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NUTRITIVE VALUE OF FISH MEAL FOR ATLANTIC SALMON (*Salmo salar*)

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

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in the Department of Health Management

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J. Stewart Anderson

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ABSTRACT

Fish meals (herring, menhaden, and anchovy), commercially available in Atlantic Canada and a Norwegian fish meal (Norse-LT94®) were evaluated for their protein quality by *in vitro* assays and growth studies with Atlantic salmon (*Salmo salar*). The *in vitro* tests included: acid-corrected pepsin digestible protein (AOAC and Torry methods), multienzyme digestibility, total volatile basic-nitrogen, available lysine, sulphydryl groups, and disulphide bonds. Pepsin digestibility by the AOAC method did not distinguish fish meals of marginal quality. Pepsin digestibility measured by the Torry method and the multienzyme assay showed a good correlation ($r=0.74$). The other chemical tests, particularly when used alone, were of limited use for measuring protein quality in fish meal.

Atlantic salmon fingerlings (initial weight 7.65±0.14 g) were used in a growth study for 70 days to evaluate the protein quality of two steam-dried herring meals and Norse-LT94®. Nine diets, containing these fish meals as the sole protein source, were formulated to contain 16, 28, or 40% protein (as-fed basis) and 15.1 MJ of digestible energy. The protein quality was evaluated on the basis of weight gain, PER, NPR, NPU, and slope assay. Fish fed Norse-LT94® gained 11 to 60% more weight than fish fed the herring meals.

Five fish meals were evaluated for their crude protein and amino acid availabilities using Atlantic salmon (initial weight 72±14 g) held in fresh water. The fish meals were: two steam- and one flame-dried herring meal, menhaden meal, anchovy meal, and Norse-LT94®. For all essential amino acids, except tryptophan, Norse-LT94® had higher apparent amino acid availabilities than the other fish meals tested, although the differences observed were not always statistically significant. Menhaden meal and anchovy meal had significantly lower amino acid availabilities than most of the herring meals tested.

A feeding experiment was conducted to determine the quantitative dietary requirement of Atlantic salmon for lysine. Groups of fish (initial weight 4.7±0.2 g) were fed diets for 70 days that were calculated to contain 1.15, 1.40, 1.65, 1.90, 2.15, 2.40, and 2.65% lysine, as-fed basis. Broken-line regression of the growth values against dietary lysine level estimated the dietary requirement of lysine to be 1.99±0.11% of the dry diet or 3.98% of the protein at 50% protein in the diet. A similar requirement value of 1.84±0.16% was obtained from a broken-line regression of expired $^{14}CO_2$ against dietary lysine concentration. Except for loss of appetite resulting in low food intake and depressed growth, no nutritional deficiency signs were observed in fish fed a lysine-deficient diet for 140 days.

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1.0 GENERAL INTRODUCTION

Proteins are the major organic material in fish tissue, making up approximately 65 to 75% of the dry-matter (Wilson 1989). Fish consume protein to obtain amino acids. The protein is digested or hydrolysed and releases free amino acids, dipeptides and polypeptides, most of which are absorbed from the intestinal tract and are transported in the blood to the organs and tissues. Fish require protein for replacement of tissue and proteinaceous products such as intestinal epithelial cells, enzymes, and hormones which are vital for the proper function of the body.

Fish, like other animals, do not have a true protein requirement but have a requirement for a well-balanced mixture of essential or indispensable and non-essential or dispensable amino acids (Wilson and Halver 1986). It has been determined that fish require ten essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Cowey and Sargent 1972). In the case of dispensable amino acids, a supply of carbon and nitrogen will allow the fish to synthesise these amino acids. Inadequate protein or the lack of an essential amino acid in the diet causes a reduction or cessation of growth and weight gain due to withdrawal of protein from less vital tissues to maintain the functions of tissues most critical for the survival of

the organism. On the other hand, if excess protein is supplied in the diet, only part of it will be used to synthesise new proteins, and the remainder will be catabolized to energy (Walton 1985).

Increase in fish production in Canada has created a higher demand for good quality fish meal for fish feed production. Since feed represents the largest single cost item (40 to 50%) of the total operating cost, it is more important now to devise strategies to reduce the feed cost and improve the efficiency of existing salmonid diets (Crampton 1985).

The main protein source (30 to 60% of the diet) in salmonid diets is of fish origin either as fish meal, raw, or ensiled fish. Other animal proteins such as blood meal, blended animal proteins, skimmed milk powder, and hydrolysed feather meal are used to a lesser extent. This is also the case with plant protein sources. Fish meal constitutes the major protein supplement in salmonid diets and directly affects the feed cost (Tacon and Jackson 1985). The reason for the high quantity of fish meal in fish diets is because fish require high levels of protein (25 to 55%) in their diet (Wilson and Halver 1986). Attempts to completely replace the fish meal component of fish diets with other ingredients of plant and animal origin have not been successful (Higgs et al. 1988). The quality of fish meal is affected by the raw material used (origin, species, season,

whole fish or fish scraps, storage conditions), processing methods (cooking, fat removal, drying, grinding, antioxidant addition), and storage conditions of the fish meal (Tarr and Biely 1972).

In most cases fish are caught specifically for the purpose of fish meal production (FAO 1975). The species usually used include herring, mackerel, anchovy, and capelin along with other species of lesser importance. Other sources of fish include the cuttings and offal of processing industries such as cod and salmon. Where the fish is specifically caught for the purpose of fish meal production, the freshness of the raw material used is of great importance. In the case of the herring industry the harvest season is short and large amounts of fish are caught. With large amounts of fish caught in a short period of time inevitably, quantities of fish are held for long periods and enzymatic and bacterial breakdown occurs. The bacterial spoilage of protein to ammonia may result in significant loss of protein and production of malodorous compounds (Farber 1965). These compounds may carry through the processing into the fish meal and affect the quality of the 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 the wet processing method (FAO 1975;

Stansby 1990). The steps involve cooking, separation, and drying. The raw material is cooked to cause the coagulation of the protein, thereby liberating bound water and oil. Separation of the water and oil is done by pr ;sing the coagulate, yielding a solid phase (presscake) containing 60-80% of the oil-free dry matter (protein and bones) and oil and a liquid phase (press water) containing the rest of the solids (oil, dissolved and suspended protein, vitamins and minerals). The main part of the solids in the press water is removed by centrifugation and the oil is subsequently removed by centrifugation. The watery phase from the centrifuges (called stickwater) is concentrated and thoroughly mixed into the presscake, which is then dehydrated. Drying of the fish meal can be accomplished by two main methods. Flame-drying, which involves a current of flue gases diluted with secondary air, in direct contact with the fish material being dried (FAO 1975). The second method is indirect steam-drying, which the meal is fed continuously into one end of the drier and is dried in direct contact with steam-heated elements (tubes, discs, coils) and the meal emerges at the other end (FAO 1975). A countercurrent stream of air is blown through the drier, to facilitate removal of water vapour. The dried material is milled and an antioxidant (usually ethoxyquin) is added and the fish meal stored in bags or bulk (FAO 1975; Stansby 1990).

During the drying conditions amino acids may be chemically altered in a way that makes them nutritionally unavailable to animals. The reduction in available amino acids reduces the protein quality of the fish meal (Bender 1972; Tarr and Biely 1972). The effects of drying conditions on the protein quality of fish meal directly affects the quality of diets produced. This is especially true when considering the high concentration of fish meal in most diets for salmonids. Fish meal quality in Atlantic Canada is known to vary from batch to batch within a processing plant and among different processors. Fish meal manufacturers require methods to measure the protein quality of fish meals that are accurate, inexpensive, rapid, and easy to perform. This allows fish meal manufacturers to monitor the quality of fish meal produced and permits production of a consistent quality fish meal.

There were four objectives in this study: 1) Since wide variation in the amino acid requirements of salmonids has been reported (Wilson 1989), a study was conducted to quantify the dietary requirement of Atlantic salmon for lysine. Lysine was selected because several requirement values have been reported for other salmonids. Moreover, the loss of lysine in processed feeds is common (Carpenter 1973) and the author wanted to insure the proper requirement of amino acids were met in the bioassays. 2) To compare different <u>in vitro</u> methods used for the measurement of

protein quality in several fish meals produced in Atlantic Canada. 3) To evaluate the protein quality of selected fish meals with growth studies using Atlantic salmon and to compare the results of the growth studies with the results of the *in vitro* assays. 4) Finally, to determine the apparent and true availabilities of protein and amino acids from various fish meals produced in Atlantic Canada and to examine the effects of manual stripping or sedimentation methods of feces collection on amino acid availability values.

2.0 QUANTITATIVE DIETARY LYSINE REQUIREMENT

2.1 Introduction

The quantitative requirement for lysine of several species of fish has been thoroughly reviewed (Wilson 1989). Most requirements were determined by the conventional dose-response growth curves and vary among species. Lysine requirements (dry matter basis, DMB) have been reported to be: for chinook salmon (Oncorhynchus tschawytscha), 20 $g \cdot kg^{-1}$ of a diet containing 40% protein (Halver et al. 1958); Japanese eel (Anguilla japonica), 20 $g \cdot kg^{-1}$ of a diet containing 37.7% protein (Nose 1979); channel catfish (Ictalurus punctatus), 15 $g \cdot kg^{-1}$ of a diet containing 30% protein (Robinson et al. 1980); gilthead bream (Sparus aurata L.), 17 $g \cdot kg^{-1}$ of a diet containing 34% protein (Luquet and Sabaut 1974); and milkfish (Chanos chanos), 20 $g \cdot kg^{-1}$ of a diet containing 50% protein (Borlongan and Benitez 1990). For rainbow trout (Oncorhynchus mykiss) several lysine requirement values have been reported: 13 $g \cdot kg^{-1}$ of a diet containing 35% protein (Kim and Kayes 1982); 19 $g \cdot kg^{-1}$ of a diet containing 45% protein (Walton et al. 1984a); and 29 $g \cdot kg^{-1}$ of a diet containing 47% protein (Ketola 1983).

Walton et al. (1984a) used an amino acid oxidation technique to estimate the lysine requirement of rainbow

trout. This method is based on the principle that when an amino acid is limiting or deficient in the diet the major portion will be utilized for protein synthesis. When an amino acid is in excess, and thus not a limiting factor for protein synthesis, the proportion of the amino acid in the free pool will increase and more will be available for oxidation. The intake level at which there is an increase in oxidation of any given amino acid, should be close to the requirement value for that amino acid. The amino acid oxidation method has been used for the determination of oxidized methionine (Walton et al. 1982), tryptophan (Walton et al. 1984b; Walton et al. 1986), lysine (Walton et al. 1984a; Walton et al. 1986), and arginine (Walton et al. 1986) in rainbow trout. Excellent agreement between estimated lysine requirements of rainbow trout determined by dose-response growth curves and the amino acid oxidation method has been observed (Walton et al. 1984a). In most studies, lysine deficiency was manifested as a reduction in the rate of weight gain. Ketola (1983) observed caudal fin erosion and high mortality in rainbow trout fed lysine-deficient diets. Walton et al. (1984a) also observed some fin erosion, but no increase in mortalities.

The purpose of the present study was to quantify the dietary requirement for lysine of Atlantic salmon (Salmo salar), examine the effects of variations in lysine intake on the oxidation of a tracer dose of L-[U-^{14}C]lysine, and

determine the effects, if any, of lysine deficiencies.

2.2 Materials and Methods

Atlantic salmon fingerlings obtained from Cobequid hatchery, Department of Fisheries and Oceans, Collingwood, Nova Scotia, were acclimated for a 2 week period in 100 L circular fibreglass tanks. During that time, they were fed a commercial diet twice daily. Three sets of seven tanks were selected and each set considered as a block in the experimental design. Within each block the seven diets were randomly assigned. The experiment was conducted according to a randomized complete block design. Fifty fish of uniform size (initial mean weight, 4.71±0.20 g (SD)) were randomly distributed to each tank. Each tank received water at approximately 2 $L \cdot min^{-1}$. The water was continuously aerated and the temperature controlled to 15±0.9°C (SD). Water temperature was controlled by the mixing of fresh water (17°C) and sea water (10°C). The salinity of the water did not exceed 10 ppt. Photoperiod was 12 hours light and 12 hours dark. During the experimental period, fish were hand fed to satiety three times per day during the week and daily on week ends. Records were kept of weekly feed consumption and daily mortalities.

Diets were formulated (Tables I and II) to contain 45% protein on an as-fed basis (AFB), and amino acid levels

(essential and non-essential, except for lysine) that mimic those found in cod-flesh protein (Walton et al. 1984a). The dietary essential amino acid levels were similar to or slightly higher than amino acid levels found in whole body tissue of Atlantic salmon (Wilson and Cowey 1985). These authors suggested that using amino acid test diets formulated to simulate the amino acid profile of whole body tissue from the fish species studied will ensure adequate weight gains. Test diets were formulated to contain lysine at 1.15, 1.40, 1.65, 1.90, 2.15, 2.40 and 2.65 % of the total diet (AFB). After mixing, these diets were steam pelleted in a Laboratory Pellet Mill (California Pellet Mill Co., Crawfordsville, IN) and stored in air tight containers at -30°C until used.

2.2.1 Sample collection and analytical procedures

Following 24 hours of food deprivation, surviving fish from each tank were weighed as a group, on days 0, 28, 56, and 70 of the feeding trial, and the average weight determined. Growth increments were estimated from the average fish weight in each of the three tanks in each dietary treatment.

Table I. Composition of basal mix used for the determination of the lysine requirement of Atlantic salmon

Ingredients	% of mix (as-fed basis)
Herring meal	12.00
Wheat gluten	30.00
Corn starch	10.00
Dextrin	8.00
Celufil[a]	3.56
Vitamin mix[b]	2.00
Mineral mix[c]	4.00
Choline chloride	0.50
EAA mix[d]	9.71
Herring oil	15.00
Total	94.77

[a] Alpha-cellulose (U.S. Biochemical Corporation, Cleveland, Ohio)

[b] Vitamin mix (mg or IU·kg^{-1} diet): vitamin A, 8000 IU; vitamin D_3, 4000 IU; vitamin E, 300 IU; vitamin K (menadione sodium bisulphite), 40; thiamin, 50; riboflavin, 70; pantothenic acid, 200; biotin, 1.5; folic acid, 20; niacin, 300; vitamin B_{12}, 0.15; pyridoxine, 20; ascorbic acid, 1200; inositol, 400; butylated hydroxytoluene, 15; butylated hydroxyanisole, 15.

[c] Mineral mix (mg·kg^{-1} diet): $MnSO_4 \cdot H_2O$, 123.1; $FeSO_4 \cdot 7H_2O$, 248.8; $CuSO_4 \cdot 5H_2O$, 39.4; $ZnSO_4 \cdot 7H_2O$, 330.4; $MgSO_4 \cdot 7H_2O$, 2010.1; KI, 6.54; Na_2SeO_3, 2.19; $CoCl_2 \cdot 6H_2O$, 60.5; NaCl, 2500; NaF, 9.96; $CaH_4(PO_4)_2 \cdot H_2O$, 14227.6; KH_2PO_4, 6579.0.

[d] EAA mix (g·kg^{-1} diet): L-arginine·HCl, 18.58; L-histidine·HCl·H_2O, 10.25; L-isoleucine, 8.12; DL-methionine, 2.74; L-phenylalanine, 4.59; L-threonine, 13.78; L-tryptophan, 2.81; L-tyrosine, 6.22; L-valine, 11.68.

Table II. Composition of experimental diets used for the determination of the lysine requirement of Atlantic salmon

Ingredients[a]	Diets						
	1	2	3	4	5	6	7
Basal mix	94.77	94.77	94.77	94.77	94.77	94.77	94.77
Celufil[b]	0.39	0.33	0.26	0.20	0.14	0.08	0.02
Non-EAA mix[c]	4.84	4.59	4.34	4.09	3.84	3.59	3.34
L-Lysine·HCl	0.00	0.31	0.63	0.94	1.25	1.56	1.87
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Lysine[a]	1.15	1.40	1.65	1.90	2.15	2.40	2.65

[a] Expressed as a percentage of the as-fed diet.

[b] Alpha-cellulose (U.S. Biochemical Corporation, Cleveland, Ohio).

[c] Non-EAA mix was prepared by mixing together (g): L-alanine, 141; L-aspartic acid, 221; glycine, 575; L-serine, 63.

Specific growth rates (SGR) (Walton et al. 1984b) were calculated by the following equation:

$$\frac{\ln(\text{final weight (g)}) - \ln(\text{initial weight (g)})}{70 \text{ days}} * 100$$

Feed conversion ratios were calculated from weight of feed consumed (g as-fed basis) divided by weight gain (g).

After 70 days, fish were starved for 48 hours. Six fish from each tank were killed and immediately frozen in liquid nitrogen and stored at -90°C until analyzed. Fish on the lowest lysine diet were maintained for an additional 70 days to allow adequate time for nutritional pathologies to develop. In preparation for chemical analysis, the fish carcasses were partially thawed and coarsely ground in a Hobart grinder, refrozen, lyophilized, and finely ground in a Wiley mill to pass through a 20 mesh screen.

Lyophilized fish carcasses and diet samples were analyzed by similar procedures as follows: Ash, by ignition at 550°C (Williams 1984); lipid, by chloroform/methanol extraction (Bligh and Dyer 1959); total nitrogen, by Dumas method (Ebeling 1968) using a Leco Nitrogen Determinator (model FP-228, Leco Corporation, St. Joseph, MI); amino acid composition, following acid hydrolysis (Gehrke et al. 1985), on a Beckman System 6300, High-Performance Amino Acid Analyzer (Beckman Instruments, 2500 Harbor Boulevard, Fullerton, CA); moisture, by weight loss after drying for 24 hours at 105°C (Williams 1984) and; gross energy of the

diets by an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL).

2.2.2 Oxidation of L-[U-^{14}C]lysine

The method of Walton et al. (1984b) was used for the oxidation procedure. A solution of L-[U-^{14}C]lysine (282 mCi·mmol^{-1}, Amersham Canada Ltd., Oakville, Ontario) was prepared in sterile 0.15 M sodium chloride so that 100 mL contained 1 µCi. After 70 days, one fish, chosen at random, from each dietary treatment was held in a 5 L polyethylene pail containing 3 L of water and 10 mM HEPES (pH 7.35) at 15.5°C. A 1 µCi portion of the radioactive solution was injected intraperitoneally into the fish at 13:00 hours. The $^{14}CO_2$ respired between then and 09:00 hours the following day (20 hours total) was collected into two CO_2 traps. The traps consisted of two 50 mL test tubes, connected in series, each containing 25 mL ethanolamine:ethylene glycol monomethyl ether (1:2 v/v). The fish were removed from the containers and the water acidified (pH 1-2) with concentrated HCl and collection of CO_2 continued for an additional 2 hours. The contents of the two CO_2 traps were then combined and made up to 100 mL with distilled water. Samples of acidified water were collected from each pail to determine residual radioactivity. This procedure was repeated for a total of three fish from each dietary

treatment. All samples were frozen at -40°C until used.

For each sample a 1.0 mL aliquot was added to 10 mL of scintillation cocktail (Beckman Ready Solv-HP, Beckman Instruments, 2500 Harbor Boulevard, Fullerton, CA) for assay of radioactivity. Radioactivity was measured on a Beckman LS 3801, Liquid Scintillation System (Beckman Instruments, 2500 Harbor Boulevard, Fullerton, CA).

2.2.3 Statistical procedures

The results were subjected to analysis of variance for a randomized block design. Dietary lysine level was considered the main effect in the model and replication the blocking variable. Orthogonal contrasting was performed to determine the effects of graded levels of dietary lysine on the various growth parameters and body composition. Differences among treatment means were determined at the 5% probability level using Duncan's new multiple-range test, as described by Steel and Torrie (1960). The statistical package SYSTAT (Systat, Inc., 1800 Sherman Ave, Evanston, IL) was used for the statistical analysis.

The lysine requirement was determined by broken-line regression analysis (Brookes et al. 1972; Zeitoun et al. 1976; Robbins et al. 1979) of percentage of weight gain against dietary lysine level (% DMB). The model used was:

$$Y = \begin{cases} L & \text{when } X \geq R \\ A + BX & \text{when } X < R \end{cases}$$

where L is the intercept, R is the lysine requirement, B is the slope (Figure 1). The lysine requirement was also determined by broken-line regression analysis of recovered $^{14}CO_2$, expressed as a percentage of the injected L-[U-^{14}C]lysine, against dietary lysine level (% DMB). The model used was:

$$Y = \begin{cases} L & \text{when } X \leq R \\ A + BX & \text{when } X > R \end{cases}$$

where L is the intercept, R is the lysine requirement, B is the slope (Figure 2).

