QUALITATIVE AND QUANTITATIVE ASPECTS OF THE PROTEIN NUTRITION OF JUVENILE CHINOOK SALMON (*Oncorhynchus tshawytscha*)

by

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ABSTRACT

Two experiments were conducted to study the effect of protein source and level in the diet of juvenile chinook salmon (Oncorhynchus tshawytscha) reared in fresh water tanks. The protein sources compared were a freeze-dried pollock muscle and euphausid mix (9:1)(FPE), three whole herring meals processed differently from the same lot of raw herring and a casein-gelatin mix supplemented with arginine and DL-methionine (CS). The protein sources were tested at three levels of dietary protein in isocaloric diets fed to satiation to duplicate groups of fish for a 42-day period. Protein was replaced by dextrin and glucose on an estimated metabolizable energy basis. The various methods employed to evaluate protein quality yielded different values relative to FPE. In terms of growth rate and assays based on body protein gain, FPE was found to be the best protein source.

Low temperature (75°C) drying of herring meal caused a slight reduction in protein quality compared to freeze-drying. High temperature (150°C) dried herring meal was found to be an extremely poor quality protein source. Although high estimates of protein quality were obtained for CS, lower food intake depressed growth in fish fed CS diets. The determination of the endogenous loss of nitrogen from fish enabled the partitioning of protein intake into the amounts used for growth, maintenance and exogenous excretion for each protein source.

In Experiment 2, two series of isocaloric diets were tested containing 17 to 47% protein, in increments of 10%, provided by FPE at two levels of dietary energy. The equation $y = -0.50699 + 0.25398x - 8.37872x^2$,(where y = specific growth rate, and x = protein energy:total energy (PE:TE)) was derived to quantify the dietary protein requirement for juvenile chinook salmon over a 105-day period. Maximum growth was achieved at a PE:TE ratio of 0.55. However, for practical purposes the PE:TE ratio required was found to lie in the range between 0.35 and 0.55. The range permits the fish culturist to consider economic efficiency in diet formulation.

Table of Contents

Table of Contents (cont'd)

Table of Contents (cont'd)

List of Tables

List of Tables (cont'd)

List of Tables (cont'd)

List of Figures

List of Figures (cont'd)

List of Appendices Tables

List of Appendices Tables (cont'd)

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CHAPTER 1

1.0 INTRODUCTION

The relationship between food and growth is one of the most important aspects of fish production. Furthermore, the ability of hatchery-raised fish to survive in the wild may be a function of their nutritional history (Burrows, 1969; Plotnikoff et al., 1984). Compared to most other cultured animal species, salmonids have high dietary protein requirements. Protein is predominantly furnished by fish meal, and is the most expensive component of fish diets. Suitable high quality protein sources derived from the British Columbia fishery are becoming increasingly scarce on a year round basis. Total replacement of fishmeal in practical diets for chinook salmon (*Oncorhynchus tshawytscha*) does not, however, appear to be a readily attainable goal (Westgate, 1979; Fowler, 1980, 1981a,b; Higgs et al., 1982, 1983). Consequently, an important objective among fish nutritionists is to maximize the utilization of dietary proteins. This could be achieved by obtaining a correct balance of all nutrients in the diet. In particular, the quality of the protein source in terms of meeting the essential amino acid requirements of the fish and the ratio of protein energy to total energy in the diet would be expected to play a major role (Cowey, 1979). Further, there is a need to develop the criteria for the biological measurement of the nutritive value of protein in fish.

The overall objectives of this study on chinook salmon fry were twofold. First, to evaluate the effect of dietary protein source and level on protein utilization (Experiment 1). Second, to determine the effect of dietary energy level on the requirements and utilization of protein for growth (Experiment 2).

The following review of literature is intended to provide the reader with a background in protein nutrition to facilitate understanding of the experimental goals and procedures employed.

CHAPTER 2

2.0 REVIEW OF LITERATURE

2.1 The utilization of dietary protein

The utilization of dietary protein is mainly dependent upon the ability of the protein source to fulfill the total protein and individual amino acid requirements of the animal. The energy content of the diet, source of dietary energy, the genetic predisposition, the environmental conditions and the physiological state of the animal also play a major role.

Most fish species of concern to fish culturists are carnivorous and have a high protein requirement (Cowey, 1979). All fish species studied require the same ten essential dietary amino acids that are needed by most other animals. These have been reviewed for over a dozen fish species (Ketola, 1982).

Carnivorous fish utilize carbohydrates poorly (Phillips, 1969; Shimeno et al., 1979; Hilton and Atkinson, 1982). Compared to most animal species raised for food, salmonids metabolize a greater proportion of dietary protein for energy (Walton and Cowey, 1982). However, the energy requirements of fish are lower than for mammals which require a considerable amount of energy to maintain body temperature. Fish are poikilothermic. Also, most of the nitrogen excreted by fish is in the form of ammonia, while birds and mammals require energy to synthesize uric acid and urea respectively for excretion (Smith et al., 1978).

Unlike carbohydrates and lipids, proteins, or the amino acids derived from them, cannot be stored as such by the animal if fed in excess of requirements. This means that no considerable inert deposits of protein or amino acids are found in the animal body comparable to glycogen granules or fat globules. For this reason the efficiency of dietary protein utilization is dependent upon the quantities and proportions of amino acids absorbed with each meal (Albanese and Orto, 1959). Protein sources vary in their digestibility and metabolic utilization by various animal species.

The quality or efficiency of utilization of dietary protein is subject to three general factors: intake, digestibility in the gastrointestinal tract, and the metabolic utilization of the digestion products. A general representation of the utilization of food nitrogen as it applies to the growing fish is depicted in Fig.1.

Once absorbed, amino acids are utilized through anabolic and catabolic enzymatic processes which give rise to nitrogen losses via the gills and kidney. Another fraction of nitrogen intake is retained and used for growth and maintenance. The endogenous nitrogen fractions (Fig.1) are of interest primarily because they play a role in the calculation of some estimators of protein quality. The feature of the endogenous nitrogen losses is that although they are indistinguishable from exogenous nitrogen wastes, they represent fractions which have actually been utilized by the animal. These and other aspects of protein

utilization and evaluation will be discussed in this thesis based on the scheme depicted in Fig.1.

2.2 General aspects of protein metabolism

In fish, assimilated amino acids enter the same pathways and are presumed to undergo the same complex biochemcial reactions as in other animals (Walton and Cowey, 1982). These are protein synthesis, deamination followed by oxidation and possibly conversion to lipid and glucose, and synthesis of various polypeptides, purines and nucleic acids. The rates of protein synthesis and breakdown are extremely sensitive to dietary protein intake and are under hormonal control.

Body proteins are in a continual state of turnover, being broken down and resynthesized. Recent studies have confirmed that, not only does each tissue and each protein have a different rate of turnover, but also the mechanisms for regulating the mass of each tissue protein may differ (Garlick, 1980). For example, changes in the rates of protein synthesis are primarily responsible for the regulation of muscle protein mass. In the liver, on the other hand, protein mass is thought to be regulated by the rate of protein breakdown. In adults, non-growing muscle maintenance results from a balance between the rate of synthesis and breakdown (Millward et al., 1978). In the growing animal protein growth results from the fact that protein synthesis exceeds protein breakdown.

