. Canadian-produced meals of West Coast and East Coast origin were evaluated in relation to Norwegian fish meal (Norse-LT94®), which is currently considered to be the premium quality meal on a world-wide basis. Also, because Chilean fish meals are now widely used by the fish feed manufacturing industry in Canada, we assessed the nutritive value of two of these meals. In addition, several herring meals were produced in the laboratory to assess the influence of processing conditions and freshness of the raw material on fish meal quality for Atlantic salmon.

MATERIALS AND METHODS

Fish meals

Samples (20-50 kg) of 11 different commercially-produced fish meals were obtained in June 1992 for the study. These included, a silver hake (Russian) meal, a groundfish meal, a menhaden meal, a mixed meal produced in Western Canada, four

herring meals (one from Western Canada - Herring meal 1, and three from Eastern Canada - Herring meals 2, 3, and 4), two Chilean meals (mackerel), and Norse-LT94®. The choice of the two Chilean meals was based on an evaluation in a chick-growth assay. The chick-growth assay evaluated one meal was good and the other was poor in quality (data not given). The mixed meal consisted of waste salmon scraps and offal from the filleting industry. Five additional herring meals, described below, were produced with a pilot-sized reduction machine at the West Vancouver Laboratory (Department of Fisheries and Oceans, West Vancouver, Canada).

The reduction machine consisted of a steam cooker, a press, and a steam-jacketed dryer with a flow through design. Desired processing temperatures for the cooker and dryer could be set and maintained by the injection of steam. Probes positioned in the cooker and at the exhaust vent of the dryer monitored temperatures.

The cooking and drying temperatures for the standard processing conditions were both set at 88°C. The retention time in the cooker was approximately 10 min and in the dryer was 60-90 min. For LT conditions, the cooking temperature was 85°C and the drying temperature was 75°C, with retention times in the cooker and dryer the same as for standard processing. In the production of both meal types, the soluble fraction was removed and collected after cooking. The lipid fraction in the solubles was separated and discarded. The remaining fraction was condensed to 250 - 350 g dry matter (DM)·kg^{-1} in a steam-jacketed bowl-cooker and approximately 100 g of condensed solubles DM·kg^{-1} of presscake DM was added during the drying phase.

The fish meals produced with this machine consisted of: 1) frozen fresh whole male herring, processed under standard conditions (HM-FM-Std); 2) frozen fresh whole male herring, processed under LT conditions (HM-FM-Lt); 3) frozen fresh female herring

with the roe removed, processed under standard conditions (HM-FF-Std); 4) frozen fresh female herring with the roe removed, processed under LT conditions (HM-FF-Lt); and 5) spoiled whole male herring, processed under standard conditions (HM-SM-Std). The frozen fresh fish were flash-frozen in ice and maintained at -20°C pending processing. The spoiled whole male herring were obtained by holding previously frozen fresh whole male herring at 15°C for five days before processing. The five meals were each ground to pass through a 1.5 mm mesh screen. Thereafter, they were stabilized with 250 mg of ethoxyquin·kg^{-1} and stored in polyethylene containers. All fish meals (commercially and laboratory produced) were stored at 15°C and relative humidity was less than 20%.

Chemical and in vitro analyses

Each of the 16 test fish meals was analysed in duplicate for their content of dry matter (Williams, 1984), crude protein (CP) (N% X 6.25) (micro-Kjeldahl Technicon AutoAnalyzer, industrial method no. 334-74W/B), ash (Williams, 1984), lipid (Bligh and Dyer, 1959), and gross energy (adiabatic bomb calorimeter). Mineral compositions were determined by inductively-coupled plasma emission spectroscopy (Helrich, 1990) and biogenic amines (cadaverine, histamine, putrescine, and tyramine) by HPLC analysis (Roisier and Van Peteghem, 1988).

Fatty acid compositions were determined by gas chromatography (Varian 3400 gas chromatograph) after acid-catalyzed transmethylation (Metcalfe et al., 1966). A fused silica capillary column (SP-2330, 30 m x 0.25 mm i.d., Supleco Canada, 1300 Aimco Blvd. Mississauga, ON) was used for fatty acid determinations under the following conditions: the initial temperature was set at 150°C for three min and then

increased at 2°C·min^{-1} to 170°C, where it was held for one min and subsequently increased at 3°C·min^{-1} to 210°C and maintained for 9.5 min. The detector was set at 240°C, and helium gas at 1 ml·min^{-1} was used as the carrier. An automated injector was used in the split mode (100/1) and heated to 220°C. Quantification of the areas for the fatty acids was performed by a Varian 4290 Integrator.

Amino acid levels in the meals were determined after 20 h of hydrolysis at 115°C in 6N HCl that contained 0.05 μl of mercaptoethanol·ml^{-1} and an excess of phenol. The values for serine and threonine were increased by 10% and 5% respectively, to compensate for their destruction during acid hydrolysis. Cysteine and cystine were determined as cysteic acid after performic acid oxidation prior to acid hydrolysis (AOAC, 1985). Tryptophan was determined by the method of Hugli and Moore (1972).

The quality of the lipid fraction in the fish meals was evaluated by determining peroxide value (Woyewoda et al., 1986), anisidine value (Windsor and Barlow, 1981), iodine value (Windsor and Barlow, 1981), and thiobarbituric acid (TBA) reactive substances (Sinnhuber and Yu, 1977).

Protein quality was evaluated in vitro by the dilute-pepsin digestibility method (Olley and Pirie, 1966) and the multi-enzyme pH-stat digestible protein method (Pedersen and Eggum, 1983). Multi-enzyme pH-stat digestible protein was calculated from the following equation: True digestibility = 76.14 + 47.77·(ml of 0.1N NaOH used). This equation represents the true digestible crude protein, as determined with rats. The percentage of acid-soluble protein was also determined by incubating a 1 g sample of fish meal in 0.075N HCl for 16 h at 45°C. This corresponds to the sample blank in the dilute-pepsin digestibility assay (Olley and Pirie, 1966). The percentage of protein that was soluble in the acid was then determined by measuring residual CP after filtration.

For the determination of TVBN a sample of meal was extracted with a magnesium sulphate solution (600 $g \cdot l^{-1}$) (Woyewoda et al., 1986) and the amount of TVBN, in a portion of the extract, was determined by the microdiffusion method of Conway (1933) as modified by Öbrink (1955).

In vivo evaluations

Apparent digestibility coefficients (%) for each of the 16 test meals were determined for crude protein (ADCP-F), gross energy (ADGE-F), and organic matter (ADOM-F) using Atlantic salmon reared in seawater. Six of the test fish meals were further assessed for their nutritive value by conducting a growth experiment using Atlantic salmon reared in seawater. Male Wistar rats were used to determine the coefficients (%) for apparent digestible crude protein (ADCP-R), apparent metabolizable crude protein (AMCP-R), and true digestible crude protein (TD) for the 16 test fish meals. The biological values (BV) and net protein utilization (NPU) values were also determined for each of the 16 test fish meals using rats.

Experimental diets

For the digestibility experiment using fish, the procedure of Cho et al. (1982) was followed. The composition of the basal diet is provided in Table 2. Each test diet contained ($g \cdot kg^{-1}$ as-fed) 300 g of a test fish meal, 695 g of the basal diet and 5 g of chromic sesquioxide (Cr_2O_3). The reference diet contained 5 g of Cr_2O_3 and 995 g of the basal $diet \cdot kg^{-1}$. All diets were steam-pelleted (California Pellet Mill) through a 3.97 mm die, forced-air dried, and stored at 4°C until used.

Table 2. Composition of the basal diet used in the determination of the nutrient and energy availability in various fish meals for Atlantic salmon reared in seawater.

Ingredient	$g \cdot kg^{-1}$ (as-fed)
Herring meal, steam dried	385
Blood meal, spray dried	50
Soybean meal	75
Brewer's dried yeast	40
Poultry by-product meal	80
Whey	90
Feather meal	62
Extruded wheat	75
Sardine oil	100
Vitamin premix[1]	10
Choline chloride (60%)	6
Ascorbic acid	1
DL-Methionine	2
Mineral premix[2]	10
Monocalcium phosphate	14
Total	1000

[1] Vitamin premix supplied the following amounts (IU or $mg \cdot kg^{-1}$ diet): Vitamin A, 6000; Vitamin D_3, 4000; Vitamin E, 350; Vitamin K (menadione), 5; thiamin, 35; riboflavin, 40; Ca d-pantothenic acid, 125; folic acid, 15; biotin, 0.8; Vitamin B_{12}, 0.05; niacin, 200; pyridoxine, 25; myo-inositol, 400; butylated hydroxytoluene, 22.

[2] Mineral premix supplied the following amounts (mg element$\cdot kg^{-1}$ diet): Mn ($MnSO_4 \cdot H_2O$), 35; Fe ($FeSO_4 \cdot 7H_2O$) 28; Zn ($ZnSO_4 \cdot 7H_2O$), 50; Mg ($MgSO_4 \cdot 7H_2O$) 40; I (KI), 10.

For the growth experiment, six diets were formulated to contain (·kg^{-1} dietary DM) 390 g CP, 220 g lipid, and 19 MJ of digestible energy (DE) (Table 3). The DE contents of the diets were estimated by ascribing values of 16.73 kJ·g^{-1} dextrin, 33.59 kJ·g^{-1} lipid, and 13.3 kJ·g^{-1} gelatinized starch (Cho and Kaushik, 1990). For Atlantic salmon in seawater, the gross energy in the fish meals was assumed to be 94.7% digestible (S.P. Lall, unpublished data). The test fish meals supplied 80% of the crude protein in the various growth diets. Diets were steam-pelleted as above and stored at -20°C until used.

Sixteen test diets were formulated for Wistar rats, with each of the test fish meals as the sole source of protein. Each diet was formulated to contain (·kg^{-1} dietary DM) 93.8 g CP, 16 g mineral premix (Eggum, 1973), and 40 g vitamin premix (AIN Vitamin Mixture 76, ICN Biomedicals Canada Ltd., St. Laurent, Quebec). The remaining portion of the diet was supplied by a nitrogen-free mixture composed of (g·kg^{-1} as-fed mixture) cane sugar (89.2 g), cellulose (52 g), corn oil (52 g), and autoclaved potato starch (806.8 g) (see Eggum, 1973 for complete details).

Animal facilities and experimental protocols

A group of 3000 Atlantic salmon smolts (mean weight approximately 50 g) was obtained from Sea Spring Salmon Farm Ltd. (Chemainus, British Columbia) and were divided into two groups of approximately 1500 fish that were each held in 4000 l tanks supplied with aerated, ambient seawater. Until the beginning of an experiment, the fish were fed by hand twice daily a practical-type diet, formulated to meet their nutritional requirements (NRC, 1981), prepared at the West Vancouver Laboratory (Department of Fisheries and Oceans, West Vancouver, Canada). Rearing facilities and the

Table 3. Ingredient and proximate composition of the diets used to assess the nutritive values (growth trial) of selected fish meals for Atlantic salmon reared in seawater.

	Diet					
Ingredient[1]	1	2	3	4	5	6
Norse-LT94®	402	-----	-----	-----	-----	-----
Groundfish meal	-----	460	-----	-----	-----	-----
Herring meal 1	-----	-----	404	-----	-----	-----
Herring meal 2	-----	-----	-----	418	-----	-----
HM-FM-Std	-----	-----	-----	-----	425	-----
HM-SM-Std	-----	-----	-----	-----	-----	412
Sardine oil	172	182	170	165	150	153
Dextrin	157	148	155	139	135	157
Alpha-cellulose	79	20	81	88	100	88
Basal mix[2]	190	190	190	190	190	190
Total	1000	1000	1000	1000	1000	1000
Proximate composition						
Dry matter	924	933	935	934	934	928
Crude protein[1]	376	385	383	389	376	395
Lipid[1]	235	233	234	222	259	213
Ash[1]	80	133	83	78	81	79
Gross energy ($MJ \cdot kg^{-1}$ DM)	23.2	21.7	22.7	23.2	23.4	23.3

[1] Expressed as $g \cdot kg^{-1}$ of the dietary dry matter.

[2] Basal mix contained ($g \cdot kg^{-1}$ of the dietary dry matter): blood meal, spray dried 21.2; soybean meal, 28.3; brewer's dried yeast, 21.2; poultry by-product meal, 24.7; whey, 21.2; feather meal, 17.7; vitamin premix[3], 21.5; choline chloride (60%), 6.8; ascorbic acid, 1.1; mineral premix[4], 11; monocalcium phosphate, 15.3.

[3] Vitamin premix supplied the following amounts (IU or $mg \cdot kg^{-1}$ diet): Vitamin A, 6000; Vitamin D_3, 4000; Vitamin E, 350; Vitamin K (menadione), 5; thiamin, 35; riboflavin, 40; Ca d-pantothenic acid, 125; folic acid, 15; biotin, 0.8; Vitamin B_{12}, 0.05; niacin, 200; pyridoxine, 25; myo-inositol, 400; butylated hydroxytoluene, 22.

[4] Mineral premix supplied the following amounts (mg element$\cdot kg^{-1}$ diet): Mn ($MnSO_4 \cdot H_2O$), 35; Fe ($FeSO_4 \cdot 7H_2O$) 28; Zn ($ZnSO_4 \cdot 7H_2O$), 50; Mg ($MgSO_4 \cdot 7H_2O$) 40; I (KI), 10.

experimental protocols for the fish had the approval of the animal care committee (Department of Fisheries and Oceans, West Vancouver, Canada).

Digestibility experiment

A total of 810 Atlantic salmon (70 ± 10 g, mean ± sd) was randomly and equally allotted to 27 modified "Guelph" digestibility tanks (Hajen et al., 1993a). Each tank (approximately 150 l) was supplied with running, filtered, ambient seawater at 7-8 $l \cdot min^{-1}$. Water temperature during the experimental period ranged from 10.0°C to 13.5°C (average 11.0 ± 0.6°C sd) and the salinity from 26‰ to 30‰. The natural photoperiod was followed (June - August, Vancouver, Canada, 49°15'N 123°10'W). Oxygen saturation of 85% or better was maintained in each tank with the aid of supplemental aeration. Fish were allowed to acclimate to the tanks for four wks. During the last wk of acclimation, eight test diets and the reference diet were assigned randomly to triplicate groups of fish. Due to the limited number of tanks, three trials using the same fish for each trial were required to complete the evaluation of all test diets. The same reference diet was used in each trial throughout the experiment.

Faeces were collected for ten days in each trial according to the procedures of Hajen et al. (1993a). Four days were allowed between trials to acclimate the fish to the new test diets. Faeces and feed were analysed for their content of moisture, ash, CP, and gross energy by the methods described previously. The concentration of chromic oxide in the test diets and faecal samples was determined by the method of Fenton and Fenton (1979).

The apparent digestibility coefficients of the dietary components and of the test fish meal components were calculated by the indicator method of difference as

described by Maynard and Loosli (1969).

Growth experiment

A total of 1350 Atlantic salmon smolts (69.2 ± 11.2 g, mean ± sd) was randomly and equally distributed into 18 oval fibreglass tanks (approximately 800 l). The tanks were supplied with running (15 $l \cdot min^{-1}$), filtered, ambient seawater, with the same temperature, salinity, and oxygen saturation as described above. The natural photoperiod, as described above, was in effect during the study. Fish were allowed to acclimate to the tanks for four wks before the start of the experiment. During this period, the fish were fed by hand twice daily to satiation a practical-type diet, formulated to meet their nutritional requirements (NRC, 1981). The diet was prepared at the West Vancouver Laboratory.

Six diets, as described above, were randomly assigned to triplicate groups of fish. Groups of 50 fish from each dietary treatment were individually identified with passively induced transponder (PIT) tags (2 mm diameter x 10 mm long, Canadian BioSonics Ltd., Sardis, BC) injected intraperitoneally.

Individual lengths and weights of the fish were taken after each 4-wk period during the 12-wk study. All fish were fed to satiation their prescribed diets by hand twice daily. Feed consumption and mortality records were kept for each 4-wk period. The feed consumption data were corrected for fish mortality within each 4-wk period. At the end of 12 wks, five fish were sampled from each tank for assessment of their health as judged by the presence of bacterial, viral, or fungal diseases. Fish that were fed the Norse-LT94®, HM-FM-Std, and HM-SM-Std diets (three fish from each diet) had samples of their liver, small intestine, and pyloric caeca removed, fixed in 10% buffered

formalin, and sent to Diagnostic Services, Atlantic Veterinary College, PEI for histological interpretation. An additional three fish from each tank, whose growths had been followed by aid of the PIT tags, were killed and stored at -20°C pending proximate analyses (moisture, lipid, CP, and ash). Fish growth and performance was described by the following variables: weight gain, specific growth rate (SGR), feed intake, feed to gain ratio, and protein efficiency ratio (PER). SGR (%·day^{-1}) was calculated from the (ln(final weight (g)) - (ln(initial weight (g))·100·number of experimental days^{-1}. Feed to gain ratio was calculated as dry feed intake (g)·wet weight gain (g)$^{-1}$. PER was calculated as wet weight gain (g)·protein intake (g)$^{-1}$.

Rat experiment

Two trials, each using 40 male Wistar rats, were designed to evaluate the quality of the protein in the various test fish meals. In each trial the rats were randomly assigned to individual metabolism cages. Initial weight was 70.3 ± 3.0 g (sd) in the first trial and 70.5 ± 2.2 g (sd) in the second. For each trial, eight test diets were randomly assigned to five rats per diet. The metabolism cage design, feeding protocol, and faecal and urinary collection protocols have been described in detail by Eggum (1973). Each rat received 10 g of dietary DM containing 150 mg of N once daily at 08:00 h (Eggum, 1973). All rats were supplied with unlimited fresh water.

The experimental diets and faeces were analysed for DM and CP by methods described previously. Urine was analysed for nitrogen content. Feed consumption and weight gain data were recorded for each rat during the experiment. ADCP-R, AMCP-R, TD, BV, and NPU values were calculated for each fish meal. For the above calculations, the values for endogenous faecal and urinary nitrogen excretion were 17.0

and 15.2 mg $N \cdot day^{-1}$, respectively. These values were determined in a feeding experiment in which rats were fed egg powder (Eggum, 1973). Experimental facilities and the experimental protocols had the approval of the animal care committee (University of British Columbia).

Statistical analyses

The fish performance data (absolute weights, weight gains, feed intakes, SGR, PER, and feed to gain ratios) were analysed by a repeated measures ANOVA model. When a significant main effect ($P<0.05$) was detected, differences among treatment means were further evaluated by orthogonal contrasts. The repeating variable was measured in each 4-wk period (three periods totalling 12 wks). The main effects in the analysis were diet and replication (Neter et al., 1985). In cases where there was an initial value, e.g. absolute weights, this was included in the data set for analysis.

The body composition data for the fish were analysed by analysis of covariance. The effect of dietary treatment on the log of the absolute amount of body component (g) was evaluated using log of the body weight (g) as the covariate (Shearer, 1994). Significant ($P<0.05$) differences among treatment means were further assessed by the Student-Newman-Keuls multiple comparison procedure ($P=0.05$) (Kleinbaum et al., 1988).

The apparent digestibility coefficient data for fish and rats and the other protein quality parameters determined with rats were analysed by two-way ANOVA with diet and replication as the main effects. Where necessary, the square root transformation was applied to achieve homogeneity of variance. Means were then further compared by Student-Newman-Keuls multiple comparison procedure ($P=0.05$). Untransformed

means are provided in all tables. Pearson correlation coefficients (r) were determined for the in vivo parameters between fish and rats and for in vitro versus in vivo estimates of protein quality and the coefficients of simple determination (r^2) have been reported. Statistical analyses were performed using Systat (Ver. 4.0, Systat, Inc., 1800 Sherman, Ave., Evanston, IL).