2.3 Results

Experimental diets were similar in protein, ash, energy, dry matter, and lipid content (Table III). Dietary lysine levels, by analysis, were 1.24, 1.76, 1.90, 2.19, 2.45, 2.68 and 2.94% on a dry weight basis (Table III). The dietary level of lysine in Diet 2 was formulated to be 1.40% (AFB), but analysis (Table III) showed that Diet 2 had a lysine content of 1.55% (AFB) or 1.76% (DMB). This value was higher than anticipated perhaps due to a mixing error, although diets were carefully mixed to avoid errors. Growth, feed utilization and body composition of fish fed

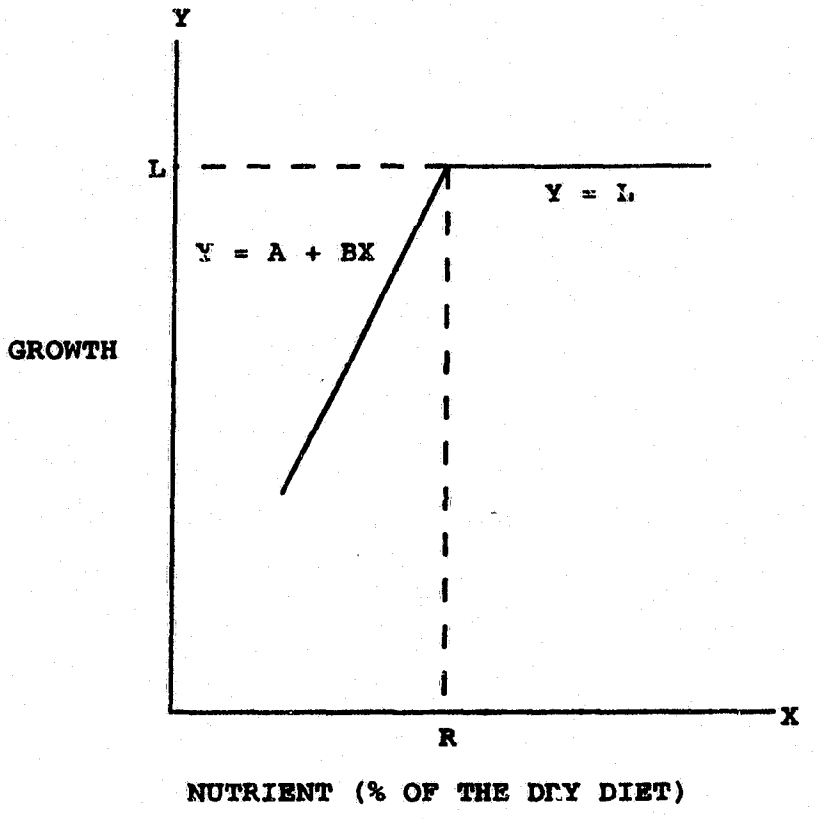

Figure 1. Broken-line regression model used for the determination of the dietary lysine requirement using weight gains against dietary lysine level

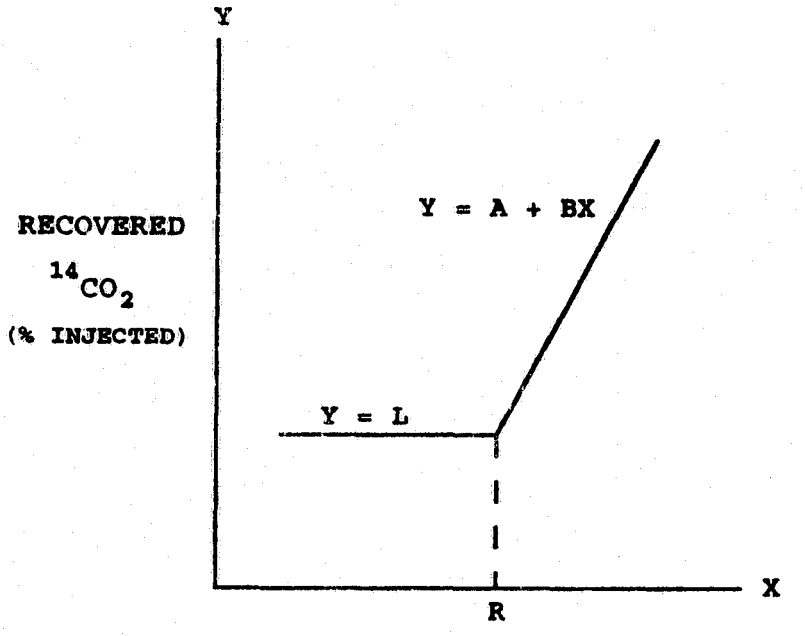

Figure 2. Broken-line regression model used for the determination of the dietary lysine requirement using recovered $^{14}CO_2$ against dietary lysine level

Table III. Chemical and amino acid composition of the experimental diets used for the determination of the lysine requirement of Atlantic salmon

	Formulated Dietary Lysine level (% as-fed diet)						
	1.15	1.40	1.65	1.90	2.15	2.40	2.65
Diet[a]	1	2	3	4	5	6	7
Protein (%)	51.0	51.7	51.1	52.0	50.7	51.4	50.6
Lipid (%)	17.6	17.7	17.3	17.5	17.3	17.9	17.2
Ash (%)	2.9	2.9	2.9	2.9	2.9	2.9	2.9
Moisture[b]	12.2	12.1	11.4	11.8	10.0	13.0	10.6
Gross Energy ($MJ \cdot kg^{-1}$)	24.2	24.2	24.1	24.1	24.2	24.1	24.1
Amino Acid[a]							
Alanine	1.71	2.07	2.01	1.97	1.90	1.92	1.97
Arginine	3.04	3.30	3.06	3.03	3.17	3.04	3.04
Aspartic acid	3.85	3.61	3.42	4.00	3.32	3.20	3.43
Glutamic acid	8.27	10.06	9.94	8.92	9.47	9.39	9.00
Glycine	4.88	4.98	4.64	4.85	4.67	3.99	4.04
Histidine	1.80	1.67	1.73	1.73	1.70	1.71	1.69
Isoleucine	2.23	2.67	2.56	2.46	2.50	2.54	2.30
Leucine	4.32	4.75	4.70	4.57	4.46	4.54	4.49
Lysine	1.24	1.76	1.90	2.19	2.45	2.68	2.94
Methionine	1.07	1.38	1.33	1.19	1.29	1.31	1.14
Phenylalanine	2.48	2.87	2.89	2.50	2.60	2.61	2.35
Proline	3.89	4.54	4.46	4.10	4.28	4.31	4.12
Serine	1.81	1.66	1.59	1.82	1.60	1.67	1.71
Threonine	3.38	3.08	2.37	3.81	3.80	2.99	3.55
Tyrosine	1.47	1.75	1.66	1.62	1.76	1.66	1.59
Valine	2.69	3.22	3.29	2.72	3.07	2.87	2.71

[a] Expressed as a percentage of the dry diet.

[b] Expressed as a percentage of the as-fed diet.

diet 2 was between those of fish fed diets 1 and 3, thus not affecting the results obtained.

Dietary lysine level had a significant quadratic effect ($P<0.05$) on percent weight gain (PWG) and SGR (Table IV). Growth gradually increased as the dietary level of lysine increased until the lysine requirement was met, when further increases in lysine intake resulted in no significant improvement in weight gain. Fish fed the lowest lysine level had significantly lower weight gains than fish fed the other diets. Fish fed the lowest level of lysine had a significantly lower SGR than those fed the other diets (Table IV). There was an increase in SGR between 1.24% and 1.90% dietary lysine (DMB) with no significant change thereafter. The broken-line regression of PWG versus dietary lysine level (% DMB) (Figure 3) gave an estimated dietary lysine requirement of 1.99±0.11% (SE) or 3.98% of the dietary protein when protein was 50.0% of the dry matter.

Dietary lysine levels had a significant ($P<0.05$) cubic response on feed consumption (Table IV). Feed consumption was significantly different ($P<0.05$) among the treatments, being highest at 1.90% dietary lysine (DMB). Dietary lysine levels had a significant ($P<0.05$) quadratic response on feed conversion ratios (Table IV). Feed conversion ratios (FCR) ranged from 1.09 to 1.84 in fish fed diets containing levels of 2.45% and 1.24% lysine (DMB), respectively. The FCR of

Table IV. Growth, feed consumption, and feed conversion of Atlantic salmon fed diets containing graded levels of lysine for 10 weeks

Dietary lysine level[a] (%)	Initial weight[b] (g)	Final weight[b] (g)	Weight gain[b] (%)	Specific growth rate[bc] (% day^{-1})	Feed consumption[bc] (g)	Feed conversion ratio[bd]
1.24	4.59±0.17	7.79±0.14	70.2± 3.7a	0.76a	253.0± 0.8a	1.84±0.09a
1.76	4.82±0.06	10.26±0.30	112.6± 5.0b	1.08b	347.4±11.6bc	1.36±0.04b
1.90	4.71±0.12	12.18±0.14	159.0± 7.2c	1.36bc	373.4± 8.2c	1.16±0.03bc
2.19	4.69±0.19	11.88±0.94	152.5±10.0bc	1.32bc	365.4±29.7bc	1.19±0.08bc
2.45	4.70±0.04	12.74±0.92	171.4±21.1c	1.40c	348.4±19.7bc	1.09±0.08c
2.68	4.84±0.18	12.09±0.57	150.2±14.3bc	1.31bc	292.2±39.0ab	1.23±0.05bc
2.94	4.71+0.22	11.85+0.72	152.3+15.6bc	1.32bc	318.1+23.9abc	1.22+0.07bc

[a] Analyzed dietary lysine concentration (% dry matter basis).

[b] Mean±SEM (n=3).

[c] $SGR = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{70 \text{ days}} \times 100.$

[d] $FCR = \frac{\text{weight of feed consumed (g as-fed basis)}}{\text{wet weight gain (g)}}$

a-c, Numbers, within the same column, not sharing the same postscript are significantly different ($P<0.05$).

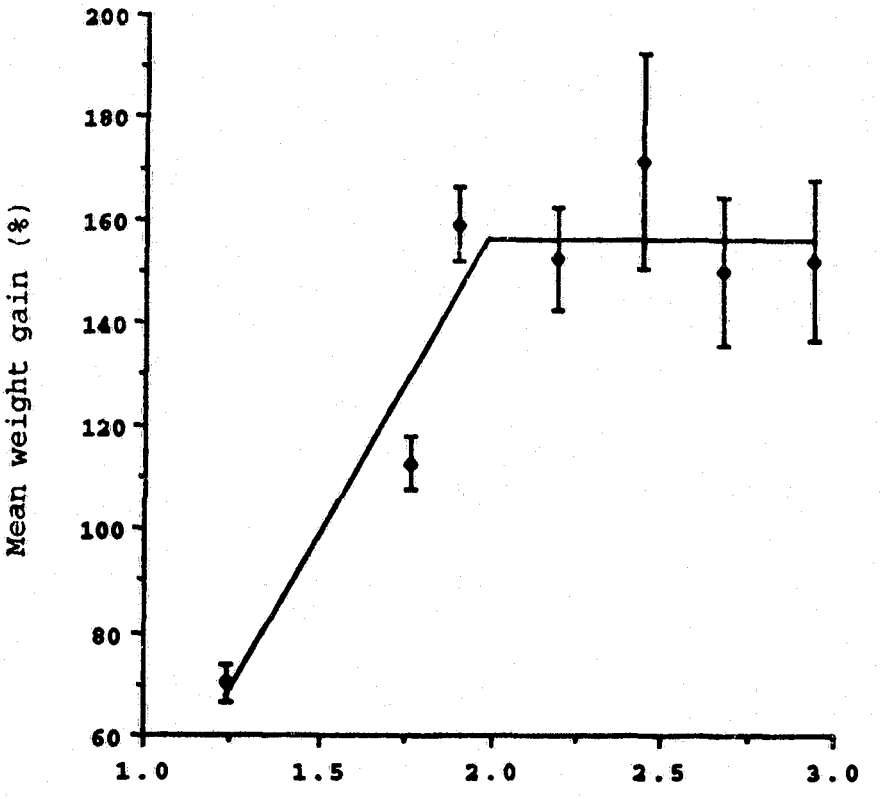

Figure 3. Broken-line regression of weight gain of Atlantic salmon against dietary lysine level. Points represent the mean and the standard error of the mean (vertical bar) for three tanks of 50 fish.

fish on low lysine diets was significantly higher ($P<0.05$) than those on lysine-adequate and lysine-excessive diets.

Except for loss of appetite, resulting in low food intake and depressed growth, no other nutritional deficiency signs such as caudal fin erosion, were observed in fish fed a lysine-deficient diet for 140 days. Mortality was variable, ranging from 8 to 30%. The high mortalities were mainly attributed to the incidence of Vibrio anguillarum during the latter part of the study rather than to dietary treatments. The use of antibiotics for the treatment of vibriosis was avoided because they are known to influence the growth rate of fish.

Recovery of $^{14}CO_2$ (Table V), expressed as a percentage of injected L-[U-^{14}C]lysine, was progressively higher from diets containing more than 1.90% lysine (DMB). This indicates that at higher levels of dietary lysine the amount of injected radiolabelled lysine oxidized to carbon dioxide also increased. Broken-line regression (Figure 4) estimated a dietary lysine requirement of 1.84±0.16% (SE) or 3.68% of the dietary protein with 50.0% protein in the dry matter.

There were no significant differences in dry matter content of carcasses from the different treatments (Table VI). Graded levels of lysine in the diet had significant ($P<0.05$) cubic effects on carcass protein, lipid, ash, and lysine levels. Carcass lipid levels of fish fed lysine levels of 1.76% and 1.90% (DMB), were significantly higher

Table V. Percent of injected L-[U-^{14}C]lysine recovered as $^{14}CO_2$ from Atlantic salmon fed graded levels of dietary lysine

Dietary lysine level[a] (%)	% of injected L-[U-^{14}C]lysine recovered as $^{14}CO_2$[b]
1.24	1.23 ± 0.30a
1.76	1.41 ± 0.15a
1.90	1.31 ± 0.12a
2.19	2.65 ± 0.13bc
2.45	2.14 ± 0.35ab
2.68	3.09 ± 0.52cd
2.94	3.93 ± 0.40d

[a] Analyzed dietary lysine concentration (% dry matter basis).

[b] Mean±SEM (n=3).

a-d, Numbers, within the same column, sharing the same postscript are not significantly different (P>0.05).

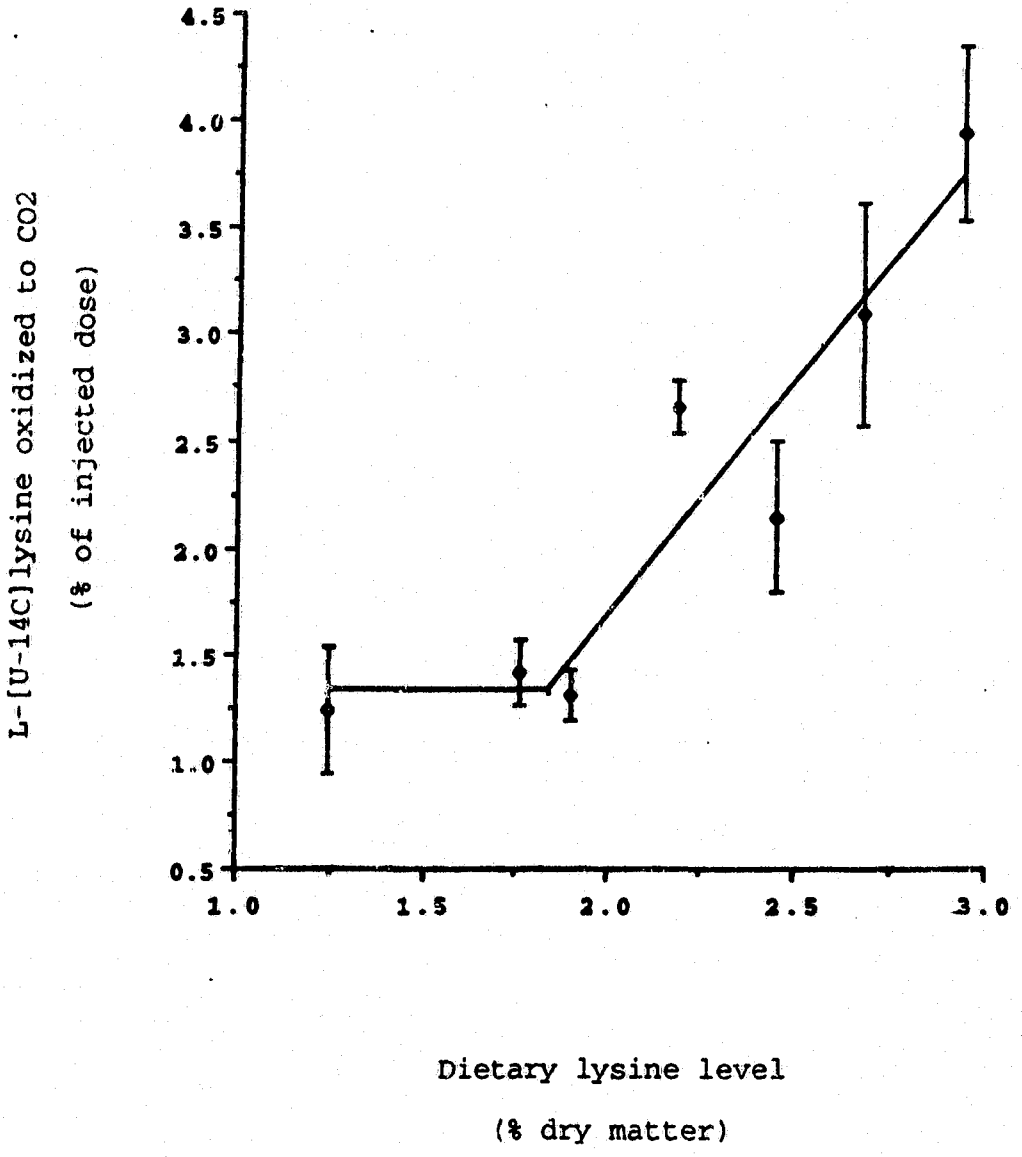

Figure 4. Broken-line regression of intraperitoneally injected L-[U-^{14}C]lysine recovered as $^{14}CO_2$ against dietary lysine level. Points represent the mean and the standard error of the mean (vertical bar) for three fish.

Table VI. Body composition of Atlantic salmon fed graded levels of dietary lysine for 10 weeks

Dietary Lysine Level[a] (%)	Dry Matter[bc] (%)	Protein[cd] (%)	Ash[cd] (%)	Lipid[cd] (%)	Lysine[cd] mg Lys/ 100 mg fish
1.24	23.3 ± 0.7a	55.3 ± 0.9cd	10.6 ± 0.2a	30.2 ± 0.03a	8.30±1.10a
1.76	24.8 ± 0.3a	52.9 ± 0.4e	9.6 ± 0.1b	33.7 ± 1.3b	5.08±0.41b
1.90	25.0 ± 0.4a	53.7 ± 0.3de	8.5 ± 0.2c	34.5 ± 0.7b	5.37±0.74b
2.19	25.0 ± 0.3a	56.5 ± 0.9bc	8.8 ± 0.1c	30.6 ± 1.0a	6.62±0.12ab
2.45	24.0 ± 1.0a	57.0 ± 0.6bc	8.4 ± 0.3c	29.0 ± 0.7a	5.50±0.40b
2.68	24.2 ± 0.2a	59.3 ± 0.4a	8.6 ± 0.2c	28.9 ± 0.9a	6.27±0.26b
2.94	23.7 ± 0.6a	58.0 ± 0.6ab	8.4 ± 0.03c	31.0 ± 1.2a	5.95±0.71b

[a] Analyzed dietary lysine concentration (% dry matter basis).

[b] Dry matter in the original carcass (as-is basis).

[c] Mean±SEM (n=3).

[d] Expressed as a percentage of the sample (dry matter basis)

a-e, Numbers, within the same column, not sharing the same postscript are significantly different ($P<0.05$).

(P<0.05) than in the other treatments. Fish fed a dietary lysine level of 1.24%, had lower carcass lipid levels (30.2%) than fish fed 1.76 or 1.90% lysine (DMB) but not significantly different from the other dietary lysine levels. Carcass protein increased with dietary lysine level, reaching a maximum at 2.68% lysine (DMB). Carcass lysine levels of fish fed 1.24% lysine were significantly higher (P<0.05) than all other treatments except the fish fed 2.19% dietary lysine (DMB).

2.4 Discussion

The dietary lysine requirement of Atlantic salmon was estimated to be 1.99±0.11% (SE) of the dry matter, or 3.98% of the protein at 50.0% protein in the dry matter. Although, no other quantitative lysine requirement of Atlantic salmon has been reported, the results are similar to the requirement found for rainbow trout by Walton et al. (1984a). In contrast to the work of Walton et al. (1984a), other reports suggest either a lower lysine requirement of 1.3% of the diet (Kim and Kayes 1982) or a higher value of 2.9 % of the diet (Ketola 1983) for rainbow trout. Our results further confirm the observation of Wilson (1989) that the value reported by Ketola (1983) is extremely high when compared with other reported values for lysine requirement.

The lysine requirement was determined on the basis of a growth response curve and the interpretation of results was based on broken-line regression rather than quadratic regression because the former yielded a smaller residual sum of squares, indicating a better fit to the data (Appendices A and B). Both methods have been used for the determination of nutrient requirements of fish (Zeitoun et al. 1976; Wilson et al. 1980; Santiago 1985). Although dose-response curves have been widely used in determination of essential amino acid requirement of fish, the following problems have been identified with the growth method: inaccurate interpretation of the growth response curve; lower growth rates are commonly observed with crystalline amino acid test diets, compared with diets containing intact proteins and, leaching of the crystalline amino acids from the test diets (Cowey and Luquet 1983).

Dietary lysine requirement determined by amino acid oxidation estimated that 1.84±0.16% (SE) (DMB) dietary lysine was necessary for optimal performance of Atlantic salmon. This value is close to the 1.99% dietary lysine determined in the growth study. Walton et al. (1984a) working with rainbow trout and using the oxidation technique found a dietary lysine requirement of 2.0% of the dry diet which compared to the value of 1.9% estimated in their growth studies. Although the amino acid oxidation technique has been used successfully with rainbow trout for the

determination of oxidized methionine (Walton et al. 1982), tryptophan (Walton et al. 1984b; Walton et al. 1986), lysine (Walton et al. 1984a; Walton et al. 1986), and arginine (Walton et al. 1986) and for the confirmation of requirements established from growth data, this method is not sufficiently precise to be solely used for the determination of amino acid requirements. The difference in the requirement levels estimated using the different methods could be due to the size or age of the fish used for the determination of the requirement. The oxidation technique, which is an acute measure, gave a lower requirement than the growth study, which was a chronic measurement over a large range of size and age.

Quantitative amino acid requirements of a given species may be influenced by many factors such as type of diet (purified, semipurified and practical) used to establish the requirement, protein level, energy level, genetic strain and size of fish, growth rate, water temperature, and salinity. However, the effects of these factors have been investigated in more detail with warm blooded animals than fish. The fish in this study were reared in water of 10 ppt salinity, which is isotonic with fish tissue and should not affect energy demands for osmoregulation. Increase in the salinity of fresh water (0 ppt) to 10 ppt had no effect on either protein requirement of coho salmon smolts (Zeitoun et al. 1974) or growth rates of coho, sockeye, and chinook salmon

(Clarke et al. 1981).