In studies concerned with whole body protein metabolism two pools of amino acids are conceptualized (Garlick, 1980). One

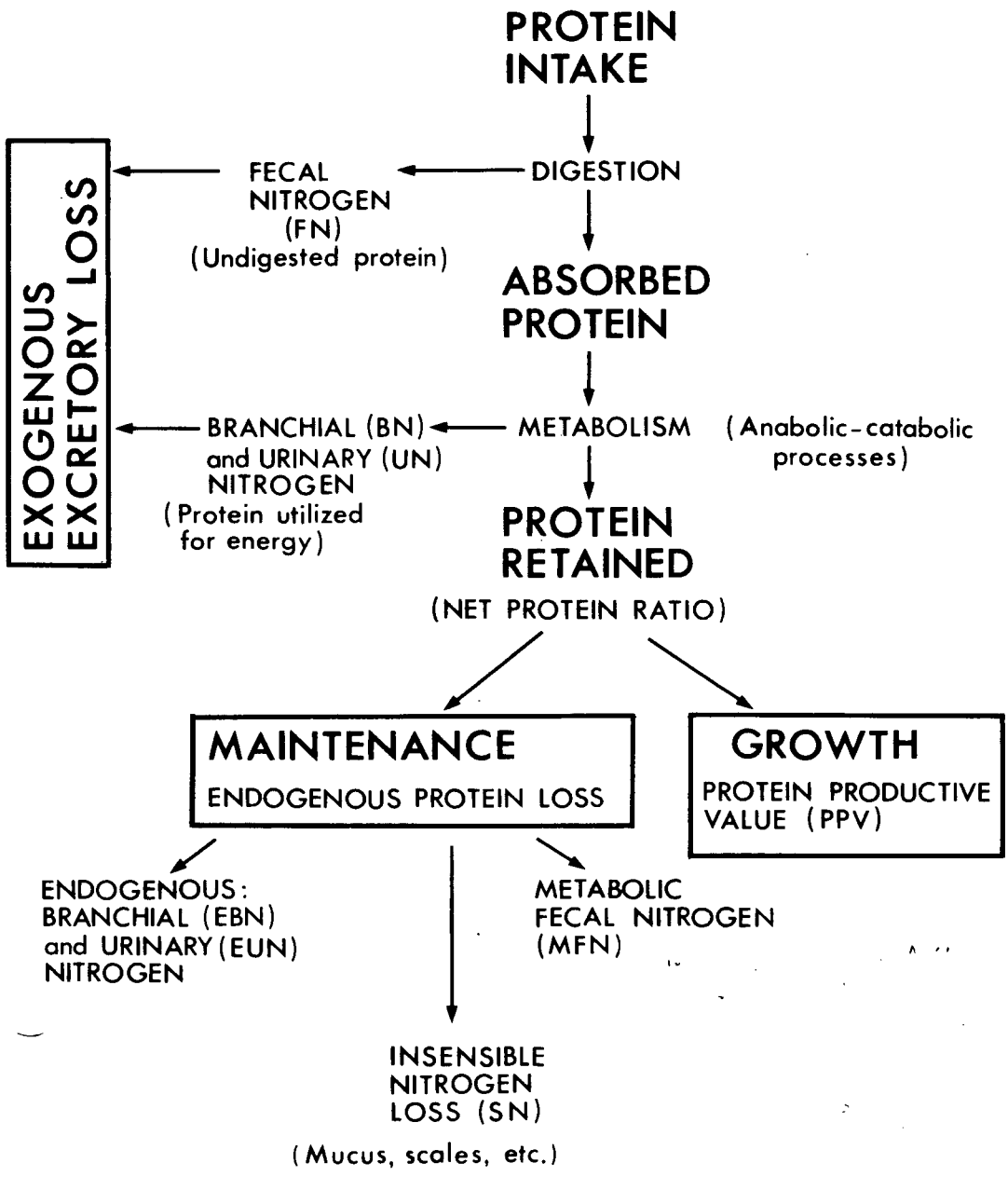


Fig. 1. Simplified scheme for the utilization of food protein in juvenile growing fish.

represents the total free amino acids of the body known as the "metabolic pool" and the other the whole body protein pool. These exist in dynamic equilibrium. The inputs into the metabolic pool are from food intake and body protein breakdown. The outputs from the metabolic pool are from protein synthesis and amino acid oxidation with subsequent nitrogen excretion.

Characteristic nitrogen excretion curves from starved fish have served to demonstrate the existence of the above compartments in teleosts (Iwata, 1970; Smith and Thorpe, 1976). The rate of depletion of body protein stores in fish have been shown to be dependent on water temperature (Savitz, 1971) and salinity (Smith and Thorpe, 1976).

When protein is provided after a period of starvation, retention of the dietary protein will be high in order to replenish the body protein stores. During regeneration, the tissues that were depleted last are probably refilled first. This may indicate that the tissue depleted last was more essential. The dynamic nature of protein metabolism has led to the concept of a metabolic pool of amino acids. The pool is not considered to be a disorganized fluid container of the body, but rather as an integrated mechanism for the transfer of amino acids from one tissue to another (Shapiro and Fisher, 1962). The above concepts of the anabolic/catabolic interrelationships provide the background for nitrogen balance studies of animals.

2.3 Nitrogen balance

The principle of nitrogen balance in nutritional studies is identical to input versus output in any other science, since its purpose is to measure net gain or loss occurring with dietary protein utilization. Nitrogen balance can be defined as the quantity of nitrogen intake that has been retained by the body. Many experimental procedures based on nitrogen balance have been devised for the measurement of the nutritive value of foods and nutrient requirements of animals.

The dynamic anabolic-catabolic state of protein metabolism is simply described by the nitrogen balance equation (Allison, 1951) as follows:

$$NB = NI - (UN + FN)$$

where NB = nitrogen balance, NI = nitrogen intake, UN = urinary nitrogen, and FN = fecal nitrogen.

If nitrogen balance is positive, the animal is gaining protein, either through the growth of new tissues or the repletion of depleted stores. All growing animals are, under normal conditions, in positive nitrogen balance. Adult animals, under normal conditions, are in nitrogen equilibrium. Maintaining nitrogen equilibrium does not mean that all tissues are in this state because some tissues may be maintained at the expense of others (Young and Scrimshaw, 1977).

The aforementioned nitrogen balance formula of Allison (1951) has not been found to be totally correct. Studies with human subjects have demonstrated that significant amounts of nitrogen are eliminated in perspiration. Also, losses from skin

and hair must be accounted for in long term nitrogen balance studies. These losses have been termed insensible nitrogen losses (SN) (Bressani, 1977). They are accounted for when the factorial procedure is employed to calculate protein requirements for maintenance in farm animals (Maynard and Loosli, 1969).

Similarly, the application of nitrogen balance to fish must consider all sources of nitrogen loss. Branchial excretion (BN) is the major mechanism for nitrogen loss in fish. Also fish are covered in mucus which functions to protect (Ingram, 1980) and aid in swimming and osmoregulation (Cameron and Endean, 1973). Epithelial mucus contains both protein and carboydrate (Ingram, 1980). Stress, as a result of infection or handling, is known to cause an increased mucus production in fish (Pickering and Macey, 1977). Savitz (1969) recognized that consideration for the unknown amount and rate of mucus nitrogen secretion would bias estimates of protein metabolism. Insensible nitrogen loss may be of significance in aquaculture where fish are crowded and frequently handled. Therefore, an appropriate nitrogen balance relationship for fish would be:

$$NB = NI - (FN + UN + BN + SN)$$

When fish are fed a series of diets which supply equal amounts of metabolizable energy, their nitrogen balances can be expected to form a curve of the type shown in Fig. 2 (McDonald et al., 1976). As intake increases from zero there is a gradual reduction in the negative balance until the point of exact equilibrium is reached. The extent to which further nitrogen