RESULTS AND DISCUSSION

Few differences were observed in levels (%) of proximate constituents and gross energy among the various test fish meals (Table 4). The ash contents of the meals made from processing wastes (groundfish and mixed meals) or species of fish high in bone content (silver hake, menhaden, and mackerel) were higher than in the various herring meals. Norse-LT94® is made mostly from capelin, a species similar to herring; thus, little difference was seen between the composition of these two meals. The small variations noted in the crude protein content among the herring meals can be explained by the processing conditions used in their production. The amount of lipid extracted and/or amount of fish solubles added to the herring presscake likely had the most influence on the crude protein and energy levels in the final meals.

The mineral levels in the meals are presented in Table 5. The meals made from raw materials with a higher bone content exhibited higher levels of calcium and phosphorus than the herring meals. Sodium content was below 17 $g \cdot kg^{-1}$ in all meals, which is well within the guidelines of < 30 $g \cdot kg^{-1}$ for high quality Norwegian fish meals (Pike et al., 1990) and recommended levels for Canadian fish meals (CWGFN, 1988). This suggests that seawater was not used to transport any of the products to the processing plants.

Table 4. Levels of proximate constituents and gross energy in the various fish meals.

Fish meal	As-is basis	Dry matter basis			
	Dry matter ($g \cdot kg^{-1}$)	Crude protein ($g \cdot kg^{-1}$)	Ash ($g \cdot kg^{-1}$)	Lipid ($g \cdot kg^{-1}$)	Gross energy ($MJ \cdot kg^{-1}$)
Herring meal 1	940	772	136	113	22.0
Herring meal 2	949	746	124	120	22.3
Herring meal 3	940	751	124	128	22.4
Herring meal 4	924	748	109	120	22.9
HM-FF-Lt	977	728	132	134	22.4
HM-FF-Std	961	741	134	120	22.4
HM-FM-Lt	943	770	121	148	22.9
HM-FM-Std	936	733	130	153	23.4
HM-SM-Std	928	757	120	150	22.9
Chilean - Good quality	911	715	155	102	20.8
Chilean - Poor quality	904	713	171	103	20.5
Groundfish meal	937	679	232	73	18.7
Menhaden meal	950	657	192	129	20.5
Mixed meal	944	656	210	136	20.5
Norse-LT94®	930	775	136	107	21.8
Silver Hake	932	720	183	99	20.2

Table 5. Mineral levels in the various fish meals (dry matter basis).

Fish meal	Ca $g \cdot kg^{-1}$	Cu $mg \cdot kg^{-1}$	Fe $mg \cdot kg^{-1}$	K $g \cdot kg^{-1}$	Mg $g \cdot kg^{-1}$	Mn $mg \cdot kg^{-1}$	Na $g \cdot kg^{-1}$	Zn $mg \cdot kg^{-1}$	P $g \cdot kg^{-1}$
Herring meal 1	41	5.3	136	2.5	1.7	10.6	10	86.2	25
Herring meal 2	30	4.2	233	6.4	3.1	12.6	11	95.9	23
Herring meal 3	24	5.0	137	13.8	2.1	7.4	13	73.4	18
Herring meal 4	28	6.5	204	8.3	2.4	8.7	7	93.0	21
HM-FF-Lt	33	7.2	196	4.5	1.6	13.3	16	80.9	21
HM-FF-Std	33	8.3	269	4.7	1.4	15.6	16	79.1	20
HM-FM-Lt	24	6.4	158	11.0	1.7	7.4	6	72.1	25
HM-FM-Std	32	7.5	470	4.6	1.5	16.0	16	104.7	26
HM-SM-Std	27	7.5	263	10.1	1.7	8.6	6	76.5	25
Chilean - Good quality	36	8.8	268	12.3	3.0	14.3	12	91.1	26
Chilean - Poor quality	41	5.5	231	12.3	2.9	7.7	13	69.7	26
Groundfish meal	79	3.2	196	4.2	2.0	16.0	7	73.6	41
Menhaden meal	59	6.3	313	8.3	2.6	22.1	9	98.9	33
Mixed meal	64	4.2	260	5.8	2.0	12.7	13	109.2	35
Norse-LT94®	28	5.4	176	15.4	2.4	5.4	11	75.3	21
Silver Hake	55	4.3	193	7.9	2.4	11.8	7	77.3	33

The fatty acid composition of the total lipid (Table 6) varied little among the various meals. Chilean - good quality meal contained a very high level of 22:6 n-3 (docosahexaenoic acid) compared with the other meals. Among the various meals, 20:1 n-9 and 22:1 n-11 had the greatest variation. Fatty acid levels of fish are influenced by fish species as well as the size and sex, reproductive status, water salinity and temperature, and level and composition of food intake (Ackman, 1982; Higgs et al., 1995b). These influences account for the differences in fatty acid composition of the test fish meals, which were obtained from greatly divergent sources. Almost all of the 20:1 and 22:1 fatty acids in marine fish are of exogenous origin. Probably, the North Pacific fish ingested euphausiids or other species of invertebrates that were low in 20:1 n-9 and 22:1 n-11 rather than the copepods that are important prey items in the North Atlantic (Ackman, 1982). This fact is clearly seen when the herring meals of East and West Coast origin are compared. One point of interest relates to Herring meal 3, which was purchased from the East Coast. This meal, based on fatty acid levels, appears to be made from fish of West Coast origin. It is not uncommon for fish meal manufacturers, during times of local shortages, to purchase other sources of fish meal and to repackage them under their own label.

For most amino acids there were no notable differences among the various fish meals (Table 7). The concentration of arginine was higher in the herring meals than in the other fish meals, whereas the opposite was true for glycine and proline. These differences for glycine and proline would be expected as the concentrations of these amino acids are higher in bone and collagen than in muscle tissue (Simon, 1989). Among the herring meals, the concentration of cysteine was lower in the laboratory produced meals than in the commercial meals. This reduction might have occurred

Table 6. Fatty acid levels in the lipid component (% of total fatty acids) of the various fish meals.

Fatty acid[1]	Herring meal 1	Herring meal 2	Herring meal 3	Herring meal 4	HM-FF-Lt	HM-FF-Std	HM-FM-Lt	HM-FM-Std	HM-SM-Std
Saturated									
14:0	5.1	6.2	5.2	5.5	5.1	4.9	5.3	4.2	5.0
16:0	19.9	17.9	22.5	14.7	19.7	20.2	21.7	18.9	21.9
18:0	4.5	2.0	3.7	1.6	2.9	3.2	2.9	3.3	3.5
Total saturated	29.5	26.1	31.4	21.8	27.7	28.3	29.9	26.4	30.4
Monounsaturated									
16:1 n-7	6.9	5.6	5.8	5.9	5.1	4.7	5.9	5.1	5.6
18:1 n-9	18.6	9.1	19.7	8.5	18.4	18.7	19.4	20.0	19.1
18:1 n-7	7.1	4.5	7.0	3.3	5.0	4.1	6.5	4.2	7.1
20:1 n-11	2.6	1.4	2.4	1.1	5.2	6.6	2.0	2.5	2.1
20:1 n-9	3.5	12.2	4.6	13.6	0.5	0.5	4.0	5.0	4.4
22:1 n-11	4.4	20.0	8.0	20.1	6.4	7.2	6.8	7.9	7.4
22:1 n-9	1.7	ND	ND	2.8	0.8	0.7	0.2	0.8	0.1
Total monounsaturated	44.8	52.8	47.5	55.3	41.4	42.5	44.8	45.5	45.8
Polyunsaturated									
18:2 n-6	1.3	1.0	1.0	1.1	0.9	1.2	1.1	0.8	1.0
18:3 n-6	ND	ND	ND	0.1	0.2	0.3	0.2	0.3	0.1
18:3 n-3	0.5	0.4	ND	0.4	0.2	0.2	0.4	0.3	0.4
18:4 n-3	0.9	0.4	0.6	1.0	0.7	0.6	0.7	0.7	0.7
20:3 n-6	0.3	ND	ND	0.2	0.2	1.4	0.3	0.3	0.3
20:4 n-6	1.1	0.4	0.7	0.4	0.8	0.8	0.8	0.8	0.7
20:5 n-3	7.8	4.8	6.5	5.3	6.8	6.2	6.7	6.8	5.9
22:4 n-6	0.3	ND	ND	0.3	0.9	1.0	0.3	1.1	0.7
22:5 n-3	0.8	0.3	0.4	0.4	0.8	0.9	0.4	0.7	0.6
22:6 n-3	5.4	6.5	6.2	5.9	8.9	7.9	6.6	8.8	6.1
Total polyunsaturated	18.4	13.8	15.4	15.1	20.4	20.5	17.5	20.6	16.5
Total n-6	3.0	1.4	1.7	2.1	3.0	4.7	2.7	3.3	2.8
Total n-3	15.5	12.4	13.7	13.0	17.4	15.8	14.8	17.3	13.7
n-6/n-3	0.2	0.1	0.1	0.2	0.2	0.3	0.2	0.2	0.2
unsat/saturated	2.1	2.6	2.0	3.2	2.2	2.2	2.1	2.5	2.0

[1] Expressed as a percentage of the total area identified (area %).

ND - Only trace amounts detected.

Table 6 (Con't). Fatty acid levels in the lipid component (% of total fatty acids) of the various fish meals.

Fatty acid[1]	Groundfish meal	Mixed meal	Menhaden meal	Silver hake	Chilean - Good quality	Chilean - Poor quality	Norse-LT94®
Saturated							
14:0	3.5	4.7	8.0	3.6	6.0	6.1	6.6
16:0	17.4	20.9	25.3	22.0	22.8	22.4	18.1
18:0	4.7	5.0	4.4	4.6	6.2	7.0	3.3
Total saturated	25.6	30.6	37.7	30.2	35.0	35.5	28.0
Monounsaturated							
16:1 n-7	5.1	6.3	8.0	5.4	4.8	6.4	3.9
18:1 n-9	17.9	18.9	11.0	16.4	10.9	15.6	11.8
18:1 n-7	6.1	7.0	5.2	6.4	4.9	4.7	3.3
20:1 n-11	2.4	2.0	0.2	1.0	1.8	2.6	9.5
20:1 n-9	3.6	3.3	3.5	6.9	0.3	0.4	0.1
22:1 n-11	4.7	3.4	4.0	6.2	0.7	1.9	14.3
22:1 n-9	0.9	0.9	1.2	ND	0.4	0.4	0.5
Total monounsaturated	40.7	41.8	33.1	42.3	23.8	32.0	43.4
Polyunsaturated							
18:2 n-6	0.8	0.9	1.5	1.2	2.5	1.2	1.5
18:3 n-6	ND	ND	0.2	ND	0.2	0.3	ND
18:3 n-3	0.3	ND	0.8	0.4	0.9	0.4	0.9
18:4 n-3	0.8	0.7	2.1	0.7	1.2	1.1	1.8
20:3 n-6	0.3	ND	0.5	0.3	0.3	0.2	0.4
20:4 n-6	1.4	1.5	0.7	1.0	1.4	1.0	0.7
20:5 n-3	9.3	8.9	9.2	7.2	8.1	7.5	5.2
22:4 n-6	1.3	ND	0.6	0.7	0.9	0.6	1.9
22:5 n-3	1.4	1.0	0.7	0.4	1.8	2.2	0.6
22:6 n-3	9.9	6.5	6.0	7.6	12.9	7.7	8.6
Total polyunsaturated	25.5	19.5	22.3	19.5	30.2	22.2	21.6
Total n-6	3.8	2.4	3.5	3.2	5.3	3.3	4.5
Total n-3	21.7	17.1	18.8	16.3	24.9	18.9	17.1
n-6/n-3	0.2	0.1	0.2	0.2	0.2	0.2	0.3
unsat/saturated	2.6	2.0	1.5	2.0	1.5	1.5	2.3

[1] Expressed as a percentage of the total area identified (area %).

ND - Only trace amounts detected.

Table 7. Amino acid levels (g·16 g N^{-1}) in the various fish meals.

Amino acid	Herring meal 1	Herring meal 2	Herring meal 3	Herring meal 4	HM-FF-Lt	HM-FF-Std	HM-FM-Lt	HM-FM-Std	HM-SM-Std
Ala	5.99	6.74	6.65	7.18	6.39	6.15	6.05	6.32	6.30
Arg	7.45	7.89	7.12	7.92	8.18	7.96	9.98	8.08	9.82
Asp	8.21	8.82	9.64	10.84	9.50	9.07	8.60	9.29	8.60
Cys	0.96	1.23	1.04	1.07	0.95	0.92	0.91	0.95	0.91
Glu	12.25	12.54	13.95	15.32	14.20	13.39	12.39	13.79	12.68
Gly	5.74	6.19	6.24	6.13	6.19	5.90	6.48	5.93	6.29
His	1.94	2.08	2.35	2.67	2.26	2.16	2.10	2.18	2.08
Ile	3.96	4.37	4.86	5.69	4.85	4.61	4.04	4.78	4.50
Leu	7.59	9.46	8.21	9.31	8.08	7.84	7.06	8.05	7.56
Lys	7.46	7.96	8.69	9.63	8.35	7.94	7.28	8.10	7.47
Met	2.51	2.70	2.78	3.22	2.78	2.68	2.51	2.65	2.59
Phe	3.56	3.90	4.25	4.91	4.30	4.07	3.69	4.14	4.04
Pro	3.81	4.10	4.17	4.89	4.62	4.44	4.44	4.05	4.55
Ser	3.96	4.33	4.30	4.77	4.29	4.16	4.18	4.22	4.16
Thr	4.10	4.58	4.69	5.30	4.64	4.48	4.40	4.60	4.41
Trp	1.00	1.33	0.73	0.54	0.68	0.66	0.68	0.85	0.75
Tyr	3.10	3.29	3.39	3.90	3.43	3.27	3.07	3.35	3.23
Val	4.69	5.12	5.50	6.00	5.36	5.24	5.17	5.36	5.27

Table 7 (Con't). Amino acid levels (g·16 g N^{-1}) in the various fish meals.

Amino acid	Groundfish meal	Mixed meal	Menhaden meal	Silver hake	Chilean - Good quality	Chilean - Poor quality	Norse-LT94®
Ala	6.70	7.16	6.68	6.60	6.34	7.21	6.73
Arg	6.70	7.23	5.93	6.98	6.14	6.85	6.48
Asp	8.46	8.89	8.43	10.29	9.00	10.19	9.54
Cys	1.07	1.07	0.97	1.09	0.98	1.00	1.06
Glu	12.65	12.85	12.13	15.06	12.95	14.63	13.80
Gly	9.46	9.15	7.55	6.50	6.22	7.15	6.81
His	1.79	1.99	2.12	2.28	3.59	3.52	2.40
Ile	3.76	4.16	3.99	5.10	4.75	5.10	4.73
Leu	7.15	7.81	7.53	8.42	7.76	8.40	7.92
Lys	7.01	7.58	7.34	8.59	7.89	8.87	8.32
Met	2.50	2.62	2.56	2.98	2.50	2.45	2.80
Phe	3.35	3.82	3.70	4.59	4.10	4.45	4.26
Pro	4.81	4.96	5.24	4.76	4.48	4.57	4.70
Ser	4.68	4.69	4.09	4.87	4.04	4.39	4.35
Thr	4.12	4.43	4.22	4.86	4.44	4.87	4.56
Trp	1.28	0.94	1.18	0.65	1.03	0.81	0.74
Tyr	2.91	3.25	3.01	3.61	3.18	3.50	3.33
Val	4.39	4.90	4.57	5.52	5.22	5.63	5.28

during the drying phase, as it has been shown that heating will partially destroy cysteine in fish meals (Opstvedt et al., 1984).

Biogenic amines result from the decarboxylation of specific amino acids, either by bacterial enzyme action and/or by enzymes naturally present in all fish. All fish have natural levels of amines present. High levels of amines suggest usage of raw material that may have been enzymatically degraded to varying degrees before processing (Pike, 1993). The ideal type of raw material would be either freshly caught fish immediately processed or immediately chilled or frozen, and then processed into meal within a short period (less than eight h). During the manufacturing of whole fish meal, it is important that the fish solubles are of the highest quality. The solubles contain high levels of soluble protein and free amino acids that can decompose in a short period (two to four h). The biogenic amine (histamine, putrescine, cadaverine, and tyramine) levels in the test fish meals varied widely (Table 8). HM-SM-Std, which was made from spoiled herring, had the highest levels of putrescine, cadaverine, and tyramine and the greatest total amine level (3092 $mg \cdot kg^{-1}$ DM). Five meals had combined amine levels greater than 2000 $mg \cdot kg^{-1}$ DM and three other meals had concentrations that varied between 1000 and 2000 $mg \cdot kg^{-1}$ DM.

In fresh fish, the sequence for production of amines is as follows: histamine, putrescine, and then cadaverine. The production of the different amines in fish that have been previously frozen shows lag phases that differ considerably from those found in non-frozen fish (cadaverine, putrescine, and then histamine) (Clancy, 1992). This suggests that meals with high histamine levels were produced from raw fish that were never frozen and had spoiled before processing. This observation is supported by the amine levels in HM-SM-Std, as the levels for putrescine, cadaverine, and tyramine were

Table 8. Biogenic amine concentrations ($mg \cdot kg^{-1}$ dry matter) in the various fish meals.

Fish meal	Histamine	Putrescine	Cadaverine	Tyramine
Herring meal 1	11	169	290	11
Herring meal 2	11	221	243	11
Herring meal 3	78	366	517	24
Herring meal 4	1655	652	626	52
HM-FF-Lt	10	475	511	110
HM-FF-Std	10	421	439	100
HM-FM-Lt	32	477	418	117
HM-FM-Std	51	332	416	49
HM-SM-Std	179	957	1590	366
Chilean - Good quality	668	233	467	121
Chilean - Poor quality	1434	271	617	98
Groundfish meal	11	85	216	11
Menhaden meal	1765	462	1007	202
Mixed meal	11	248	479	11
Norse-LT94®	242	585	1373	257
Silver hake	11	39	11	11

the highest, but the histamine level was low compared to the levels observed in some of the other fish meals. Dietary levels of putrescine up to 4 $g \cdot kg^{-1}$ have not been found to influence the growth of 4 g rainbow trout (Cowey and Cho, 1992). Also, in another study with 5.5 g trout, Fairgrieve et al. (1994) found that diets containing 2.0, 0.5, and 0.5 $g \cdot kg^{-1}$ DM of histamine, putrescine, and cadaverine, respectively, had no effect on fish growth or feed consumption. Therefore, it would appear that high levels (approximately 3 $g \cdot kg^{-1}$) of amines probably do not adversely affect fish growth. Consequently, the amine levels observed in this study are likely poor predictors of protein quality. This conclusion is further supported by the high levels of amines that were found in Norse-LT94® (2457 $mg \cdot kg^{-1}$ DM), which is known to support good growth in fish.

The quality of the lipid component in the fish meals was evaluated by several chemical assays (Table 9), the iodine value (as an indication of the degree of unsaturation) and three lipid quality assays (peroxide value, anisidine value, and TBA). The lipid quality assays were performed to evaluate the general breakdown products of lipid oxidation in the meals. These products include: peroxides, malonaldehyde, and aldehydes. The production of each of these compounds has different lag phases. Peroxides are produced first followed by malonaldehyde and finally by aldehydes (Hung and Slinger, 1981; Frankel, 1991), although many different products (ketones, alcohols, alkanes, alkenes) are also produced. All of the test meals were found to have undetectable levels of peroxides. The iodine values for the test meals ranged from 100 to 131, and were within acceptable limits for good quality meals (CWGFN, 1988; Stansby, 1990).

The anisidine values of the meals ranged from approximately 11 to 19. This test

Table 9. Levels of lipid quality variables in the various fish meals.