The high feed conversion ratio (1.84) of feed containing 1.24% dietary lysine, reflects the poor utilization of the low lysine diet. There was a significant improvement in feed conversion by fish fed 1.76 or 1.90% lysine; however, further increase in the dietary lysine level did not show a beneficial response. Fish fed diets containing 2.68 and 2.94% lysine showed a lower feed conversion than those fed 2.45% lysine. The reason for this response is not clear. It is possible that another amino acid, such as methionine or tryptophan, was limiting or there was an antagonism between the excess lysine and another amino acid, such as arginine. A relationship between arginine and lysine has been well documented in warm blooded animals: excessive levels of dietary lysine depress growth in poultry (Allen et al. 1972) and rats (Acheampong-Mensah and Hill 1970). In catfish, Robinson et al. (1981) could not detect this antagonism when either excess lysine was fed in diets adequate or marginal in arginine or vice versa. Although excess lysine did not affect growth rates of rainbow trout fed low levels of arginine (Kim et al. 1983), a metabolic antagonism may exist between lysine and arginine in rainbow trout. Kaushik and Fauconneau (1984) found that higher level of dietary lysine caused a decrease in the rate of arginine degradation, which was reflected in an increase in plasma and urea lysine

levels and a lower ammonia excretion. Antagonisms between different dietary amino acids have not been investigated in Atlantic salmon.

The mean carcass protein of fish fed 1.24% lysine was 55.3%; the mean carcass protein of fish fed 1.76% lysine was 52.9%. This suggests that the lowest lysine diet (1.24%), coupled with the reduced feed consumption caused a starvation-like condition. At low dietary lysine levels fish will synthesise little protein, and protein will be conserved. It appears that Atlantic salmon fingerlings utilized lipid reserves to meet demands for energy, since fish fed 1.24% lysine had lower carcass lipid levels than fish fed lysine levels of 1.76% or 1.90%. It is well known that when fish are starved, they catabolize tissue reserves to meet the demand for energy: fat deposits first (Robinson and Mead 1973; Hepher 1988), followed by protein stored in muscle. Carbohydrate stores are depleted once other preferred energy sources are used.

The fish fed 1.76% and 1.90% dietary lysine showed lower carcass protein levels and higher lipid levels than the other fish. In these two groups, lysine could be used for protein synthesis but there was insufficient amount in the diets to allow maximal growth. The feed consumption of these two groups was not different from that of fish fed the higher lysine-supplemented diets, therefore ensuring adequate energy was consumed. It is possible that excess

dietary protein was deaminated and the carbon residues used for energy or synthesis of other biological compounds via an alternate pathway such as lipogenesis or gluconeogenesis (Walton 1985). At the lowest lysine level (1.24%) the fish were in a starvation-like state and most likely their body lipid reserves were used to spare protein to meet energy requirements. In fish fed higher levels of lysine the excess dietary lysine would have been catabolized (Walton 1985). Except in fish fed 1.24% lysine, carcass lysine levels were similar to the value (9.28%) obtained by Wilson and Cowey (1985).

Lysine deficiency caused a reduction in weight gain of Atlantic salmon but no other deficiency signs were apparent. Ketola (1983) observed high mortality and caudal fin erosion in rainbow trout fed the lysine-deficient diet, however, Walton et al. (1984a) were not able confirm these lesions in the same species. Fin erosion or any other pathological signs of lysine deficiency have also not been reported in channel catfish (Wilson et al. 1977; Robinson et al. 1980), chinook salmon (Halver et al. 1958), chum salmon (Oncorhynchus keta) (Akiyama 1985), common carp (Cyprinus carpio L.) (Nose 1979) and Japanese eel (Nose 1979).

To conclude, the quantitative dietary requirement for lysine was estimated to be 1.99% of the dry diet or 3.98% of the protein at 50% protein in the dry diet. Broken-line regression of a tracer dose of L-[U-^{14}C]lysine estimated a

dietary requirement for lysine to be 1.84% of the dry diet. Except for loss of appetite, resulting in reduced weight gain, no nutritional pathologies were seen in Atlantic salmon fed a lysine deficient diet for 140 days.

3.0 COMPARISON OF IN VITRO PROTEIN QUALITY ASSAYS USED TO EVALUATE FISH MEAL QUALITY

3.1 Introduction

Increase in aquaculture production in Canada has created a higher demand for good quality fish meal for fish feed production. Since feed represents the largest single cost item (40 to 50%) of the total operating cost, it is important to devise strategies to reduce the feed cost and improve the efficiency of existing salmonid diets. Fish meal constitutes the major protein supplement in salmonid diets and directly affects the feed cost. Attempts to completely replace the fish meal component of fish diets with other ingredients of plant and animal origin have not been fully successful (Higgs et al. 1988). The reason for the high quantity of fish meal in fish diets arises from the need of fish to have high levels of protein (25 to 55%) in their diet (Wilson and Halver 1986).

There is large variation in the protein quality among lots of fish meals. Freshness of the raw material, amount of residual lipid, drying process and temperature, and whether or not the meal was made from skins, viscera, and/or bones as opposed to whole fish or muscle, drastically changes the quality and nutrient composition of the fish meal (Tarr and Biely 1972). One component of fish meal

processing that has helped to maintain protein quality is the addition of ethoxyquin. Ethoxyquin reduces lipid peroxidation and oxidation of amino acids by peroxidation products (Scott et al. 1982). Although the quality of fish meal produced in Atlantic Canada has improved significantly in the past few years, it is not up to the standard of that produced in Norway. Norwegian fish meal is produced under the strict guidelines of Norwegian Herring Oil and Meal Institute (Appendix D, page 119). During the production of Norwegian fish meal the freshness of raw material and temperatures used to dry the fish meal are strictly controlled. A Norwegian fish meal, Norse-LT94® has been shown to be of excellent quality in growth studies with Atlantic salmon. This fish meal produced a 15% greater increase in growth rates compared to fish fed a steam-dried fish meal (Pike et al. 1990). No mandatory guidelines are used in Canada but voluntary guidelines for fish meal quality have been suggested by the Canadian Working Group for Finfish Nutrition in co-operation with Canadian feed manufacturers (Appendix E, page 120).

There exist a number of *in vitro* test systems (Olley and Pirie 1966; Hsu et al. 1977; Satterlee et al. 1977; Williams 1984) using individual proteolytic enzymes or a combination of them (Hsu et al. 1977; Pedersen and Eggum 1983), all of which are conducted under artificial conditions. The *in vitro* method of assessing protein

digestibility depends on the estimation of the extent of proteolysis. Generally, the in vitro digestion is made in a closed system. The reaction is arrested either by boiling the digestion mixture or precipitation of the protein with a strong acid followed by filtration or centrifugation. The assessment of protein digestibility in these methods is usually determined by nitrogen or amino acid analysis of the different digestion fractions. Therefore, in such methods, the rate of proteolysis measured varies according to the procedure used. Also, the use of a closed system may result in inhibition of the reaction by the accumulating digestion products or the resultant drop in pH that occurs with the release of carboxyl groups from proteolysis. The enzymatic digestion at a constant pH (Pedersen and Eggum 1983) appears to offer some advantages. The measurement of NaOH required to keep the pH at 7.98 during a 10 min period eliminates the need for separation of the digestion fractions and subsequent analysis. The activity of the proteolytic enzymes used are dependent on the pH, and by keeping the pH constant during the digestion uniform enzyme activities are ensured in the different samples, as well as during the incubation period (Pedersen and Eggum 1983).

A tentative assumption was made that protein damage due to over heating during the drying of fish meal would be manifested by losses in the availability of certain amino acids. The loss of these amino acids would contribute to

decreased protein quality. Since lysine is nutritionally marginal in many proteins, and is involved in the Maillard reaction, lysine availability has been studied extensively in relation to the effects of processing (Carpenter 1973). In many studies a high correlation has been found between the ranking of samples according to available lysine and according to feeding tests, in particular, where samples have been subjected to extreme temperature treatments. In some other studies, with commercial fish meals, there was not a good correlation (Carpenter 1973).

Sulphydryl groups and disulphide bonds are important in maintaining structure and functions of native proteins and play important roles in functional properties of proteinaceous foods (Ledward 1979). Heating that affected the status of cysteine/cystine residues has been found to reduce protein utilization by animals (Waibel et al. 1977) and fish (Obstvedt et al. 1984) and it has been assumed that disulphide cross-linkages hamper the attack by proteolytic enzymes (Friedman et al. 1982), thus decreasing protein digestibility.

Microbiological activity and biochemical changes during storage of raw fish are responsible for the progressive decline of organoleptic quality. Specifically, enzymatic (bacterial and natural) breakdown of both proteins and the osmoregulatory agent trimethylamineoxide present in marine species (Love 1970) results in the production of ammonia,

monomethylamine, trimethylamine, and other volatile amines. Total volatile basic-nitrogen measuring low molecular weight volatile bases and amine compounds produced by decarboxylation of amino acids has been commonly used for assessment of fresh fish quality (Farber 1965; Woyewoda et al. 1986).

Manufacturers of fish meals need quality control methods which will help them produce meals of consistent nutritional quality. Such methods are required to be conducted accurately at the processing site in a relatively short time period, compared to the 4 to 10 weeks required for animal bioassays.

The objective of this study was to compare different *in vitro* tests (available lysine, total volatile basic-nitrogen, pepsin digestibility, multienzyme digestibility, and sulphydryl groups and disulphide bonds) for the measurement of protein quality in fish meals available in Atlantic Canada.

3.2 Materials and Methods

3.2.1 Sources and production of fish meals

Forty kilogram samples of herring (International Feed Number (IFN) 5-02-000), menhaden (IFN 5-02-009), and anchovy meals (IFN 5-01-985) were obtained from commercial fish meal or fish feed manufacturers in Atlantic Canada (Table VII).

Table VII. Drying conditions and the amount of antioxidant added for the fish meals used for the various chemical assays to measure protein quality

Fish meal	Drying Conditions	Antioxidant[a] ($mg \cdot kg^{-1}$)
Herring meal 1	Steam	200
Herring meal 2	Steam	200
Herring meal 3	Steam	200
Herring meal 4	Steam	200
Herring meal 5[b]	Steam	200
Herring meal 6[b]	Flame	200
Roe Herring[c]	Freeze dried	0
Menhaden[b]	Steam	200
Anchovy	Steam	200
Norse-LT94®[d]	Steam	400[e]

[a] The antioxidant (ethoxyquin) was added after the drying process.

[b] Made by the same fish meal manufacturer.

[c] Made in the laboratory from fresh raw herring collected from the roe herring industry.

[d] Made from whole capelin.

[e] Half of the amount added before drying and the remainder after drying.

The herring meals were freshly processed and obtained in September of 1990 from various fish meal manufacturers: Commeau Sea Products Ltd., Connor Brothers Ltd., Karlsen Shipping Company Ltd., Laurence Sweeney Fisheries Ltd., and National Sea Products Ltd (Appendix F, page 121). The meals were made from whole herring and cuttings and contained the solubles from the press-water. Approximately 40 kg of fresh herring were obtained, ground, and freeze dried for comparison. The fresh herring sample consisted mostly of sexually mature males that are waste from the roe herring industry. Menhaden and anchovy meal samples were obtained at the same time as the herring meals. The exact processing date of the anchovy meal was not known, but assumed to be 1990. All commercial meals contained 200 $mg \cdot kg^{-1}$ of an antioxidant, ethoxyquin. A 20 kg sample of a Norwegian fish meal (Norse-LT94®), produced by Norsemeal Ltd, Norway, was supplied by Dr. O. Torrissen (Directorate of Fisheries, Matre, Norway). All fish meal samples were stored at -30°C until used.

3.2.2 Analytical procedures

Duplicate samples of the fish meals (as-received basis) were subjected to chemical analysis. Dry matters were determined by weight loss after drying samples at 105°C for 24 hours (Williams 1984). Crude protein (% N x 6.25) was

determined by the Dumas method (Ebeling 1968) using a Leco Nitrogen Determinator (model FP-228, Leco Corporation, St. Joseph, MI). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL). Percentage of lipid in the fish meals was determined by the method of Bligh and Dyer (1959). Fish meal samples were analyzed for calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) content by inductively coupled argon atomic absorption spectrophotometry (Jarrell-Ash Model 9000, Franklin, MA).

Amino acid content of the fish meals was determined with a Beckman System 6300 High-Performance Amino Acid Analyzer. Sample material was subjected to acid hydrolysis using the procedure of Gehrke et al. (1985). Cysteine and cystine were determined as cysteic acid after performic acid oxidation of the sample (AOAC 1985). Tryptophan was determined after alkali hydrolysis (Williams 1984).

3.2.3 Protein quality assays

Six protein quality tests were performed in duplicate on the fish meal samples. The tests included:

1) Available lysine by the method of Carpenter (1960) as modified by Booth (1971).

2) Sulphydryl (SH) groups and disulphide (S-S) bonds by

the procedure of Sedlak and Lindsay (1968) as modified by Opstvedt et al. (1984).

3) Acid-corrected pepsin digestible protein by the AOAC method (Williams 1984, method 7.053) using a 0.2% solution of pepsin. The preliminary grinding and defatting of the samples was omitted because all the fish meal samples were obtained in finely ground form and to avoid removal of protein during the lipid extraction procedure.

4) Acid-corrected pepsin digestible protein by the Torry method (Olley and Pirie 1966). This method used a 0.0002% solution of pepsin under the same conditions of the AOAC method, omitting the preliminary grinding and defatting of the samples.

5) Total volatile basic-nitrogen (TVBN) determined by the method of Woyewoda et al. (1986). Test materials were extracted with a 60% (m/v) magnesium sulphate solution.

6) In vitro multienzyme pH-stat digestibility by the method of Pedersen and Eggum (1983). The following regression equation, based on rat digestibilities, was then used to determine the digestibility;

percent true digestibility (TD) = 79.28 + 40.74X

were X = ml of 0.1 N NaOH added to protein and enzyme solution.

3.2.4 Statistical procedures

Data were analyzed for homogeneity of variance by plotting residuals against estimated values. In cases of heterogeneity of variance, data were transformed by the use of the arcsin $\sqrt{Y}$ ($0 \leq Y \leq 1$) or $\log_e$ transformation before statistical analysis. This was done to meet requirements for equal error variances across categories for analysis of variance and multiple-comparison procedures. One-way analysis of variance, with fish meal as the main effect, was performed on the various protein quality tests. Differences among fish meals were assessed by Student Newman-Keuls multiple comparison procedure (Steel and Torrie 1960) at the 5% probability level. Correlation coefficients between various protein quality tests were determined. Statistical analysis of the data was conducted using SYSTAT (Systat, Inc., 1800 Sherman Ave., Evanston, IL). All means and standard errors presented are untransformed.

3.3 Results and Discussion

There were significant differences in the chemical composition of the various fish meals (Table VIII). The moisture content of the fish meals ranged from 2.0 to 10.4%. The protein content of the herring meals, except for herring meal 1, and Norse-LT94® was approximately 78%. The protein

Table VIII. Chemical and mineral composition of various fish meals

	Herring meal						Roe Herring[a]	Menhaden meal	Anchovy meal	Norse -LT94®
	1	2	3	4	5	6				
Chemical analysis[b]										
Dry matter (%)	97.3	93.9	93.4	96.3	96.4	95.4	98.0	96.2	89.6	91.6
Protein (%)	83.7	78.5	78.6	78.4	79.5	77.8	58.8	67.7	70.4	80.6
Lipid.(%)	9.4	11.5	14.3	9.6	10.2	15.0	33.3	10.7	11.4	12.0
Ash	11.0	14.6	12.8	14.0	11.6	13.1	9.3	21.5	17.5	13.1
Gross energy ($MJ \cdot kg^{-1}$)	22.9	21.4	22.4	21.7	22.4	22.5	26.8	20.2	21.3	21.9
Mineral analysis[b]										
Ca (%)	2.79	2.81	3.01	3.94	2.52	2.16	1.42	6.89	4.13	2.39
P (%)	2.06	2.17	2.19	2.44	1.80	1.69	1.35	3.65	2.60	2.07
Mg (%)	0.23	0.26	0.20	0.30	0.20	0.20	0.14	0.27	0.28	0.19
K (%)	0.76	1.05	0.96	0.43	0.70	0.97	1.09	0.54	0.79	1.64
Na (%)	0.46	1.57	0.71	0.69	0.73	1.18	0.67	0.67	1.07	0.83
Cu (ppm)	5.7	5.8	6.5	4.9	4.7	3.2	5.9	7.6	4.4	7.5
Fe (ppm)	76	168	181	220	116	153	73	249	300	263
Mn (ppm)	9	7	15	16	10	10	7	27	16	9
Zn (ppm)	120	79	86	107	88	78	58	122	69	108

[a] Fresh freeze-dried fish, consisting mostly of sexually mature males.

[b] Expressed as a percentage of the fish meal (as-fed basis).

content of herring meal 1 (83.7%) was higher than that of the other fish meals, which had a range from 58.8 to 79.5%. When expressed on a moisture and lipid free basis there was a 6% difference (86% to 92%) in values among the herring meals. Menhaden meal and anchovy meal had approximately 75% protein when expressed on a moisture and lipid free basis. The lower protein levels of menhaden meal and anchovy meal can be explained by the higher content of ash in these two meals. The lipid content of the various fish meals ranged from 9.4 to 33.3%. The lipid content in the herring meals ranged from 9.4 to 15.0%. Menhaden meal and anchovy meal had a higher ash content than any of herring meals. The high ash content of herring meals 2 and 6 can possibly be explained by the addition of salt to the meals. This was reflected by the higher sodium contents in these two meals. The higher ash value of herring meal 4 appears to be due to the use of filleted fish racks as the amount of Ca, P, and Mg was higher in this meal compared to other herring meals, this was also supported by the fact that the percentage of protein in the meal was low (86.3%) compared to an average of 88% for the other herring meals when expressed on a moisture and lipid free basis. The differences in the chemical composition of the various fish meals was due to the amount of residual lipid and moisture retained by these meals, which is largely due to differences in processing conditions, although some difference can be attributed to

fish species (herring versus menhaden and anchovy). Generally, the processing conditions (cooking, centrifugation, and drying conditions) for fish meals varies among fish meal producers and between batches within a production facility.

There were significant differences in amino acid composition among the different fish meals (Table IX). Herring meals 1 and 3 and anchovy meal were significantly lower in cysteine than the other fish meals. Menhaden meal and anchovy meal were also low in tryptophan. Herring meals 1, 2, and 6, menhaden meal and anchovy meal were lower in lysine than the other fish meals. When amino acid composition was expressed on a percentage of the protein basis (Appendix G, page 122) lower lysine contents were observed in herring meals 1 and 6 compared to the other fish meals. Cysteine content was also lower in herring meal 1 and anchovy meal than the other fish meals. These differences in amino acid composition could be due to the loss of these amino acids during processing or some deterioration of the raw material prior to fish meal production. The over heating of fish meal during the drying process and oxidation of amino acids during processing and storage may have caused the loss of these amino acids (Bender, 1972).

The chemical composition of the fish meals did not reflect the protein quality of these meals. The

Table IX. Amino acid composition of various fish meals

	Herring Meal						Roe Herring[a]	Menhaden meal	Anchovy meal	Norse -LT94®
	1	2	3	4	5	6				
Amino acid[b]										
Ala	5.26	4.54	4.68	5.17	5.32	4.74	3.78	4.11	4.88	5.00
Arg	6.23	5.75	6.17	6.55	6.16	5.03	5.05	4.99	4.56	5.55
Asp	8.98	8.43	9.40	9.12	10.23	8.01	8.20	8.50	8.40	9.10
Cys	0.21	0.66	0.26	0.81	0.51	0.47	ND[c]	0.63	0.12	0.50
Glu	9.97	8.66	8.98	10.18	10.32	8.63	7.54	7.52	8.29	9.39
Gly	5.10	6.18	5.52	5.32	5.43	5.06	4.43	5.49	4.91	5.57
His	1.86	1.42	1.68	1.88	1.92	1.55	1.57	1.46	1.86	1.74
Ile	4.13	3.42	4.06	4.27	4.41	3.51	3.63	3.62	3.95	4.04
Leu	7.30	5.52	6.36	7.26	7.33	6.04	5.15	5.61	6.28	6.68
Lys	6.42	6.34	7.45	7.27	7.66	5.81	6.94	6.12	6.15	6.94
Met	2.65	2.00	2.18	2.67	2.57	2.14	1.70	2.35	2.13	2.43
Phe	3.61	2.91	3.28	3.63	3.72	3.03	2.72	2.93	3.35	3.42
Pro	4.00	4.08	4.22	4.28	4.66	4.41	3.54	4.45	3.67	4.02
Ser	3.49	3.58	3.42	3.49	3.33	3.16	2.64	3.19	2.67	3.14
Thr	3.00	2.89	2.82	3.14	3.28	2.87	1.73	3.20	2.78	2.65
Trp	0.79	ND	ND	ND	0.80	1.18	ND	0.43	0.26	0.75
Tyr	2.94	2.31	2.64	3.02	3.11	2.38	2.10	2.44	2.46	2.52
Val	4.80	3.75	4.26	4.90	4.85	3.99	3.66	4.25	4.11	4.38

[a] Fresh freeze-dried fish, consisting mostly of sexually mature males.

[b] Expressed as a percentage of the fish meal (dry matter basis).

[c] ND (not determined).

determination of protein and amino acid composition involves chemical reactions that are much harsher than those occurring during digestive reactions. These harsher reactions will free nutrients otherwise unavailable through digestive reactions in the animal.

The acid-corrected pepsin digestible protein (Table X) determined by the AOAC, generally failed to differentiate the protein quality of the fish meals. Generally, high pepsin values reflects a better quality fish meal. The Torry method ranked the menhaden and anchovy meals lower than the AOAC method. A major concern about the pepsin test is the strength of pepsin solution used (Lovern et al. 1964; March and Biely 1967). The AOAC and Torry methods are identical with the exception that the former recommends a 0.2% pepsin solution and the latter a 0.0002% solution. It is apparent from the results that the Torry method gives a wider range of pepsin digestibility values than the AOAC method, thus, small differences in the meal quality were easily detected. The AOAC method recommends a much stronger concentration of pepsin which only detects very large differences in protein quality.