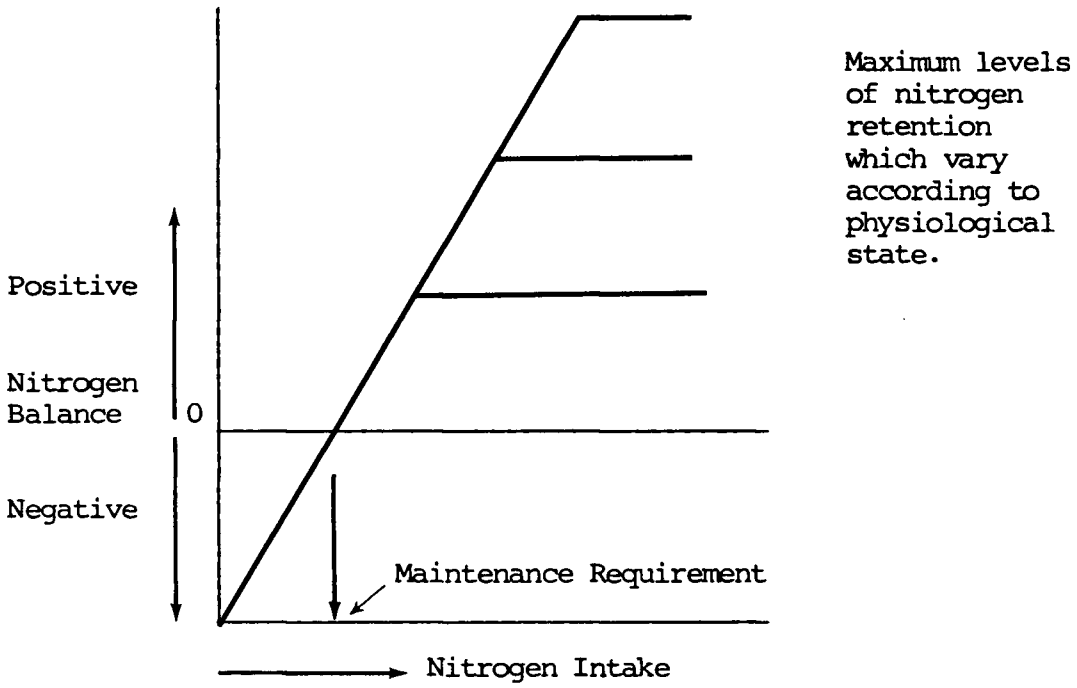


Fig. (2). Stylized representation of nitrogen balance. Taken from McDonald et al.(1976).

Figure 2 intake promotes tissue growth depends on the growth potential of the animal, the quality of the protein and the supply of other nutrients. The curve becomes horizontal when further nitrogen intake fails to promote additional nitrogen retention.

The information derived from nitrogen balance studies has been used since 1909 (Thomas, cited by Mitchell, 1962) in the calculation of the Biological Value of proteins. Biological Value (BV) is defined as the percentage of absorbed nitrogen which is retained by the organism (Mitchell, 1924):

$$BV = \frac{\text{Retained food N}}{\text{Absorbed food N}} \times 100$$

where: Absorbed food N = Food N - fecal food N

Fecal food N = Fecal N - Metabolic N of feces

Retained food N = Absorbed N - Excreted food N

Excreted food N = Urine N - Endogenous urinary N

With respect to fish nutrition research, the formula for biological value reported by Castell and Tiews (1979) was:

$$BV = \frac{NI - (FN - MFN) - (UN - EUN) - (BN - EBN)}{NI - (FN - MFN)} \times 100$$

where: MFN = Metabolic Fecal Nitrogen

EUN = Endogenous Urinary Nitrogen

EBN = Endogenous Branchial Nitrogen

MFN, EUN, and EBN are nitrogen fractions whose excretion is independent of food nitrogen (Fig.1). Their existence is demonstrated by the nitrogen excreted when an animal is given a protein-free diet. They represent fractions which have actually

been utilized by the animal even though they appear as excretions. The numerator in the above equation represents total nitrogen utilized for both maintenance and tissue growth. It is noteworthy that Castell and Tiews (1979) have not accounted for mucus nitrogen loss.

2.4 Endogenous nitrogen excretion

Endogenous nitrogen excretion originating from amino acid catabolism represents the maintenance fraction of nitrogen metabolism and is independent of dietary protein intake. In mammals the principal constituent of endogenous nitrogen excretion is urea, which arises from the breakdown of protein and other nitrogenous compounds which are catabolized to yield ammonia. The major end product of nitrogen metabolism in teleost fish is ammonia, though urea, uric acid, trimethylamine oxide, amino acids, creatinine and creatine are also excreted (Watts and Watts, 1974). The origin of urea and uric acid is from purine metabolism and creatinine is formed from the breakdown of creatine in muscle action (Forster and Goldstein, 1969).

Endogenous urinary nitrogen excretion varies in homeothermous animals and is related to body weight in a manner similar to caloric metabolism (Brody, 1945). Basal metabolism and endogenous nitrogen excretion are related to one another and both are a function of metabolic body size. In mammals the metabolic rate is proportional to the three-fourths power of body weight ($W^{0.75}$) (Maynard and Loosli, 1969,).

Gerking (1955) reported that endogenous nitrogen excretion in the bluegill sunfish (Lepomis macrochirus) decreased with increasing size by $W^{0.54}$. Since then, the exponent applicable to fish has been reported to vary from 0.34 to 1.0 (Brett and Groves, 1979). Smith et al.(1978) employed a method of direct calorimetry to study the effect of fish size on metabolic rate in four species of salmonids. Their results indicated that 1.0 to 4.0g fish have a metabolic rate proportional to $W^{1.0}$. Fish from 4.0g to 50.0g in weight have a metabolic rate proportional to $W^{0.63}$.

McDonald et al.(1976) state a generally accepted value of 2 mg of endogenous urinary nitrogen excreted per kcal at basal metabolism for warm blooded animals. Gerking (1955) obtained a value of 7 mg of endogenous nitrogen excreted per kcal in the bluegill sunfish. This agrees with a value of 7.2 mg/kcal obtained by Bonnet (1933) with frogs. The foregoing results suggest that fish and amphibians excrete three and a half times more endogenous nitrogen per basal kcal than do warm blooded animals. This established that a high rate of nitrogen excretion is characteristic of poikilothermous animals. Hence, a large portion of the energy requirements of fish are derived from protein, whereas homeotherms can use large proportions of carbohydrate and fat for this purpose.

The daily rates of endogenous nitrogen excretion have been shown to increase with rising environmental temperature in bluegill sunfish (Savitz, 1971) and carp (Ogino et al., 1973). At low temperatures nitrogen excretion rates were found not to

fluctuate, suggesting that sunfish have the ability for capacity adaptation in cold climates (Savitz, 1971).

Smith and Thorpe (1971) employed nitrogen balance to study the association of protein metabolism with the smoltification process in trout. Endogenous nitrogen excretion was found to increase with the onset of smoltification and continue in sea water. By contrast, post smolts decreased their rate of nitrogen excretion when retained in fresh water. These results indicate that the protein requirements for maintenance of trout may be greater in sea water than in fresh water (Zeitoun, 1973).

Another component of endogenous nitrogen loss is metabolic fecal nitrogen (MFN) or that proportion of fecal nitrogen that does not originate from undigested food protein (Fig.1). It arises from enzyme and cell residues sloughed off the digestive tract. MFN output increases with bulk food intake because the higher the intake, the greater the secretion of digestive juices and abrasion of the intestinal epithelial cells.