Fish meal	Iodine value	Anisidine value	TBA[1] μmol·g^{-1} fish meal (as-is)
Herring meal 1	107	17.6	0.05
Herring meal 2	115	13.4	0.04
Herring meal 3	115	12.3	0.12
Herring meal 4	104	17.4	0.26
HM-FF-Lt	115	18.7	1.29
HM-FF-Std	113	18.7	0.23
HM-FM-Lt	131	16.5	0.27
HM-FM-Std	116	19.2	0.09
HM-SM-Std	117	14.9	0.06
Chilean - Good quality	114	13.8	0.55
Chilean - Poor quality	120	10.9	0.08
Groundfish meal	100	12.7	0.02
Menhaden meal	114	15.2	0.11
Mixed meal	105	14.2	0.03
Norse-LT94®	113	19.4	0.05
Silver hake	113	16.9	2.28

[1] Thiobarbituric acid test.

measures the quantity of alpha-beta unsaturated aldehydes, which are intermediate products of lipid oxidation. The noted range for anisidine value was narrow and does not indicate any great difference in the quality of the lipid component of the fish meals. All anisidine values were below the recommended maximum value of 20 (Windsor and Barlow, 1981).

Although, TBA values were highest for silver hake (2.28 $\mu mol \cdot g^{-1}$) and HM-FF-Lt (1.29 $\mu mol \cdot g^{-1}$), it is doubtful that these values indicate poor lipid quality. This conclusion is supported by the virtual absence of peroxides and the low anisidine values found in these fish meals. The TBA test measures the amount of malonaldehyde and, indirectly, the hydroperoxides that produce malonaldehyde. This test has been shown to give erroneously high values due to reaction with other components in the test material (Sinnhuber and Yu, 1977; Hoyland and Taylor, 1991).

The results of various in vitro measures of the quality of the protein component in the fish meals are provided in Table 10. The values for dilute-pepsin digestible protein ranged from 69% to 95% for the various fish meals tested. The Chilean - poor quality, Herring meal 2, groundfish meal, and three of the laboratory-made meals had the lowest values. No obvious trend was detected among the different test meals.

Values for the percentage of the crude protein that was soluble in a weak acid solution (0.075N HCl) ranged from 17% to 45%. It was interesting to note that Norse-LT94® had the highest value. Further, among the laboratory-made herring meals, the LT meals had higher values than the corresponding meals processed under standard conditions. The low values for Herring meals 1 (21.5%), 2 (21.4%), and 4 (17.3%) and silver hake (17.7%) suggest that over heating may have occurred during meal preparation. However, the corresponding values for dilute-pepsin digestibility do not

Table 10. In vitro measures of protein quality in the various fish meals.

Fish meal	Dilute-pepsin digestibility %	Soluble protein[1] %	TVBN[2] mg N·100 g^{-1} fish meal (as-is)	Multi-enzyme pH-stat digestibility %
Herring meal 1	85.1	21.5	27.6	87.5
Herring meal 2	72.8	21.4	40.4	89.8
Herring meal 3	88.3	35.6	32.6	95.5
Herring meal 4	90.9	17.3	59.6	93.2
HM-FF-Lt	81.8	35.9	48.2	89.2
HM-FF-Std	83.4	30.4	49.2	89.5
HM-FM-Lt	68.7	38.4	30.1	89.1
HM-FM-Std	70.4	35.8	46.3	91.1
HM-SM-Std	70.5	35.8	67.2	89.7
Chilean - Good quality	95.2	35.2	59.6	92.9
Chilean - Poor quality	76.5	35.8	70.1	87.5
Groundfish meal	74.7	24.7	25.1	90.7
Menhaden meal	82.7	29.8	77.5	89.1
Mixed meal	80.1	36.7	49.8	95.9
Norse-LT94®	93.2	45.0	50.2	90.8
Silver hake	84.2	17.7	14.0	90.7

[1] The percentage of protein soluble in a weak acid solution (0.075 N HCl).

[2] Total volatile basic-nitrogen. Values for the raw fish used to make the HM-FF (fresh female) and HM-FM (fresh males) meals were 20.0 and 21.6 mg N·100 g^{-1} raw fish, respectively. The value for the spoiled male herring used to make HM-SM-Std was 112.7 mg N·100 g^{-1} raw fish.

support this premise. This former variable was determined because it was thought that it might indicate the degree of denaturation of the proteins in the test fish meals. It is well known that long periods of frozen storage or heat treatment reduce the solubility of proteins (Eggum, 1989; de Koning and Mol, 1991).

Five meals had high TVBN values. These included, Herring meal 4 (60), HM-SM-Std (67), Chilean - good quality (60), Chilean - poor quality (70), and menhaden meal (77). It is noteworthy that all values were below the recommended maximum level of 200 mg·100 g^{-1} fish meal (CWGFN, 1988). TVBN values in the raw fresh male and raw fresh female herring were respectively, 21.6 and 20.0 mg N·100 g^{-1} as-is fish. The TVBN value for the spoiled male herring was 112.7 mg N·100 g^{-1} as-is fish. TVBN measures low molecular weight volatile nitrogenous compounds, such as mono-, di-, and trimethylamines and ammonia. High quantities of these compounds in a fish meal suggest the use of spoiled fish in meal manufacture, which is seen with HM-SM-Std. Although this test is useful for indicating relative amounts of volatile non-protein nitrogenous compounds, it has not been shown to be a good indicator of fish meal quality (Clancy, 1992; Anderson et al., 1993; Romero et al., 1994).

The multi-enzyme pH-stat digestibility values ranged from 87% to 96%. This range is small and no obvious trend was detected.

In the marine environment, fish drink seawater (Smith, 1930; Evans, 1979), absorbing the water and monovalent ions but not a significant portion of the divalent ions (Smith, 1930, 1983, 1989); therefore, the ash and thus the DM content of the faeces is significantly increased. Thus, in this study, digestibility of the whole diet was measured as organic matter digestibility rather than DM digestibility. Values for ADOM-F differed little among the various fish meals (Table 11). The ADOM-F values ranged

Table 11. Apparent digestibility coefficients and digestible energy values for the various fish meals determined with Atlantic salmon reared in seawater.

Fish meal	ADOM-F %	ADCP-F %	ADGE-F %	DE $MJ \cdot kg^{-1}$ DM
Herring meal 1	93.3ab	91.5c	94.6abc	21.1cd
Herring meal 2	95.2ab	93.6abc	95.9abc	21.4bcd
Herring meal 3	97.9a	95.4abc	99.2a	22.2ab
Herring meal 4	96.6ab	95.9abc	97.9ab	22.4a
HM-FF-Lt	91.8ab	93.2bc	92.3cd	20.7d
HM-FF-Std	86.7c	86.5d	89.4d	20.0e
HM-FM-Lt	93.9ab	92.4bc	94.6abc	21.7bc
HM-FM-Std	90.3bc	86.0d	91.8cd	21.5bcd
HM-SM-Std	93.1ab	91.9c	93.8bc	21.5bcd
Chilean - Good	91.8ab	94.0abc	94.5abc	19.6ef
Chilean - Poor	93.6ab	96.5ab	94.3bc	19.3ef
Groundfish meal	93.7ab	92.1bc	95.2abc	17.8g
Menhaden meal	92.0ab	91.7c	92.7cd	19.0f
Mixed meal	93.6ab	92.3bc	95.5abc	19.6ef
Norse-LT94®	98.2a	97.6a	99.2a	21.6bc
Silver hake	93.5ab	92.9bc	95.0abc	19.2ef
Pooled sd[1]	2.2	1.6	1.7	0.4

ADOM-F: The apparent digestibility coefficient for organic matter.
ADCP-F: The apparent digestibility coefficient for crude protein.
ADGE-F: The apparent digestibility coefficient for gross energy.
DE: apparent digestible energy content.

Means (n=3) within a column sharing a common superscript letter are not significantly different (P>0.05).

[1] Pooled standard deviation (square root of the error mean square).

from 87% to 98%, with the value for HM-FF-Std being significantly ($P<0.05$) lower than those of the other fish meals.

Values for ADCP-F ranged from 86% to 98% (Table 11). HM-FF-Std and HM-FM-Std, had the lowest values ($P<0.05$) at 86.5 and 86.0%, respectively. An interesting trend was seen between the two processes of standard and LT drying temperatures used for production of the laboratory fish meals. In both cases, for meals made from males or females, values for ADCP-F of the LT meals were significantly higher ($P<0.05$) than the values found for the corresponding standard meals. Possibly, the preceding trend can be explained by an effect of the higher temperatures used in standard processing on the lipid component of the fish meals. For instance, it is well known that excessive temperature can cause the formation of lipid hydroperoxides. These in turn can react with proteins, reducing their availability (Hsieh and Kinsella, 1989; Davidek et al., 1990; Aunourg, 1993).

The formation of lipid hydroperoxides in the laboratory meals processed under standard drying conditions is supported by the trend towards a reduction in values for ADGE-F from 92.3 to 89.4% and 94.6 to 91.8% for HM-FF-Lt and HM-FF-Std and HM-FM-Lt and HM-FM-Std, respectively. The production of hydroperoxides that might have reacted with proteins during the cooking and drying phases in processing would not have been detected in any of the lipid quality assays that were conducted. The lipid quality assays would have only detected unreacted lipid oxidation products produced during manufacturing or storage and not the oxidation products covalently bonded to proteins (Hsieh and Kinsella, 1989; Aunourg, 1993). This explanation accounts for the differences seen in the crude protein and gross energy digestibilities of the two processing types of the laboratory-produced herring meals. The DE values for the

various fish meals ranged from 17.8 $MJ \cdot kg^{-1}$ DM to 22.4 $MJ \cdot kg^{-1}$ DM. No trends were observed, since the concentration of DE was more a function of the gross energy concentration in the meals and not its digestibility. Interestingly, Norse-LT94® had the highest apparent digestibility coefficients for all nutrients, with Herring meal 3 a close second.

During the growth experiment, the average fish mortality for the 12-wk period was 6.4%. The occurrence of high mortalities in fish fed diets containing Herring meal 1 and HM-FM-Std (Table 12) could not be attributed to bacterial or viral causes. For fish fed HM-SM-Std, most mortality occurred during the last four weeks of the experiment. The etiology of the mortality was not found.

No abnormalities were seen in the samples of liver, small intestine, and pyloric caeca sent for histological interpretation. The present findings support those of Fairgrieve et al. (1994) who did not observe any histological abnormalities in the liver, kidney, or stomach of rainbow trout fed diets containing high levels of amines (histamine, putrescine, and cadaverine).

The weight gains, growth rates, feed consumptions and feed and protein utilization values of the fish fed the experimental diets are presented in Table 12. The values in each case represent the average fish performance in relation to diet treatment for the entire 12-wk experiment. Statistical analyses and inferences were performed on the information obtained after each 4-wk period, as stated above. Fish fed the diet containing HM-SM-Std had significantly ($P<0.05$) lower absolute weights than fish fed diets containing the other test meals - Herring meals 1 and 2, groundfish meal, and Norse-LT94® (Table 12; Figure 3). This trend was first seen at the end of the first 4-wk period and was significant by the end of the second 4-wk period (Figure 3). At the end

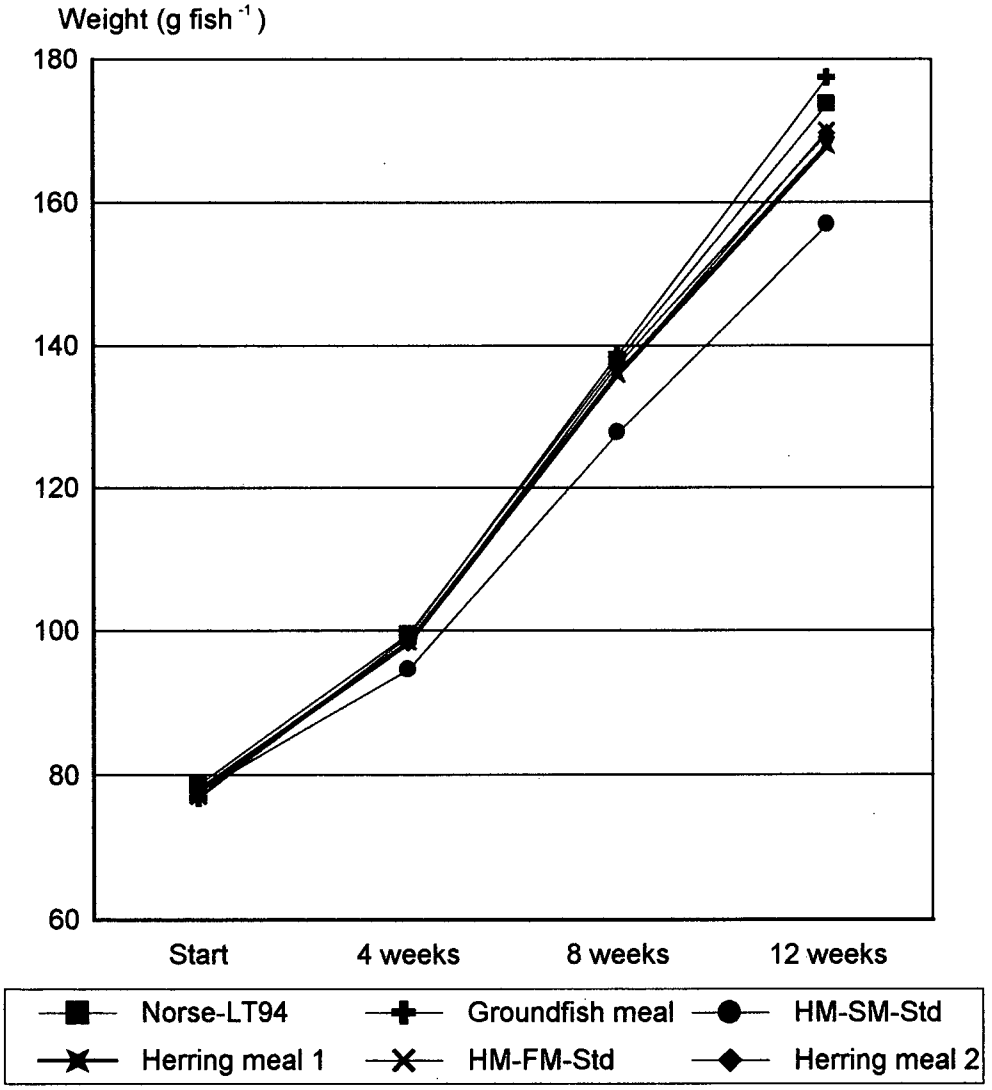
Table 12. Performance of Atlantic salmon fed diets containing various fish meals as the major protein source for 12 weeks.

Diet	Initial weight[1] ($g \cdot fish^{-1}$)	Final weight ($g \cdot fish^{-1}$)	12 week weight gain ($g \cdot fish^{-1}$)	Specific growth rate[2] ($\% \cdot day^{-1}$)	12 week dry feed consumption[2] ($g \cdot tank^{-1}$)	Feed consumption[2] ($g \cdot kg^{-1}$ BW$\cdot day^{-1}$)	Feed to gain ratio[2,3]	PER[2]	Mortality[4] %
Herring meal 1	77.8	167.9	90.1	0.92	6813.7	8.3	1.03	2.49	11.2
Herring meal 2	78.3	169.7	91.4	0.92	6952.3	8.3	1.03	2.55	4.0
HM-FM-Std	77.1	170.0	92.9	0.95	7084.1	8.6	1.03	2.52	12.9
HM-SM-Std	77.8	157.7	79.2	0.85	5914.9	7.9	1.08	2.40	4.4
Groundfish	76.8	177.2	100.5	0.99	7275.4	8.6	0.99	2.62	4.0
Norse-LT94®	78.7	174.1	95.0	0.93	6956.7	8.6	1.04	2.55	1.8
Pooled sd[5]	9.9	19.7	6.7	0.04	325.6	0.2	0.06	0.11	--------

See text for explanation of statistical analyses and inferences.

[1] Means (n=225).
[2] Means (n=3).
[3] Feed to gain ratio = dry feed consumption (g)·wet weight gain $(g)^{-1}$.
[4] Each diet treatment had 225 fish initially (three tanks of 75 fish each).
[5] Pooled standard deviation (square root of the error mean square).

Figure 3. The effect of feeding diets containing different fish meals on the absolute weights of Atlantic salmon reared in sea water.



of the third 4-wk period, fish fed the diet based on groundfish meal had the highest absolute weights and weight gains, although the differences were not significant, except as noted above. If the experiment had been conducted over a longer period, it is likely that the fish fed the groundfish meal diet would have significantly out-performed the fish on all other diets, based upon the observed differences in the slopes of weights of the groups over time (Figure 3). The weight gains of the salmon over the 12-wk experiment showed a quadratic response (Figure 4). Overall, the fish fed the groundfish meal diet had the highest weight gain, and those fed the HM-SM-Std diet the lowest ($P<0.05$). Values for SGR (Figure 5) showed the same quadratic response as weight gain, with the fish fed the groundfish meal diet having the best performance and those fed the HM-SM-Std diet the poorest rates of growth. This trend was significant at the end of the second period (eight wks).

During the third 4-wk period of the study, fish fed all diets had reduced feed consumption (Figure 6). Likely the reduced feed consumption, and thus reduced weight gains and SGR, were caused by increased fluctuations of the seawater temperatures during that period (August). A daily fluctuation of 2°C was observed compared to 1°C for the other periods, although the average temperature did not change.

There were no significant differences among feed to gain ratios during any of the 4-wk periods, although fish fed the groundfish meal diet had the lowest (best) value (0.98) and fish fed the HM-SM-Std diet had the highest (1.08). This result introduces a very interesting point; the fish fed the HM-SM-Std diet had utilization for weight gain (protein and/or fat) similar to that for fish fed the other diets. However, the fish did not eat sufficient feed to produce maximal growth. Possibly, the HM-SM-Std diet was not as palatable. Palatability of fish diets has been shown to significantly influence feed

Figure 4. The effect of diets containing different fish meals on the weight gains of Atlantic salmon reared in sea water during each 4-week period of the study.