The in vitro multienzyme pH-stat digestibility (Table X) had digestibility values that ranged approximately from 80 to 88% for the various fish meals tested. Most of the herring meals had higher digestibilities than menhaden meal or anchovy meal. The enzymatic digestion at a constant pH

Table X. Protein quality evaluation of various fish meals by *in vitro* assays

Fish meals	Acid corrected pepsin digestible protein[a] AOAC (%)	Acid corrected pepsin digestible protein[a] Torry (%)	In vitro three-enzyme pH-stat digestibility[a] (%)	Total volatile basic nitrogen[a] ($mg \cdot 100\ g^{-1}$)
Herring meal 1	91.3±0.5a	81.9±0.7c	80.3±0.6d	64.8± 0.0a
Herring meal 2	96.5±0.6b	93.6±0.7ab	88.1±0.0a	123.2± 4.8c
Herring meal 3	96.4±0.7b	93.0±0.3ab	88.0±1.0a	67.5± 0.0a
Herring meal 4	96.7±0.5b	89.2±0.5ab	81.9±0.03d	81.8± 1.3ab
Herring meal 5	98.1±0.4b	93.5±0.3ab	86.0±0.1b	45.5± 1.1a
Herring meal 6	96.7±1.0b	91.9±0.7ab	86.0±0.2b	155.5±12.5c
Roe Herring[b]	94.1±0.5c	95.9±3.4a	87.9±0.2a	27.8± 0.0a
Menhaden	97.7±0.6b	84.0±1.2bc	81.7±0.0d	99.5± 4.7b
Anchovy	96.8±0.5b	87.4±0.02bc	84.0±0.3c	55.6± 7.4c
Norse-LT94®	98.5±0.2b	96.8±0.01a	85.2±0.2bc	98.1± 0.0b

[a] Mean and SEM (n=2).

[b] Fresh freeze-dried fish.

a-d, Numbers, within the same column, not sharing the same postscript are significantly different ($P<0.05$).

(Pedersen and Eggum 1983) appears to offer some advantages. The use of a closed system may result in inhibition of the reaction by the accumulating digestion products or the resultant drop in pH that occurs with the release of carboxyl groups from proteolysis. The activity of the proteolytic enzymes used are dependent on the pH, and by keeping the pH constant during the digestion uniform enzyme activities are ensured in the different samples, as well as during the incubation period (Pedersen and Eggum 1983). The measurement of NaOH required to keep the pH at 7.98 during a 10 min period eliminates the need for separation of the digestion fractions and subsequent analysis.

TVBN values showed large differences (27.8 to 155.6 $mg \cdot 100\ g^{-1}$ sample) among various fish meal samples (Table X, page 49). The TVBN method measures the amount of low molecular weight volatile basic compounds and amines. If severe damage occurs during the drying process of the fish meal, such volatile compounds are driven off or react with oxidation products, thus giving an erroneously low value. This was best observed with herring meal 1 because although it as ranked low in quality by the pepsin tests, it had a low TVBN value (64.8 $mg \cdot 100\ g^{-1}$ sample). A high TVBN value in fish meal is not considered desirable because it constitutes a non-protein nitrogen fraction, which increases ammonia production and excretion by fish. This test is best suited to determine the freshness of raw material used in

fish meal production. The bacterial spoilage of fish results in an increased production of volatile compounds and amines.

Available lysine (Table XI), which reflects the amount of free amino groups at the terminal end of lysine, was calculated by the following two methods to determine the available lysine: as a concentration and as a percentage of the total lysine in the fish meal. The meals were not significantly different in their content of total lysine except for the roe herring, which was significantly ($P<0.05$) higher than all other fish meals. Herring meal 1 (7.13 g·16 g^{-1} N) and herring meal 5 (9.00 g·16 g^{-1} N) were significantly different ($P<0.05$) for the quantity of available lysine. The significantly ($P<0.05$) lower value, for percentage of available lysine, for roe herring (70.3%) was attributed to the fact that none of the lipid was removed and no antioxidants were added to the sample. This resulted in lipid oxidation and the loss of available lysine by the binding of lipid oxidation products with the free epsilon group of lysine (Davidek et al. 1990).

The SH groups and S-S bonds were determined for the fish meals (Table XII) but this test has limited ability to detect the differences in protein quality of fish meal. Opstvedt et al. (1984), working with fish meals made from pollock and mackerel, showed that as temperature increased from 40 to 115°C during the drying process, there was a

Table XI. Total lysine and available lysine content of various fish meals

Fish meals	Total Lysine[a] ($g \cdot 16\ g^{-1}$ N)	Available Lysine[a] ($g \cdot 16\ g^{-1}$ N)	Percent Available Lysine[a] (%)
Herring meal 1	7.67±0.09a	7.13±0.20a	92.9±2.7a
Herring meal 2	8.07±0.06a	7.54±0.16ab	93.4±2.0a
Herring meal 3	9.48±1.07a	8.80±0.18ab	92.9±1.9a
Herring meal 4	9.28±0.91a	8.39±0.43ab	90.4±4.6a
Herring meal 5	9.63±0.01a	9.00±0.50b	93.5±5.1a
Herring meal 6	7.46±0.06a	7.45±0.50ab	96.6±3.4a
Roe herring[b]	11.81±0.42b	8.30±0.16ab	70.3±1.4b
Menhaden	9.05±0.19a	7.42±0.12ab	82.0±1.3a
Anchovy	8.73±0.35a	7.68±0.08ab	88.0±0.9a
Norse-LT94®	8.62±0.62a	7.82±0.11ab	90.4±0.9a

[a] Mean and SEM (n=2).

[b] Fresh freeze-dried fish.

a-b, Numbers, within the same column, not sharing the same postscript are significantly different ($P<0.05$).

Table XII. Sulphydryl groups, disulphide bonds, and cysteine content of various fish meals

Fish meals	Sulphydryl[a]	Sulphydryl and (Disulphide*2)[ab]	(Disulphide*2)[abc]	Cysteine[d]
Herring meal 1	1.23±0.02a	1.86±0.02a	0.63±0.01a	1.98±0.58a
Herring meal 2	4.19±0.06b	5.62±0.21ab	1.43±0.15a	6.93±0.58b
Herring meal 3	0.48±0.02c	2.10±0.09a	1.62±0.11a	2.72±0.25a
Herring meal 4	1.86±0.06de	3.61±0.61ab	1.75±0.67a	8.58±0.50b
Herring meal 5	1.44±0.08ae	4.34±0.75ab	2.90±0.82a	5.28±0.33c
Herring meal 6	2.14±0.06df	5.48±0.67ab	3.34±0.61a	5.03±0.41c
Roe Herring[e]	2.37±0.30f	8.43±3.05b	6.06±2.75a	ND[f]
Menhaden	1.21±0.04a	3.11±1.01ab	1.90±0.97a	7.67±0.41b
Anchovy	1.33±0.10a	1.87±0.12a	0.54±0.22a	1.40±0.17a
Norse-LT94®	1.83±0.09de	2.85±0.76ab	1.02±0.67a	5.20±0.33c

[a] Expressed as $mM \cdot 16\ g^{-1}$ N.

[b] Disulphide bonds were determined as sulphydryl groups.

[c] Determined by difference.

[d] Determined by amino acid analysis.

[e] Fresh freeze-dried fish.

[f] ND (not determined).

a-f, Mean and SEM (n=2), within the same column, not sharing the same postscript are significantly different ($P<0.05$).

decrease in the number of sulphydryl (SH) groups and an increase in the number of disulphide (S-S) bonds. When samples were held at 115°C for 20 minutes there was a net loss of SH, probably in the form of hydrogen sulphide. Opstvedt et al. (1984) showed that as the number of S-S bonds increased, the digestibility of the protein for rainbow trout decreased. There were significant reductions (2.5 - 5.0%) in amino acid digestibilities from drum-dried samples compared to freeze-dried samples. Cysteine/cystine digestibility was reduced by approximately 10% when drum-drying was used. In the fish meals studied here it can not be concluded whether the small increase in S-S formation would have any detrimental effect on protein digestion.

Pepsin digestibility by the AOAC method did not have any significant ($P>0.05$) correlations (Table XIII) with the remaining protein quality tests. The Torry pepsin digestibility method showed significant positive correlations with the multienzyme digestibility and the concentration of total SH and S-S bonds. The multienzyme digestibility had significant positive correlations with total SH and S-S bonds and S-S bonds. This does not support the work of Opstvedt et al. (1984), who found that as S-S bond formation increased, digestibility of the protein and amino acids for rainbow trout decreased. TVBN showed a strong negative correlation with total lysine and available lysine ($r = -0.64$ and -0.53, respectively), as TVBN

Table XIII. Pearson correlation coefficients between tests used to measure protein quality in fish meals

Quality tests[a]	(AOAC)	(TORRY)	(MULTIENZ)	(TVBN)	(T-LYS)	(A-LYS)	(%A-LYS)	(SH)	(SHSS)	(SS)
(TORRY)	0.332 0.153[b]									
(MULTIENZ)	0.174 0.463	0.743 0.000								
(TVBN)	0.293 0.207	-0.226 0.339	-0.085 0.721							
(T-LYS)	0.025 0.916	0.441 0.051	0.350 0.130	-0.635 0.003						
(A-LYS)	0.238 0.313	0.394 0.085	0.428 0.060	-0.529 0.017	0.476 0.034					
(%A-LYS)	0.167 0.482	-0.189 0.425	-0.059 0.805	0.389 0.090	-0.730 0.000	0.096 0.686				
(SH)	-0.001 0.996	0.379 0.099	0.387 0.092	0.224 0.342	-0.077 0.747	-0.256 0.275	-0.038 0.874			
(SHSS)	0.027 0.911	0.567 0.009	0.495 0.027	-0.159 0.504	0.294 0.208	0.180 0.446	-0.256 0.277	0.657 0.002		
(SS)	-0.008 0.975	0.431 0.058	0.446 0.049	-0.338 0.145	0.359 0.120	0.407 0.075	-0.240 0.309	0.213 0.367	0.851 0.000	
(CYS)[c]	0.427 0.077	0.163 0.519	-0.043 0.866	-0.070 0.783	0.203 0.419	0.074 0.771	-0.190 0.450	0.458 0.056	0.618 0.006	0.439 0.069

[a] (AOAC), AOAC pepsin digestibility; (TORRY), dilute pepsin digestibility; (MULTIENZ), Multienzyme digestibility; (TVBN), total volatile basic nitrogen; (T-LYS), total lysine; (A-LYS), available lysine; (%A-LYS), percent of the total lysine available; (SH), sulphydryl groups; (SHSS), sulphydryl groups and disulphide bonds; (SS), disulphide bonds; (CYS), cysteine.

[b] The probability that r=0 (n=20).

[c] For cysteine n=18.

decreased, total and available lysine increased. The negative correlation between available lysine and TVBN suggests that lysine was damaged through bacterial spoilage thus this type of fish meal would have a high TVBN and low available lysine. Fish meals made from fresh fish and dried with low temperatures would have low to medium TVBN values and high total and available lysine contents. Meals of poor quality would have both low TVBN's and available lysine values, as was observed with herring meal 1. Total lysine and SH groups were positively correlated. This indicates that both amino acids were lost with an increase in drying temperature of the fish meals or in use of poor quality raw fish. Although significant correlations occurred between other test, such as cysteine and sulphydryl groups and disulphide bonds, they are of little scientific importance because they describe related parameters and high correlations are expected.

These protein quality tests also differ widely in terms of simplicity and time required to perform a test. The pepsin tests are easily performed with a minimal amount of equipment but require Kjeldahl protein determinations and about 48 hours to run 10 samples in duplicate. The multienzyme digestibility requires a pH meter and takes about 30 min per sample. TVBN can be completed in 30 min per sample and also requires distillation equipment. Available lysine requires toxic chemicals (1-fluoro-2,4-

dinitrobenzene) and a 16 hour reflux. SH groups and S-S bonds require 3 hours due to reaction times for the chemicals. Both available lysine and SH groups and S-S require the use of a spectrophotometer.

In summary, the pepsin digestibility procedure of the AOAC was not a good indicator of fish meal quality. The AOAC method did not distinguish marginal quality meals and did not correlate with any of the other quality tests performed. TVBN was of marginal use as a protein quality measurement unless used with another test to evaluate fish meal. SH groups and S-S bonds show little use in measuring protein quality until a SH group to S-S bond ratio or the relationship between S-S bonds and digestibility can be determined for the measurement of protein quality. The Torry pepsin digestibility and multienzyme method correlate well in determining <u>in vitro</u> protein quality in fish meals.

4.0 BIOLOGICAL EVALUATION OF THE PROTEIN QUALITY OF FISH MEALS

4.1 Introduction

Fish meal is the principal source of protein in commercial salmonid diets. Fish meal levels range from 20 to 60% of the complete feed (Tacon and Jackson 1985). Fish meal is incorporated in the complete diet at such high levels because fish require high levels of protein (25 to 55%) (Wilson and Halver 1986). The protein quality in fish meals produced in Atlantic Canada has been known to vary considerably. This variability is caused by the raw material used (origin, species, season, whole fish or fish scraps, and storage conditions), processing methods (cooking, fat removal, drying, grinding, and antioxidant addition), and storage conditions of the fish meal (Tarr and Biely 1972).

Norse-LT94® is a Norwegian fish meal that is produced under the strict guidelines of the Norwegian Herring Oil and Meal Institute (reviewed by Pike et al. 1990). The freshness of raw material (total volatile nitrogen measurement of < 50 mg·100 g^{-1} sample) and drying temperatures in processing are controlled ($< 80°C$). Norse-LT94® has been shown to produce, approximately 15% higher growth rates in Atlantic salmon compared with steam dried

(85-100°C drying temperature) fish meal (Pike et al. 1990).

The present study was conducted to compare the protein quality of two herring meals commercially available in Atlantic Canada with that of Norse-LT94® for Atlantic salmon. The evaluation of the protein quality was carried out at several concentrations of dietary protein and the use of a protein-free diet. The use of a protein-free diet allowed protein quality tests to be used which consider the use of dietary protein for maintenance as well as for growth. The bioassays included weight gains, protein efficiency ratio, net protein retention, net protein utilization, and slope assay (Hegsted and Chang 1965; March et al. 1985; McCallum and Higgs 1989).

4.2 Materials and Methods

4.2.1 Diet formulation

Nine diets were formulated using either Norse-LT94®, herring meal 1 or herring meal 5 (Table XIV) as the sole protein source. These meals were previously evaluated using in vitro techniques. Each fish meal was included in the diet at levels necessary to obtain dietary protein concentrations of 16, 28, or 40%. The diets were formulated so they contained 15.1 MJ (3600 kcal) $\cdot kg^{-1}$ of digestible energy (DE). The DE values of the diets were calculated using DE ($MJ \cdot kg^{-1}$) values of 16.7 for dextrin,

Table XIV. Formulation and chemical composition of the experimental diets use for the biological determination of protein quality in various fish meals

Ingredients[a]	Calculated dietary protein levels (as-fed basis)									
	16.0			28.0			40.0			Protein Free
Norse-LT94®	21.7	0.0	0.0	38.0	0.0	0.0	54.2	0.0	0.0	0.0
Herring meal 1	0.0	19.7	0.0	0.0	34.4	0.0	0.0	49.1	0.0	0.0
Herring meal 5	0.0	0.0	20.9	0.0	0.0	36.5	0.0	0.0	52.2	0.0
Herring oil	14.1	14.7	14.5	12.3	13.4	12.9	10.5	12.0	11.4	18.0
Dextrin	25.0	25.0	25.0	10.0	10.0	10.0	0.0	0.0	0.0	40.0
Pre-gelatinized starch	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	0.0
Corn starch	12.9	15.1	13.4	13.5	17.3	14.3	4.0	9.6	5.3	18.4
Cellufil[b]	13.2	12.4	13.2	13.9	12.6	13.9	19.7	17.7	19.7	15.5
Vitamin Premix[c]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral Premix[d]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Monocalcium phosphate	1.6	1.6	1.6	0.8	0.8	0.8	0.0	0.0	0.0	1.6
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chemical analysis[a]										
Protein (%)	16.3	16.3	16.3	29.9	28.0	29.2	41.2	40.1	40.9	1.2
Lipid (%)	15.0	15.4	15.7	15.8	15.7	15.5	15.6	16.2	15.9	17.8
Ash (%)	5.1	4.7	4.9	6.4	5.5	5.9	7.7	6.4	6.9	2.7
Moisture (%)	7.0	6.7	6.3	6.4	6.4	6.7	6.2	5.9	5.6	6.0
Gross energy ($MJ \cdot kg^{-1}$)	19.4	19.9	19.9	20.2	20.7	20.5	20.6	21.2	21.1	19.7

[a] Expressed as a percentage of the diet (as-fed basis).

[b] Alpha-cellulose (US biochemical Corporation, Cleveland, Ohio)

[c] Vitamin premix supplied (mg or $IU \cdot kg^{-1}$ diet): vitamin A, 8000 IU; vitamin D_3, 4000 IU; vitamin E, 300 IU; vitamin K (menadione sodium bisulphite), 40; thiamin, 50; riboflavin, 70; pantothenate, 200; biotin, 1.5; folic acid, 20; vitamin B_{12}, 0.15; niacin, 300; pyridoxine, 20; ascorbic acid, 1200; inositol, 400; butylated hydroxytoluene, 15; butylated hydroxyanisole, 15.

[d] Mineral premix supplied ($mg \cdot kg^{-1}$ diet): $MnSO_4 \cdot H_2O$, 153.9; $FeSO_4 \cdot 7H_2O$, 497.5; $CuSO_4 \cdot 5H_2O$, 59.1; $ZnSO_4 \cdot 7H_2O$, 440.5; $MgSO_4 \cdot 7H_2O$, 2525.3; KI, 6.5; Na_2SeO_3, 2.2; $CoCl_2 \cdot 6H_2O$, 60.5; NaCl, 2500; NaF, 10.0; $CaH_4(PO_4) \cdot H_2O$, 12195.1.

13.3 for pre-gelatinized starch, and 8.6 for corn starch (Cho and Kaushik 1990). A DE value for protein was calculated from the gross energy of protein (23.6 $MJ \cdot kg^{-1}$) by applying a digestibility coefficient of 87%. A DE value for lipid was calculated from the gross energy of lipid (39.5 $MJ \cdot kg^{-1}$) by applying a digestibility coefficient of 85%. The apparent digestibility coefficients used for the calculation of DE for protein and lipid were those determined for Atlantic salmon at the Department of Fisheries and Oceans, Halifax, Nova Scotia. A protein free diet was formulated and fed to fish to measure endogenous protein loss. Chemical analyses (moisture, protein, lipid, ash, and energy) of the diets (Table XIV, page 60) were performed by the same methods described for the fish meals (Section 3.2.2, page 40). Mineral and amino acid analyses were omitted.

4.2.2 Fish husbandry and experimental procedures

Atlantic salmon fingerlings obtained from Cobequid hatchery, Department of Fisheries and Oceans, Collingwood, Nova Scotia, underwent a 2 week acclimation period in 100 L circular fibreglass tanks. During this period the fish were fed to satiation twice daily, on a standard commercial diet.

Fish were assigned to treatments according to a two-way randomized block design. Three sets of nine tanks each were

selected and each set was considered to be a block in the experimental design. Within each block, the nine diets were randomly assigned. Fish in two tanks were fed the protein free diet. A total of 1363 fish (initial mean weight, 7.65±0.14 g (SD)) of uniform size were randomly distributed to 29 tanks with 47 fish per tank. Each tank received fresh water at a flow rate of approximately 2 $L \cdot min^{-1}$. The water was continuously aerated and the temperature controlled to 15.4±0.5°C (SD). A photoperiod of 12 hours of light and 12 hours of dark was maintained.

The feeding regime during the experimental period (70 days), involved hand feeding to satiety three times per day for 5 days followed by 2 days during which fish were fed once a day. Records of weekly feed consumption and daily mortalities were kept. On days 0, 28, 56, and 70 of the feeding trial, following 24 hours of food deprivation, each tank of fish was weighed. Fish in a tank were weighed as a group and then the average weight was determined. On day 0, a group of 10 fish that represented the entire population was randomly selected, killed with 2-phenoxy ethanol (2 $mL \cdot L^{-1}$) and stored at -40°C until analysis. On day 70, five fish from each tank were randomly collected, killed with 2-phenoxy ethanol (2 $mL \cdot L^{-1}$) and stored at -40°C until used. Prior to chemical analyses, the frozen fish bodies were semi-thawed and ground in a Hobart grinder, refrozen, and then freeze-dried. After freeze-dying the bodies were

reground to pass through a 1 mm mesh screen. Chemical analyses (protein, lipid, and ash) of the freeze-dried fish, except for moisture, were performed by the same methods described for the fish meals (Section 3.2.2, page 40). Moisture was determined by weight loss after freeze-dying and then corrected for moisture after drying in an oven at 105°C for 24 hours.

4.2.3 Protein quality assays

Specific growth rates (SGR) were calculated by the formula of Walton et al. (1984b). Feed efficiency (FE) was calculated by wet weight gain ($g \cdot fish^{-1}$) divided by feed consumed ($g \cdot fish^{-1}$ as-fed basis). Protein efficiency ratio (PER) was calculated as weight gain (g) divided by protein intake (g). Net protein ratio (NPR) was calculated by the equation:

$$\frac{\text{weight gain (g) of the test group} + \text{Weight loss (g) of the protein free group}}{\text{weight of protein consumed by the test group (g)}}$$

Net protein utilization (NPU) was calculated by the equation:

$$\frac{\text{Body nitrogen test fish (g)} - \text{Body nitrogen fish fed a protein free diet (g)}}{\text{Nitrogen consumed (g) by the test fish}} \times 100$$

Slope assays were determined by regression analysis (Hegsted

and Chang 1965; March et al. 1985; McCallum and Higgs 1989).