Mitchell and Bert (1954) determined MFN with a nitrogen-free ration or with rations containing low levels of almost totally digestible protein. They estimated that MFN is approximately 0.1g/100g dry matter consumed for rats, pigs and man. Titus (1927) plotted total nitrogen intake against total nitrogen excretion of steers fed at a fixed ration level with diets of varying protein content. He arrived at the estimated MFN excreted at any level of food intake by straight line extrapolation to the point of zero protein intake. Mitchell and

Bert (1954) obtained good agreement with the Titus (1927) method by direct determination for several species.

Ogino et al.(1973) followed a similar approach to the above to determine MFN excretion in carp. From the relationship between protein content of the diet and the amount of nitrogen excreted in the feces, they obtained MFN excretion values at different water temperatures. These values were within the range of values obtained when a non-protein diet was employed. Ogino et al.(1973) showed that MFN excretion in carp increases as water temperature increases. Skrede et al.(1980) obtained a value for MFN in rainbow trout of 180 mg/100g of dietary dry matter when a protein-free diet was fed.

2.5 Methods used to determine protein quality

The purpose of measuring protein quality is to evaluate the efficacy of different protein sources to support rapid growth. Then the economic return of a protein can be estimated. The quality of a protein refers to how well the available concentration and balance of its constituent essential amino acids correspond to the needs of a given species. As mentioned earlier, there are major quantitative differences in the amino acid requirements of fish species. The determination of amino acids in a feedstuff is an expensive procedure which is often subject to considerable inter-laboratory variation. Alternatively, the reliance on tabulated amino acid composition tables (NRC, 1973,1981) often leads to erroneous estimates (Payne,1972). Furthermore, amino acid composition data give no

indication of amino acid availability and hence usefulness to the animal when included in the final diet.

Methods commonly used to assess protein quality may be divided into chemical methods, microbiological assay systems, animal feeding experiments and procedures based on metabolic indices of protein metabolism. These methods have been critically reviewed by Bodwell (1977), Satterlee et al. (1977), Evans and Witty (1978) and Von der Decken (1983).

Studies with fish have usually employed growth trials coupled with carcass analysis to evaluate the protein quality of feedstuffs. The use of metabolic indices was attempted by Cowey et al.(1981) who found no consistent relationship between the presumed equilibrium point of opposing hepatic enzyme activities and maximum weight gain by trout fed diets containing fishmeal, casein or corn gluten. Nose (1972) and Kaushik and Luquet (1979) demonstrated a correspondence between the proportions of essential amino acids in the diet and the plasma free amino acid pattern. However, these techniques require further refinement before they can be employed successfully.

A common estimate of protein quality is protein efficiency ratio (PER). This is weight gain of an animal per gram of protein intake. Osborne et al.(1919) carried out PER determinations at several protein levels and accepted the highest value as the PER for that protein. PER estimates do not allow for maintenance needs and assume that all food is used for growth. Net protein ratio (NPR) (Bender and Doell, 1957) is similar in concept to PER except for the inclusion of a group

fed a diet devoid of protein. NPR is defined by the following equation:

$$NPR = \frac{\text{gain in weight of test group} + \text{weight loss of group fed a non-protein diet}}{\text{protein intake}}$$

Investigators with fish routinely report PER (Cowey and Sargent, 1972; Steffens, 1981; Pfeffer, 1982), but not NPR because of the problems associated with maintaining fish on a protein free diet (Zeitoun et al., 1973). Usually PER is estimated only at one dietary protein level usually at the recommended dietary protein level for maximum growth of a given species at a particular stage of life history.

The use of weight gain as a criterion for estabilishing protein quality has been criticized (Maynard and Loosli, 1969) because it may not reflect true growth, since gain in weight may result from deposition of fat rather than elaboration of new tissue. The addition of protein sources to animal diets alters the overall mineral, vitamin and fatty acid profiles of these diets, and these effects cannot be discounted (Evans and Witty, 1978). Diet acceptability and the presence of anti-nutritional factors in protein sources, particularly those of plant origin, will also alter growth rate (Higgs et al., 1979). Fish feeding trials may be more beneficial to the assessment of protein quality when an attempt is made to measure the fate of ingested protein. Techniques utilizing nitrogen balance depict the causes for variation in protein sources, for example impaired digestion and absorption or altered retention (Evans and Witty, 1978).

Some investigators have modified the simpler Mitchell formula for BV for fish studies and called it the gross efficiency of nitrogen utilization (Birkett, 1969; Iwata, 1970;Brett and Zala, 1975; Smith and Thorpe, 1976). Endogenous nitrogen loss has been determined from the nitrogen excretion rate of starved fish. Iwata (1970) and Brett and Groves (1979) cite Storer (1967) as evidence that the rate of nitrogen excretion of starved fish provides a measure of endogenous nitrogen excretion. Stover (1967) reported that the rate of protein catabolism did not increase with the onset of starvation. However, Savitz (1969,1971) found that the rate of nitrogen excretion in starved bluegill sunfish was higher than in controls fed glucose alone. Walton and Cowey (1982) present considerable evidence that starvation in fish, as it does in mammals, leads to increases in the levels of amino acid degrading enzymes. Under normal conditions a major portion of the caloric requirement of fishes is derived from protein (Gerking 1955). Savitz (1971) showed by analysis of body composition, that this situation changes in starvation when fat is relied upon heavily to satisfy energy requirements.

Nutrient balance studies are used extensively in evaluating feedstuffs for domestic animals (Maynard and Loosli, 1969). Quantitative collection of feces and excretory products are fraught with problems in fish studies because they must be separated from large volumes of water. Indirect measurement using an indigestible marker such as chromic oxide has been used extensively to determine the digestibility of feedstuffs for

fish (Nose, 1960; Smith and Lovell, 1973; Cho and Slinger, 1979; Windell et al., 1978; Wilson et al., 1981; Pfeffer, 1982). By determining the ratio of the concentration of the marker to that of a nutrient in the food and the same ratio in the feces resulting from the food, the digestibility of the nutrient can be estimated without having to measure food intake or feces output. Urinary and gill excretions are not collected and only digestibility can be calculated. Difficulties in obtaining a representative sample of feces and in avoiding nutrient leaching (Windell et al., 1978; Choubert et al., 1979) are the major problems of this method.

Total collection of feces and nitrogenous excretions facilitates the determination of digestibility, nitrogen balance and metabolizable energy. These methods vary from simply scooping or siphoning feces from the fish tank and analysing the water for nitrogen (Gerking, 1955; Birkett, 1969; Iwata, 1970; Rychly and Spanhoff, 1979), to the development of automated metabolism chambers for fish (Cho et al., 1975). With these methods the assumption is made that all insoluble matter is fecal in origin and that soluble material is excreted. Ogino (1973) developed an apparatus that filters effluent tank water to precipitate, separate and preserve feces with cupric hydroxide and chloroform. Nitrogen excretions are recovered from the effluent water with an ion exchange column. Smith (1971,1976) employed an apparatus that permitted the separate collection of feces, urinary and gill excretions. However, the fish were restrained in such a way that the determination of the

nutritive value of feedstuffs was made when the fish were under considerable stress and in a state of negative nitrogen balance. Data on the digestibility of metabolizable energy contents of foodstuffs determined by these methods are tabulated in tables of nutrient requirements for fishes (NRC,1981).

The utilization of a protein by an animal will depend on its digestibility as well as its biological value. Net protein utilization (NPU) is the product of these two values :

$$NPU = BV \times (\% \text{ digestibility})$$

Miller and Bender (1953) devised NPU to assess the efficiency of nitrogen utilization of a test protein. Their method is less cumbersome for the determination of NPU in small animals than is the procedure involving the collection of excretory products. It is based on a comparison of the body nitrogen content resulting from a test protein source with that resulting over the same period of time from a nitrogen-free diet:

$$NPU = \frac{\text{body N of test group} - \text{body N of group fed a non-protein diet}}{\text{N consumed by test group}} \times 100$$

Ogino et al (1980) described a method that is particularly suited to fish studies. The endogenous loss of nitrogen was determined by carcass analysis after feeding a protein-free diet during the experimental period. They found that the value for the endogenous loss of nitrogen in rainbow trout was 9.5 mg/100g body weight/day and changed in direct proportion to body weight.