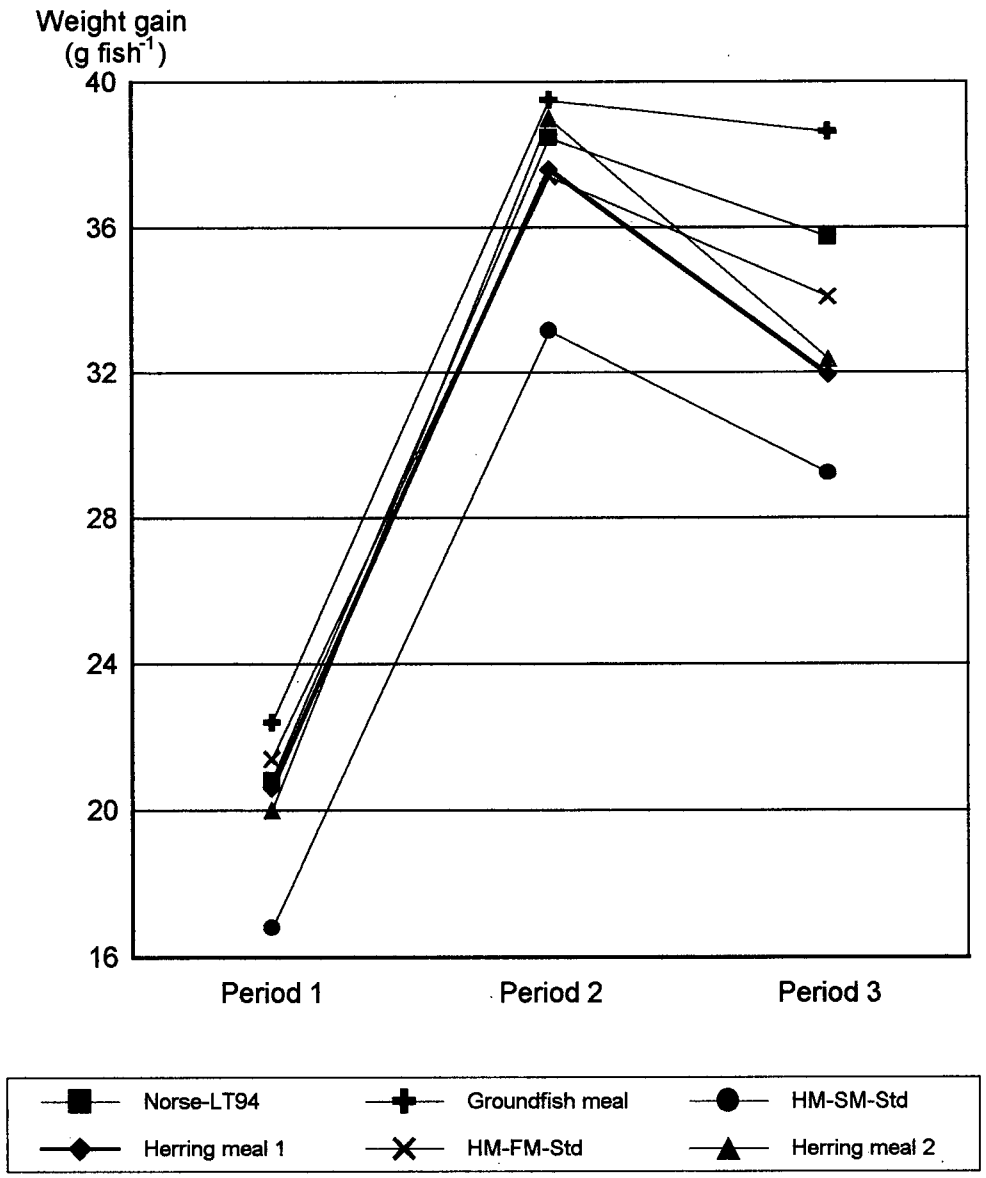


Figure 5. The effect of different fish meals in the diet on the specific growth rates of Atlantic salmon reared in sea water during each 4-week period of the study.

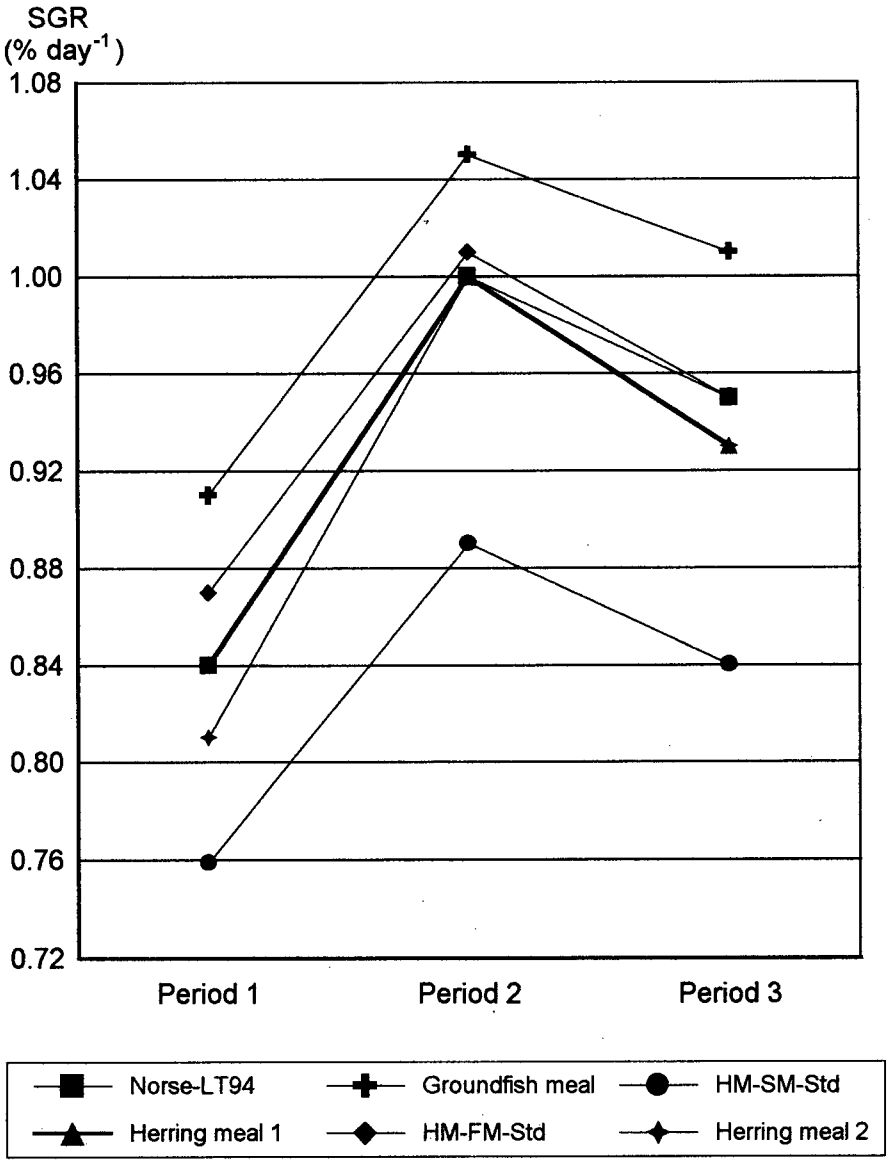
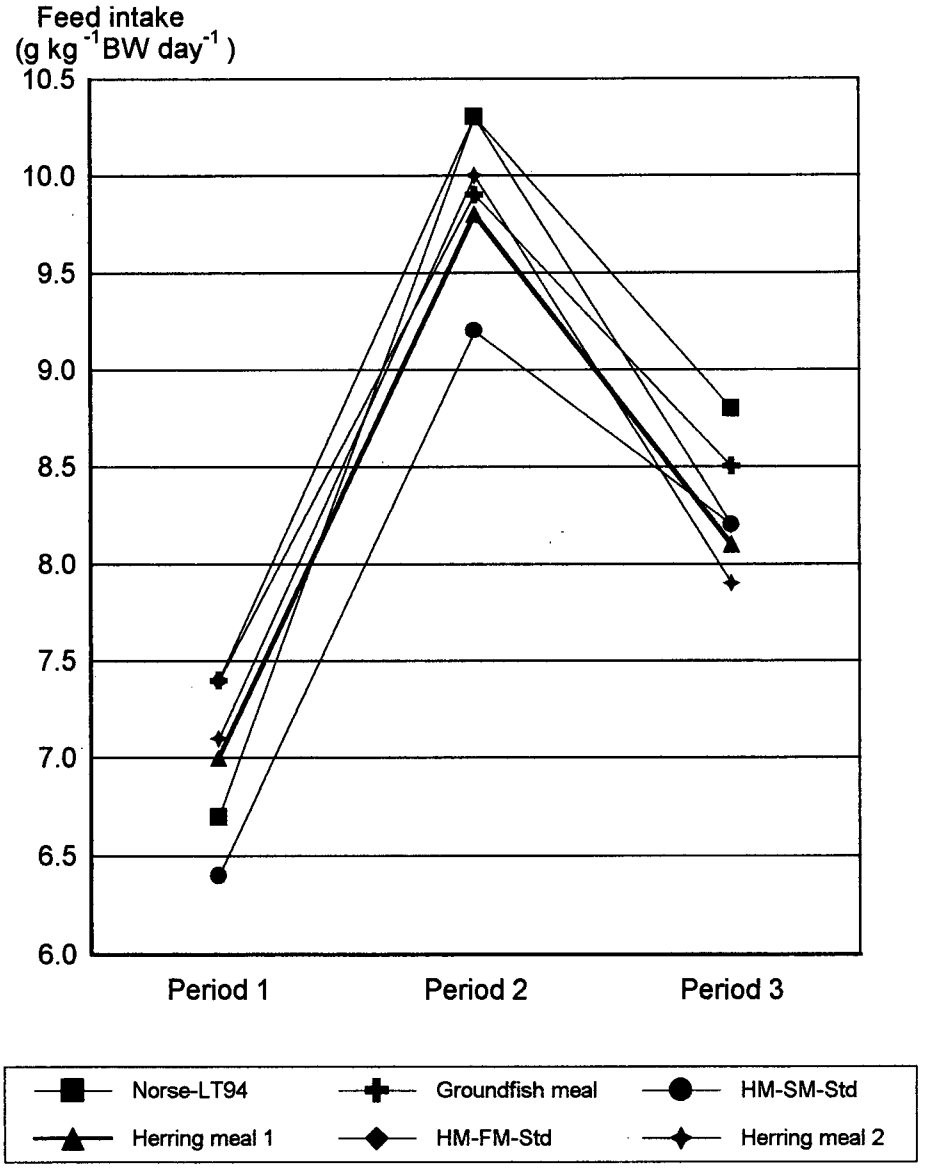


Figure 6. The effect of feeding diets containing different fish meals on feed intakes of Atlantic salmon reared in sea water during each 4-week period of the study.



consumption (Adron and Mackie, 1978; Jones, 1990). Hughes (1991) assessed the effects of various chemical compounds in the diets of first-feeding chinook salmon (0.4 g) and found that trimethylamine was a strong inhibitor of feed consumption. This might have been the case for the fish fed the HM-SM-Std diet, as the TVBN value (67 mg·100 g^{-1}) and biogenic amine levels were the highest among the fish meals evaluated. This effect may have been due to another compound(s), either of protein or lipid origin, produced during the spoilage process. Evidence for reduced feed consumption in salmonids because of high dietary levels of biogenic amines, at least those analysed, is not clear-cut. High levels of amines were also present in the sample of Norse-LT94® (Table 8, page 67), yet the fish did not exhibit reduced feed consumption. This suggests that the amines might not have affected feed consumption. Cowey and Cho (1992) using putrescine and Fairgrieve et al. (1994) using histamine, putrescine, and cadaverine, also did not find any influence of dietary amine levels on the feed consumption of rainbow trout.

A significant negative linear ($P<0.05$) response was observed for PER values over the three 4-wk periods, although there were no significant differences among the dietary treatments. PER values for the salmon averaged over all diets at each 4-wk period, were 2.74, 2.60, and 2.32 at four, eight, and 12 wks, respectively. These results again suggest that the quantity of protein ingested by the fish fed the HM-SM-Std diet was utilized well for growth, but that these fish did not ingest enough of the diet to obtain comparable growth to that obtained for fish fed the other test diets.

Analysis of covariance, with the log of the absolute body weight (g) as the covariate, was used to correct for differences in levels of proximate constituents because of dissimilar weights of the fish among the various treatment groups (Shearer,

1994). The fish fed the diet with Norse-LT94® had significantly lower lipid levels than the other fish (Table 13). The various fish meals tested in the growth experiment did not have any significant effect on carcass protein levels. This again supports the premise that the protein in HM-SM-Std was utilized as efficiently as the protein from the other fish meals.

When HM-FM-Lt and HM-SM-Std provided the sole source of protein for rats, significantly ($P<0.001$) lower AMCP-R, BV, and NPU values were found compared with the other fish meals (Table 14). When Norse-LT94® was fed to rats as the sole dietary protein source, consistently higher values (not significant) were observed for most of the parameters evaluated (Table 14). For the other fish meals, there were no obvious trends in the differences for ADCP-R, AMCP-R, TD, BV, or NPU values.

Correlations between the protein quality or protein utilization variables for the in vitro and in vivo assays and between the different in vivo assays using fish or rats as the test animals were performed. Of the various in vitro tests, only dilute-pepsin digestibility was found to have significant correlations with AMCP-R ($r^2=0.433$,$n=16$, $P=0.006$), BV ($r^2=0.261$, $n=16$, $P=0.04$), and NPU ($r^2=0.372$, $n=16$, $P=0.01$). No significant correlations were noted for any other fish versus rat comparison. When the lowest two ADCP-F values were removed from the correlation analysis, then the correlation between ADCP-F and AMCP-R became significant ($r^2=0.359$, $n=14$, $P=0.024$). The removal of these two points can be justified because they are associated with two laboratory-made meals and these meals had significantly reduced values for ADCP-F compared with all other meals. Although there were other significant correlations, such as TVBN and soluble protein, they were between related measurements and thus held no significant meaning. The lack of correlation between

Table 13. Whole body proximate compositions ($g \cdot kg^{-1}$ wet basis) of Atlantic salmon fed diets containing different fish meals as the major protein source.

Diet	Moisture	Crude protein	Lipid	Ash
Herring meal 1	727ab	172	79a	21
Herring meal 2	726ab	170	81a	23
HM-FM-Std	722b	172	81a	23
HM-SM-Std	735a	167	77a	22
Groundfish meal	728ab	172	79a	21
Norse-LT94®	736a	172	68b	22
Pooled sd[1]	10	4	9	3

[1] Pooled standard deviation (square root of the error mean square).

Means (n=9) within a column that share or do not have a common postscript letter are not significantly different (P>0.05).

Table 14. Estimates of the protein quality of various fish meals using rats as the test animal.

Diet	ADCP-R (%)	AMCP-R (%)	TD (%)	BV	NPU
Herring meal 1	80.7cd	64.8a	92.9abc	0.94a	0.88ab
Herring meal 2	79.6cde	64.5a	91.1c	0.95a	0.86ab
Herring meal 3	83.4ab	66.8a	94.7ab	0.93a	0.88ab
Herring meal 4	80.8cd	66.2a	91.9c	0.95a	0.87ab
HM-FF-Lt	80.4cd	63.5a	92.0c	0.93a	0.86ab
HM-FF-Std	81.0cd	64.1a	92.6bc	0.93a	0.86ab
HM-FM-Lt	81.8bc	56.4b	93.1abc	0.84b	0.78c
HM-FM-Std	80.1cde	65.2a	91.6c	0.95a	0.87ab
HM-SM-Std	81.3cd	56.3b	92.4bc	0.84b	0.77c
Chilean - Good	78.5de	62.2a	90.5c	0.94a	0.85ab
Chilean - Poor	79.5cde	63.7a	91.0c	0.94a	0.86ab
Groundfish meal	79.7cde	62.1a	91.0c	0.92a	0.84ab
Menhaden	77.6e	61.5a	88.5d	0.93a	0.82b
Mixed meal	77.5e	61.8a	90.7c	0.96a	0.87ab
Norse-LT94®	83.5a	67.5a	95.0a	0.94a	0.89a
Silver hake	79.7cde	66.2a	90.8c	0.96a	0.87ab
Pooled sd[1]	1.3	2.9	1.3	0.03	0.03

[1] Pooled standard deviation (square root of the error mean square).

Means (n=5) within a column sharing a common postscript letter are not significantly different (P>0.05).

ADCP-R: (apparent digestibility coefficient for crude protein) = (PN-FNE) ·100·PN^{-1}.
AMCP-R: (apparent metabolizable coefficient for crude protein) = (PN-(FNE+UNE)) ·100·PN^{-1}.
TD: (true digestibility coefficient for crude protein) = (PN-(FNE-EFNE))·100·PN^{-1}.
BV: (biological value) = (PN-(FNE-EFNE)-(UNE-EUNE))·100·(PN-(FNE-EFNE))$^{-1}$.
NPU: (net protein utilization) = TD·BV

Where: PN = nitrogen intake; FNE = faecal nitrogen excretion; UNE = urinary nitrogen excretion; EFNE = endogenous faecal nitrogen excretion; and EUNE = endogenous urinary nitrogen excretion.

any in vitro assay and in vivo protein quality variable determined with fish was not encouraging. Although it was not significant, the best correlation was found between dilute-pepsin digestibility and ADCP-F (r^2=0.195, n=16, P=0.087).

Since the test fish meals were made from raw material from such divergent species of fish, the various herring meals and Norse-LT94® (which is made from a species similar in composition to herring) were inspected again for correlation after grouping separately from the remaining fish meals (mixed meal, groundfish meal, Chilean meals, silver hake, and menhaden). Only slight improvements in the correlations between dilute-pepsin digestibility and AMCP-R (r^2=0.545,n=10, P=0.02) and NPU (r^2=0.521, n=10, P=0.02) were seen and the correlation with BV became non-significant. Again, when the lowest two ADCP-F values were removed from the correlation analysis, the correlation between dilute-pepsin digestibility and ADCP-F became significant (r^2=0.549, n=8, P=0.036) and a weak correlation between pH-stat and ADCP-F was detected (r^2=0.453, n=8, P=0.068). The correlation between ADCP-F and AMCP-R (r^2=0.487, n=8, P=0.054) was not improved by grouping the fish meals. No correlations between any variables were found with the other group of meals.

The lack of a correlation between the in vitro pH-stat values and any protein quality measurement, especially TD (r^2=0.012, n=16, P=0.68), determined with rats was very surprising. The pH-stat calculates the TD of the test protein based on an equation derived from work with rats (Pedersen and Eggum, 1983). The reason for this lack of correlation is unknown. Previous work by the author (Anderson et al., 1993) found a correlation between the dilute-pepsin digestibility and pH-stat methods (r^2=0.552, n=20, P<0.000). In this study, no correlation was found (r^2=0.138, n=16, P=0.16) between dilute-pepsin digestibility and pH-stat values. Grouping of the fish meals made no

difference to the outcome. Work by Romero et al. (1994) also found poor correlations between protein digestibility coefficients determined with rainbow trout and in vitro assays (in vitro digestibility, available lysine, and chemical analyses).

CONCLUSIONS

From the work presented here, it appears that dilute-pepsin digestibility is the preferred in vitro test to evaluate the protein quality of herring meals for Atlantic salmon. The dilute-pepsin digestibility and the multi-enzyme pH-stat digestion appear to correlate poorly with the results from in vivo assays when other types of fish meals are included. AMCP-R (apparent metabolizable crude protein) using rats as the test animal was the only non-fish in vivo assay that was found to correlate (but poorly) the entire range of fish meals with assays using fish. Of the various commercial herring meals tested, all but one were equal to Norse-LT94® and the Chilean meals with respect to their apparent digestibility of crude protein for fish. The fish fed diets based on the two commercial herring meals in the growth experiment performed as well as those fed the Norse-LT94® diet. A significant finding was the observation that Atlantic salmon fed a diet with groundfish meal as the major protein source (80% of dietary protein) performed as well as fish fed Norse-LT94®. This represents a cost saving to fish farmers. Based on the similar feed to gain ratios noted for salmon ingesting the diets with herring meal or groundfish meal, at current fish meal prices ($885 and $620 $\cdot$tonne^{-1}, FOB Vancouver, July 1995 prices for herring and groundfish meal, respectively) the cost saving of replacing herring meal with groundfish meal in a salmon diet would be approximately 0.08\cdot$kg^{-1} diet.

CHAPTER FOUR - THE EFFECT OF VARYING THE DIETARY DIGESTIBLE PROTEIN TO LIPID RATIO ON THE GROWTH AND WHOLE BODY COMPOSITION OF ATLANTIC SALMON (0.5 - 1.2 kg) REARED IN SEAWATER.