4.2.4 Statistical procedures

Differences among means of the growth parameters were assessed using a two-way randomized block ANOVA. The blocking variable was tank row. Main effects in the ANOVA were fish meal and dietary protein level. Where appropriate, to meet requirements for equal variances, data were transformed using the arcsin $\sqrt{Y}$ ($0 \leq Y \leq 1$) or log transformation before statistical analysis. Differences among parameter means were assessed at the 5% probability level by Student Newman-Keuls multiple comparison procedure (Steel and Torrie 1960). The pooled standard deviation was calculated from the mean square error of the analysis of variance for an estimate of the overall variation in the parameter. All means and standard errors presented in the tables are untransformed.

Slope assays (Hegsted and Chang 1965) were determined by the regression of body protein gains ($g \cdot fish^{-1}$) or weight gains ($g \cdot fish^{-1}$) on protein intake ($g \cdot fish^{-1}$). The regressions included the information from the fish fed the protein free diet. Statistical differences among slopes were accessed by Scheffé's multiple comparison at the 5% probability level (Steel and Torrie 1960). Statistical analysis was performed using SYSTAT (Systat, Inc., 1800

Sherman Ave., Evanston, IL).

4.3 Results

There were significant differences among means of the chemical composition of the various fish meals (Table VIII, page 44). The moisture content of the fish meals ranged from 2.7 to 8.4%. The protein content of herring meal 1 (83.7%) was higher than that of the other fish meals. The difference in the chemical composition of the fish meals can be attributed to the amount of residual lipid and moisture present in these meals. Protein, expressed on a moisture and lipid free basis, ranged from 92 to 88%. Generally, the processing conditions for fish meals may vary among fish meal plants and among batches within a production facility. Consequently the extent of lipid removal and drying of the fish meals produced differs.

There were significant differences in amino acid composition among the different fish meals (Table IX, page 47). Herring meal 1 was significantly lower in cysteine than the other two fish meals. When amino acid composition was expressed on a percentage of the protein (Appendix G, page 122) a lower lysine content was seen in herring meal 1 compared to the other fish meals. These differences in amino acid composition could be due to effects of processing or the freshness of the raw product used. The over heating

of fish meal during drying and oxidation of amino acids during processing and storage may have caused the loss of these amino acids.

Evaluation of the protein quality, by chemical tests (Table X, page 49; Table XI, page 52), was determined in an attempt to evaluate the extent of protein damage. The AOAC and Torry pepsin digestibility tests ranked the fish meals in order of quality as: Norse-LT94®, best; herring meal 5, intermediate and; herring meal 1, poorest. The multienzyme digestibility and available lysine ranked the quality as: Herring meal 5, best; Norse-LT94®, intermediate and; herring meal 1 poorest. The quantity of available lysine in the fish meals well exceeded the estimated lysine requirement of 3.98% of the protein for Atlantic salmon (Section 2.4, page 27) and the reported values of 3.7 to 6.1% of the dietary protein stated for rainbow trout (Kim and Kayes 1982; Ketola 1983).

The growth of Atlantic salmon increased in relation to dietary protein level (Table XV; Figure 5). All groups receiving 40% protein in their diet had significantly different ($P<0.05$) growth rates from each other. When the dietary protein concentration was at 40% fish fed Norse-LT94® gained approximately 10% and 18% more weight than fish on the other meals. Fish fed Norse-LT94® gained approximately 28% to 38% more weight than fish fed the other fish meals at 28% dietary protein. Norse-LT94® showed

Table XV. Growth performance and protein utilization of Atlantic salmon fed various fish meals as sole protein sources at different dietary protein levels for 70 days

	Protein Free	Norse-LT94®			Herring meal 1			Herring meal 5			Pooled SD[a]
		16	28	40	16	28	40	16	28	40	
Initial weight (g)	7.51	7.86	7.41	7.56	7.63	7.70	7.60	7.67	7.82	7.58	
Weight gain (g)	-0.80	9.04d	16.16ab	17.53a	4.76e	9.95d	14.43b	5.38e	11.69c	15.69b	0.88
SGR (%·day^{-1})	-0.16	1.09e	1.65ab	1.72a	0.69f	1.18e	1.52e	0.76f	1.31d	1.60bc	0.83
Feed consumption (g)	7.59	13.82ab	15.07a	15.02a	12.17bc	12.98abc	11.92bc	11.14c	13.15abc	13.03abc	0.05
Feed efficiency	-0.12	0.65c	1.07a	1.17a	0.39d	0.77bc	1.23a	0.48d	0.89b	1.20a	0.09
PER	----	4.00a	3.59a	2.83b	2.39b	2.74b	2.99b	2.95b	3.05b	2.94b	0.26
NPR	----	4.37a	3.77b	2.96c	2.81c	2.96c	3.15c	3.40bc	3.27bc	3.09c	0.26
NPU	----	68.77a	62.01ab	55.64bc	47.24c	50.82c	57.13bc	55.58bc	55.22bc	57.45bc	3.93
Protein consumption (g)	0.00	2.26f	4.51c	6.19a	1.98g	3.64e	4.90c	1.82g	3.84d	5.33b	0.33
Protein gain (g)	-0.24	1.30f	2.54c	3.19a	0.68f	1.59e	2.51bc	0.76f	1.86e	2.80b	0.14

[a] Square root of the appropriate mean square error from the analysis of variance.

a-g, Numbers, within a row, not sharing the same postscript are significantly different ($P<0.05$).

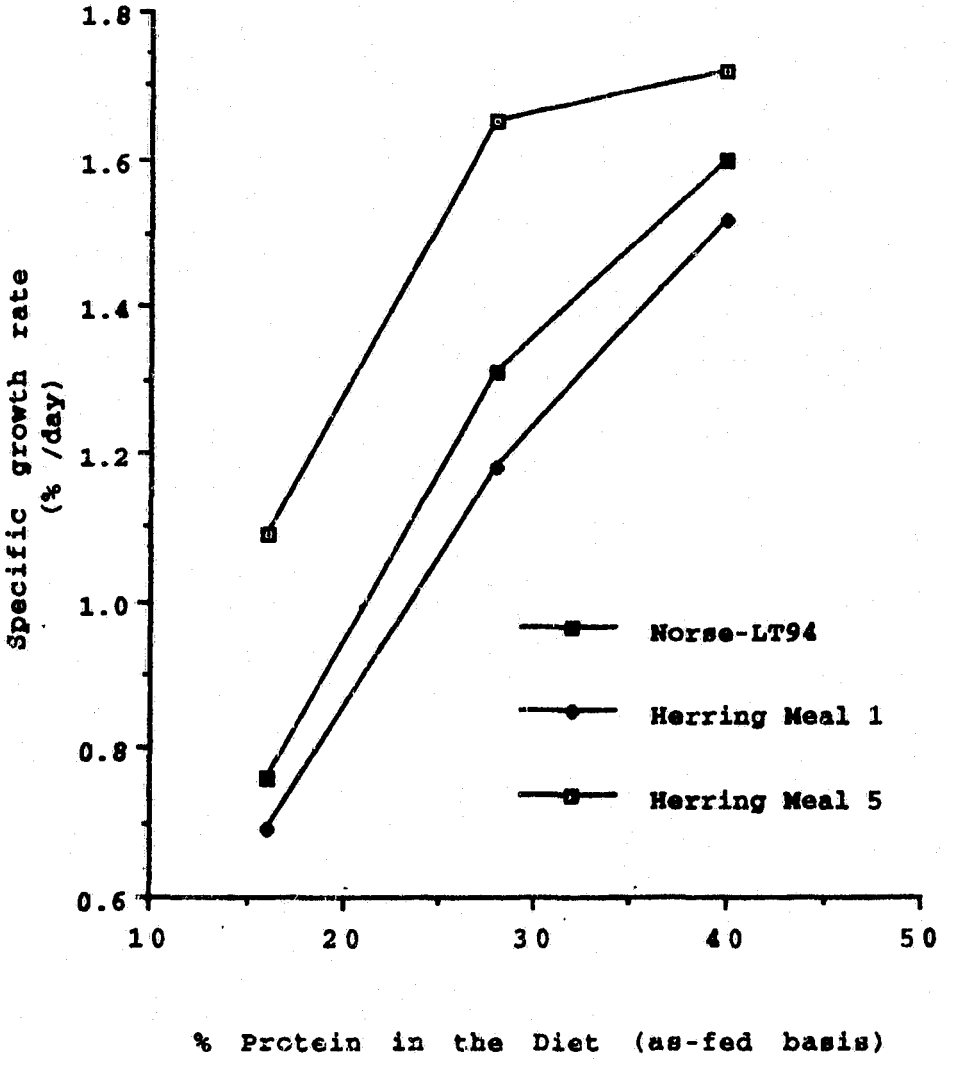

Figure 5. Specific growth rates of Atlantic salmon fed various fish meals at different dietary protein concentrations.

significantly ($P>0.05$) higher PER, NPR, and NPU values at 16% and higher PER and NPR values at 28% dietary protein than the other fish meals tested. The biological tests for protein quality consistently ranked the fish meals in order of quality as: Norse-LT94®, best; herring meal 5, intermediate and; herring meal 1, poorest. The relationship between PER and dietary protein concentration for herring meal 5 revealed no dose-dependent pattern (Table XV, page 67; Figure 6). Norse-LT94® had a significant ($P<0.05$) negative correlation ($r = -0.96$) for PER with the range of dietary protein levels used. By contrast, PER values for fish fed diets containing herring meal 1 were positively correlated with dietary protein level ($r = 0.62$, $P=0.08$).

The fish fed Norse-LT94® at 40% dietary protein plateaued in their growth (Figure 5, page 68). For this reason this data from this group of fish were not included in the slope assays. The slopes for body protein gain against protein intake were clearly different for the fish meals (Table XVI), indicating different gains due to protein source (fish meal). By slope assay procedures the following sequence for protein quality emerged: Norse-LT94® > herring meal 5 > herring meal 1.

Level of protein in the diet had a significant effect ($P<0.05$) on body composition (Table XVII). As protein concentration in the diet increased the amount of protein in the body increased. There was an inverse effect on body

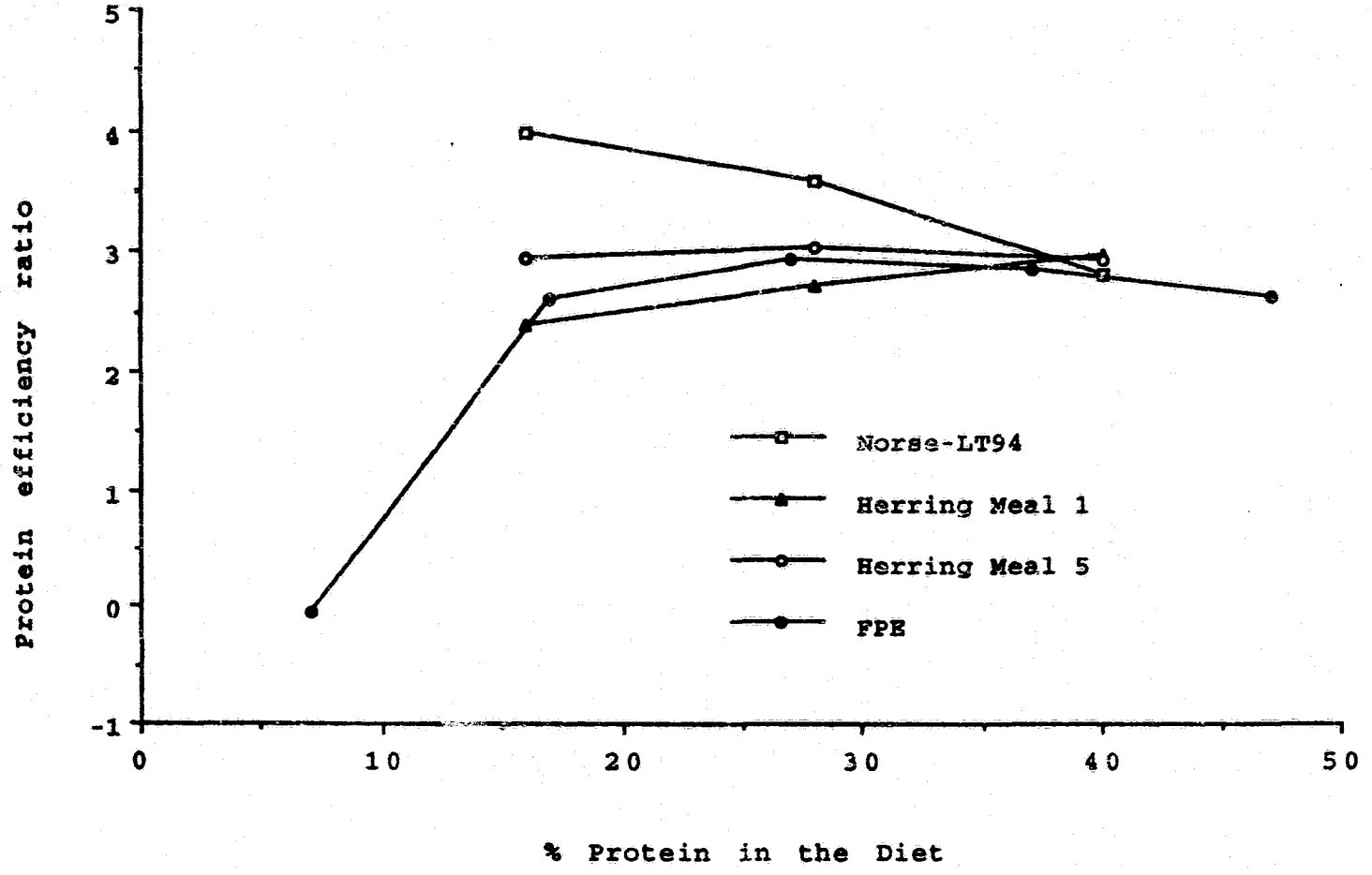

Figure 6. Effect of dietary protein level on protein efficiency ratio of Atlantic salmon fed different fish meals and chinook salmon fry fed freeze dried pollock and euphausiid (FPE) (McCallum and Higgs 1989).

Table XVI. Slope ratios of body protein gains or weight gains against protein intake for groups of Atlantic salmon fed various fish meals at different protein levels

Protein source		Slope[a] of body protein	Slope[a] of body weight
Norse-Lt94®	Slope	0.612[b]a	3.721[b]a
	SE of slope	0.019	0.156
	Intercept	-0.177	-0.194
	Correlation coefficient	0.997	0.995
	Relative slope	(100)	(100)
	Ranking	1	1
Herring Meal 1	Slope	0.516b	2.859a
	SE of slope	0.014	0.084
	Intercept	-0.289	-0.781
	Correlation coefficient	0.997	0.997
	Relative slope	(84)	(77)
	Ranking	3	3
Herring Meal 5	Slope	0.569a	3.091a
	SE of slope	0.018	0.107
	Intercept	-0.272	-0.482
	Correlation coefficient	0.995	0.996
	Relative slope	(93)	(83)
	Ranking	2	2

[a] The slopes were calculated including points obtained from groups of fish fed a protein free diet.

[b] Data from the fish fed Norse-LT94® at 40% dietary protein were omitted from the regression analysis to meet the requirements of linearity for the slope assay.

a-b Slopes, within the same column, not sharing the same postscript are significantly different ($P<0.05$; Scheffé's test).

Table XVII. Body composition of Atlantic salmon fed diets containing various fish meals at different dietary protein levels

Protein Source	Protein Level (%)	Moisture[a] (%)	Protein[a] (%)	Lipid[a] (%)	Ash[a] (%)
Norse-LT94®	16	72.6a	14.8d	9.8a	2.4a
	28	72.7a	15.9c	9.0abc	2.3a
	40	73.4a	17.5a	7.5c	2.4a
Herring meal 1	16	72.2a	15.3d	9.9a	2.4a
	28	73.0a	15.8c	8.8abc	2.4a
	40	73.1a	16.9b	8.2bc	2.4a
Herring meal 5	16	72.5a	15.1d	9.8a	2.4a
	28	72.4a	15.7c	9.3ab	2.3a
	40	73.1a	17.3a	8.0bc	2.4a
Protein free	0	77.0b	14.2e	6.1d	2.8b
Pooled SD[b]		0.5	0.2	0.6	0.04

[a] Expressed as a percentage of the sample (as-is basis).

[b] Square root of the appropriate mean square error from the analysis of variance.

Body composition (as-is basis) at the beginning of the experiment was: moisture, 75.7%; protein, 15.9%; lipid, 6.1% and; ash, 2.4%.

a-e, Means (n=3), within the same column, not sharing the same postscript are significantly different ($P < 0.05$).

lipid stores, as dietary protein level increased body lipid levels decreased. There was no effect of protein level or source on body moisture or ash levels. Mortality for the entire experiment did not exceed 1% of the entire population of fish.

4.4 Discussion

Norse-LT94® supported excellent growth and highly efficient protein utilization compared to the herring meals tested here. Herring meal 5 had amino acid concentrations that were higher than those for Norse-LT94® (Table IX, page 47), yet the growth of fish fed herring meal 5 was significantly less, suggesting that the availability of the amino acids might have been affected. Cowey et al. (1972) observed that freeze-dried and low temperature dried (30°C) cod meals were almost identical in their essential amino acid contents, yet they supported different growth rates in plaice. McCallum and Higgs (1989) working with chinook salmon found that freeze-dried pollock and euphausiid mix gave better growth rates than freeze-drie herring. They thought that, because whole herring was used, some of the integumental proteins may not have been assimilated completely. This was not the reason for the differences in growth rates in this study, since whole fish were used in production of all the fish meals.

It is well established that excessive heat can cause severe loss of protein quality (March et al. 1966; Soares et al. 1971; Tarr and Biely 1972; March and Hickling 1982). Norse-LT94® is made from fresh material and is dried with temperatures less than 80°C. The two herring meals used were steam-dried products and drying temperatures could have ranged from 80 to 100°C. Damage to essential amino acids may arise from the formation of cross-linkages which can not be broken by digestive enzymes (Bender 1972), oxidation of amino acids, especially methionine, cysteine, and tryptophan (Davidek et al. 1990), or reaction of lipid oxidation products with amino acids (Gardner 1979; Nielsen et al. 1985; Davidek et al. 1990). This damage was also shown by some of the chemical tests used to measure the protein quality of these fish meals.

Not all of the chemical tests for protein quality ranked the fish meals in the same order of quality as the biological assays and the use of only three fish meals limited the conclusions that could be drawn. Total agreement in the ranking of the fish meals among the various tests used to measure protein quality was not expected. The various chemical tests measured different elements of the quality of protein in the fish meals. The *in vitro* digestibilities (pepsin and multienzyme) measured overall amino acid availability. The test for available lysine is useful for the measurement of protein quality only when

lysine is the first limiting amino acid in the protein or final diet (Bender 1982). Threonine has been calculated to be the first limiting amino acid in herring meals by the chemical score and essential amino acid index (Tacon and Jackson 1985; Hepher 1988). Those calculations were based on the use of dietary essential amino acid requirements of rainbow trout or the amino acid pattern of whole fish eggs as the reference protein.

The relationship between PER and dietary protein concentration for herring meal 5 revealed no dose-dependent pattern (Figure 6, page 70). This was unlike that reported for rats (Hegsted and Chang 1965) and chinook salmon (McCallum and Higgs 1989). A reason for this could be due to the range of protein concentrations used for this fish meal as Norse-LT94® and herring meal 1 had dose-dependant patterns. The fish fed Norse-LT94® at 40% dietary protein showed departure from linearity following the procedure for the slope assay (Hegsted et al. 1968). For that reason the body protein gain and weight gain for this group of fish was removed from the regression analysis. This departure from linearity could be due to the significantly ($P<0.05$) large decrease in protein efficiency that occurred with the increasing level of protein in the diet for Norse-LT94®. This decrease in protein efficiency could be due to a relatively larger amount of protein used for maintenance requirements or catabolism for energy purposes instead of

protein synthesis. The correlation coefficients obtained for each slope (Table XVI, page 71), with the data removed for fish fed Norse-LT94® at 40% dietary protein, indicated that, under the conditions of the present study, the relationship between protein gain and protein intake satisfied the requirements for linearity in a slope assay (Hegsted et al. 1968). Linearity was evaluated by the use of orthogonal contrasts and visual examination of regression lines. A linear relationship between protein intake and body protein gain has been shown in previous fish studies (Nose 1963; Iwata 1970; Gerking 1971; Rychly 1980; McCallum and Higgs 1989). The slope assay for body protein gains against protein intake were a better estimate of protein quality than were body weight gains against protein intake (McCallum and Higgs 1989). Although the slope assay using weight gains ranked the quality of the fish meals the same as the slope assay using protein intake, there were no significant differences among the slopes for weight gains against protein intake, thus suggesting no difference in the protein quality of the different fish meals tested.

It can be seen from the body composition data that as dietary protein level increased so did body protein levels while body lipid levels were negatively correlated. This indicates that when the protein/energy ratio increased more energy was used for the accretion of protein and less for lipid synthesis. This has also been shown to occur in

rainbow trout (Lee and Putnam 1973) and channel catfish (Page and Andrews 1973).

The results of this experiment indicate that the protein quality of the Canadian fish meals tested was not equal to that of Norse-LT94®. The pepsin and multienzyme digestibilities ranked the fish meals in the same order as the biological tests, but the biological testing of feedstuffs to determine protein quality is recommended as the final method of comparison.

5.0 APPARENT AND TRUE AVAILABILITIES OF AMINO ACIDS FROM VARIOUS FISH MEALS DETERMINED WITH MANUAL STRIPPING OR SEDIMENTATION OF FECES

5.1 Introduction

Sources of dietary proteins are not identical in their nutritional and biological values. The biological value of a protein varies with its digestibility and amino acid composition. Deficiency of an essential amino acid creates poor utilization of the dietary protein and consequently reduced growth and decreased feed efficiency ratio.

The high percentage of protein (40-50%) from fish meals used in Atlantic salmon diets accounts for the high cost of feed (Crampton 1985). However, the scarcity of information on amino acid requirements for various fish species, the availability of amino acids from different sources, and the interactions among amino acids, are a few of the factors that have hindered the successful replacement of fish meal in commercial feeds with less costly proteins such as soybean or other oilseed meals (Higgs et al. 1988).