Accordingly, NPU for rainbow trout was calculated as follows:

$$NPU = \frac{\text{Body N gain of test group (g)} + \frac{W_1 + W_2}{2}\text{ (g)} \times \frac{9.5}{100} \times 10^{-3} \times d}{\text{Nitrogen intake of test group(g)}} \times 100$$

Where: W_1 = initial body weight

W_2 = final body weight

d = days of feeding

By this method the principles of nitrogen balance can be readily adapted to studies with fish by carcass analysis. The difficult task of recovering nitrogenous wastes from the water can be entirely avoided. Furthermore, the determination of initial and final body nitrogen would be more accurate because it would account for all nitrogen losses including the difficult task of measuring insensible losses.

Several workers in fish nutrition have estimated the efficiency of protein utilization by the ratio of body protein gained to protein fed (Gerking, 1971; Zeitoun et al., 1973; Rumsey and Ketola, 1975; De la Higuera et al., 1977; Higgs et al., 1979; Pfeffer, 1982). This has been termed apparent net protein utilization or protein productive value (PPV):

$$(\%)PPV = \frac{\text{carcass protein gained}}{\text{protein fed}} \times 100$$

This method has the advantage of being simple and sufficiently reproducible to provide relative ratings to various proteins when conducted under standardized experimental conditions (Cowey and Sargent, 1979). However, PPV does not make allowances for maintenance requirements and assumes that

all food protein is used for growth. As both maintenance and growth demand protein, maintenance receives first priority.

Allison (1959) proposed that the slope of the line measuring the rate of change of nitrogen balance with protein intake be used to compare proteins. Hegsted and Chang (1965a) described an assay with rats using the slope of the regression of weight gain on nitrogen intake as a percentage of the slope obtained with rats fed lactalbumin. This method was also undertaken with nitrogen gain replacing weight gain (Hegsted and Chang 1965b). These methods are slope-ratio assays and have been critically reviewed by McLaughlan (1979) with respect to linearity and origin of the intercepts of the dose-response curves. Slope-ratio assay protocols have been proposed both with and without use of a zero protein fed group (Hegsted and Chang, 1965a; Samonds and Hegsted, 1977; McLaughlan and Keith, 1977).

2.6 The protein requirements of salmonids with respect to dietary energy.

The gross protein requirement has been the subject of many studies in fish nutrition. As mentioned earlier, the value of a protein is chiefly determined by its ability to satisfy the amino acid requirements of the animal under consideration. Perhaps of equal importance in the design of economic feeding programs are the dietary concentrations of protein and energy. About 70% of the energy in natural foods of salmon is provided in the form of protein and most of the remainder is supplied by lipid (Gulbrandsen and Utne, 1977). Most commercial salmon

diets, however, contain far less of the total energy as protein and lipid. There is considerable reliance on carbohydrate as an energy source.

The objective of protein requirement studies is to find the minimum amount of protein required to produce maximal growth. Delong et al.(1958) conducted one of the first of these studies with chinook salmon. The fish were fed a diet in which the protein was supplied by a mixture of casein, gelatin, and crystalline amino acids with an overall essential amino acid composition simulating that of whole chicken egg protein. This composition was thought to contain an excess of indispensable amino acids. A series of diets containing different levels of protein was formulated by substituting digestible carbohydrate (dextrin) for protein in an attempt to maintain the diets isocaloric on a gross energy basis. After a ten-week feeding period Delong et al.(1958) found that weight gains of chinook salmon were highest when the diet contained protein at levels of 40 and 55% when water temperature was 8.3 and 14.5°C respectively.

Employing similar diets, Zeitoun et al.(1974) found that the minimum protein requirement of coho salmon maintained at either 10 or 20 ppt salinity was approximately 40%. By contrast, the protein requirement of juvenile rainbow trout was found to be directly related to water salinity and increased from 40% to 45% as salinity was raised from 10 to 20 ppt (Zeitoun et al., 1973). Satia (1974) conducted a study to determine the protein requirements of a particular genetic strain of rainbow trout.

This strain is noted for its fast growth rate (Donaldson and Olson, 1957). These fish were reared at high temperatures of 16 to 27°C and they were fed diets ranging in protein content from 30% to 50%. The diets were formulated to be isocaloric by adjusting dextrin in relation to protein from fishmeal on a metabolizable energy basis (Phillips, 1969). The fish were fed at a rate of 4.5% of body weight per day. Based on food conversion data, Satia (1974) showed that the protein requirements of the trout dropped from 50% to 40% as the fish grew over 20g in weight.

The major uncertainty of all of the foregoing studies relates to the caloric values assigned by individual investigators to the feedstuffs. The metabolizable energy values of protein, lipid and carbohydrate are usually estimated from the gross energy content of each component which is then corrected for digestibility and in the case of protein, for energy lost in nitrogenous excretory products (Phillips, 1969; Brett and Groves, 1979). Unfortunately, there is a lack of standardization in these procedures and the values employed differ between investigators. For example, Smith (1971) has shown that 4.5 kcal/g is a more appropriate metabolizable energy value for protein than the more frequently used value of 3.9 kcal/g of Phillips (1969). This is because fish primarily excrete ammonia. Thus, the non-metabolizable energy fraction of ingested protein should be accounted for by the heat of combustion value for ammonia rather than that of urea. Also, with respect to carbohydrate the picture is complicated further

because for example, increased amounts of starch or dextrin in the diet result in lowered digestibility (Singh and Nose, 1967). Hence, the metabolizable energy values of the various dietary components can be expected to vary with the level of dietary carbohydrate. Jobling (1983) pointed out that the estimated metabolizable energy content of a diet will depend not only upon diet composition, but also upon ration level.

The minimum dietary protein level for optimum weight gain or feed efficiency has generally been stated as a percentage of the dry diet (Delong, 1958; Luquet, 1971; Satia, 1974; Cowey, 1975; Zeitoun et al., 1976). Phillips (1969), Ringrose (1971), Lee and Putman (1973), Takeda et al.(1975) and Cowey et al.(1975) have reported that the non-protein energy components of the diet can promote the utilization of dietary protein and have a protein sparing effect. Protein acts both as a source of energy and of amino acids for tissue synthesis. Fish have been shown to eat to satisfy their energy requirements (Lee and Putman, 1973; Brett and Groves, 1979). Recently, Cowey and Sargent (1979) have stated that since the ultimate objective of protein requirement studies is to estimate the dietary protein level in relation to that of energy, the protein content of the diet can best be expressed in terms of the proportion of energy that it contributes.

Previous work with chinook salmon has indicated that the best ratio of protein energy to total energy (PE:TE) in the diet is 0.50 (Combs et al., 1962; Fowler et al., 1964). Gulbrandsen and Utne (1977) reported that dietary PE:TE ratios

varying between 0.37 and 0.41 were optimal for maximal growth of rainbow trout. They further noted that practical Norwegian dry feeds for trout had excessively high PE:TE ratios. The lipid content of the diet employed by Gulbrandsen and Utne (1977) was approximately 20%. The lipid component, supplied by capelin oil, accounted for 40% to 42% of the total dietary energy content. Gulbrandsen and Utne (1977) found that lipid levels higher than this caused a depression in growth. Unlike most other studies on fish, the level of energy furnished by digestible carbohydrate (dextrin) was kept at 20% of the total energy in the diet and consequently the energy supplied by protein was balanced by varying dietary levels of fish oil. Whether protein energy is replaced by energy from lipid or from carbohydrate could be of great significance to the outcome of a protein requirement experiment. Recent evidence (Shimeno et al, 1979; Hilton and Atkinson, 1982) supports the statement of Phillips (1969) that carnivorous fish are not able to tolerate digestible carbohydrate levels above 14% of the diet.