INTRODUCTION

The dietary protein and energy requirements of salmonids vary with stage of development as well as with species (NRC, 1993). Many studies on this topic have been conducted on small juvenile fish in freshwater. For instance, research has been conducted on 0.9 g chum salmon, Oncorhynchus keta (Akiyama et al., 1981), 2.2 g rainbow trout, Oncorhynchus mykiss (Takeuchi et al., 1978), 3.5 g coho salmon, Oncorhynchus kisutch (Clarke and Higgs, 1984), 3 and 18 g Arctic charr, Salvelinus alpinus (Jobling and Wandsvik, 1983; Tabachek, 1986), and 1.0 g brown trout, Salmo trutta (Arzel et al., 1995). By contrast, few studies have been conducted to determine the dietary protein and energy needs of large salmonids reared in seawater. To date, research has been conducted on 57 - 600 g Atlantic salmon, Salmo salar (Lall and Bishop, 1977; Hillestad and Johnsen, 1994; Hemre et al., 1995), 50 - 450 g rainbow trout (Lall and Bishop, 1979; Brauge et al., 1994), and 110 - 300 g chinook salmon, Oncorhynchus tshawytscha (Silver et al., 1993).

Salmonids preferentially use proteins and lipids for energy purposes, although carbohydrate may, to some degree, satisfy their energy needs (Lee and Putnam, 1973; Pieper and Pfeffer, 1980). The protein-sparing action of non-protein energy sources has been studied in several salmonid species (Lee and Putnam, 1973; Reinitz et al., 1978; Takeuchi et al., 1978; Pieper and Pfeffer, 1980; Kaushik and de Oliva Teles, 1985; Kaushik et al., 1989; Kim and Kaushik, 1992; Garcia-Riera et al., 1993; Brauge et

al., 1994, 1995; Hemre et al., 1995). Suggested levels of energy and protein in diets for salmonids are 14 - 17 MJ of digestible energy (DE)·kg^{-1} diet, with 4 - 7 MJ coming from lipid, and 22 - 25 g digestible protein (DP)·MJ^{-1} DE (310 - 430 g DP·kg^{-1} diet) (Cho and Kaushik, 1990).

The following study was undertaken to estimate the optimal dietary ratio of DP to DE (g·MJ^{-1}) for rearing Atlantic salmon in seawater over a size range of 0.5 - 1.2 kg. The accomplishment of this goal required conducting both a growth experiment and a digestibility experiment.

MATERIALS AND METHODS

Experimental diets

Nine experimental diets were formulated to contain 360, 410, or 460 g of DP·kg^{-1} dry matter (DM) (Table 15). In all diets, herring meal supplied 80% of the dietary protein. The ratios of feedstuffs supplying protein in the diets were kept constant across each protein level to maintain equivalent amino acid ratios. Digestible lipid levels in the diets were adjusted to be 180, 230, or 280 g·kg^{-1} DM at each protein level. All diets were manufactured by an extrusion processing (Pro Form Feeds Inc., Chilliwack, BC) and the supplemental lipid (menhaden oil) was added by spraying on the required amount after extrusion. Two sizes of pellets, 5.0 and 6.5 mm, were manufactured for each diet, so that particle size suited fish size during the study. Each diet was formulated to contain 50 mg of canthaxanthin·kg^{-1} DM to determine whether alterations in the dietary protein to lipid ratio would influence the extent of canthaxanthin deposition in the flesh of the salmon. For diet formulation, the apparent digestibility coefficients (ADC, %) used for crude protein (CP), lipid, and gross energy (GE) were,

Table 15. Ingredient levels (g·kg^{-1} DM) and calculated and analysed nutrient and energy levels (g or MJ·kg^{-1} DM) in diets used to evaluate the optimal dietary ratio of digestible protein to digestible energy for rearing Atlantic salmon in seawater.

Ingredients	Diets								
	1	2	3	4	5	6	7	8	9
Herring meal, steam dried[1]	427.0	427.0	427.0	486.3	486.3	486.3	545.6	545.6	545.6
Wheat middlings[2]	72.2	72.2	72.2	82.2	82.2	82.2	92.3	92.3	92.3
Poultry by-product meal[3]	106.1	106.1	106.1	120.9	120.9	120.9	135.6	135.6	135.6
Pregelatinized wheat starch	246.9	194.3	141.6	176.1	123.5	70.6	105.3	52.7	0.0
Menhaden oil	114.7	167.3	220.0	104.3	156.9	209.8	93.9	146.5	199.2
Mineral premix[4]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin premix[5]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
DL-Methionine	4.0	4.0	4.0	4.6	4.6	4.6	5.2	5.2	5.2
Ascorbyl polyphosphate (15%)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Choline chloride (70%)	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Calcium phosphate, dibasic	7.0	7.0	7.0	3.5	3.5	3.5	0.0	0.0	0.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated values									
Crude protein	398	398	398	453	453	453	509	509	509
Lipid	190	242	295	190	242	295	190	242	295
Digestible crude protein	360	360	360	410	410	410	460	460	460
Digestible lipid	180	230	280	180	230	280	180	230	280
Digestible energy	19.7	21.0	22.2	19.9	21.2	22.4	20.2	21.4	22.7
Digestible protein to energy ratio	18.3	17.2	16.2	20.6	19.4	18.3	22.8	21.5	20.3
Chemical analyses									
Dry matter (g·kg^{-1} As fed)	917	928	935	932	940	948	941	948	949
Protein	399	373	366	409	404	401	483	449	470
Lipid	222	275	278	234	262	313	222	267	308
Starch	279	261	257	265	222	176	184	163	128
Ash	96	82	80	89	86	87	100	96	92
Gross energy	23.6	23.9	23.9	23.5	24.0	24.9	26.5	24.1	25.6

[1] Herring meal contained by analysis (g·kg^{-1} as-fed): DM, 955; crude protein, 705; lipid, 127; gross energy, 21.0 MJ·kg^{-1}.
[2] Wheat middlings contained by analysis (g·kg^{-1} as-fed): DM, 894; crude protein, 133; lipid, 36.1; gross energy, 17.6 MJ·kg^{-1}.
[3] Poultry by-product meal contained by analysis (g·kg^{-1} as-fed): DM, 961; crude protein, 632; lipid, 138; gross energy, 20.4 MJ·kg^{-1}.
[4] The mineral premix provided (mg compound·kg^{-1} dietary DM):$MnSO_4 \cdot H_2O$, 123; $FeSO_4 \cdot 7H_2O$, 249; $CuSO_4 \cdot 5H_2O$, 39.4; $ZnSO_4 \cdot 7H_2O$, 330; KI, 6.5; Na_2SeO_3, 0.66; $CoSO_4$, 13; NaF, 10.0.
[5] The vitamin premix provided (mg or IU·kg^{-1} dietary DM):vitamin A, 2500; vitamin D_3, 2400; vitamin E, 250; vitamin K (menadione sodium bisulphite), 5; thiamin, 20; riboflavin, 50, Ca d-pantothenate, 80; biotin, 1; folic acid, 15; vitamin B_{12}, 0.05; niacin, 200; pyridoxine, 50; myo-inositol, 400, canthaxanthin, 50.

respectively, 91.3, 95.0, and 95.0 for herring meal; 85.7, 95.0, and 89.0 for poultry by-product meal; and 91.8, 94.2, and 60.7 for wheat middlings (S.P. Lall, unpublished results for Atlantic salmon in seawater). The pregelatinized wheat starch was assumed to be 75% digestible, providing 12.9 MJ DE·kg^{-1}. The menhaden oil was assumed to be 95.0% digestible, providing 37.5 MJ DE·kg^{-1}. The diets were formulated to contain equal amounts of phosphorus at 7.5 g·kg^{-1} DM and required levels of other minerals and vitamins (NRC, 1993). The phosphorus level of 7.5 g·kg^{-1} DM was chosen as it represented the amount provided in the diets of highest protein content, which contained no supplemental phosphorus. The methionine content in all diets was adjusted to be 40 g·kg^{-1} DP.

Animal facilities and experimental protocols

A group of 550 Atlantic salmon maintained for one winter in aerated ambient seawater at the West Vancouver Laboratory (Department of Fisheries and Oceans, West Vancouver, Canada) was used in the experiment. Until the beginning of the experiment, the fish were fed by hand twice daily a commercial Atlantic salmon diet (Moore-Clark, Vancouver, Canada). Rearing facilities and the experimental protocols for the fish had the approval from the animal care committee.

Growth experiment

A total of 459 Atlantic salmon smolts (initial weight, 550 g ± 89 g, mean ± sd) was randomly and equally distributed into 27 oval fibreglass tanks (approximately 1100 l). The tanks were supplied with running (15 l·min^{-1}), filtered, ambient seawater. The natural photoperiod was followed (June 3 - November 28, 1994, Vancouver, Canada,

49°15'N 123°10'W). Fish were allowed to acclimate to the tanks for four wks before the start of the experiment. During this initial period, the fish were fed by hand to satiation twice daily with a commercial Atlantic salmon diet (Moore-Clark, Vancouver, Canada). The water temperature ranged from 8.2°C to 14.2°C during the experimental period (July 28 - November 28) and the average temperature was 10.5 ± 0.5°C (mean±sd). Salinity of the water during this period ranged from 26‰ to 32‰ with an average of 28‰. Oxygen saturation in the water was maintained above 85% with the aid of supplemental aeration in each tank.

The experimental design was a two-way randomised block, with nine tanks per block. Within each block, the nine diets were randomly assigned.

All fish were individually identified by injecting each fish intraperitoneally with a passively induced transponder (PIT) tag (2 mm diameter x 10 mm length, Canadian BioSonics Ltd., Sardis, BC). Individual lengths and weights were taken after each 4-wk period during the 16-wk study. All fish were fed their prescribed diets by hand twice daily to satiation. The 5.0 mm pellets were fed for four wks and the 6.5 mm pellets thereafter. Feed consumption and mortality records were kept for each 4-wk period.

At the beginning of the experiment, five fish were selected at random, killed, and stored at -20°C pending whole body proximate analysis. At the end of eight and 16 wks, two and three fish, respectively, whose growths had been followed by aid of the PIT tags, were removed from each tank and killed. Subsequently, the fish were stored at -20°C pending whole body proximate analysis. Before sampling the fish, feed was withdrawn for 24 h.

Fish growth and performance were described and assessed by the following variables: initial and final absolute weights, weight gains, specific growth rates (SGR,

%·day^{-1}), dry feed consumption (g·kg^{-1} body weight·day^{-1}), feed to gain ratios (dry feed consumption (g)·wet weight gain (g)$^{-1}$), protein efficiency ratios (PER) (wet weight gain (g)·protein consumption (g)$^{-1}$). SGR was calculated as (ln(final weight (g)) - (ln(initial weight (g))·100·number of experimental days^{-1}. Other variables used to calculate dietary protein and energy utilization were protein productive value (PPV, %) and gross energy utilization (GEU, %). PPV was calculated as body protein gain·100·protein intake^{-1}. GEU was calculated in a similar fashion except intake and gain of gross energy were followed.

In addition to the above, at the beginning of the experiment five fish in total were selected and killed and at the end of the experiment three fish from each tank were selected at random and killed. Subsequently, for the two groups of fish, the liver from each fish was rapidly removed, weighed, frozen in liquid nitrogen, and stored at -70°C pending glycogen analysis. The remaining portion of each fish was gutted and weighed, and then the fillets were removed, vacuum packed, and stored at -20°C until analysed. Estimates of hepatosomatic index (HSI, %) and dressing percentage were obtained for each fish. HSI was calculated as the liver weight·100·total fish weight^{-1}. Dressing percentage was calculated as eviscerated carcass weight, including gills, ·100·total fish weight^{-1}. Liver and white muscle samples from each of these fish were analysed for glycogen content. The fillets were used to evaluate the effects of different ratios of dietary protein to lipid on the extent of canthaxanthin deposition in the flesh. Also, the influence of these dietary variables on the fatty acid composition of the flesh lipids was followed. These results will be reported elsewhere (Chapter Five).

Digestibility experiment

A total of 360 Atlantic salmon (71.0 ± 14.6 g, mean ± sd) was randomly and equally allotted to 18 modified "Guelph" digestibility tanks (Hajen et al., 1993a). Each tank was supplied with running (5 - 6 $l \cdot min^{-1}$), filtered, ambient seawater. Water temperature during the experimental period ranged from 10.0°C to 12.4°C (average 10.8 ± 0.14°C sd). The natural photoperiod was followed (August, Vancouver, Canada, 49°15'N 123°10'W). The seawater salinity ranged from 28‰ to 32‰ and dissolved oxygen was maintained above 85% saturation. Fish were allowed to acclimate to the tanks for four wks, during which time they were maintained on a commercial diet. The nine test diets (the 6.0 mm pellets from the growth trial, crumbled by hand to a 3.0 mm size) were assigned randomly to duplicate groups of fish and the fish were fed for three days before collection of faeces began.

Faeces were collected for five days according to the procedures of Hajen et al. (1993a). Faeces were freeze-dried and analysed for moisture, ash, CP, starch, and gross energy by the methods described below. Hydrolysis-resistant organic matter (HROM) was used as the indicator in the diets and faeces. The ADC's for CP, starch, and GE were calculated by the indicator method as described by Maynard and Loosli (1969).

Chemical analyses

The diets (both sizes of pellets) and freeze-dried faeces were analysed in duplicate for dry matter (Williams, 1984), CP (N% X 6.25) (micro-Kjeldahl Technicon AutoAnalyzer, industrial method no. 334-74W/B), ash (Williams, 1984) and the GE content was determined by calorimetry (adiabatic bomb calorimeter). The starch

content in the diets and faeces was determined by the method of Thivend et al. (1972) and HROM was measured by the method of Crampton and Maynard (1938) as modified by Buddington (1980). The lipid content of the diets was measured by the method of Bligh and Dyer (1959).

The individual whole fish, from each sampling period, i.e. initial, eight wks (middle), and 16 wks (final), were partly thawed, chopped into pieces, and thoroughly homogenized in a food processor (Braun® K1000, type 3210). Samples of the homogenate were then analysed in duplicate for proximate composition by the methods described above. Samples of the homogenate, for the initial and final fish only, were freeze-dried and then the dried material was used for determination of GE content. Residual moisture in the freeze-dried material was also measured to correct GE values to 100% DM. Samples of muscle and liver were analysed for glycogen content by the method of Murat and Serfaty (1974), except that glucose was measured by the enzymatic hexokinase reaction (Kit 16-100, Sigma, St. Louis, MO).

Statistical analyses

The fish performance data (absolute weights, weight gains, feed consumption, SGR, PER, and feed to gain ratios) were analysed by a repeated measures ANOVA model to evaluate the responses over time. The repeating variable was measured in each 4-wk period (four periods totalling 16 wks) with the main effects of protein level, lipid level, and block (Neter et al., 1985). The interaction term of protein level by lipid level was included in the ANOVA. For absolute weights, there were five measurements taken and all were used in the repeated measures ANOVA. Individual fish that had values for absolute weights, weight gains, and SGR for each period were analysed by

the repeated measures ANOVA (n was approximately 25 for each dietary treatment).

In addition to the above, the values for SGR, feed consumption, PER, and feed to gain ratio were calculated for the entire experimental period (112 days) and analysed by ANOVA, with the same main effects and interactions as described above and the responses were averaged on a tank basis (n=3 for each dietary treatment). When significant effects were found ($P<0.05$), differences among treatment means were further assessed by the Student-Newman-Keuls multiple comparison procedure ($P=0.05$) (Kleinbaum et al., 1988).

The whole body proximate composition data were analysed by analysis of covariance. The data set used for statistical analysis contained the proximate composition data for the initial, middle, and final samplings of fish (n=20 for each diet). 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 covariate, was performed (Shearer, 1994). The main effects in the ANOVA were protein level, lipid level, sampling day (0, 56, or 112), and block. The interaction terms for protein level by lipid level, protein level by day, and lipid level by day were included in the ANOVA model. When significant effects were found ($P<0.05$), differences among treatment means were further assessed by the Student-Newman-Keuls multiple comparison procedure ($P=0.05$) (Kleinbaum et al., 1988).

The GE content of the whole fish bodies for the final sampling time only, and estimates for PPV, GEU, HSI, dressing percentage, and muscle and liver glycogen concentrations were analysed by ANOVA (n=9 for each diet). The main effects in the ANOVA were protein level, lipid level, and block. The interaction term for protein level by lipid level was included in the ANOVA model. When significant effects were found

($P<0.05$), differences among treatment means were further assessed by the Student-Newman-Keuls multiple comparison procedure ($P=0.05$) (Kleinbaum et al., 1988).

ADC's were analysed by ANOVA (n=2 for each diet). The main effects in the ANOVA were protein level and lipid level and their interaction term was included in the analysis. When significant effects were found ($P<0.05$), differences among treatment means were further assessed by the Student-Newman-Keuls multiple comparison procedure ($P=0.05$) (Kleinbaum et al., 1988).

In all statistical analyses, data outliers and homogeneity of variance were assessed. Outliers were determined by analysis of studentized residuals from the respective ANOVA's and significant outliers were removed from the data sets. If homogeneity of variance was not met, the data were transformed by taking the square root before the ANOVA was performed. The pooled standard deviation was calculated from the error mean square of the ANOVA for an estimate of the overall variation of the parameter. Untransformed means are provided in all tables. Statistical analyses were performed using Systat (Ver. 4.0, Systat, Inc., 1800 Sherman, Ave., Evanston, IL).

RESULTS AND DISCUSSION

The proximate composition of the two pellet sizes for each diet were not appreciably different. Consequently, the levels of proximate constituents in the 6.0 mm diets are shown in Table 15 (page 90). The determined amounts of DP in the diets ranged from 326 $g \cdot kg^{-1}$ DM to 446 $g \cdot kg^{-1}$ DM and the DE contents ranged from 20.4 $MJ \cdot kg^{-1}$ DM to 23.9 $MJ \cdot kg^{-1}$ DM (Table 16). Unfortunately, in many instances, the determined DP levels were not as close to the calculated values as expected, thus a linear increase in DP was not achieved and a very narrow range for the DP to DE ratio

Table 16. Apparent digestibility coefficients (%), digestible protein and energy levels (g or MJ·kg^{-1} DM), and the digestible protein to digestible energy ratios (g·MJ^{-1}) for diets used to evaluate the optimal dietary ratio of digestible protein to digestible energy for rearing Atlantic salmon in seawater.

Diet	Apparent digestibility of crude protein	Apparent digestibility of starch	Apparent digestibility of gross energy	Digestible protein	Digestible energy	Digestible protein to energy ratio
1	91.0a	77.1a	87.9a	363	20.7	17.5
2	90.6ab	75.6a	86.8b	338	20.7	16.3
3	89.2c	73.7a	85.2c	326	20.4	16.0
4	90.4b	78.5a	87.2d	370	20.5	18.0
5	89.1c	79.0a	86.4e	360	20.7	17.4
6	87.3d	76.8a	84.6f	350	21.1	16.6
7	92.3e	81.4a	90.0g	446	23.9	18.7
8	91.0a	81.2a	89.5h	409	21.6	18.9
9	91.1a	78.7a	88.6i	428	22.7	18.9
Pooled sd[1]	0.2	1.3	0.2	-----	-----	-----
			Main effects			
Protein level[2]						
1 (342:258)	90.3a	75.5a	86.6a	-----	-----	-----
2 (360:270)	88.9b	78.1b	86.1b	-----	-----	-----
3 (428:266)	91.5c	80.4c	89.4c	-----	-----	-----
Lipid level[2]						
1 (393:226)	91.2a	79.0a	88.4a	-----	-----	-----
2 (369:268)	90.2b	78.6a	87.6b	-----	-----	-----
3 (368:300)	89.2c	76.4b	86.2c	-----	-----	-----

[1] Pooled standard deviation (square root of the error mean square).

[2] The determined dietary digestible protein:lipid levels (g·kg^{-1} DM) are shown in parentheses.