Although the digestibility of proteins from several sources has been reported for rainbow trout (Rychly and Spannhof 1979; Rychly 1980; NRC 1981; Jauncey 1982; Cho and Kaushik 1990), the values may vary due to experimental conditions (water temperature and salinity and feeding

levels) (Cho and Kaushik 1990). Apparent and true availabilities of amino acids from several common feed ingredients have been determined for channel catfish (Wilson et al. 1981), common carp (Hossain and Jauncey 1989), and rainbow trout (Hilton and Slinger 1986).

In fish, the collection of feces requires much effort. A method for quantitative separate collection of metabolic excretions from individual fish has been developed (Post et al. 1965; Smith 1971). This method has the disadvantage of the need for special equipment, the necessity of force feeding and the physiological stress caused by confinement. To overcome the disadvantages mentioned, indirect methods for quantifying fecal output have been used, these experiments frequently use an inert marker substance, normally chromic sesquioxide, included in the diet. If samples of feces are taken by stripping, there is the danger of interfering with digestive processes in the hindgut and thus yielding artificially low figures for digestibility (Austreng 1978). On the other hand, for feces voided into the water a considerable leaching may occur which may lead to serious over-estimations of digestibility (Windell et al. 1978; Choubert et al. 1979). Comparative determinations of digestibility from stripped or netted feces in most cases resulted in higher values from netted feces, usually 10 percentage units higher (Inaba et al. 1962; Windell et al. 1978; Cho and Slinger 1979).

The present study was designed to measure the apparent and true availabilities of amino acids to Atlantic salmon from 5 fish meals produced in Atlantic Canada. A second objective was to evaluate the difference between manual stripping and sedimentation collection of feces ("Guelph system" - Cho et al. 1975) on the determination of amino acid availabilities. The fish meals tested were: Norse-LT94, two steam- and one flame-dried herring meal, menhaden meal, and anchovy meal.

5.2 Materials and Methods

Experimental diets were formulated (Table XVIII) for the determination of amino acid availabilities using fish meal as the sole protein source. The fish meals used were: herring meals 1, 5, and 6, menhaden meal, and anchovy meal. Herring meal 6 was a flame-dried fish meal, all other fish meals used were steam-dried. The fish meals were combined with purified ingredients and chromic oxide was added as an inert marker. The test fish meals were incorporated at 45.0% of the diet (as-fed basis). A protein-free diet was made by incorporating alpha-cellulose at 45.0% of the diet, in place of fish meal.

Table XVIII. Composition of the experimental diets used for the determination of amino acid availabilities

Ingredients	% (as-fed basis)
Corn starch	10.6
Dextrin	10.0
D-Glucose	6.0
Vitamin premix[a]	2.0
Mineral premix[b]	4.0
Choline chloride	0.2
Chromic oxide	0.7
Herring oil	10.0
Celufil[c]	11.5
Test protein	45.0

[a] Vitamin premix supplied the following (mg or IU·kg^{-1} diet): Vitamin A, 8000 IU; Vitamin D_3, 4000 IU; Vitamin E, 300 IU; Vitamin K (menadione sodium bisulphite), 40; thiamin HCl, 50, riboflavin, 70; d-calcium pantothenate, 200; biotin, 1.5; folic acid, 20; Vitamin B_{12}, 0.15; niacin, 300; pyridoxin HCl, 20; ascorbic acid, 1200; inositol, 400; butylated hydroxytoluene, 15 and; butylated hydroxyanisole, 15.

[b] Mineral premix supplied (mg·kg^{-1} diet): $MnSO_4 \cdot H_2O$, 123.1; $FeSO_4 \cdot 7H_2O$, 248.8; $CuSO_4 \cdot 5H_2O$, 39.4; $ZnSO_4 \cdot 7H_2O$, 330.4; $MgSO_4 \cdot 7H_2O$, 4040.4; KI, 6.54; Na_2SeO_3, 2.19; $CoCl_2 \cdot 6H_2O$, 60.5; NaCl, 2500; NaF, 9.96; $CaH_4(PO_4) \cdot H_2O$, 12195; KH_2PO_4, 6579.

[c] Alpha-cellulose (US Biochemical Corporation, Cleveland, Ohio).

5.2.1 Experimental design and fish rearing systems

Manual Stripping: three hundred and sixty salmon, average weight 72.1±13.8 g (SD), were randomly allotted to 12 circular holding tanks. Each tank was supplied with fresh water with an average temperature of 19.3±1.8°C (SD) at approximately 2 $L \cdot min^{-1}$ and fish were subjected to 12 hours of light and 12 hours of dark. Fish were fed to satiation three times daily. A protein-free diet was fed to fish in two tanks. The protein-free diet was fed in order to determine endogenous fecal amino acids. Diets were randomly assigned in duplicate to the tanks.

The experiment lasted 30 days. On days 16, 23, and 30, feces were collected between 6 to 8 hours after feeding. Feces were pooled for each tank. Fish were anesthetized in a solution of 2-phenoxy ethanol (0.5 $mL \cdot L^{-1}$) and gentle pressure was applied from the ventral fins to the anal region to strip the fecal material into a container. Fecal samples were stored at -30°C until used.

Sedimentation: two hundred and forty salmon, average weight 93.7±4.7 g (SD), were randomly allotted to 4 sets of Guelph CYAQ-2 digestibility systems (Cho et al. 1975). Each system consisted of three tanks with a common drain to a feces settling column. Each tank within a set held 20 fish and was supplied with fresh water with an average temperature of 19.3±1.8°C (SD) at approximately 2 $L \cdot min^{-1}$

and subjected to 12 hours of light and 12 hours of dark. The fish were fed the same experimental diets as those used for manual stripping. Diets were randomly assigned to the tanks in two runs. This was due to the limited number of tanks available. Diets 1 through 4 were fed then diets 5, 6, and two sets of three tanks for the protein-free diet. Fish were fed to satiation three times a day.

The experiment lasted 34 days. Fish were allowed to acclimate to the tanks and diets for 15 days. During the fecal collection period fish were fed 3 times daily. They were fed to satiation twice and then under-fed on the last feeding prior to fecal collection. Feeding was done in this manner to eliminate any feed wastage. The tanks and collection system were cleaned to remove any residual fecal and feed materials prior to collection of feces. Fecal samples were collected via the sedimentation column after 12 hours. Fecal collection occurred on days 16 through 19, the feeding and cleaning protocol was followed on each collection day. On day 20, diets were changed and fish were allowed to adjust for 10 days. On days 31 through 34, fecal samples were collected following the above protocol. Fecal samples for each diet were pooled and frozen at -30°C until used.

5.2.2 Analytical procedures

Fecal samples from both experiments were freeze-dried before analysis. Duplicate samples of test diets and fecal material from both experiments were subjected to chemical (moisture and crude protein) and amino acid analyses (acid and alkali hydrolysis) by the methods described earlier for the fish meals (Section 3.2.2, page 40). Chromium in the test diets and fecal samples was determined by the procedure of Arthur (1970).

Apparent amino acid availability (AAAA) was calculated, as a percentage, by the formula of Wilson et al. (1981). True amino acid availability (TAAA) was calculated by correcting the AAAA data for endogenous fecal amino acids, which were determined from fish fed the protein-free diet.

5.2.3 Statistical procedures

Two-way analysis of variance was performed on the apparent and true availabilities for protein and individual dietary amino acids. The main effects in the ANOVA were fish meals and tank replicate. Differences between protein and individual amino acid means among fish meals were assessed by Student Newman-Keuls multiple comparison procedure (Steel and Torrie 1960) at the 5% probability level. The paired t-test was used to determine differences

between manual stripping and sedimentation collection for protein and individual amino acids. Pairing was done on fish, as the same fish were used for the two collection methods. Statistical analysis was performed using SYSTAT (Systat, Inc., 1800 Sherman Ave., Evanston, IL).

5.3 Results and Discussion

There were significant differences in the chemical composition of the various fish meals (Table VIII, page 44). The moisture content of the fish meals ranged from 2.7 to 10.4%. The protein content of herring meal 1 (83.7%) was higher than that of the other fish meals. Except for herring meal 6 the lipid content of the fish meals was close to 11%. Menhaden meal and anchovy meal had higher ash content than all the herring meals. The difference in the chemical composition of the fish meals was attributed to the amount of residual lipid and moisture present in these meals, although some difference can be attributed to fish species. When expressed on a moisture and lipid free basis there was range of 92 to 88% protein for the herring meals and Norse-LT94®. Menhaden meal and anchovy meal has approximately 75% protein expressed on a moisture and lipid free basis. This lower protein level can be explained by the higher content of ash in these two meals. Generally, the processing conditions for fish meals may vary among fish

meal producers and between batches within a production facility and consequently the extent of lipid removal and amount of drying differs.

There were significant differences in amino acid composition among the different fish meals (Table IX, page 47). Herring meal 1 and anchovy meal were significantly lower in cysteine than the other fish meals. Anchovy meal was also low in tryptophan. Herring meal 6 was lower in lysine than the other fish meals. When amino acid composition was expressed on a percentage of the protein basis (Appendix G, page 122) lower lysine contents are seen in herring meals 1 and 6 compared to the other fish meals. Cysteine content was also lower in herring meal 1 and anchovy meal than the other fish meals. These differences in amino acid composition could be due to effects of processing or the freshness of the raw product used. The over heating of fish meal during drying and oxidation of amino acids during processing and storage may have caused the loss of these amino acids.

Apparent crude protein digestibility calculated with values obtained from feces manually stripping (Table XIX) showed that Norse-LT94® was not significantly higher than any of the herring meals tested but was higher than either the anchovy and menhaden meals. Herring meal 5 was significantly higher ($P<0.05$) than the menhaden meal and anchovy meal. The low crude protein digestibilities for

Table XIX. Apparent crude protein and amino acid availabilities calculated with values obtained from feces collected by manual stripping of fish

Amino acid	Norse-LT94®	Herring meal 1	Herring meal 5	Herring meal 6	Menhaden meal	Anchovy meal
Ala	94.2±0.4a	86.1±0.4b	92.3±0.4a	87.7±0.3b	87.9±0.03b	81.1±2.4c
Arg	95.9±0.7a	90.8±0.1a	95.7±0.7a	91.6±1.2a	91.3±0.43a	81.5±2.4b
Asp	86.3±1.4a	83.2±0.2a	83.9±2.4a	79.0±2.5a	69.9±2.5b	78.7±1.1a
Glu	94.4±0.7a	86.3±0.4d	91.4±0.4b	88.1±0.3c	85.5±0.1d	81.5±0.7e
Gly	85.1±0.7a	81.1±0.4a	82.2±0.8a	80.1±4.9a	72.1±6.2a	82.3±2.2a
His	96.4±2.1a	74.6±1.3c	89.5±0.9b	90.3±1.5b	85.4±0.7b	75.9±1.7c
Ile	94.9±0.8a	84.9±0.2ab	93.1±0.1a	90.8±0.2ab	90.6±0.3ab	81.6±4.4b
Leu	95.3±0.4a	86.3±0.1ab	93.0±0.2a	91.0±0.1ab	89.3±0.1ab	82.3±4.2b
Lys	94.6±0.8a	86.2±0.1c	90.9±1.1b	87.7±1.0c	84.1±0.1d	80.6±0.5e
Phe	94.4±0.9a	84.0±0.0b	92.4±0.02a	89.5±0.3a	89.2±0.3a	80.2±2.7b
Pro	89.3±0.8a	83.0±0.4b	88.9±0.9a	79.8±0.8b	83.8±0.4b	73.6±2.0c
Ser	94.2±1.5a	81.9±1.3b	90.2±2.3ab	85.8±1.8ab	85.6±2.5ab	81.1±1.0b
Thr	93.2±1.3a	80.8±0.4c	89.4±1.2ab	87.4±0.6b	85.1±1.1b	80.7±1.5c
Trp	85.9±1.0ab	83.5±0.8b	88.1±0.6ab	93.3±0.1a	72.7±2.3c	81.3±3.3b
Tyr	97.1±0.7a	88.5±0.03a	96.8±0.3a	82.4±5.0a	86.2±5.1a	86.6±2.5a
Val	94.8±0.6a	85.8±0.07ab	92.6±0.04a	89.7±0.2ab	89.7±0.2ab	81.5±3.9b
Crude Protein	87.0±1.1a	80.6±2.3abc	85.1±0.8a	82.6±0.3ab	78.2±0.7bc	76.7±1.2c

a-e, Means±SEM (n=2), within a row, not sharing the same postscript are significantly different ($P<0.05$).

Herring meal 1 and 6 are possibly due to processing effects. It is well established that excessive heat damage can cause severe damage to the protein quality (Soares et al. 1971; Tarr and Biely 1972; March and Hickling 1982). Cowey et al. (1972) observed that freeze-dried and low temperature dried (30°C) cod meals were almost identical in their essential amino acid contents, yet they supported different growth rates in plaice. The low crude protein digestibilities for menhaden meal and anchovy meal could be due to the binding of the proteins to lipid oxidation products, as these two fish species contain higher levels of polyunsaturated fatty acids that are prone to oxidation (Stansby 1990). The duration of storage, amount of fat extraction, and quality of raw material may affect protein quality and amino acid availability (Rand et al. 1959).

Norse-LT94® had significantly ($P<0.05$) higher apparent availabilities of histidine and lysine than the other fish meals tested (Table XIX, page 87). Apparent availability of threonine for Norse-LT94® was higher ($P<0.05$) than all other fish meals except herring meal 5. For most amino acids, Herring meal 1, menhaden meal, and anchovy meal had significantly lower availabilities than herring meal 5 or Norse-LT94®.

The damage to essential amino acids may arise from the formation of cross-linkages that cannot be broken by digestive enzymes (Bender 1972), oxidation of amino acids

(especially methionine, cysteine, and tryptophan) (Davidek et al. 1990), or reaction of lipid oxidation products with amino acids (Gardner 1979; Nielsen et al. 1985; Davidek et al. 1990). This could explain the low protein and amino acid availabilities for menhaden meal and anchovy meal: these fish species contain higher levels of polyunsaturated fatty acids than herring (Stansby 1990).

The apparent amino acid availabilities presented here are approximately 5% higher than those determined with Atlantic salmon reared in salt water and fed comparable fish meals (Anderson et al. 1992). This difference can be explained by the water salinity, which has been shown to influence the digestibility of protein supplements (Lall and Bishop 1979; Lall et al. 1984; Uki et al. 1985).

Apparent crude protein digestibility for Norse-LT94®, determined by sedimentation (Table XX), was similar to Herring meal 5. and both of these meals were significantly higher than all other meals tested. Herring meal 1 and menhaden meal were significantly lower than the other fish meals. There was only approximately 5% difference among all of the crude protein digestibilities. Norse-LT94® had significantly ($P<0.05$) higher apparent availabilities for isoleucine, leucine, and valine than the other fish meals (Table XX). Herring meal 1 had significantly lower availabilities for isoleucine, leucine, lysine, phenylalanine, and valine than the other fish meals. A

Table XX. Apparent crude protein and amino acid availabilities calculated with values obtained from feces collected by sedimentation

Amino acid	Norse-LT94©	Herring meal 1	Herring meal 5	Herring meal 6	Menhaden meal	Anchovy meal
Ala	96.9±0.4a	92.9±0.02b	96.4±0.2a	96.0±0.7a	94.9±0.6ab	94.2±0.5ab
Arg	98.5±0.3a	95.3±0.7a	98.5±0.1a	98.3±0.8a	96.4±1.1a	95.6±0.8a
Asp	96.2±0.3a	91.9±0.4b	95.1±0.1a	95.6±0.5a	90.0±1.5b	92.3±0.4b
Glu	97.9±0.2a	93.6±0.5c	97.0±0.1ab	97.1±0.4ab	94.9±0.9bc	95.1±0.4bc
Gly	92.5±0.9a	90.2±0.3ab	94.1±0.1a	92.3±1.6a	85.4±1.5b	88.3±1.0ab
His	------[a]	95.1±1.8a	------	------	99.2±0.7a	97.9±0.5a
Ile	98.2±0.1a	92.6±0.3d	96.7±0.03b	97.0±0.4b	95.8±0.6bc	94.8±0.1c
Leu	97.9±0.1a	93.4±0.1d	96.7±0.1b	96.6±0.3b	95.3±0.5c	94.7±0.1c
Lys	99.8±---[b]a	95.9±0.4b	99.7±0.00a	99.3±0.7ab	98.0±0.6ab	97.1±0.4ab
Phe	97.7±0.2a	90.5±0.7c	95.4±0.1ab	96.3±0.6ab	94.6±1.0b	93.6±0.4b
Pro	94.6±0.9a	91.7±0.02a	95.6±0.2a	93.7±1.2a	93.5±0.9a	90.9±0.9a
Ser	98.5±0.7a	94.7±0.7a	97.4±0.5a	97.8±1.1a	96.5±1.2a	96.7±1.1a
Thr	97.6±0.2a	93.2±0.2a	96.2±0.5a	97.1±1.0a	96.1±0.8a	95.4±0.6a
Trp	98.1±0.1a	96.3±0.1b	96.6±0.7ab	------	89.4±0.3c	86.0±0.1d
Tyr	------	93.9±0.4a	97.6±0.1a	98.2±1.5a	92.3±3.3a	95.7±0.8a
Val	97.9±0.1a	93.0±0.2d	96.6±0.1b	96.8±0.4b	95.4±0.6c	94.6±0.1c
Crude Protein	95.0±0.04a	90.5±0.1c	94.8±0.1a	92.6±0.1b	90.5±0.04c	92.7±0.16b

[a] No peak detected during analysis.

[b] Only one observation (no peak detected for the duplicate sample during amino acid analysis).

a-e, Means±SEM (n=2), within a row, not sharing the same postscript are significantly different ($P<0.05$).

number of amino acids were not detected in the fecal samples thus availabilities could not be calculated. The reason for this could be due to the leaching of these amino acids from the feces.

AAAA's determined by sedimentation were on average 9% higher than those determined by manual stripping (Table XXI). All amino acids, except for histidine, were significantly different ($P<0.05$) when AAAA's calculated with values obtained from the two fecal collection methods were compared by paired t-test (Table XXI). The difference in the number of observations in the paired t-tests was a result of the inability to calculate some amino acid availabilities for certain fish meals. Windell et al. (1978) compared feces collected by manual stripping of fish to feces collected from water (sedimentation). They found that, after the feces remained for 16 hours in the water, there was a 11.5, 10.0 and 3.7% difference in dry matter, protein, and lipid apparent digestibility coefficients, respectively, and significant leaching occurred within 1 hour.

The water environment of the fish poses problems for the collection of feces for the determination of nutrient digestibility coefficients. Fecal material is semi-liquid and subject to leaching once passed. Feces may be collected by sedimentation (Cho et al. 1975; Rychly and Spannhof 1979; Cho and Kaushik 1990), although this procedure may result in

Table XXI. Manual stripping versus sedimentation collection of feces on the calculation of apparent amino acid availabilities

Amino acid	Manual Stripping	Sedimentation	n
Ala	88.2±1.3[a]a	[illegible].2±0.4b	12
Arg	91.1±1.5a	97.1±0.5b	12
Asp	80.2±1.7a	93.5±0.7b	12
Glu	87.9±1.3a	[illegible].9±0.5b	12
Gly	80.5±1.6a	[illegible].5±0.9b	12
His	85.3±2.4a	[illegible].4±0.9a	6
Ile	89.3±1.5a	[illegible].9±0.6b	12
Leu	89.5±1.4a	[illegible].8±0.5b	12
Lys	87.4±1.4a	[illegible].2±0.5b	11
Phe	88.3±1.5a	[illegible].7±0.7b	12
Pro	83.1±1.7a	[illegible].3±0.5b	12
Ser	86.5±1.5a	[illegible].9±0.5b	12
Thr	86.1±1.4a	[illegible].9±0.5b	12
Trp	84.1±2.0a	[illegible]3.3±1.6b	10
Tyr	89.6±1.9a	95.5±0.9b	10
Val	89.0±1.4a	95.7±0.5b	12
Crude Protein	81.7±1.2a	92.7±0.5b	12

[a] Mean±SEM for all fish meals tested for each collection method.

a-b, Means±SEM, within a row, not sharing the same postscript are significantly different ($P<0.05$).

leaching of soluble nitrogenous material and consequently result in higher availabilities (Windell et al. 1978; Smith et al. 1980).

True crude protein digestibilities (Table XXII) calculated with values obtained from feces collected by manual stripping showed no significant differences among the fish meals. TAAA's for histidine, phenylalanine, and valine were significantly higher in Norse-LT94® than herring meal 1 or anchovy meal. Norse-LT94® had a significantly higher TAAA for lysine than all other fish meals. TAAA's increased from 2 to 20% over apparent values which is in the range of the found by Wilson et al. (1981) for channel catfish. The reason that herring meal 1 and anchovy meal had lower TAAA's could be due to processing. The duration of storage, amount of fat extraction, and quality of raw material may affect protein quality and amino acid availability (Rand et al. 1959). The damage to amino acids may arise from oxidation or the formation of cross-linkages which can not be broken by digestive enzymes (Bender 1972; Gardner 1979; Nielsen et al. 1985; Davidek et al. 1990).