Despite the reports indicating that carnivorous fish are inferior to omnivorous fish in their ability to utilize carbohydrate effectively, some experiments have shown that the former do adapt to high dietary levels of carbohydrate (Shimeno et al., 1979). For example, Buhler and Halver (1961) fed juvenile chinook salmon a series of diets in which the protein level was decreased from 71% to 40% and levels of dextrin were increased to 43%. They found that protein efficiency ratios were increased by substituting dextrin for protein whereas

growth rate remained similar among fish receiving the different dietary treatments. Similarly, Bergot (1979a) found in trout that elevating the dietary level of glucose from 15% to 30% while maintaining equivalent protein intake resulted in improved growth and protein utilization. Therefore, these experiments have demonstrated that carbohydrate may have a protein sparing action in fish, perhaps through reducing the extent of gluconeogenesis. Additional studies directed to determine the optimal protein/energy ratio (Lee and Putman, 1973; Ringrose, 1971; Garling and Wilson, 1977; and Cowey et al., 1953 in diets of fish also support the concept that to some extent dietary carbohydrate spares protein.

In summary, proper definition of the correct level of protein in relation to the energy content of a salmonid diet is difficult owing to the different nutrient interactions which can occur. Therefore, the protein requirements of fish may have to be continuously re-evaluated as more fundamental knowledge is gained in fish nutrition.

CHAPTER 3

EXPERIMENT 1

3.0 Protein utilization and the measurement of protein quality in diets for chinook salmon fry.

3.1 Introduction

Recent evidence suggests that the survival of hatchery-reared chinook salmon in the ocean is directly related to their size at the time of seawater entry (Fowler et al., 1980; Bilton et al., 1982). Therefore it is essential that juvenile chinook salmon realize their full growth potential during the freshwater stage of their life history through the best possible nutrition. Also, it is well recognized that the successful development of salmon farming in British Columbia is partly dependent on the supply of profitable fish feeds. In particular, the quality of proteins comprising salmonid foods is critical to fish performance and cost effectiveness of salmon culture. Hence, in this study, the first objective was an understanding of which protein sources have the highest nutritive value for chinook salmon and how processing conditions either enhance or diminish protein quality.

Fishmeal is the principal source of protein in commercial salmonid diets, and not only provides protein to supply essential amino acids, but also contributes lipids, vitamins, and minerals. The protein quality of fishmeal presently

available in British Columbia is known to vary considerably. This variability is a function of the nature of the raw material (origin, species, season, whole fish or fish scraps, storage conditions), processing methods (cooking, drying, grinding, antioxidant stabilization) and storage conditions of the final product. Several fish species are commonly processed into fishmeal and of these herring and pollock were selected for a comparison of nutritive value in this study.

Both herring and pollock muscle were freeze-dried to maintain amino acid availability. Moreover, the pollock was blended with a small portion of euphausids, a species of marine zooplankton, in an attempt to promote appetite and improve amino acid balance. In this regard it was observed that the amino acid profile of freeze-dried pollock muscle and euphausids compared favourably to that of stated essential amino acid requirements of chinook salmon (Table 1). Also, it is noteworthy, that Cowey et al.(1971,1972) obtained excellent results in studies with plaice using freeze-dried cod muscle as a protein source. To evaluate the effects of different processing methods alone, on protein quality, a common batch of raw herring was cooked and then dried under different conditions. A tentative assumption was made that damage due to heat during drying would be manifested by losses in the availability of certain amino acids, especially lysine, and this would contribute to decreased protein quality.

The quality of all of the foregoing protein sources was compared to that of a mix of casein and gelatin fortified with

Table 1. Amino acid requirements for chinook salmon (NRC,1981), composition of eggs and the analyzed essential amino acid composition of freeze-dried pollock muscle, freeze-dried whole euphausids and vitamin-free casein.

		g amino acid / 16g nitrogen			
Amino acid	Requirements (NRC, 1981)	Freeze-dried[1] eyed chinook eggs	Freeze-dried[3] pollock muscle	Freeze-dried[3] whole euphausids	Vitamin-free[3] casein
Arginine	6.0	6.39	9.30	5.09	3.83
Histidine	1.8	2.88	2.15	2.00	2.95
Isoleucine	2.2	5.94	4.34	3.65	4.10
Leucine	3.9	9.74	8.61	5.81	7.60
Lysine	5.0	8.65	12.92	6.17	7.39
Methionine		2.51	4.10	4.27	3.01
Cystine[2]	4.0	1.56	1.68	0.99	0.74
Phenylalanine		5.39	4.39	3.74	4.91
Tyrosine[2]	5.1	4.62	4.13	3.60	5.59
Threonine	2.2	5.02	5.13	3.55	3.83
Tryptophan	0.5	1.42	1.22	1.00	1.23
Valine	3.2	7.32	4.26	3.75	5.39

1. Determined by A.A.A. Laboratory, Seattle, Wash.
2. Cystine and tryosine are dispensable, but spare requirements for methionine and phenylalanine respectively.
3. Determined at the Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada.

arginine and methionine to meet the essential amino acid requirements of chinook salmon (NRC, 1981). This was done because a blend of casein and gelatin has been considered to be an excellent source of protein for juvenile salmon and other fish species in previous nutrition studies, and indeed, diets based on casein and gelatin have been used extensively to study the nutritional requirements of fish (Cowey, 1976; Halver, 1982). Also, the case for a standard reference diet containing casein and gelatin as a protein source has been proposed to permit direct comparison of results among laboratories (Castell and Tiews, 1980).

Owing to the paucity of standardized techniques for the evaluation of food proteins in fish (Cowey and Sargent, 1979), the second goal of this study was to assess the merits versus demerits of several protein quality bioassay procedures used in assessment of the nutritive value of protein in fish diets. This necessitated the evaluation of protein quality at several concentrations of dietary protein. As part of this goal, estimates were made of endogenous nitrogen loss by the different groups of fish during the experimental period to permit measurement of protein quality by methods which consider use of dietary protein for maintenance as well as growth. This approach enabled determination of the amounts of protein utilized for maintenance, growth and that lost from exogenous excretions by fish ingesting proteins of varying quality.

3.2 Materials and methods

3.2.1 Test protein sources.

Frozen pollock (Theragra chalcogramma) fillets were partially thawed, comminuted through a meat grinder (6.35 mm grinder plate), loosely spread over trays, refrozen and freeze-dried. The freeze-dried pollock muscle was ground (Fitzmill, model JT) so that particles would pass 100% through a size U.S. 20 sieve. Ethoxyquin (.025%)was added to prevent oxidative rancidity. Whole frozen euphausids (Euphasia pacifica) were prepared in an identical manner. The proximate compositions of freeze-dried pollock and euphausids were respectively: moisture, 1.80% and 3.40%; ash, 4.97% and 12.10%; lipid, 2.01% and 16.45%; and crude protein, 89.74% and 60.01%. Nine parts of of the former were combined with one part of the latter on a dry weight basis to produce freeze-dried pollock muscle and whole euphausid meal (FPE).