Means within a column sharing a common postscript letter are not significantly different (P>0.05). Columns without postscript letters were not analysed statistically.

that the ADC for lipid decreased from 81.5 to 76.8% (although not significantly) with an increase in lipid from 104 to 144g $\cdot kg^{-1}$ DM. Further, increasing dietary lipid levels have been shown to decrease gastric evacuation rates of lipid (Windell et al., 1972). Other work (reviewed by Fänge and Grove, 1979) has also detected decreased gastric evacuation rates with less digestible foodstuffs. The findings of Cho and Kaushik (1990) suggest that as the melting point of a fat increases the digestibility decreases. Also, work by Sigurgisladottir et al. (1992) has shown that an increase in the saturation of a fatty acid decreases its digestibility. Although not measured, the digestibility of the lipid fraction of the diets may have been inversely related to its dietary concentration. Menhaden oil contains a higher amount of saturated fatty acids (approximately 40%) than found in the oil from herring, capelin, or mackerel (approximately 25%) or anchovy, pilchard or sardines (approximately 35%) (Ackman, 1980, 1982; Windsor and Barlow, 1981). This would explain the trend for reduced dietary energy digestibility coefficients as the lipid levels in the diets were increased.

Fish in all groups had statistically equivalent mean weights at the beginning of the study. Those fed diet 5 (360 g DP and 17.4g DP$\cdot MJ^{-1}$ DE) had the highest absolute mean weight by the end of the study (Table 17). These fish also had the highest average weight gain, although this was not significantly different from the gains noted for fish fed diets 1, 3, or 9. SGR's of the fish were not significantly influenced by dietary treatment and values ranged from 0.63%$\cdot day^{-1}$ to 0.70%$\cdot day^{-1}$.

Feed consumptions (g DM$\cdot kg^{-1}$ body weight$\cdot day^{-1}$) were influenced by dietary lipid levels (Table 17). Fish fed diets containing intermediate lipid levels (268 g$\cdot kg^{-1}$ DM, diets 2, 5, and 8) also had significantly lower intakes (mean = 7.77 g$\cdot kg^{-1}$ body weight$\cdot day^{-1}$) than those fed the diets with the other two lipid levels (average intake of

Table 17. Growth and other aspects of performance of Atlantic salmon reared in seawater when fed diets containing different ratios of digestible protein to lipid.

Diet[1]	Initial weight ($g \cdot fish^{-1}$)	Final weight ($g \cdot fish^{-1}$)	Weight gain ($g \cdot fish^{-1}$)	SGR ($\%\cdot day^{-1}$)	Dry feed consumption ($g \cdot kg^{-1}$ body weight$\cdot day^{-1}$)	Feed to gain ratio	PER	Mortality ($n \cdot diet^{-1}$)
1 (363:222)	527.4	1158.5ab	631.1ab	0.70	8.23b	1.27	1.82a	12
2 (338:275)	581.6	1182.5ab	600.8bcd	0.70	8.68b	1.46	1.71a	15
3 (326:278)	569.7	1189.7ab	620.0abc	0.68	9.00b	1.42	1.85a	15
4 (370:234)	549.4	1100.3b	550.9c	0.68	8.43b	1.43	1.60a	15
5 (360:262)	570.8	1261.0a	680.6a	0.65	7.24b	1.15	2.03a	9
6 (350:313)	531.8	1130.2ab	598.4bcd	0.64	9.12b	1.43	1.71a	15
7 (446:222)	566.1	1181.6ab	607.5bcd	0.64	11.08a	1.68	1.18b	27
8 (409:267)	543.2	1109.5b	566.3cd	0.63	7.37b	1.23	1.72a	12
9 (428:308)	546.0	1193.3ab	652.8ab	0.63	7.70b	1.24	1.65a	12
Pooled sd[2]	89.0	194.6	167.2	0.13	1.10	0.19	0.20	----
Protein level	NS[3]	NS	NS	NS	NS	NS	P<0.05	----
Lipid level	NS	NS	NS	NS	P<0.05	NS	p<0.05	----

[1] The determined dietary digestible protein:lipid levels ($g \cdot kg^{-1}$ DM) are shown in parentheses.

[2] Pooled standard deviation (square root of the error mean square).

[3] NS (not significant, P>0.05).

SGR = (ln(final weight (g)) - ln(initial weight (g))·100·days on experiment^{-1}.

Dry feed consumption = mean daily dry feed consumption per fish·1000·mean body weight^{-1}.

Feed to gain ratio = dry feed consumption (g)·wet weight gain (g)$^{-1}$.

PER = wet weight gain (g)·protein consumed (g)$^{-1}$.

Means within a column that do not have or share a common postscript letter are not significantly different (P>0.05).

9.25 g·kg^{-1} body weight·day^{-1}, diets 1, 4, and 7 and 8.60 g·kg^{-1} body weight·day^{-1}, diets 3, 6, and 9). The higher feed consumption noted for fish fed diet 7 was the one exception to the above trend and it is also noteworthy that this diet also contained the highest DE level. The above findings cannot be readily explained by differences between diets in DE content or in ratios of DP to DE. Juvenile salmonids generally consume organoleptically acceptable diets to satisfy energy demands, but the feed consumption of salmon in this study did not bear any consistent relationship to dietary DE content. To confuse matters more, for fish maintained on diets with the lowest protein level (diets 1, 2, and 3) there appears to be a trend for increasing feed consumption with increasing lipid levels, although there were no apparent differences in the DE content of any of these diets. Kaushik and Médale (1994), in a review, presented data that suggest voluntary feed consumption is influenced by DE levels, although they also state that more precise and reliable studies are required.

Salmon fed diet 5 exhibited the lowest feed to gain ratio (1.15) and the highest PER value (2.03), although these values were not significantly ($P>0.05$) different from those groups fed many of the other diets.

Some of the trends for growth, feed consumption, and other aspects of performance in relation to dietary treatments may have been more clear-cut if fish mortality (Table 17) had been lower, although these parameters were adjusted for the mortalities. The cause of the high mortality was related to a subclinical infection with furunculosis (Aeromonas salmonicida), which the stress of periodic weighing exacerbated.

Diets containing varying amounts of DP and lipid had a significant ($P<0.05$) influence on whole body proximate compositions (Table 18). Fish fed diets with the

Table 18. The effect of feeding diets containing different ratios of digestible protein to lipid on the whole body composition (g or $MJ \cdot kg^{-1}$ wet basis) of Atlantic salmon reared in seawater.

	Moisture	Crude protein	Lipid	Ash	Gross energy
		Initial sample fish			
	715	185	79	25.2	7.33
Diet[1]		Middle (56 days) sample fish			
1 (363:222)	706ab	184	98bc	22.7	----
2 (338:275)	695abc	180	108abc	22.7	----
3 (326:278)	690c	180	116a	24.1	----
4 (370:234)	699abc	183	101bc	21.7	----
5 (360:262)	698ab	182	102c	21.7	----
6 (350:313)	699abc	179	110ab	23.6	----
7 (446:222)	705a	182	91c	21.3	----
8 (409:267)	709ab	180	93bc	21.1	----
9 (428:308)	693b	180	103bc	24.8	----
Diet[1]		Final (112 days) sample fish			
1 (363:222)	685ab	177	114d	20.0	8.83
2 (338:275)	681cd	178	124bc	23.1	8.94
3 (326:278)	661e	177	141a	18.8	9.28
4 (370:234)	681bc	181	115cd	19.9	8.62
5 (360:262)	673cd	179	136abc	20.1	9.21
6 (350:313)	668de	175	138ab	22.7	9.28
7 (446:222)	689a	182	109d	22.3	8.62
8 (409:267)	680ab	178	117cd	22.6	8.67
9 (428:308)	669de	177	130abc	21.3	9.13
Pooled sd	10	10	11	11.5	1.17

[1] The determined dietary digestible protein:lipid levels ($g \cdot kg^{-1}$ DM) are shown in parentheses.

[2] Pooled standard deviation (square root of the error mean square).

Means within a column, for each sampling period, that do not have or that share a common postscript letter are not significantly different ($P>0.05$).

Table 18 (Con't). The effect of feeding diets containing different ratios of digestible protein to lipid on the whole body composition (g or MJ·kg^{-1} wet basis) of Atlantic salmon reared in seawater.

	Moisture	Crude protein	Lipid	Ash	Gross energy
Main effects					
Protein level[1]					
1 (342:258)	701a	182a	99a	23.4a	7.96a
2 (360:270)	701a	182a	99a	23.4a	7.94a
3 (428:266)	702b	182a	94b	23.7a	7.87a
Lipid level[1]					
1 (393:226)	704a	183a	92a	23.2a	7.82a
2 (369:268)	702b	182ab	97b	23.5a	7.91a
3 (368:300)	698c	181b	102c	23.7a	8.04b
Day					
1 (0)	715a	185a	79a	25.2a	----
2 (56)	699b	181a	102a	22.6b	----
3 (112)	677c	178a	125b	21.2c	----
Interaction terms					
PL*LL	P<0.05	NS[2]	NS	NS	NS
PL*day	P<0.05	NS	P<0.05	NS	----
LL*day	P<0.001	NS	P<0.001	NS	----

[1] The determined dietary digestible protein:lipid levels (g·kg^{-1} DM) are shown in parentheses.

[2] NS (not significant, P>0.05).

Means within a column sharing a common postscript letter are not significantly different (P>0.05).

highest DP levels had lower whole body lipid levels than those fed diets of lower DP content. Fish fed diets with increasing lipid levels showed a trend towards increasing whole body lipid levels and decreasing protein and moisture levels. Fish fed the diets containing the highest lipid level had significantly higher whole body GE levels than fish fed the diets with lower levels of lipid. At each sampling time (day 56 or 112) no specific diet influenced whole body protein content. A significant influence was seen in levels of whole body lipid at each sample time, with body lipid levels increasing during the entire experimental period. However, whole body moisture and ash levels were noted to decline. The foregoing trends generally mirrored those reported by other investigators who have conducted research on this theme (for a review see Shearer, 1994).

Dietary treatments did not significantly influence white muscle glycogen levels (Table 19). Increasing the dietary DP levels significantly ($P<0.05$) decreased liver glycogen levels and HSI values. The starch added to the diets to replace the protein energy was the cause of this effect. These effects have been noted by many authors (Lee and Putnam, 1973; Bergot, 1979; Lall and Bishop, 1979; Kim and Kaushik, 1992).

The highest level of DP in the diets significantly elevated the dressing percentages (Table 19). Fish fed the diets with the lowest lipid levels also showed this effect. In both cases, these groups of fish had leaner whole bodies, suggesting less fat deposited into the viscera. These findings support those of Hillestad and Johnsen (1994) who found that as dietary protein level was decreased and lipid level was increased the dressing percentage for post-smolt Atlantic salmon (600 g) declined.

PPV and GEU were calculated using average tank data (n=3) and data from the three fish selected from each tank (n=9) for proximate analysis. There was no significant difference between the values calculated by each method for any diet. Table

Table 19. The effect of feeding diets containing varying ratios of digestible protein to lipid on the muscle and liver glycogen levels, hepatosomatic index, dressing percentage, and the retention of dietary protein and energy of Atlantic salmon reared in seawater.

	Muscle glycogen ($mg \cdot g^{-1}$ as-is)	Liver glycogen ($mg \cdot g^{-1}$ as-is)	HSI %	Dressing percentage %	PPV %	GEU %
Initial	6.97	42	0.96	90.4	----	----
Diet[1]						
1 (363:222)	8.93	146b	1.98ab	87.3bc	43.7a	40.4a
2 (338:275)	7.40	191a	2.20a	86.0c	31.1ab	27.8ab
3 (326:278)	8.62	143b	1.92abc	87.0bc	32.1ab	30.3ab
4 (370:234)	8.97	151b	1.69bcde	87.2bc	34.1ab	29.9ab
5 (360:262)	9.20	115bc	1.78bcd	86.3c	43.4a	38.9a
6 (350:313)	7.65	137b	1.88abcd	86.6bc	34.3ab	32.8ab
7 (446:222)	8.62	94c	1.48de	88.8a	25.2b	22.9b
8 (409:267)	8.23	93c	1.53cde	88.3ab	37.2ab	35.8a
9 (428:308)	9.62	87c	1.34e	85.9c	29.5b	28.8ab
Pooled sd[2]	2.17	29	0.33	1.3	9.2	8.7
			Main effects			
Protein level[1]						
1 (342:258)	8.30	159a	2.03a	86.8a	35.6a	32.8
2 (360:270)	8.64	135b	1.78b	86.7a	37.3a	33.8
3 (428:266)	8.82	91c	1.45c	87.6b	30.6b	29.2
Lipid level[1]						
1 (393:226)	8.84	130	1.72	87.8a	34.3	31.0
2 (369:268)	8.28	132	1.82	86.9b	37.2	34.2
3 (368:300)	8.67	122	1.71	86.5b	31.9	30.7

[1] The determined dietary digestible protein:lipid levels ($g \cdot kg^{-1}$ DM) are shown in parentheses.

[2] Pooled standard deviation (square root of the error mean square).

Dressing percentage = eviscerated body weight (with gills on) (g)·100·total body weight $(g)^{-1}$.

HSI (hepatosomatic index) = liver weight (g)·100·total body weight $(g)^{-1}$.

PPV (protein productive value) = protein retained in the body (g)·100·protein consumed $(g)^{-1}$.

GEU (gross energy utilization) = gross energy retained in the body (MJ)·100·gross energy consumed $(MJ)^{-1}$.

Pooled sd = Square root of the error means square from the ANOVA.

Means within a column that do not have or that share a common postscript letter are not significantly different ($P>0.05$).

Therefore, the values presented (Table 19) were calculated using the individual fish values. Fish fed diets 1 and 5 had the highest protein retention (PPV = 43.7 and 43.4%, respectively) and gross energy retention (GEU = 40.4 and 38.9%, respectively), while those fed diet 8 were second in this regard (Table 19). Diets with intermediate DP levels (diets 4, 5, and 6) resulted in the best retention of protein, as did the intermediate lipid level (diets 2, 5, and 8), although differences were not significant. The values for PPV and GEU found for fish fed diets 1 and 5 in this study are higher than those noted by Silver et al. (1993) for chinook salmon (100 - 300 g) (PPV = 33.1% and GEU = 29.5%) reared in seawater even though similar SGR's were observed for both species. The results for Atlantic salmon in this study, however, are lower than those found by Hillestad and Johnsen (1994) for post-smolt Atlantic salmon (100 - 600 g). Fish in the latter study had PPV and GEU values of 51 and 47%, respectively, and likely the higher values reflect the restricted ration protocol that was used by Hillestad and Johnsen (1994). However, Hemre et al. (1995) working with Atlantic salmon (100 - 480 g) in seawater found PPV and GEU values of 49% and 52%, respectively when diets were fed ad libitum. By contrast, Brauge et al. (1994) working with rainbow trout in seawater (230 - 450 g) obtained PPV and GEU values of 28.8% and 33.4%, respectively.

One explanation for the differences in PPV's between studies can be attributed to the quality of the different protein sources that were employed. The protein sources in the various experimental diets ranged from blends of animal and plant meals (Silver et al., 1993), to herring meal (Brauge et al., 1994), Norse-LT94® (Hillestad and Johnsen, 1994), and to fresh fish fillets and squid mantle (Hemre et al., 1995). The quality of the protein used in the various test diets will undoubtedly have a direct effect on the extent of protein deposition (Cowey and Walton, 1989; McCallum and Higgs, 1989). Other

important factors affecting the PPV and GEU values found in the various studies include the source, level, and quality of the non-protein energy supplied.

CONCLUSIONS

The growth and the protein and energy utilization data in this study indicate that 17.4 g DP·MJ^{-1} DE in the diet is optimal for rearing Atlantic salmon in seawater over a size range of 0.5 kg to 1.2 kg. This amount of DP·MJ^{-1} DE can be supplied by a diet containing 360 g DP (404 g crude protein), 220 - 260 g lipid, and up to 220 g of pregelatinized starch, with 20.7 MJ DE (24.0 MJ gross energy) ·kg^{-1} DM. These conclusions are similar to those reached by Hillestad and Johnsen (1994) who found that a diet containing 440 g crude protein, 290 g lipid, and 24.9 MJ gross energy produced the best growth in Atlantic salmon over a size range of 100 g to 600 g.

With increasing dietary lipid levels there appears to be a decrease in the apparent digestibility other dietary components (CP, GE, and starch). This finding may be related to the fatty acid composition of the supplemental menhaden oil used in this study (high saturated fatty acid content). It would also appear under the conditions of this study that Atlantic salmon are able to tolerate up to 279 g starch·kg^{-1} DM without any detrimental effect on growth, although liver glycogen and HSI values were elevated. Additional research is required to establish acceptable dietary concentrations of digestible carbohydrate for culturing Atlantic salmon in seawater at different stages of the life history. Further, it is apparent that additional work, preferably of much longer duration, is needed to confirm and extend the results of the present study considering fish of even larger size than those used in this study.

CHAPTER FIVE - THE EFFECTS OF CHANGING THE DIETARY PROTEIN TO LIPID RATIO ON THE PROXIMATE COMPOSITION, FATTY ACID LEVELS, AND PIGMENTATION IN ATLANTIC SALMON FILLETS.

INTRODUCTION

The fatty acid composition of fish responds to complex influences, including nutritional status (diet composition and feed consumption), water salinity and temperature, stage of development, and spawning migration (Greene and Selivonchick, 1987; Higgs et al., 1995b). The gross fatty acid composition of edible salmon parts was the subject of an extensive study by Ackman (1989). Several other studies have investigated methods for influencing the n-3 fatty acid content of farmed salmon (Hardy and King, 1989, Kim et al., 1989; Turner et al., 1990; Bell et al., 1991, Waagbo et al., 1991, 1993; Polvi and Ackman, 1992).

Some plant oils, notably linseed, canola, and soybean, contain appreciable amounts of α-linolenic acid (18:3 n-3), but marine phytoplankton and zooplankton are rich sources of the longer chain (20 and 22 carbon) polyunsaturated fatty acids (PUFA). The role of PUFA's, especially 20:5 n-3 and 22:6 n-3, in human health is a controversial issue, but their essentiality in the human diet is being increasingly demonstrated and recognized (Singh and Chandra, 1988; Weaver and Holob, 1988; Lands, 1989; Innis, 1991; Nelson et al., 1991).

The first objective of this study was to investigate the influence of the dietary ratio of protein to lipid on the proximate composition and fatty acid profile of the total (polar and neutral) lipid in the fillets from Atlantic salmon reared in seawater. The second objective was to investigate the influence of the same dietary factors on the extent of

canthaxanthin deposition in the fillets of these fish.

MATERIALS AND METHODS

Experimental diets

Nine experimental diets were formulated to contain 360, 410, or 460 g of digestible protein (DP)·kg^{-1} dry matter (DM). At each protein level, digestible lipid levels were varied so that the diets contained 180, 230, or 280 g·kg^{-1} DM. All diets were manufactured by an extrusion process (Pro Form Feeds Inc., Chilliwack, BC) and the lipid level was adjusted by spraying on the required amount of fish oil after extrusion. Most of the lipid in the diets originated from herring meal and the supplemental marine oil (menhaden). Each diet was formulated to contain 50 mg of canthaxanthin·kg^{-1} DM. For complete details of the ingredients and chemical compositions of the diets see Table 15 (page 90).

Animal facilities and experimental protocols

A group of 550 Atlantic salmon that had been maintained for one winter in seawater at the West Vancouver Laboratory (Department of Fisheries and Oceans, West Vancouver, Canada) was used. The stock fish were maintained in two groups of approximately 275 fish that were each held in 4000 l tanks supplied with aerated, ambient seawater. Until the beginning of the experiment, the fish were fed by hand twice daily a commercial Atlantic salmon diet that did not contain supplemental carotenoid pigments (Moore-Clark, Vancouver Canada). Rearing facilities and the experimental protocols for the fish had the approval of the animal care committee.