Calculation of the chromic oxide ratio of the protein-free feces showed a dilution of chromic oxide. This not only suggests that the protein-free diet was not digested but also that other components were also recovered. Consequently, amino acids with true availabilities greater than 100% (Table XXII) were calculated. This is one of the

Table XXII. True crude protein and amino acid availabilities calculated from feces collected by manual stripping of fish

Amino acid	Norse-LT94®	Herring meal 1	Herring meal 5	Herring meal 6	Menhaden meal	Anchovy meal
Ala	99.5±0.6a	91.1±0.4cd	97.0±0.4ab	93.1±0.6bc	93.8±0.2bc	87.4±2.1d
Arg	99.9±0.0[a]a	94.4±0.1a	99.2±0.7a	96.2±1.4a	96.4±0.3a	87.0±2.2b
Asp	94.4±1.6a	90.3±0.3ab	90.9±2.4ab	87.2±2.0ab	83.7±2.1b	89.6±0.7ab
Glu	99.9±0.0[a]a	92.4±0.3d	97.6±0.4b	95.1±0.1c	94.5±0.2c	90.3±0.3e
Gly	92.9±0.9a	88.8±0.3a	90.2±0.8a	88.7±4.4a	84.8±5.8a	92.5±2.6a
His	97.9±2.2a	76.5±1.3c	90.7±0.9b	91.9±1.6b	86.5±0.7b	77.2±1.7c
Ile	99.9±0.0[a]a	91.3±0.3a	98.8±0.1a	97.9±0.2a	97.4±0.1a	89.7±4.1a
Leu	99.9±0.0[a]a	92.8±0.1a	99.0±0.2a	98.3±0.3a	97.3±0.1a	91.2±3.9a
Lys	98.0±1.0a	89.3±0.1c	94.0±1.1b	91.2±0.8c	88.8±0.04c	85.2±0.3d
Phe	99.9±0.0[a]a	90.4±0.03b	98.0±0.01a	96.5±0.6a	95.7±0.1a	87.9±2.4b
Pro	99.9±0.0[a]a	98.0±0.3a	99.9±0.0[a]a	96.4±1.7a	98.1±0.1a	92.8±2.7a
Ser	99.9±0.0[a]a	87.2±1.3b	95.0±2.3ab	92.9±2.1ab	91.8±2.7ab	87.8±0.8b
Thr	99.9±0.0[a]a	96.6±0.2a	99.9±0.0[a]a	99.9±0.0[a]a	99.9±0.0[a]a	99.9±0.0[a]a
Trp	89.1±0.8a	86.4±0.7b	90.8±0.6ab	95.4±0.1a	79.0±2.1c	91.2±2.9ab
Tyr	99.9±0.0[a]a	95.0±0.04a	99.9±0.0[a]a	95.1±4.4a	99.9±0.0[a]a	95.3±2.8a
Val	99.9±0.0[a]a	92.5±0.01b	98.9±0.1ab	97.3±0.2ab	97.5±0.1ab	90.9±3.5b
Protein	99.9±0.0[a]a	93.2±2.4a	97.8±0.7a	97.7±0.5a	96.5±0.1a	93.7±1.8a

[a] Values were calculated to be greater than 100% and rounded down to 99.9%.

a-d, Means±SEM (n=2), within a row, not sharing the same postscript are significantly different ($P<0.05$).

unresolved problems in determination of true nutrient availabilities with carnivorous fish. Salmonids generally refuse to eat low-protein or protein-free diets. Such diets contain extremely high levels of carbohydrate that may affect rate of passage (Spannhof and Plantikow 1983) and thus endogenous fecal patterns. Force feeding of a protein-free diet has also been used (Wilson et al. 1981), but this procedure subjects fish to stress and endogenous nitrogen output is affected. In the past, Atlantic salmon did not accept the protein-free diet readily and attempts to force feed them did not show a consistent pattern of fecal amino acids. Therefore in this study feces were collected only from fish which were actively feeding. This was relatively easy to determine because fish which were not feeding had almost no fecal material present and the green color of chromic oxide could not be detected. Another source of error could be the addition of urine to the fecal samples during collection. However, Forster and Goldstein (1969) established that only a small fraction of the total nitrogen excreted by fish appears in the urine and Smith (1971) found that about 80% of non-fecal waste nitrogen was excreted as ammonia through the gills of fish. Therefore, if contamination of the fecal sample occurred the amount of nitrogen contributed by urine could be considered to be small, and all samples have the possibility of equal contamination.

Amino acid analysis of feces from fish fed protein-free diet collected by sedimentation revealed detectable levels of glutamic acid, glycine, leucine, and proline only (Appendix H, page 123). All other amino acids were not detected in the feces from fish fed the protein-free diet, this suggest that leaching occurred from the feces. For those reasons true crude protein and amino acid availabilities calculated from values obtained from feces collected by sedimentation were not tabulated here (Appendix I, page 124).

The availability of amino acids from fish meals produced in Atlantic Canada was only slightly less than that of Norse-LT94®. The 10% difference between values calculated from feces obtained by either manual stripping or sedimentation reported in the literature (Windell et al. 1978; Smith et al. 1980) and was observed in this study.

In summary, these data indicate that protein digestibility assay has limited use in feed formulation. Amino acid availability data for common feedstuffs is needed for the most efficient use of feedstuffs. In addition this study showed that the individual amino acid availability of a feed ingredient is quite variable and a valid estimate of endogenous amino acid excretion are necessary for an accurate determination of true amino acid availabilities.

6.0 GENERAL DISCUSSION

Amino acid requirements have been estimated for chinook salmon and rainbow trout (Wilson 1989) but not for Atlantic salmon. A lysine requirement, based on growth response, was assessed using broken-line analysis (Robbins et al. 1979) and estimated to be 1.99±0.11% (SE) of the diet (DMB) or 3.98% of the protein at 50% protein in the diet (DMB). A dietary lysine requirement was also estimated based on the oxidation of L-[U-^{14}C]lysine. Broken-line analysis of oxidized L-[U-^{14}C]lysine estimated the dietary lysine requirement to be 1.84±0.16% (SE) of the diet (DMB) or 3.68% of the protein at 50% protein in the diet (DMB).

The difference in the requirement levels estimated using the different methods could be due to the size or age of the fish used for the determination of the requirement. The oxidation technique, which is an acute measure, gave a lower requirement than the growth study, which was a chronic measurement over a large range of size (4.7 to 12.7 g) and age. Both estimated lysine requirements for Atlantic salmon are similar to those reported for other salmonids (Wilson 1989).

Protein is required to provide amino acids and energy to fish. If a protein is of poor quality a relatively larger proportion of amino acids is used for energy or synthesis of biological compounds other than protein (Walton

1985), this "waste" of protein is very inefficient. The inefficient use of protein leads to the production of ammonia, which reduces water quality, and the production of solid nitrogenous wastes which are a pollution problem in the water surrounding fish rearing sites. As protein is one of the most expensive nutrients in fish diets, the use of high quality fish meals leads to the reduction of feed costs and pollution, as the protein is used more efficiently by the fish.

Several chemical and biological approaches have been used to determine the availability of amino acids in a protein. Biological availabilities of amino acids are seldom determined because the required growth assays are time consuming and expensive. In vitro measurement of protein quality allows for the rapid screening of animal by-product quality. However, chemical tests fail to predict correctly the protein quality of some products when compared to biological assays (Cowey and Sargent 1979). The in vitro tests employed in this study measured several aspects of the protein quality in the fish meals. The pepsin tests and multienzyme measured overall amino acid availability. Available lysine and sulphydryl groups and disulphide bonds measured specific amino acids and their "availability". Total volatile basic-nitrogen measured the quantity of volatile amine compound present. Total volatile nitrogen is the best assay for determination of the freshness of the raw

material used to make fish meal, as the production of volatile amines is indicative of bacterial spoilage prior to meal production.

The pepsin tests used in this study were the recommended AOAC method (Williams 1984) and the Torry method (Olley and Pirie 1966). The only difference in the two methods was the concentration of pepsin solution used for incubation of the samples. In the AOAC method a 0.2% solution of pepsin was used, whereas the Torry method requires a 0.0002% solution. At the higher pepsin concentration differences among meals may be masked due to the strength of pepsin used. Pepsin concentration has been clearly shown to affect the sensitivity of the _in vitro_ assay (March and Biely 1967; March and Hickling 1982).

Available lysine and the sulphydryl groups and disulphide bonds, although they measure protein damage, were not good indicators of protein quality for the fish meals used in this study. _In vitro_ available lysine did not have a significant correlation with apparent or true lysine availabilities (Table XIII, page 55). Available lysine only relates to the biological estimate of protein quality if lysine is the first limiting amino acids in the fish meal (Bender 1982). Threonine and tryptophan have been calculated to be the limiting amino acids in herring meal (Hepher 1988; Halver 1989); thus available lysine does show protein damage, but it did not necessarily rank the fish

meals in the same order of quality as biological tests. It is clear that a single chemical test alone can not be used for the measurement of protein quality in fish meals. The use of an number of tests in combination, such as the Torry pepsin assay and multienzyme digestibility, would give a better estimate of the protein quality. The best method for evaluation of protein quality is an animal assay. Chemical tests usually involve treatments that are much harsher than what occurs in the digestive tract. These tests usually do not evaluate the effects of other nutrients, in the protein source or diet, that may affect availability.

Evaluation of the protein quality, by growth assays using Atlantic salmon, of two Canadian herring meal and Norse-LT94® revealed that at lower dietary levels, protein utilization was significantly poorer in the Canadian herring meals tested (Table XV, page 67). The two herring meals tested were steam dried meals, but herring meal 1 appeared to be an over-dried meal, based on the results from the growth assays and in vitro protein evaluation.

Evaluation of the protein quality by chemical tests usually classified herring meal 1 as poor quality. It has been shown that damage to essential amino acids may arise from the formation of cross-linages which can not be broken by digestive enzymes (Bender 1972), but the protein hydrolysis during amino acid analysis would cause the release of such cross-linked amino acids. This was

supported by the amino acid composition (Table IX, page 47) and amino acid availabilities (Table XIX, page 87; Table XXII, page 94) where for most essential amino acids herring meal 1 had lower availabilities than Norse-LT94® but had higher concentrations of amino acids between the two fish meals.

Both pepsin digestibility assays and the multienzyme assay (Table X, page 49) showed that the three fish meals tested were of different quality and this was further confirmed by the biological assays. The reason for this appears to be due to the difference in the processing conditions of the various meals evaluated. Norse-LT94® is produced from fresh fish (TVBN < 50 mg·100 g^{-1} sample) with drying temperatures of less than 75°C. Although the fish meals used in this study were freshly produced products, the freshness of the raw material used was unknown and the drying temperatures used could have ranged from 80 to 100°C (steam drying), thus affecting protein quality. Since only three meals (a very good, a good, and a poor quality) were tested due to limited availability of tanks, these conclusions are based on limited observations.

The weight gain of fish fed Norse-LT94® at 28% dietary protein was similar to fish fed diets containing 40% protein using the other fish meals (Table XV, page 67). The protein intake for fish fed Norse-LT94® at 28% dietary protein was less than the fish fed diets containing 40% protein. These

results confirm that a high quality fish meal will produce equal or better weight gains fed at lower levels in the diet compared to a poor quality fish meal include at higher levels in the diet. The efficiency at which the protein will be used will be higher for the high quality meal. This was supported by the high PER values for Norse-LT94® (Table XV, page 67). The high efficiency of protein use was also shown by the high amino acid availabilities of Norse-LT94®.

Apparent and true availability of histidine and lysine, determined from feces collected by manual stripping, were significantly higher in Norse-LT94® than the other fish meals tested (Table XIX, page 87; Table XXII, page 94). Threonine availabilities for Norse-LT94® were also higher than the other fish meals except for herring meal 5. For all essential amino acids, except tryptophan, Norse-LT94® had higher apparent availabilities, although not always significantly, than the other fish meal (Table XIX, page 87). Herring meal 5 had the second highest value except in the case of histidine. Calculation of the true amino acid availabilities, in some cases, led to values greater than 100% (Table XXII, page 94). As an amino acid can not be greater than 100% available, this error results from the over estimation of digestibility due to the fecal marker used or in the determination of the endogenous fecal amino acids. The inclusion of high amounts of starch in protein-free diets may cause a significant change in the endogenous

fecal pattern. The high amounts of starch increase the rate of passage and possibly the amount of sloughed intestinal material, increasing endogenous nitrogen and amino acids. Carnivorous fish usually do not readily accept protein-free diets. This makes collection of feces difficult.

7.0 CONCLUSIONS

A quantitative dietary lysine requirement for juvenile pre-smolt Atlantic salmon determined by growth studies was estimated to be 1.99±0.11% (SE) of the diet (dry matter basis) or 3.98% of the protein at 50% protein in the dry matter. Broken-line analysis of oxidized L-[U-^{14}C]lysine estimated the dietary lysine requirement to be 1.84±0.16% (SE) of the diet (DMB) or 3.68% of the protein at 50% protein in the diet (DMB).

The pepsin digestibility procedure of the AOAC did not distinguish marginal quality meals and did not correlate with the other chemical assays (Torry pepsin digestibility, TVBN, available lysine, and sulphydryl groups and disulphide bonds) used to determine protein quality. The Torry pepsin digestibility and multienzyme method gave an accurate estimate of the protein quality for the fish meals used in the growth study.

The comparison of two herring meals with Norse-LT94® revealed that the herring meals used were of lower quality. Norse-LT94® was shown to be of excellent quality in growth studies with Atlantic salmon in this study. Norse-LT94® had weight gains approximately 47, 38, and 18% greater than the herring meals tested when compared at 16, 28, and 40% protein in the diet, respectively.

The availability of amino acids from various fish meals

produced in Atlantic Canada was less than those for Norse-LT94®. Calculation of amino acid availabilities from feces collected by sedimentation was found to be approximately 9% higher than availabilities calculated from feces collected by manual stripping. Calculation of true amino acid availabilities was found to be hindered by use of high amount of starch in the protein-free diet and the refusal of fish to ingest the diet.

The use of a single chemical assay for the determination of protein quality is not recommended. A number of tests need to be performed to give an accurate estimate of the protein quality. The tests recommended to be use are: total volatile basic nitrogen, for the evaluation of the freshness of raw material; pepsin digestible protein, by the Torry method (0.0002% solution of pepsin) and the multienzyme digestibility. These two latter *in vitro* assays are recommended because evaluated the quality of the fish meals the same as in the biological assays used in this study. The two *in vitro* assays also correlated well ($r=0.74$) when used to evaluate the protein quality of the fish meals used in this study.

The possible results of using chemical assays to evaluate the quality of fish meals for the fish meal manufacturing industry could be to help determine if processing conditions are optimal for the production of a high quality product and reduce variation that occurs among

different batches of fish meals from the same manufacturer. The control of processing conditions (freshness of raw material and especially drying temperatures and times) will allow Atlantic Canadian fish meal manufacturers to produce products equivalent in quality to Norse-LT94®.

8.0 REFERENCES

ACHEAMPONG-MENSAH DK, HILL DC. Effect of excess dietary lysine on the weanling rat. Nutr Rep Int 1970; 2: 2-17.

AKIYAMA T, ARAI S, MURAI T, NOSE T. Threonine, histidine and lysine requirements of chum salmon fry. Bull Jpn Soc Sci Fish 1985; 51: 635-639.

ALLEN NK, BAKER DH, SCOTT HM, NORTON HW. Quantitative effect of excess lysine on the ability of arginine to promote chick weight gain. J Nutr 1972; 102: 171-180.

ANDERSON JS, ANDERSON DM, LALL SP, MCNIVEN MA. Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (Salmo salar) reared in sea water. Aquaculture 1992; (In Press).

ARTHUR D. The determination of chromium in animal feed and excreta by atomic absorption spectrophotometry. Can Spectroscopy 1970; 15: 134-136,140.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC). Sulfur amino acids in food and feed ingredients. J Assoc Off Anal Chem 1985; 68: 398.

AUSTRENG E. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture 1978; 13: 265-272.

BENDER AE. Evaluation of protein quality: methodological considerations. Proc Nutr Soc 1982; 41: 267-276.

BENDER AE. Processing damage to protein food: a review. J Food Technol 1972; 7: 239-250.

BLIGH EG, DYER WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37: 911-917.

BOOTH VH. Problems in the determination of FDNB-available lysine. J Sci Food Agric 1971; 22: 658-666.

BORLONGAN IG, BENITEZ LV. Quantitative lysine requirement of milkfish (Chanos chanos) juveniles. Aquaculture 1990; 87: 341-347.

BROOKES IM, OWENS FN, GARRIGUS US. Influence of amino acid level in the diet upon amino acid oxidation by the rat. J Nutr 1972; 102: 27-36.

KIM KI, KAYES TB, AMUNDSON CH. Protein and arginine requirements of rainbow trout. Fed Proc, Fed Am Soc Exp Biol 1983; 42: 667, Abstr. 2198.

LALL SP, BISHOP FJ. Studies on the nutrient requirements of rainbow trout, Salmo gairdneri, grown in sea water and fres water. In: Pillay TVR, Dill WA, eds. Advances in aquaculture. Rome, Italy: Food and Agriculture Organization, 1979: 580-584.

LALL SP, ADAMS NJ, HINES JA. Digestibility measurement in feedstuffs for Atlantic salmon in freshwater and seawater. International Symposium: Feeding and nutrition in fish, University of Aberdeen, Abstracts of papers, Poster No. 11, 1984.

LEDWARD DA. Proteins. In: Priestley RJ, ed. Effects of heating on foodstuffs. London: Applied Science Publishers Ltd., 1979: 1-34.

LEE DJ, PUTNAM GB. The response of rainbow trout to varying protein/energy ratios in a test diet. J Nutr 1973; 103: 916-922.

LOVE RM. The chemical biology of fishes. New York: Academic Press, 1970.

LOVERN JA, OLLEY J, PIRIE R. Pepsin digestibility: As an index of quality in fish meal: Part II. International Fisheries News 1964; 3(4): 310-318.

LUQUET P, SABAUT JJ. Nutrition azotee et croissance chez la daurade et la truite. Acetes Coll, Coll Aquacult, Brest 1974; 1: 243-253.

MARCH BE, BIELY J. Nutritional evaluation of fishmeals for poultry feeding. Cornell Nutr Conf for Feed Mfrs 1967; pp. 133-135.

MARCH BE, HICKLING DR. Assessment of heat damage to protein digestibility from fish meals by in vitro pepsin solubilization at different temperatures. Can J Animal Sci 1982; 62: 657-660.

MARCH BE, MACMILLAN C, MING FW. Techniques for evaluation of dietary protein quality for the rainbow trout (Salmo gairdneri). Aquaculture 1985; 47: 275-292.

MARCH BE, BIELY J, GOUDIE C, TARR HLA. Protein nutritive quality of commercial fish meals as assessed by biological and chemical methods. J Fish Res Board Can 1966; 23: 395-414.

HEGSTED DM, NEFF R, WORCESTER J. Determination of the relative nutritive value of proteins factors affecting precision and validity. J Agri Food Chem 1968; 16: 190-195.

HEPHER B. Nutrition of pond fishes. New York: Cambridge University Press, 1988.

HIGGS DA, MCBRIDE JR, DOSANJH BS, CLARKE WC, ARCHDEKIN, C, HAMMONS A -M. Nutritive value of plant protein sources for fish with special emphasis on canola products. Aquaculture International Congress & Exposition, Congress Proceedings, Sept. 6-9, 1988, Vancouver, British Columbia, Canada, 1988: 427-435.

HILTON JW, SLINGER SJ. Digestibility and utilization of canola meal in practical-type diets for rainbow trout (Salmo gairdneri). Can J Fish Aquat Sci 1986; 43: 1149-1155.

HOSSAIN MA, JAUNCEY K. Studies on the protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for common carp (Cyprinus carpio L.). Aquaculture 1989; 83: 59-72.

HSU HW, VAVAK DL, SATTERLEE LD, MILLER GA. A multienzyme technique for estimating protein digestibility. J Food Sci 1977; 42: 1269-1273.

INABA D, OGINO C, TAKAMATSU C, SUGANO S, HATA H. Digestibility of dietary components in fishes I. Digestibility of dietary proteins in rainbow trout. Bull Jpn Soc Sci Fish 1962; 28: 267-370.

IWATA K. Relationship between food and growth in young crucian carps, Carassius auratus curieri, as determined by the nitrogen balance. Jpn J Limnol 1970; 31: 129-151.

JAUNCEY K. The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (Sarotherodon mossambicus). Aquaculture 1982; 27: 43-54.

KAUSHIK SJ, FAUCONNEAU B. Effects of lysine administration on plasma arginine and on some nitrogenous catabolites in rainbow trout. Comp Biochem Physiol 1984; 79A: 459-462.

KETOLA HG. Requirement for dietary lysine and arginine by fry of rainbow trout. J Anim Sci 1983; 56: 101-107.

KIM KI, KAYES TB. Test diet development and lysine requirement of rainbow trout. Fed Proc, Fed Am Soc Exp Biol 1982; 41: 392, Abstr. 716.

CARPENTER KJ. Damage to lysine in food processing: its measurement and its significance. Nutr Abstr Rev 1973; 43: 423-451.

CARPENTER KJ. The estimation of the available lysine in animal-protein foods. Biochem J 1960; 77: 604-610.

CHO CY, KAUSHIK SJ. Nutritional energetics in fish: Energy and protein utilization in rainbow trout (Salmo gairdneri). World Rev Nutr Dietetics 1990; 61: 132-172.

CHO CY, SLINGER SJ. Apparent digestibility measurement in feedstuffs for rainbow trout. In: Halver JE, Tiews K, eds. Finfish nutrition and fishfeed technology, Vol. 2. Berlin: Heenemann GmbH and Co., 1979: 239-247.

CHO CY, BAYLEY HS, SLINGER SJ. An automated fish respirometer for nutrition studies. Proc. 28th Ann. Meeting of Can. Conf. for Fish. Res., Vancouver, B.C., 1975.

CHOUBERT G JR, DE LA NOUE J, LUQUET P. Continuous quantitative automatic collector for fish feces. Prog Fish-Cult 1979; 41: 64-67.

CLARKE WC, SHELBOURN JE, BRETT JR. Effect of artificial photoperiod cycles, temperature, and salinity on growth and smolting in underyearling coho (Oncorhynchus kisutch), chinook (O. tshawytscha), and sockeye (O. nerka) salmon. Aquaculture 1981; 22: 105-116.

COWEY CB, LUQUET P. Physiological basis of protein requirements of fishes. Critical analysis of allowances. In: Arnal M, Pion R, Bonin D, eds. Reports of the 4th International Symposium on Protein Metabolism and Nutrition, Clermont-Ferrand, France, Vol. I. Paris: INRA, 1983: 365-384.