The herring meals were prepared from a single batch of herring (Clupea harangus pallasi) caught on January 8, 1981, off Ladysmith, B.C. The herring was transported on ice and kept deep frozen (-20°C) in airtight plastic bags for not more than a month while being processed. Freeze-dried whole raw herring (FRH) was prepared in a similar manner to the pollock muscle except that ethoxyquin was added to the mixed herring at a level of 0.005% before freeze-drying.

The heat-dried herring meals were prepared in a continous pilot fish meal manufacturing machine (Chemical Research Organization, Esbjerg, Denmark). The machine consists of a

steam-jacketed cooker, screw press and steam-jacketed rotary dryer. The temperature of the cooker and dryer can be controlled. Minced, whole herring was cooked at 75°C. After pressing, the oil in the press liquid was decanted and the aqueous fraction was condensed to approximately 30% solids in a steam-jacketed bowl cooker. The condensed solubles were added to the presscake in the rotary dryer. The dryer was set to operate at 75°C to produce low temperature dried herring meal (LTH). The high temperature dried herring meal (HTH) was mainly dried in the rotary dryer at 120°C and further oven-dried at 150°C for one hour. Because both the LTH and HTH products contained an excess of lipid, it was necessary to partially extract lipid from the meals with hexane (5:1 v/w) to permit grinding and diet formulation.

After mixing and allowing to stand for 30 minutes, the solvent and meal slurry was filtered through a Buchner funnel over Whatman No.1 filter paper. This procedure was repeated three times. The final herring meal products were placed in wire mesh trays over a current of ambient air for five hours to remove any traces of residual hexane. Ethoxyquin (0.025%) was added to the hexane extracted products which were then ground through a size U.S. 20 screen with a hammer mill. Table 2 shows the compositions of the fish protein sources.

The casein-gelatin mix supplemented with 1.4% L-arginine and 0.57% DL-methionine (CS) was a modified version of the Oregon Test Diet outlined by NRC (1973)(Swarok and Higgs, 1981).

Table 2. Composition of protein sources (values in parenthesis show percentages for each proximate constituent and lysine expressed on a dry matter basis).

	Code	% Moisture	% Protein	% Lipid	% Ash	% Available lysine	Available lysine (% of crude protein)
Freeze-dried pollock muscle & freeze-dried euphausids[1]	FPE	1.96	86.72 (88.45)	3.43 (3.50)	5.69 (5.80)	7.42 (7.57)	8.56
Freeze-dried raw herring (hexane extracted)	FRH	8.11	70.56 (76.79)	10.48 (11.40)	11.49 (12.50)	3.86 (4.20)	5.47
Low temperature dried herring meal (hexane extracted)	LTH	7.77	63.95 (69.34)	16.98 (18.41)	10.21 (11.07)	3.45 (3.74)	5.39
High temperature dried herring meal (hexane extracted)	HTH	3.83	72.13 (75.00)	13.12 (13.64)	11.15 (11.59)	3.28 (3.41)	4.55
Casein mix[2]	CS	2.61	90.82 (93.25)			6.69 (6.87)	7.37

1. 90% freeze-dried pollock and 10% freeze-dried euphausids on a dry weight basis.
2. Contains 88% casein, 10% gelatin, 1.4% L-arginine, 0.57% DL-methionine (I.C.N., St. Louis, MO.)

Table 3. Composition of diets (g/kg diet on a dry matter basis).

	Diet Code (Protein source and % dietary protein)								
Ingredients	PF 0	FPE 7	FPE 17	FRH 17	LTH 17	HTH 17	CS 17	FPE 27	FRH 27
Freeze-dried pollock-euphausid	-	79.14	192.20	-	-	-	-	305.26	-
Freeze-dried raw herring	-	-	-	221.38	-	-	-	-	351.61
Low temp dried herring meal	-	-	-	-	245.17	-	-	-	-
High temp dried herring meal	-	-	-	-	-	226.67	-	-	-
Casein-gelatin, arginine, and methionine mix	-	-	-	-	-	-	182.31	-	-
Herring oil[1]	130.0	127.22	123.22	104.76	84.86	99.08	130.0	119.32	89.92
Mineral mix[2]	83.7	79.6	71.1	50.4	50.4	50.4	83.7	62.7	28.1
Ground cellulose	85.5	69.24	85.5	91.66	87.77	92.05	105.49	127.52	110.57
Dextrin	346.0	307.0	250.5	250.5	250.5	250.5	250.5	194.5	194.5
Glucose	346.0	307.0	250.5	250.5	250.5	250.5	250.5	194.5	194.5
Carboxy-methyl cellulose	20	20	20	20	20	20	20	20	20
Vitamin mix[3]	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Choline chloride (50%)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5

1. Stablized with BHA-BHT (1:1) 0.33%.
2. Mineral mix formulated so that each diet contained (g/kg dry diet): Ca 12.6, P 9.0, Mg 1.8, Fe 0.2, Na 2.4, Al 0.05, Cu 0.01, Mn 0.03, Co 0.01, Zn 0.08, K 8.3, I 0.004, Na:K 0.29, Ca:Mg 7, P:Mg 5, Ca:P 1.4. Calcium levels in FRH, LTH and HTH containing diets were calculated to be 13.9 and 19.6 g/kg for diets containing 27 and 37% protein. Similarly, phosphorus levels were 10.0 and 14.0 respectively to maintain equivalent Ca:P in all diets. Sources of minerals were potassium phosphate monobasic, potassium phosphate dibasic, sodium phosphate monobasic, calcium phosphate dibasic, calcium carbonate, magnesium sulphate, ferric oxide, zinc oxide, sodium chloride, manganese sulphate, cobalt chloride, cuprous chloride, potassium iodide and aluminum chlorhydrate.
3. Vitamin mix to supply (mg/kg dry diet unless otherwise indicated): ascorbic acid 1200, inositol 400, niacin 300, Ca-pantothenate 150, riboflavin 60, menadione 80, pyridoxine HCL 30, thiamin HCL 30, folic acid 20, biotin 3, vitamin A 10,000 IU, vitamin D3 1,000 IU, vitamin E 600 IU.

Table 3. cont'd.....

Ingredients	Diet Code (Protein source and % dietary protein)								
	LTH 27	HTH 27	CS 27	FPE 37	FRH 37	LTH 37	HTH 37	CS 37	FPE 47
Freeze-dried pollock-euphausid	-	-	-	418.32	-	-	-	-	531.37
Freeze-dried raw herring	-	-	-	-	481.83	-	-	-	-
Low temp dried herring meal	389.39	-	-	-	-	533.60	-	-	-
High temp dried herring meal	-	360.0	-	-	-	-	493.33	-	-
Casein-gelatin, arginine, and methionine mix	-	-	289.54	-	-	-	-	396.78	-
Herring oil[1]	58.31	80.90	130	115.36	75.07	31.76	62.71	130	111.40
Mineral mix[2]	28.1	28.1	83.7	56.00	17.04	17.4	17.4	83.7	47.4
Ground cellulose	104.40	11.20	132.56	142.12	118.90	110.44	119.76	149.02	115.43
Dextrin	194.5	194.5	194.5	138	138	138	138	138	82
Glucose	194.5	194.5	194.5	138	138	138	138	138	82
Carboxy-methyl cellulose	20	20	20	20	20	20	20	20	20
Vitamin mix[3]	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Choline chloride (50%)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5

1. Stablized with BHA-BHT (1:1) 0.33%.
2. Mineral mix formulated so that each diet contained (g/kg dry diet): Ca 12.6, P 9.0, Mg 1.8, Fe 0.2, Na 2.4, Al 0.05, Cu 0.01, Mn 0.03, Co 0.01, Zn 0.08, K 8.3, I 0.004, Na:K 0.29, Ca:Mg 7, P:Mg 5, Ca:P 1.4. Calcium levels in FRH, LTH and HTH containing diets were calculated to be 13.9 and 19.6 g/kg for diets containing 27 and 37% protein. Similarly, phosphorus levels were 10.0 and 14.0 respectively to maintain equivalent Ca:P in all diets. Sources of minerals were potassium phosphate monobasic, potassium phosphate dibasic, sodium phosphate monobasic, calcium phosphate dibasic, calcium carbonate, magnesium sulphate, ferric oxide, zinc oxide, sodium chloride, manganese sulphate, cobalt chloride, cuprous chloride, potassium iodide and aluminum chlorhydrate.
3. Vitamin mix to supply (mg/kg dry diet unless otherwise indicated): ascorbic acid 1200, inositol 400, niacin 300, Ca-pantothenate 150, riboflavin 60, menadione 80, pyridoxine HCL 30, thiamin HCL 30, folic acid 20, biotin 3, vitamin A 10,000 IU, vitamin D3 1,000 IU, vitamin E 600 IU.