Growth experiment

A total of 459 Atlantic salmon (weight 550 g ± 89 g (sd)) was randomly and equally distributed into 27 oval 1100 l fibreglass tanks. The tanks were supplied with running (15 $l \cdot min^{-1}$), filtered, ambient seawater. The natural photoperiod was followed (June 3 - November 28, 1994, Vancouver, Canada, 49°15'N 123°10'W). Fish were allowed to acclimate to the experimental tanks and conditions for four wks before the start of the experiment. During this initial period, the fish were fed by hand to satiation twice daily with the same commercial Atlantic salmon diet described above.

During the experimental period (July 28 - November 28), the water temperature ranged from 8.2°C to 14.2°C (mean ± sd,10.5 ± 0.5°C). Also, salinity ranged from 28‰ to 32‰ and dissolved oxygen remained above 85% saturation.

The experimental design was a two-way randomised block. A group of nine tanks was selected as a block. The nine diets were randomly assigned within each block.

All fish from each dietary treatment were individually identified with passively induced transponder (PIT) tags (2 mm diameter x 10 mm length, Canadian BioSonics Ltd., Sardis, BC) injected intraperitoneally. Individual lengths and weights of the fish were taken after each 4-wk period during the 16-wk study. All fish were fed their prescribed diets by hand twice daily to satiation. Records of feed consumption and mortality were kept for each 4-wk period. At the end of 16 wks, three fish from each tank, whose growths had been followed with the aid of the PIT tags, were killed and both fillets were removed. Fillets were vacuum packed and retained at -20°C pending determination of proximate constituents (moisture, lipid, CP, and ash) according to the procedures described below. Fish growth was evaluated using the following variables:

initial and final absolute wet weights, weight gains, and specific growth rates (SGR). SGR (%·day^{-1}) was calculated as follows: (ln(final weight (g)) - (ln(initial weight (g))·100·number of experimental days^{-1}. For complete details of the effects of the dietary treatments on fish performance and whole body proximate compositions, and for an estimate of the optimal dietary ratio of digestible protein to digestible energy for rearing the Atlantic salmon employed in this study in seawater see Chapter Four.

Chemical analyses

The skin was removed from both fillets from each fish. Then the fillets were combined and homogenized. Each of the fillet homogenates was analysed in duplicate for their content of dry matter (Williams, 1984), crude protein (CP) (N% X 6.25) (micro-Kjeldahl Technicon AutoAnalyzer, industrial method no. 334-74W/B), ash (Williams, 1984), and lipid (Bligh and Dyer, 1959).

The fatty acid compositions of the diets and fillets were determined by gas chromatography (Varian 3400 gas chromatograph) after base-catalyzed (0.5M $NaOCH_3$:CH_3OH) transmethylation (Christie, 1982; Medina et al., 1992). A fused silica capillary column (SP-2330, 30 m x 0.25 mm i.d., Supleco Canada, 1300 Aimco Blvd. Mississauga, ON) was used for fatty acid determinations under the following conditions: the initial temperature was set at 150°C for three min and was then increased at 2°C·min^{-1} to 170°C, where it was held for one min and subsequently increased at 3°C·min^{-1} to 210°C and maintained for 9.5 min. The detector was set at 240°C, and helium gas at 1 ml·min^{-1} was used as the carrier. An automated injector was used in the split mode (100/1) and heated to 220°C. Quantification of the areas for the fatty acids was performed by a Varian 4290 Integrator.

Canthaxanthin in the diets and fillets was separated and quantified by HPLC. The lipid portion, containing carotenoid pigments, of a 0.5 g sample of diet or 2 g sample of fillet (white muscle only) was extracted by the method of Bligh and Dyer (1959) and used for canthaxanthin analysis. After extraction, the solvent was evaporated and the remaining lipid was diluted in 1 ml of hexane and this was used directly for analysis. For canthaxanthin analysis, a 10 μl portion for diets or a 50 μl portion for fillets was injected onto a silica column (μPorasil, 3.9 mm x 30 cm, Millipore Corp., Milford, MA) that had been previously treated with ortho-phosphoric acid in methanol (1 g·100 ml^{-1}). Canthaxanthin was eluted with a solution of hexane:acetone (86:14 v/v) delivered at 1 ml·min^{-1}. The absorbency of this pigment was determined at 472 nm. For quantification, pure canthaxanthin (Hoffmann-La Roche Ltd., Mississauga, ON) was used as the standard. Relative amounts were calculated by determining the area of each absorbency peak.

Statistical analyses

Dietary fatty acid levels were analysed by two-way ANOVA. Dietary protein and lipid levels constituted the main effects and the interaction of these two effects was included in the ANOVA model.

Fillet fatty acid levels, fish growth data (absolute weights, weight gains, and SGR), and fillet pigment levels were analysed by a two-way randomised block ANOVA. Dietary protein level, lipid level, and block were the main effects (Neter et al., 1985). The interaction term (protein level by lipid level) was also included in the ANOVA model.

Since there was no dietary effect on final fish weights or weight gains, the fillet composition data, on a wet basis, were analysed with the same ANOVA model that was

used for the fish performance data (Shearer, 1994).

In cases where homogeneity of variance was not observed, the data were transformed by taking the square root before the ANOVA was performed. When the ANOVA detected a significant main effect ($P<0.05$), the differences among treatments means were further evaluated by Student-Newman-Keuls multiple comparison procedure ($P=0.05$). The pooled standard deviation was calculated from the error mean square of the ANOVA for an estimate of the overall variation of the parameter. Untransformed means are provided in all tables. Statistical analyses were performed using Systat (Ver. 4.0, Systat, Inc., 1800 Sherman, Ave., Evanston, IL).

RESULTS AND DISCUSSION

Among the diets, there were significant differences in the percentages of some of the fatty acids (Table 20). With the increase in the amount of protein supplied by the herring meal, there was a concomitant increase in the amount 18:1 n-9. The addition of menhaden oil significantly reduced the levels of 18:1 n-9 and 18:2 n-6 in the diets and affected the ratios of n-6/n-3 in the diets. On the other hand, the addition of the menhaden oil increased the levels of 20:5 n-3. There was a significant interaction effect on dietary levels of 18:1 n-9 and 18:2 n-6. Since the herring meal was of West Coast origin, it contained high levels of 18:1 n-9 and low levels of the 20:1 and 22:1 series (Table 6, page 62). Almost all 20:1 and 22:1 fatty acids in marine fish oils are of exogenous origin (Ackman, 1982). The lipid component of the herring meal consists mostly of triglycerides with a significant portion supplied by phospholipids, whereas triglycerides would be the major lipid class supplied by the added dietary menhaden oil. Differences exist between the fatty acid composition of individual phospholipids but the

Table 20. The fatty acid levels (% of total fatty acids) of diets containing different ratios of digestible protein to digestible lipid ($g \cdot kg^{-1}$ DM).

	Diets[1]									Pooled
	1	2	3	4	5	6	7	8	9	sd[2]
Digestible protein	360	360	360	410	410	410	460	460	460	
Digestible lipid	180	230	280	180	230	280	180	230	280	
Fatty acid										
14:0	15.3	15.6	14.3	15.4	14.1	14.3	14.9	14.6	15.5	0.59
15:0	0.6	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.6	0.03
16:0	24.0	23.4	23.3	23.9	23.0	22.7	23.6	22.7	23.8	0.84
18:0	2.7	2.4	2.6	2.6	2.9	2.6	2.4	2.4	2.5	0.24
Total saturates	42.6	42.0	40.8	42.4	40.6	40.2	41.5	40.3	42.4	1.18
16:1 n-7	14.2	14.1	13.8	13.5	12.9	13.2	13.3	13.3	13.2	0.83
18:1 n-9	10.9 a	9.9 b	9.8 b	10.9 a	10.7 a	9.9 b	10.9 a	10.8 a	10.1 ab	0.26
18:1 n-7	4.0	3.9	3.9	4.1	4.0	3.8	4.0	4.0	3.9	0.19
20:1 n-9	1.5	1.6	1.4	1.6	1.8	1.4	1.4	1.5	1.5	0.27
22:1 n-11	0.6	0.7	0.7	0.6	0.7	0.7	0.5	0.6	0.6	0.09
Total monounsaturates	31.2	30.2	29.6	30.7	30.1	29.0	30.1	30.2	29.3	0.87
18:2 n-6	2.1 a	1.8 bc	1.6 c	2.1 a	1.9 abc	1.7 c	1.8 bc	2.0 ab	1.8 bc	0.08
18:3 n-6	0.1	0.1	0.3	0.5	0.4	0.3	0.4	0.3	0.1	0.14
18:3 n-3	0.6	0.5	0.6	0.5	0.6	0.6	0.5	0.6	0.6	0.04
18:4 n-3	1.5	1.5	1.5	1.4	1.5	1.4	1.4	1.4	1.6	0.29
20:4 n-6	1.0	0.8	1.1	1.0	1.0	1.0	0.8	0.9	1.0	0.15
20:5 n-3	10.4	10.7	11.0	10.2	10.8	11.2	9.8	10.5	10.5	0.40
22:4 n-6	0.4	0.5	0.5	0.5	0.4	0.5	0.4	0.4	0.5	0.06
22:5 n-3	1.0	0.8	1.1	0.9	1.2	1.3	1.1	1.1	1.0	0.13
22:6 n-3	3.1	3.5	3.5	3.3	3.7	3.5	3.6	4.1	3.5	0.30
Total polyunsaturates	20.2	20.2	21.2	20.4	21.5	21.5	19.8	21.3	20.6	0.70
Total n-6	3.6	3.2	3.5	4.1	3.7	3.5	3.4	3.6	3.4	0.29
Total n-3	16.6	17.0	17.7	16.3	17.8	18.0	16.4	17.7	17.2	0.81
n-6/n-3	0.22	0.19	0.20	0.25	0.21	0.19	0.21	0.20	0.20	0.02
unsat/sat	1.21	1.20	1.25	1.20	1.27	1.26	1.20	1.28	1.18	0.03

[1] Refer to Chapter Four for details of the determined levels of digestible protein and lipid in the diets.

[2] Pooled standard deviation calculated from the error mean square from the ANOVA.

Means (n=2) within a row that share a common postscript letter are not significantly different (P>0.05).

Table 20 (Con't). The fatty acid levels (% of total fatty acids) of diets containing different ratios of digestible protein to digestible lipid ($g \cdot kg^{-1}$ DM).

Fatty acid	Main effects[3]		
	Protein level		
	360	410	460
18:1 n-9	10.2 a	10.5 b	10.6 b
	Lipid level		
	180	230	280
18:1 n-9	10.9 a	10.5 b	10.0 c
18:2 n-6	2.0 a	1.9 b	1.7 b
20:5 n-3	10.1 a	10.7 b	10.9 b
n-6/n-3	0.23 a	0.20 b	0.20 b

[3] Fatty acids that were significantly influenced by the main effects of dietary protein or lipid level (ANOVA indicated $P<0.05$).

For each main effect, means (n=27) within a row that share a common postscript letter are not significantly different ($P>0.05$).

major fatty acids contained in them are 16:0, 18:0, 18:1 n-9, 20:5 n-3, and 22:6 n-3. A notable exception is phosphatidylinositiol, which is characterised by its high proportion of 18:0 and 20:4 n-6 (Bell et al., 1983, 1985).

All diets contained similar amounts of canthaxanthin (53.6 ± 2.7 (sd) $mg \cdot kg^{-1}$ DM).

There were no significant effects of the dietary treatments on growth or most other aspects of the performance of the fish that were selected for fillet removal (Table 21). However, there was a significant dietary influence on the average growth and performance of all the fish in the study (Table 17, page 100). Also, there was a significant influence of the dietary lipid levels on dry feed consumption (g $DM \cdot kg^{-1}$ body $weight \cdot day^{-1}$) and consequently on lipid intake of the fish. Fish consuming the diets with the highest lipid levels (300 $g \cdot kg^{-1}$ DM) had significantly higher lipid intake (2.58 $g \cdot kg^{-1}$ body $weight \cdot day^{-1}$) than either of the other diets (2.08 $g \cdot kg^{-1}$ body $weight \cdot day^{-1}$ respectively).

The proximate compositions of the fillets were influenced by the dietary lipid levels, but not by dietary protein levels, and except for ash levels, there was not a significant interaction between dietary protein and dietary lipid level (Table 22). As dietary lipid level increased, crude protein, ash, and moisture content in the fillets decreased, but lipid content increased. This finding was expected, because the increase in deposition of lipid from dietary sources would tend to dilute the other fillet components. Other investigators have also found similar effects of dietary protein to lipid ratio on fillet proximate compositions of salmonids (Takeuchi et al., 1978; Silver et al., 1993).

Table 21. The effect of different dietary ratios of digestible protein to digestible lipid on the performance and feed consumption of Atlantic salmon reared in seawater.

Diet[1]	Digestible protein level $g \cdot kg^{-1}$ DM	Digestible lipid level $g \cdot kg^{-1}$ DM	Initial weight g	Final weight g	Weight gain g	Specific growth rate $\%\cdot day^{-1}$	Dry feed consumption $g \cdot kg^{-1}$ body weight$\cdot day^{-1}$	Lipid consumption $g \cdot kg^{-1}$ body weight$\cdot day^{-1}$
1	360	180	530	1188	657	0.72	8.23b	1.83b
2	360	230	587	1255	668	0.68	8.68b	2.39ab
3	360	280	579	1295	716	0.72	9.00b	2.50ab
4	410	180	541	1247	705	0.75	8.43b	1.97b
5	410	230	580	1366	786	0.77	7.24b	1.90b
6	410	280	546	1194	648	0.70	9.12b	2.86a
7	460	180	630	1303	673	0.65	11.08a	2.46ab
8	460	230	566	1195	629	0.67	7.37b	1.97b
9	460	280	523	1215	692	0.75	7.70b	2.37ab
Pooled sd			75	185	145	0.11	1.10	0.29

[1] Refer to Chapter Four for details of the determined levels of digestible protein and lipid in the diets.

Means (n=9) within a column that do not have any postscript or that share a common postscript letter are not significantly different ($P>0.05$).

Lipid consumptions were calculated from the feed consumption data and took the actual dietary lipid levels into account, see Chapter Four for values.

Table 22. The effect of different dietary ratios of digestible protein to digestible lipid ($g \cdot kg^{-1}$ DM) on the proximate compositions ($g \cdot kg^{-1}$ wet weight) of the fillets from Atlantic salmon reared in seawater.

Diet	Digestible protein level	Digestible lipid level	Moisture	Ash	Lipid	Crude protein
1	360	180	707	22ab	65	210
2	360	230	705	20b	71	204
3	360	280	699	23ab	84	198
4	410	180	711	22ab	66	205
5	410	230	707	21b	73	206
6	410	280	705	21b	78	199
7	460	180	715	24a	58	207
8	460	230	713	23ab	63	206
9	460	280	702	20b	73	203
Pooled sd	----	----	11	2	13	6
Dietary protein level			NS	NS	NS	NS
Dietary lipid level						
1	----	180	711a	23a	63a	207a
2	----	230	708a	21b	69a	206a
3	----	280	702b	21b	80b	200b
Initial fish[1]	----	----	739	24	34	213

[1] Average value for five fish.

Means (n=9 for dietary treatments and n=27 for main effects) within a column that do not have any postscript or share a common postscript letter are not significantly different (P>0.05).

Fillet fatty acid compositions were significantly affected by dietary protein and lipid levels (Table 23). Increasing the dietary protein level decreased the percentages of 14:0, 18:4 n-3, and 20:5 n-3 in the fillets. The fish fed the diets with the highest protein level (460 g DP·kg^{-1} DM) had significantly higher levels of 18:0,18:1 n-9, 18:2 n-6, and total monounsaturates than the fish fed the diets with the lower protein levels. The levels of 18:1 n-7, 18:3 n-6, and total n-6 in the fillets were also positively correlated with dietary protein level. Fish fed the diets with the highest protein level also consumed significantly more protein (4.1 g·kg body weight^{-1}·day^{-1}) than those fed diets with the other two protein levels (3.3 g·kg body weight^{-1}·day^{-1} each). Since the major fatty acids contained in the phospholipids of fish are 16:0, 18:0, 18:1 n-9, 20:5 n-3, and 22:6 n-3 (Bell et al., 1983, 1985), this could explain why the levels of some of these fatty acids increased in the fillets as the dietary level of protein was increased. The changes in the level of 18:3 n-6 cannot be explained by its dietary concentration or on the basis of dietary levels of 18:2 n-6 or 18:3 n-3. Although there was an increase in the percentage of 18:2 n-6 in the fillets of the fish fed the diets with the highest protein level, the fillets from fish fed the diet containing the intermediate protein level had the highest 18:3 n-6 level. There was no dietary influence on the levels of 18:3 n-3, which might affect 18:3 n-6 levels by influencing its synthesis from 18:2 n-6, since the preference for substrates of the Δ6 desaturase enzyme is n-3 > n-6 > n-9 (Tocher and Dick, 1990).

With an increase in the dietary lipid level, there were decreases in the concentrations of 16:0, 18:0, 18:1 n-9, 18:2 n-6, total monounsaturates, total n-6, and in the ratios of n-6 to n-3 in the fillets. The same decreases in total monounsaturates and in the ratio of n-6 to n-3 were observed by Silver et al. (1993) in chinook salmon that were fed diets with different levels of lipid at each of two protein levels. These changes

Table 23. The effect of different dietary ratios of digestible protein to digestible lipid ($g \cdot kg^{-1}$ DM) on the total fatty acid levels (% of total fatty acids) of fillets from Atlantic salmon reared in seawater.

	Initial	Diet[1]									Pooled sd[2]
		1	2	3	4	5	6	7	8	9	
Digestible protein		360	360	360	410	410	410	460	460	460	
Digestible lipid		180	230	280	180	230	280	180	230	280	
Fatty acid											
14:0	9.3	11.1	11.7	11.9	10.9	11.2	10.9	10.4	10.8	11.3	0.90
15:0	0.62	0.46 ab	0.46 ab	0.47 ab	0.47 ab	0.48 ab	0.45 ab	0.48 ab	0.44 b	0.49 a	0.03
16:0	20.2	20.9	20.4	20.2	20.8	19.9	19.5	21.0	20.6	19.9	1.15
18:0	3.6	3.6	3.1	3.2	3.3	3.2	3.2	3.6	3.5	3.2	0.17
Total saturates	33.7	36.0	35.7	35.8	35.5	34.8	34.0	35.5	35.3	34.9	2.18
16:1 n-7	10.4	10.9	11.8	11.9	11.5	11.6	11.5	11.2	11.2	12.0	0.69
18:1 n-9	10.4	13.8	12.7	12.5	13.7	12.8	12.6	14.6	13.8	12.9	0.76
18:1 n-7	4.4	4.8	4.8	4.9	4.7	4.7	4.8	5.1	5.0	4.8	0.30
20:1 n-9	1.3	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.7	1.7	0.22
22:1 n-11	1.1	1.0	1.1	1.0	1.0	1.1	1.1	1.0	1.0	1.1	0.10
Total monounsaturates	27.6	32.1	32.0	31.9	32.5	31.8	31.6	33.6	32.7	32.5	1.17
18:2 n-6	1.8	2.4	2.2	1.9	2.4	2.2	2.0	2.5	2.3	2.3	0.16
18:3 n-6	1.02	0.35	0.44	0.36	0.40	0.45	0.40	0.35	0.39	0.42	0.05
18:3 n-3	0.81	0.61	0.63	0.60	0.62	0.56	0.57	0.63	0.60	0.61	0.01
18:4 n-3	0.98	0.94 b	1.11 a	1.10 a	0.96 b	1.06 a	1.06 a	0.92 b	0.92 b	1.08 a	0.07
20:4 n-6	1.15	0.96	0.96	0.97	0.92	0.97	0.96	0.97	0.95	0.98	0.09
20:5 n-3	7.3	7.8	8.2	8.6	7.8	8.7	8.4	7.3	7.9	8.5	0.48
22:4 n-6	0.69	0.59	0.58	0.64	0.57	0.68	0.68	0.58	0.61	0.62	0.09
22:5 n-3	3.55	2.48	2.80	2.58	2.56	2.87	3.18	2.51	2.43	2.62	0.51
22:6 n-3	14.9	7.7	7.0	7.1	7.6	7.3	7.6	8.2	7.6	7.2	1.29
Total polyunsaturates	32.2	23.8	24.9	23.8	23.8	24.8	24.8	24.0	23.7	24.3	1.62
Total n-6	4.7	4.3 ab	4.2 abc	3.9 c	4.3 ab	4.3 a	4.0 bc	4.4 ab	4.2 ab	4.3 ab	0.26
Total n-3	27.5	19.5	20.7	19.9	19.5	20.5	20.8	19.6	19.5	20.0	1.54
n-6/n-3	0.17	0.22	0.20	0.20	0.22	0.21	0.19	0.22	0.22	0.22	0.02
unsat/sat	1.77	1.55	1.59	1.56	1.59	1.63	1.66	1.62	1.60	1.63	0.12

[1] Refer to Chapter Four for details of the determined levels of digestible protein and lipid in the diets.