COWEY CB, SARGENT JR. Nutrition. In: Hoar WS, Randall DJ, Brett JR, eds. Fish physiology, Vol. VIII. New York: Academic Press, 1979: 1-69.

COWEY CB, SARGENT JR. Fish nutrition. Adv Mar Biol 1972; 10: 383-492.

COWEY CB, POPE JA, ADRON JW, BLAIR A. Studies on the nutrition of marine flatfish. The protein requirements on plaice (Pleuronectes platessa). Br J Nutr 1972; 28: 447-456.

CRAMPTON VO. The application of nutritional findings to the formulation of practical diets. In: Cowey CB, Mackie AJ, Bell G, eds. Nutrition and feeding in fish. London: Academic Press, 1985: 447-464.

DAVIDEK J, VELISEK J, POKORNY J, comps. Chemical changes during food processing. New York, NY: Elsevier Science Publishing Company, Inc., 1990.

EBELING ME. The Dumas method for nitrogen in feeds. J Assoc Off Anal Chem 1968; 51: 766-770.

FARBER L. Freshness tests. In: Borgstrom G, ed. Fish as food. New York: Academic Press, 1965.

FOOD AND AGRICULTURE ORGANIZATION (FAO). The production of fish meal and oil. Rome: FAO Fish Tech Pap 1975; 142.

FORSTER RP, GOLDSTEIN L. Formation of excretory products. In: Hoar WS, Randall DJ, eds. Fish physiology, Vol. 1. New York: Academic Press, 1969: 313-350.

FRIEDMAN M, GROSJEAN O -KK, ZAHNLEY JC. Inactivation of soya bean trypsin inhibitors by thiols. J Sci Food Agric 1982; 33: 165-172.

GARDNER HW. Lipid hydroperoxide reactivity with proteins and amino acids: a review. J Agric Food Chem 1979; 27: 220-229.

GEHRKE CW, WALL LL SR, ABSHEER JS, KAISER FE, ZUMWALT RW. Sample preparation for chromatography of amino acids: Acid hydrolysis of proteins. J Assoc Off Anal Chem 1985; 68: 811-821.

GERKING SD. Influence of rate of feeding and body weight on protein metabolism of bluegill sunfish. Physiol Zool 1971; 44: 9-19.

HALVER JE, comp. Fish nutrition. 2nd ed. New York: Academic Press, 1989.

HALVER JE, DELONG DC, MERTZ ET. Threonine and lysine requirements of chinook salmon. Fed Proc, Fed Am Soc Exp Biol 1958; 17: 478, Abstr. 1873.

HEGSTED DM, CHANG Y -O. Protein Utilization in growing rats. I. Relative growth index as a bioassay procedure. J Nutr 1965; 85: 159-168.

MCCALLUM IM, HIGGS DA. An assessment of processing effects on the nutritive value of marine protein sources for juvenile chinook salmon (Oncorhynchus tshawytscha). Aquaculture 1989; 77: 181-200.

NATIONAL RESEARCH COUNCIL (NRC). Nutrient requirements of coldwater fishes. Washington, D.C.: National Academy of Science, 1981.

NIELSEN HK, FINOT PA, HURRELL RF. Reactions of proteins with oxidizing lipids 2. Influence on protein quality and on the bioavailability of lysine, methionine, cyst(e)ine and tryptophan as measured in rat assays. Br J Nutr 1985; 53: 75-86.

NOSE T. Summary report on the requirements of essential amino acids for carp. In: Halver JE, Tiews K, eds. Finfish nutrition and fishfeed technology, Vol. I. Berlin: Heenemann GmbH and Co., 1979: 145-156.

NOSE T. Determination of nutritive value of food protein on fish -II. Effect of amino acid composition of high protein diets on growth and protein utilization of the rainbow trout. Bull Freshwater Fish Res Lab, Tokyo 1963; 13: 41-50.

OLLEY J, PIRIE R. The pepsin digestibility method at low pepsin strengths. International Fisheries News 1966; 5(4): 27-29.

OPSTVEDT J, MILLER R, HARDY RW, SPINELLI J. Heat-induced changes in sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (Salmo gairdneri). J Agric Food Chem 1984; 32: 929-935.

PAGE JW, ANDREWS JW. Interactions of dietary levels of protein and energy on channel catfish (Ictalurus punctatus). J Nutr 1973; 103: 1339-1346.

PEDERSEN B, EGGUM BO. Prediction of protein digestibility by an in vitro enzymatic pH-stat procedure. Z Tierphysiol, Tierernahrg u Futtermittelkde 1983; 49: 265-277.

PIKE IH, ANDORSDOTTIR G, MUNDHEIM H. The role of fish meal in diets for salmonids. Hertfordshire, England: International Association of Fish Meal Manufacturers, 1990; 24.

POST G, SHANKS WE, SMITH RR. A method for collecting metabolic excretions from fish. Prog Fish-Cult 1965; 27: 108-111.

RAND NT, COLLINS VK, VARNER DS, MOSSER JD. Biological evaluation of the factors affecting the protein quality of fish meals. Poultry Sci 1959; 39: 45-53.

ROBBINS KR, NORTON HW, BAKER DH. Estimation of nutrient requirements from growth data. J Nutr 1979; 109: 1710-1714.

ROBINSON EH, WILSON RP, POE WE. Arginine requirement and apparent absence of lysine-arginine antagonist in fingerling channel catfish. J Nutr 1981; 111: 46-52.

ROBINSON EH, WILSON RP, POE WE. Re-evaluation of the lysine requirement and lysine utilization by fingerling channel catfish. J Nutr 1980; 110: 2313-2316.

ROBINSON JS, MEAD JF. Lipid absorption and deposition in rainbow trout (Salmo gairdnerii). Can J Biochem 1973; 51: 1050-1058.

RYCHLY J. Nitrogen balance in trout. II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. Aquaculture 1980; 20: 343-350.

RYCHLY J, SPANNHOF L. Nitrogen balance in trout I. Digestibility of diets containing varying levels of protein and carbohydrate. Aquaculture 1979; 16: 39-46.

SANTIAGO CB. Amino acid requirements of nile tilapia. Ph.D. Thesis. Auburn, Alabama, USA: Auburn University, 1985.

SATTERLEE LD, KENDRICK JG, MILLER GA. Rapid in-vitro assays for estimating protein quality. Food Technol 1977; 31: 78-81.

SCOTT ML, NESHEIM MC, YOUNG RJ. Feedstuffs for poultry. In: Scott ML, Nesheim MC, Young RJ, eds. Nutrition of the chicken. Ithaca, New York: M.L. Scott and Associates, 1982: 431-549.

SEDLAK J, LINDSAY RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968; 25: 192-205.

SMITH RR. A method for measuring digestibility and metabolizable energy of fish feeds. Prog Fish-Cult 1971; 33: 132-134.

SMITH RR, PETERSON MC, ALLRED AC. Effect of leaching on apparent digestion coefficients of feedstuffs for salmonids. Prog Fish-Cult 1980; 42: 195-199.

SOARES JH JR, MILLER D, FITZ N, SANDERS M. Some factors affecting the biological availability of amino acids in fish protein. Poultry Sci 1971; 50: 1134-1143.

SPANNHOF L, PLANTIKOW H. Studies on carbohydrate digestion in rainbow trout. Aquaculture 1983; 30: 95-108.

STANSBY ME, comp. Fish oils in nutrition. New York: Van Nostrand Reinhold, 1990.

STEEL RGD, TORRIE JH. Principles and procedures of statistics. New York: McGraw-Hill Book Company, Inc., 1960.

TACON AGJ, JACKSON AJ. Utilization of conventional and unconventional protein sources in practical fish feeds. In: Cowey CB, Mackie AM, Bell JG, eds. Nutrition and feeding in fish. London: Academic Press, 1985: 119-145.

TARR HLA, BIELY J. Effect of processing on the nutritional value of fish meal and related products. In: Effect of processing on the nutritional value of feeds. Washington, D.C.: National Academy of Sciences, 1972: 252-281.

UKI N, KEMUYAMA A, WATANABE T. Nutritional evaluation of several protein sources in diets for abalone Haliotis discus hannai. Bull Jpn Soc Sci Fish 1985; 51: 1835-1839.

WAIBEL PE, CUPERLOVIC M, HURRELL RF, CARPENTER KJ. Processing damage to lysine and other amino acids in the manufacture of blood meal. J Agric Food Sci 1977; 25: 171-175.

WALTON MJ. Aspects of amino acid metabolism in teleost fish. In: Cowey CB, Mackie AM, Bell JG, eds. Nutrition and feeding in fish. New York: Academic Press, 1985: 47-67.

WALTON MJ, COWEY CB, ADRON JW. The effect of dietary lysine levels on growth and metabolism of rainbow trout (Salmo gairdneri). Br J Nutr 1984a; 52: 115-122.

WALTON MJ, COWEY CB, ADRON JW. Methionine metabolism in rainbow trout fed diets of differing methionine and cystine content. J Nutr 1982; 112: 1525-1535.

WALTON MJ, COWEY CB, COLOSO RM, ADRON JW. Dietary requirements of rainbow trout for tryptophan, lysine and arginine determined by growth and biochemical measurements. Fish Physiol Biochem 1986; 2: 161-169.

WALTON MJ, COLOSO RM, COWEY CB, ADRON JW, KNOX D. The effects of dietary tryptophan levels on growth and metabolism of rainbow trout (Salmo gairdneri). Br J Nutr 1984b; 51: 279-287.

WILLIAMS S. Official methods of analysis of the association of official analytical chemists, 14th ed. Arlington, VA: Association of Official Analytical Chemists (AOAC), 1984.

WILSON RP. Amino Acids and Proteins. In: Halver JE, ed. Fish nutrition. 2nd ed. New York: Academic Press, 1989: 112-153.

WILSON RP, COWEY CB. Amino acid composition of whole body tissue of rainbow trout and Atlantic salmon. Aquaculture 1985; 48: 373-376.

WILSON RP, HALVER JE. Protein and amino acid requirements of fishes. Ann Rev Nutr 1986; 6: 225-244.

WILSON RP, HARDING DE, GARLING DL JR. Effect of dietary pH on amino acid utilization and the lysine requirement of fingerling channel catfish. J Nutr 1977; 107: 166-170.

WILSON RP, POE WE, ROBINSON EH. Leucine, isoleucine, valine, and histidine requirements of fingerling channel catfish. J Nutr 1980; 110: 627-633.

WILSON RP, ROBINSON EH, POE WE. Apparent and true availability of amino acids from common feed ingredients for channel catfish. J Nutr 1981; 111: 923-929.

WINDELL EG, FOLTZ JW, SAROKON JA. Methods of fecal collection and nutrient leaching in digestibility studies. Prog Fish-Cult 1978; 40: 51-55.

WOYEWODA AD, SHAW SJ, KE PJ, BURNS BG. Recommended Laboratory methods for assessment of fish quality. Can Tech Rep Fish Aquatic Sci 1986; No. 1448: 9-14.

ZEITOUN IH, ULLREY DE, HALVER JE, TACK PJ, MAGEE WT. Influence of salinity on protein requirements of coho salmon (Oncorhynchus kisutch) smolts. J Fish Res Board Can 1974; 31: 1145-1148.

ZEITOUN IH, ULLREY DE, MAGEE WT, GILL JL, BERGEN WG. Quantifying Nutrient Requirements of fish. Fish Res Board Can 1976; 33: 167-172.

APPENDIX A - Systat commands and printout for broken-line analysis of percent weight gain against dietary lysine level

Note:

MWG - Percentage weight gain
LLD - Dietary lysine level (dry matter basis)

COMMANDS USED IN NONLIN MODULE:

```
MODEL MWG = BO + B1 * LLD * (LLD < R) + B1 * R * (LLD > R)

EST /START -82,120,2.0
```

PRINTOUT OF ANALYSIS:

ITERATION	LOSS	PARAMETER VALUES		
0	.8757996D+04	-.8200D+02	.1200D+03	.2000D+01
1	.8733199D+04	-.8200D+02	.1200D+03	.1987D+01
2	.8733169D+04	-.8203D+02	.1200D+03	.1988D+01
3	.8733007D+04	-.8210D+02	.1200D+03	.1988D+01
4	.8732975D+04	-.8222D+02	.1201D+03	.1988D+01
5	.8732972D+04	-.8234D+02	.1202D+03	.1988D+01
6	.8732971D+04	-.8234D+02	.1202D+03	.1988D+01

DEPENDENT VARIABLE IS MWG

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE
REGRESSION	421416.698	3	140472.233
RESIDUAL	8732.971	18	485 165

PARAMETER	ESTIMATE	STANDARD ERROR
B0	-82.338	42.785
B1	120.163	25.779
R	1.988	0.111

APPENDIX B - Systat commands and printout for quadratic analysis of percent weight gain against dietary lysine level

Note:

MWG - Percentage weight gain
LLD - Dietary lysine level (dry matter basis)

COMMANDS USED IN MGLH MODULE:

```
MODEL MWG = CONSTANT + LLD + LLD^2

EST
```

PRINTOUT OF ANALYSIS:

```
DEP VAR: MWG
N:                                21
MULTIPLE R:                       0.828
SQUARED MULTIPLE R:               0.686
ADJUSTED SQUARED MULTIPLE R:      0.651
STANDARD ERROR OF ESTIMATE:      22.334
```

VARIABLE	COEFFICIENT	STD ERROR	STD COEF	TOLERANCE	T	P(2 TAIL)
CONSTANT	-213.102	72.897	0.000	0.0	-2.923	0.009
LLD	302.016	71.954	4.419	0.0157	4.197	0.001
LLD^2	-60.764	16.972	-3.769	0.0157	-3.580	0.002

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REGRESSION	19578.192	2	9789.096	19.626	0.000
RESIDUAL	8978.148	18	498.786		

APPENDIX C - Systat commands and printout for broken-line analysis of percent recovery of $^{14}CO_2$ against dietary lysine level

Note:

DOSE - Percentage of injected $^{14}CO_2$ recovered
LYS - Dietary lysine level (dry matter basis)

COMMANDS USED IN NONLIN MODULE:

```
MODEL DOSE = B0 + B1 * R * (LYS < R) + B1 * LYS * (LYS > R)

EST /START -2.7, 2.18, 1.84
```

PRINTOUT OF ANALYSIS:

ITERATION	LOSS	PARAMETER VALUES		
0	.2326201D+02	-.2700D+01	.2180D+01	.1840D+01
1	.2325567D+02	-.2697D+01	.2187D+01	.1842D+01
2	.2325556D+02	-.2697D+01	.2188D+01	.1839D+01
3	.2325434D+02	-.2699D+01	.2187D+01	.1840D+01
4	.2325407D+02	-.2700D+01	.2186D+01	.1840D+01
5	.2325407D+02	-.2700D+01	.2186D+01	.1840D+01

DEPENDENT VARIABLE IS DOSE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE
REGRESSION	231.459	3	77.153
RESIDUAL	23.254	38	0.612

INDEX	LABEL	ESTIMATE	STANDARD ERROR
1	B0	-2.700	1.016
2	B1	2.186	0.416
3	R	1.840	0.162

APPENDIX D - Quality standards for NorSeaMink® and Norse-LT94®

	NorSeaMink®	Norse-LT94®
Raw material	No preservatives 90 mg TVN/100 g	No preservatives 50 mg TVN/100 g
Meal		
Protein	>70%	>68%
Moisture	>5% <10%	>5% <10%
Fat, Soxhlet	<11.5%	<11.5%
Ash - salt	<14.0	<14.0
Ammonia - N	< 0.18%	< 0.16%
Salt	< 3.0%	< 3.0%
Pepsin soluble protein	-	>94.0%
Dimethyl nitrosamine	ND[1]	ND
Salmonella	ND	ND
Added Antioxidant (ethoxyquin)	400 ppm	400 ppm[2]

[1] Not detected.

[2] 200 ppm added before and 200 ppm added after the dryer.

Reviewed by Pike et al. (1990).

APPENDIX E - Voluntary quality standards for fish meals recommended by the Canadian Working Group for Finfish Nutrition in co-operation with Canadian feed manufacturers

Parameters		Recommended levels	
		whole Herring meal[1,2]	white fish meal[1,2]
Protein		> 68%	> 60%
Fat, crude		> 5%	> 3%
Ash, total		< 16%	< 20%
Moisture	Min.	> 5%	> 5%
	Max.	< 10%	< 10%
Salt (NaCl)		< 3%	< 3%
Total volatile nitrogen		< 0.2%	< 0.2%
Antioxidant (ethoxyquin)		200 ppm	200 ppm
Salmonella		not detected	not detected
Steam processed, preferable ground finer than 0.25 mm			

[1] Digestible protein > 90%.
[2] Term adapted from Agriculture Canada, feed ingredient list.

Taken from: Minutes of a special meeting with Canadian fish feed manufacturers. Canadian Working Group for Finfish Nutrition, 7th Annual meeting, Quebec City, October 3, 1988. Can be obtained from Dr. S.P. Lall, Department of Fisheries and Oceans, Halifax Fisheries Research Laboratory, 1707 Lower Water Street, P.O. Box 550, Halifax, Nova Scotia, B3J 2S7

APPENDIX F - Addresses of fish meal manufacturers that supplied products

Commeau Sea Products Ltd.
P.O. Box 39
Saulnierville, Digby County, Nova Scotia
B0W 2Z0
Phone: (902) 769-2101

Connors Brothers Ltd.
Blacks Harbour, New Brunswick
E0G 1H0
Phone: (506) 456-3391

Karlsen Shipping Company Ltd.
R.R.#1, Hubbards, Lunenberg County, Nova Scotia
B0J 1T0
Phone: (902) 228-2558

Laurence Sweeney Fisheries Ltd.
Suite 500
1668 Barrington Street, Halifax, Nova Scotia
B3J 2A2
Phone: (902) 423-3809

National Sea Products Ltd.
1959 Upper Water Street, Halifax, Nova Scotia
B3J 3B7
Phone: (902) 422-9381

APPENDIX G - Amino acid composition of various fish meals expressed as a percentage of the protein

	Herring Meal						Roe Herring[a]	Menhaden meal	Anchovy meal	Norse -LT94®
	1	2	3	4	5	6				
Amino acid[b]										
Ala	6.29	5.78	5.95	6.60	6.69	6.09	6.43	6.07	6.92	6.21
Arg	7.44	7.32	7.85	8.36	7.75	6.47	8.59	7.38	6.48	6.89
Asp	10.73	10.74	11.96	11.64	12.86	10.30	13.96	12.57	11.93	11.31
Cys	0.24	0.84	0.33	1.04	0.64	0.61	ND[c]	0.93	0.17	0.63
Glu	11.91	11.03	11.43	12.99	12.98	11.09	12.83	11.11	11.77	11.66
Gly	6.09	7.87	7.03	6.79	6.83	6.51	7.54	8.11	6.97	6.92
His	2.22	1.80	2.14	2.40	2.41	2.00	2.67	2.15	2.64	2.16
Ile	4.94	4.35	5.16	5.44	5.55	4.51	6.18	5.34	5.61	5.02
Leu	8.72	7.02	8.09	9.26	9.23	7.76	8.77	8.30	8.92	8.30
Lys	7.67	8.07	9.48	9.28	9.63	7.46	11.81	9.05	8.73	8.62
Met	3.17	2.55	2.78	3.41	3.23	2.75	2.90	3.48	3.02	3.02
Phe	4.31	3.70	4.17	4.64	4.68	3.90	4.63	4.34	4.75	4.24
Pro	4.78	5.19	5.37	5.46	5.86	5.67	6.02	6.57	5.21	4.99
Ser	4.17	4.56	4.35	4.45	4.19	4.05	4.50	4.72	3.78	3.91
Thr	3.59	3.68	3.58	4.00	4.12	3.69	2.96	4.74	3.94	3.30
Trp	0.95	ND	ND	ND	1.01	1.52	ND	0.63	0.37	0.93
Tyr	3.51	2.94	3.37	3.86	3.92	3.06	3.58	3.61	3.48	3.13
Val	5.73	4.77	5.42	6.25	6.11	5.13	6.23	6.28	5.83	5.44

[a] Fresh freeze-dried fish, consisting mostly of sexually mature males.

[b] Expressed as g amino acid·16 g^{-1} N.

[c] ND (not determined).

APPENDIX H - Endogenous fecal amino acids in feces collected by manual stripping or sedimentation from fish fed a protein-free diet

Amino acid[a]	Fecal Collection Method	
	Manual stripping	Sedimentation
Ala	0.256	-----[b]
Arg	0.175	-----
Asp	0.600	-----
Glu	0.705	0.023
Gly	0.377	0.143
His	0.020	-----
Ile	0.229	-----
Leu	0.415	0.024
Lys	0.204	-----
Phe	0.182	-----
Pro	0.479	0.067
Ser	0.122	-----
Thr	0.494	-----
Trp	0.024	-----
Tyr	0.120	-----
Val	0.312	-----
Crude protein (%N x 6.25)	10.93	1.49

[a] Expressed as a percentage of the sample (100% dry matter basis).

[b] Values were not detectable.

APPENDIX I - True crude protein and amino acid availabilities calculated with values obtained from feces collected by sedimentation

Amino acid[a]	Norse-LT94®	Herring meal 1	Herring meal 5	Herring meal 6	Menhaden meal	Anchovy meal
Glu	98.4±0.2a	94.0±0.5b	97.5±0.1a	97.5±0.4a	95.4±0.9ab	95.7±0.4ab
Gly	98.3±0.9a	96.2±0.3a	100.5±0.1a	98.4±1.6a	93.7±1.5a	95.2±1.0a
Leu	98.8±0.1a	94.2±0.1d	97.5±0.1b	97.4±0.3b	96.1±0.6c	95.6±0.1c
Pro	98.9±0.9a	96.0±0.02a	99.8±0.2a	98.0±1.2a	96.9±0.9a	95.7±0.9a
Crude Protein						
	98.7±0.1a	94.0±0.02e	98.5±0.1a	96.4±0.[illegible]	94.8±0.2d	96.8±0.2b

[a] The other amino acids did not have detectable levels in the protein-free feces.

a-d, Means±SEM (n=2), within a row, not sharing the same postscript are significantly different ($P<0.05$).