[2] Pooled standard deviation calculated from the error mean square from the ANOVA. Means (n=9) within a row that do not have a postscript or share a common postscript letter are not significantly different (P>0.05).

Table 23 (Con't). The effect of different dietary ratios of digestible protein to digestible lipid ($g \cdot kg^{-1}$ DM) on the fatty acid levels (% of total fatty acids) of fillets from Atlantic salmon reared in seawater.

Fatty acid	Main effects[3]		
	Protein level		
	360	410	460
14:0	11.6 a	11.0 b	10.8 b
18:0	3.3 a	3.2 a	3.4 b
18:1 n-9	13.0 a	13.0 a	13.8 b
18:1 n-7	4.8 ab	4.7 b	5.0 a
18:2 n-6	2.2 a	2.2 a	2.3 b
18:3 n-6	0.38 a	0.42 b	0.39 ab
18:4 n-3	1.05 a	1.02 a	0.98 b
20:5 n-3	8.3 a	8.3 a	7.9 b
Total monounsaturates	32.1 a	32.1 a	33.2 b
Total n-6	4.1 a	4.2 b	4.3 b
	Lipid level		
	180	230	280
16:0	20.9 a	20.3 ab	19.9 b
16:1 n-7	11.2 a	11.5 ab	11.8 b
18:0	3.5 a	3.3 b	3.2 c
18:1 n-9	14.0 a	13.1 b	12.7 b
18:2 n-6	2.4 a	2.2 b	2.1 c
18:3 n-6	0.36 a	0.43 b	0.39 a
18:4 n-3	0.94 a	1.03 b	1.08 c
22:1 n-11	0.99 a	1.05 b	1.07 b
20:5 n-3	7.6 a	8.3 b	8.5 b
22:4 n-6	0.58 a	0.62 ab	0.65 b
Total monounsaturates	33.1 a	32.3 b	32.0 b
Total n-6	4.2 a	4.2 a	4.1 b
n-6/n-3	0.22 a	0.22 a	0.20 b

[3] Fatty acids that were significantly influenced by the main effects of dietary protein or lipid level (ANOVA indicated $P<0.05$).

For each main effect, means (n=27) within a row that share a common postscript letter are not significantly different ($P>0.05$).

in the fillet fatty acids were likely due to the effect of the dietary lipid levels, as most of these same trends were seen with the fatty acid levels in the diets. Increases in the concentrations of 16:1 n-7, 18:3 n-6, 18:4 n-3, 20:5 n-3, and 22:4 n-6 were observed with increasing dietary lipid levels. The increase in 20:5 n-3 content can be related to lipid intake and dietary levels of 20:5 n-3. The reasons for the increases in 18:3 n-6 and 18:4 n-3 cannot be explained. The increase in 22:4 n-6 may have resulted from desaturation and elongation of 18:3 n-6, although no changes in 20:4 n-6 were observed.

Fillets from the five fish selected at the beginning of the experiment contained no canthaxanthin. After 112 days of feeding the test diets, the fillet canthaxanthin levels ranged from 2.7 $mg \cdot kg^{-1}$ to 3.7 $mg \cdot kg^{-1}$ wet flesh (Table 24) and the average for all groups was 3.2 ± 0.4 (sd) $mg \cdot kg^{-1}$ wet flesh. Dietary protein and lipid levels did not significantly influence the quantity of canthaxanthin in the fillets of Atlantic salmon under the test conditions employed in this study. The amount of pigment found in the fillets in this study agreed with values found in other work with this species (Storebakken et al., 1987: Torrissen and Naevdal, 1988) and with results obtained with rainbow trout (Torrissen, 1986, 1989). Some previous studies have reported that dietary lipid level influences carotenoid pigment deposition in salmonids, but others have not been able to confirm this relationship. Abdul-Malak et al. (1975) and Choubert and Luquet (1983), for instance, did not observe any significant effect of dietary lipid level on carotenoid pigment deposition in the flesh of rainbow trout, while Seurman et al. (1985), Torrissen (1985) and Tibaldi and Ballestrazzi (1990) found a moderate increase in carotenoid pigment deposition in rainbow trout as the dietary lipid level was increased. By contrast, Spinelli (1979) observed a large (33%) increase in the deposition of carotenoid pigment

Table 24. The effect of different dietary ratios of digestible protein to digestible lipid on flesh canthaxanthin levels of Atlantic salmon reared in seawater.

Diet	Digestible protein level $g \cdot kg^{-1}$ DM	Digestible lipid level $g \cdot kg^{-1}$ DM	Fillet canthaxanthin[1] $mg \cdot kg^{-1}$ wet fillet
1	360	180	3.0
2	360	230	3.4
3	360	280	3.7
4	410	180	3.8
5	410	230	3.4
6	410	280	2.8
7	460	180	3.5
8	460	230	2.8
9	460	280	2.7
Pooled sd			1.2

[1] The means (n=9) for each dietary treatment were not significantly different ($P>0.05$).

in the flesh of rainbow trout by elevating the dietary lipid level from 10 to 15%. In this study, the average retention efficiency of canthaxanthin in the muscle was found to be 6.5 ± 1.0% (sd) and this value is comparable to the range of 6% to 10% suggested by Torrissen et al. (1990) for salmonids.

CONCLUSIONS

The total lipid content in fillets from Atlantic salmon fed diets varying in levels of digestible protein and lipid were significantly influenced by the dietary lipid level. The increased lipid deposition observed in the fillets of salmon fed the diets of high lipid level diluted the concentration of the other proximate constituents in the fillets. The fatty acid profile of the fillets was not only affected by the dietary lipid level and/or the ratio of digestible protein to lipid, but also by the quantity of residual lipid contained in the fish meal portion of the diet. Although the total n-3 percentages were not significantly changed by alterations of the dietary protein or lipid levels, the percentage of 20:5 n-3 was increased in direct relation to the dietary lipid content. As the health benefits of PUFA in the human diet become more clearly defined the marketability of salmon with higher PUFA levels, especially 20:5 n-3 and 22:6 n-3, should increase.

CHAPTER SIX - GENERAL DISCUSSION

If a protein is of poor nutritive quality (heat damaged, low amino acid availabilities, or an unbalanced amino acid profile) a relatively large proportion of amino acids is used as energy or for synthesis of biological compounds other than protein (Walton, 1985); from a nutritional point of view, this "waste" of protein is very inefficient. The use of protein for energy purposes leads to an increased production of ammonia, which can create a pollution problem in the effluent water. The use of high quality fish meals, at the appropriate dietary level, improves protein utilization, which in turn reduces nitrogen excretion.

Information derived from the various chemical analyses that are normally performed on fish meals (proximate composition, mineral profiles, amino acid profiles, and fatty acid profiles), although important for diet formulation, provides little information about the nutritional quality (availability of the nutrients) of the meal. However, the proximate compositions and mineral profiles are useful as indicators of the nature of the raw material used (eg. whole fish versus processing wastes) and, to a very limited extent, the conditions used during processing. The variation noted in the crude protein content among the herring meals can be explained by the processing conditions used in their production. The amount of lipid extracted and/or the amount of fish solubles added to the presscake likely has the most influence on the crude protein and energy levels of the final meals.

Biogenic amines result from the decarboxylation of specific amino acids, either by bacterial enzyme action and/or by enzymes naturally present in fish. High levels of amines suggest usage of raw material that may have been enzymatically degraded to

varying degrees before processing (Pike, 1993). Yet, results from the work by Cowey and Cho (1992), Fairgrieve et al. (1994), and from the present study do not show any clear influence of high dietary amine levels on fish growth or feed consumption. The present findings also support those of Fairgrieve et al. (1994) who did not observe any histological abnormalities in the liver, kidney, or stomach of rainbow trout fed diets containing high levels of amines (histamine, putrescine, and cadaverine).

Performance parameters (PER, feed to gain ratio, body composition) indicate that salmon fed the diet containing herring meal made from spoiled male herring (HM-SM-Std) utilized the dietary protein for weight gain (protein and/or fat) similar to that for fish fed the other diets (Table 12, page 76). However, these fish did not eat sufficient feed to produce maximal growth. The various fish meals tested in the growth experiment had no significant effect on carcass protein levels. This again supports the premise that the protein in HM-SM-Std was utilized as efficiently as the protein from the other fish meals. As indicated by the feed consumption data, it appears that the HM-SM-Std diet was not palatable to the fish. Palatability of fish diets has been shown to significantly influence feed consumption (Adron and Mackie, 1978; Jones, 1990). Hughes (1991) assessed the effects of various chemical compounds in the diets of first-feeding chinook salmon (0.4 g) and found that trimethylamine was a strong inhibitor of feed intake. Trimethylamine levels in the various fish meals were not measured, but it is one of the nitrogenous compounds measured by TVBN. The TVBN value (67 mg·100 g^{-1}) and biogenic amine levels for HM-SM-Std were the highest among the fish meals evaluated. However, it cannot be ruled out that the reduced feed consumption may have been due to another compound(s), either of protein or lipid origin, produced during the spoilage process.

Of the various chemical and in vitro assays, only dilute-pepsin digestibility was found to have a significant correlation with apparent metabolizable crude protein, BV, and NPU determined using rats. No significant correlations were observed between fish and rat in vivo assays. Since the test fish meals were from such divergent species of fish, the various herring meals and Norse-LT94® (which is made from capelin, a species similar in composition to herring) were grouped separately from the remaining fish meals (mixed meal, groundfish meal, Chilean meals, silver hake, and menhaden) and both groups were inspected again for correlations. No correlations were found between any of the variables with the latter group of meals. For the herring meals, dilute-pepsin digestibility was the only assay that correlated (r^2=0.549, P=0.036) with apparent crude protein digestibility values determined using fish. Work by Romero et al. (1994) also found poor correlations between protein digestibility coefficients determined with rainbow trout and in vitro digestibility, available lysine, or chemical analyses. Unfortunately, few details of the methodologies of the assays were given.

In the study to evaluate the optimal dietary ratio of digestible protein to digestible energy for Atlantic salmon reared in seawater, significant differences were found for the ADC's for CP and GE (Table 16, page 98), even though there was not more than a five percentage unit range among values in both cases. Starch digestibility coefficients increased in direct relation to dietary protein level. This trend was attributed to progressive reductions in the starch content of the diets as dietary protein levels were raised. Other researchers, notably Hemre et al. (1995) working with Atlantic salmon and Pieper and Pfeffer (1980) and Henrichfreise and Pfeffer (1992) working with rainbow trout, have also noted that starch digestibility is inversely related to its dietary concentration over a range of 0 $g \cdot kg^{-1}$ to 310$g \cdot kg^{-1}$ DM. For salmonids, the digestibility

of starch is affected by the source and dietary level (Kaushik and Médale, 1994). Dietary treatments did not significantly influence muscle glycogen level (Table 19, page 104), although increasing the dietary DP level did significantly ($P<0.05$) decrease liver glycogen levels and HSI values. The starch added to the diets to replace the protein energy was the cause of this effect. These effects have been noted by many authors (Lee and Putnam, 1973; Bergot, 1979; Lall and Bishop, 1979; Kim and Kaushik, 1992).

As the dietary lipid level increased, the ADC's for CP, GE, and starch decreased, although the spread of absolute values was only approximately two percentage units. Brauge et al. (1994), working with rainbow trout (230 g) in seawater, observed that the ADC for lipid decreased from 81.5 to 76.8% (although not significantly) with an increase in lipid from 104 to 144g $\cdot kg^{-1}$ DM. Cho and Kaushik (1990) give evidence far an inverse relationship between the melting point of a fat and its digestibility and work by Sigurgisladottir et al. (1992) has shown that the absorption of saturated fatty acids is less than that of unsaturated fatty acids. The menhaden oil, used in the present experiment, contained a relatively higher amount of saturated fatty acids (approximately 40%) compared to values reported for oils from herring, capelin, and mackerel (approximately 25%) or anchovy, pilchard and sardines (approximately 35%) (Ackman, 1980, 1982; Windsor and Barlow, 1981). This could explain the trend for reduced dietary energy digestibility coefficients as the lipid levels in the diets were increased.

Some of the trends noted for growth, feed consumption, and other aspects of performance in relation to dietary treatments may have been more clear-cut if fish mortality (Table 17, page 100) had been lower, although these parameters were adjusted for fish mortality. The cause of the high mortality was due to a subclinical infection with furunculosis (Aeromonas salmonicida), which the stress of periodic

weighing probably exacerbated.

Increasing dietary lipid levels were associated with a trend towards an increase in whole body lipid content and a decrease in protein and moisture content (Table 18, page 102). Also, whole body lipid content increased with time (age of fish). However, whole body moisture and ash levels declined with time. The foregoing trends generally mirrored those reported by other investigators who have conducted research on this subject (for a review see Shearer, 1994).

Retention of protein (PPV) and energy (GEU) was influenced by dietary treatments. Important factors affecting the PPV and GEU values found in this study and other studies include the source, level, and quality of the non-protein energy supplied. The protein sources in the various experimental diets have ranged from blends of animal and plant meals (Silver et al., 1993), to herring meal (Brauge et al., 1994), Norse-LT94® (Hillestad and Johnsen, 1994), and to fresh fish fillets and squid mantle (Hemre et al., 1995).

Fillet fatty acid compositions were significantly affected by dietary protein and lipid levels (Table 23, page 120). With an increase in the dietary lipid level, there were decreases in the concentration of 16:0, 18:0, 18:1 n-9, 18:2 n-6, total monounsaturates, total n-6, and in the ratio of n-6 to n-3 in the fillets. The same decreases in total monounsaturated percentages and in the ratio of n-6 to n-3 were observed by Silver et al. (1993) in chinook salmon that were fed diets with different levels of lipid (150, 200, or 250 $g \cdot kg^{-1}$ DM) at each of two protein levels (350 or 450 $g \cdot kg^{-1}$ DM). These changes in the levels (%) of fillet fatty acids were likely due to the effect of the dietary lipid levels, as most of these same trends were seen with the fatty acid levels in the diets.

Dietary protein and lipid levels did not significantly influence the quantity of

canthaxanthin in the fillets of Atlantic salmon under the test conditions employed in this study. The amount of pigment found in the fillets agreed with values reported by other workers with this species (Storebakken et al., 1987; Torrissen and Naevdal, 1988) and with results obtained with rainbow trout (Torrissen, 1986, 1989). Although, the present study on Atlantic salmon and some on rainbow trout (Abdul-Malak et al., 1975; Choubert and Luquet, 1983) have not observed any significant effect of dietary lipid level on carotenoid pigment deposition in the flesh, other studies have found an effect. Seurman et al. (1985), Torrissen (1985) and Tibaldi and Ballestrazzi (1990) reported only a moderate increase in carotenoid pigment deposition in rainbow trout as the dietary lipid level was increased. In contrast, Spinelli (1979) observed a large (33%) increase in the deposition of carotenoid pigment in the flesh of rainbow trout by elevating the dietary lipid level from 10 to 15%.

CHAPTER SEVEN - GENERAL CONCLUSIONS AND RECOMMENDATIONS

Although there are numerous chemical and in vitro methods that can be used for the evaluation of the protein quality of dietary components for fish, there is still much information needed on how well the values obtained with these methods correlate with nutrient availability and growth of fish. From the work presented here, it appears that dilute-pepsin digestibility (Olley and Pirie, 1966) is the preferred in vitro assay to evaluate the protein quality of herring meals for fish. The test appears to correlate poorly with results from in vivo assays when other types of fish meals are included. The pH-stat method using an enzyme fraction from fish pyloric caeca (Dimes and Haard, 1994) has potential as an accurate way to estimate the digestibility and biological value of alternate protein sources for salmonids. Until more purified digestive enzymes of fish origin become commercially available, mammalian enzymes will have to be utilized for such tests. Further evaluation of the usefulness of homoeothermic animal models, especially mink, for predicting protein quality for fish is needed due to the limited number of studies in which direct comparisons have been made. The only non-fish assay that was found to correlate the entire range of fish meals with an assay using fish was AMCP-R (apparent metabolizable crude protein) using rats as the test animal.

A significant finding was the observation that Atlantic salmon fed a diet containing groundfish meal as the major (80%) protein source performed as well as fish fed Norse-LT94®. Confirmation of this finding under commercial conditions would significantly reduce the cost of salmon diets. Another noteworthy finding was the apparent equal utilization of protein provided by spoiled versus unspoiled herring meal for salmon growth and protein deposition (PER, feed to gain ratio, and whole body

composition). Yet, little information is available on how to best overcome the palatability problem associated with a meal made from spoiled herring and this area needs further study.

The growth and protein and energy retention data in this study suggest that 17.4 g DP·MJ^{-1} DE in the diet is optimal for rearing Atlantic salmon in seawater over a size range of 0.5 kg to 1.2 kg. This amount of DP·MJ^{-1} DE can be supplied by a diet containing 360 g DP (404 g crude protein), 220 - 260 g lipid, and up to 220 g of pregelatinized starch, with 20.7 MJ DE (24.0 MJ gross energy) ·kg^{-1} DM. These conclusions are similar to those reached by Hillestad and Johnsen (1994) who found that a diet containing 440 g crude protein, 290 g lipid, and 24.9 MJ gross energy produced the best growth in Atlantic salmon over a size range of 100 g to 600 g. Additional work, preferably of much longer duration, is needed to confirm and extend the results of the present study, considering fish of even larger size than those used in this study.

As dietary lipid levels were increased there appeared to be a decreases in the apparent digestibility of the lipid. This finding may be related to the fatty acid composition of the supplemental menhaden oil used in this study (high saturated fatty acid content).

It would also appear from this study that Atlantic salmon are able to tolerate up to 279 g starch·kg^{-1} DM without any detrimental effects on growth, although liver glycogen and HSI values were elevated. Additional research is required to establish acceptable dietary concentrations of digestible carbohydrate for culturing Atlantic salmon in seawater at different stages of the life history.

The total lipid levels in fillets from Atlantic salmon fed diets varying in levels of

digestible protein and lipid were significantly influenced by the dietary lipid level. The observed increase in lipid deposition with increasing dietary lipid concentrations diluted the concentrations of the other proximate constituents in the fillets. The fatty acid profile of the fillets was affected not only by the dietary lipid level and/or the ratio of digestible protein to lipid, but also by the quantity of residual phospholipids contained in the fish meal portion of the diet. Although the total n-3 fatty acid percentages were not significantly changed by alterations in dietary protein or lipid levels, the percentage of 20:5 n-3 was increased in direct relation to the dietary lipid content.

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