3.2.2 Diets

The formulae of the experimental diets are shown in Table 3. Each protein source was included in the diets as the sole source of protein. Diets were formulated to contain 17%, 27%, and 37% of dry matter as crude protein (N x 6.25). An additional two diets were prepared containing FPE at levels of 7% and 47% protein. In order to estimate endogenous and metabolic protein loss a non-protein diet was offered to two groups of fish. Basal ingredients were proportionately common to all diets. Mineral supplements were prepared to equalize the mineral composition of all diets (Table 3).

All experimental diets were formulated to contain 4000 kcal/kg by ascribing metabolizable energy (ME) values of 4.5 kcal/g crude protein, 9.5 kcal/g lipid and 4.0 kcal/g for dextrin and glucose. The high values employed for fish oil, dextrin and glucose are considered valid because each has been found to be highly digestible in rainbow trout diets (Cho and Slinger, 1979). The value for protein was derived by deducting the heat of combustion of excreted ammonia nitrogen (0.95 kcal/g of protein) from the gross energy of protein (5.66 kcal/g)(Brett and Groves, 1979) and applying a digestibility coefficient for protein of 95% for casein-gelatin (Smith, 1971) and FPE. The calculated energy values of the diets based on analysis are shown in Table 4.

Lastly, two groups of fish received a commercial diet, Oregon Moist Pellets (OMP) which is the standard hatchery diet for chinook and coho salmon in British Columbia. Values of 4.2

Table 4. Proximate composition of diets (% dry-matter basis) and calculated available lysine and energy values based on the analyses.

Analysis	PF 0	FPE 7	FPE 17	FRH 17	LTH 17	HTH 17	CS 17	FPE 27	FRH 27
Crude protein (% N x 6.25)	1.07	6.73	16.94	18.01	16.78	17.00	18.55	27.24	27.59
Available lysine	-	0.58	1.45	0.99	0.90	0.77	1.37	2.33	1.51
Total crude lipid	14.08	13.22	12.40	12.46	13.33	11.95	11.61	12.31	12.81
Lipid contributed by fish meal	-	(.28)	(.67)	(2.52)	(4.51)	(3.09)	-	(1.07)	(4.01)
Ash	7.46	7.38	7.15	6.67	7.13	7.06	7.73	7.37	7.34
Digestible carbohydrate	69.2	61.4	50.1	50.1	50.1	50.1	50.1	38.9	38.9
Moisture	9.17	10.44	7.89	9.20	8.12	6.10	8.88	7.72	10.95
Energy (kcal/kg)									
Protein:									
ME (4.5 kcal/g)	48	303	762	811	755	765	835	1126	1242
GE (5.7 kcal/g)	61	384	966	1027	956	969	1057	1553	1573
Lipid:									
ME & GE (9.5 kcal/g)	1338	1256	1178	1184	1266	1135	1103	1169	1217
Carbohydrate:									
ME & GE (4.0 kcal/g)	2768	2456	2004	2004	2004	2004	2004	1556	1556
Total (ME)	4154	4015	3495	3998	4026	3904	3942	3951	4015
Total (GE)	4167	4096	4148	4215	4226	4108	4164	4278	4346
mg protein/kcal (ME)	2.58	16.8	42.9	45.0	41.7	43.5	47.1	68.9	68.7
Protein energy:total energy (ME)	0.01	0.08	0.19	0.20	0.19	0.20	0.21	0.28	0.31

Table 4. cont'd....

Analysis	LTH 27	HTH 27	CS 27	FPE 37	FRH 37	LTH 37	HTH 37	CS 37	FPE 47	OMP
Crude Protein (% N x 6.25)	28.45	27.38	26.40	35.96	39.58	38.42	39.33	35.99	46.15	49.40
Available lysine	1.53	1.25	1.95	3.08	2.17	2.07	1.79	2.65	3.95	-
Total crude lipid	11.43	12.60	12.60	11.79	12.20	11.81	11.29	11.52	12.95	14.53
Lipid contributed by fish meal	(7.17)	(4.91)	-	(1.46)	(5.49)	(9.82)	(6.73)	-	(1.86)	-
Ash	7.78	7.11	7.21	7.61	8.07	7.50	7.84	7.30	8.30	11.92
Digestible carbohydrate	38.9	38.9	38.9	27.6	27.6	27.6	27.6	27.6	16.4	16.4
Moisture	8.36	6.28	8.32	6.72	9.12	8.19	6.73	8.59	6.03	24.62
Energy (kcal/kg)										
Protein:										
ME (4.5 kcal/g)	1281	1232	1188	1618	1781	1729	1797	1619	2077	2075[1]
GE (5.7 kcal/g)	1622	1561	1595	2050	2256	2190	2276	2051	2631	2816
Lipid:										
ME & GE (9.5 kcal/g)	1086	1197	1146	1120	1159	1122	1073	1094	1230	2380
Carbohydrate:										
ME & GE (4.0 kcal/g)	1556	1556	1556	1104	1104	1104	1104	1104	656	2662[2]
Total (ME)	3922	3985	3890	4044	3955	3974	3818	3818	3963	3721
Total (GE)	4264	4314	4207	4274	4519	4416	4453	4249	4517	4462
mg protein/cal (ME)	72.5	68.7	67.9	93.6	97.9	97.1	100.5	94.3	116.5	132.8
Protein energy:total energy (ME)	0.33	0.31	0.31	0.42	0.44	0.44	0.45	0.42	0.52	0.56

1. Based on 4.2 kcal/g protein.
2. Based on 1.6 kcal/g carbohydrate.

kcal/g of protein and 1.6 kcal/g of nitrogen-free extract were employed to estimate the ME content of OMP because they more adequately reflect the digestibility of protein and raw starch in this diet (Table 4).

The experimental feeds were prepared by mixing the ingredients with a portion of the oil for 20 minutes in a Hobart mixer. The mix was then cold pelleted in a California model CL-type 2 laboratory pellet mill with 1.59 mm die. Subsequently, the pellets were crumbled with a rolling pin and hand screened to obtain the appropriate sized crumbles. The remainder of the herring oil was sprayed, by means of a hand held syringe (needle size 18), into the crumbles. The diets were mixed thoroughly and stored refrigerated in airtight containers during the study. The dietary treatment codes, respective protein sources and protein concentrations are summarized in Table 5. This code may be used to interpret the results of this study.

3.2.3 Aquarium facility

The experimental aquarium facility contained two rows of fiberglass tanks. Each tank contained 150 litres of aerated 10.5°C well-water. Flow rate was 4 to 6 litres/minute/tank. A natural photoperiod was provided by a series of fluorescent lights (Vitalite, Durotest 40w) controlled by an astronomical time clock.

Table 5. Summary of dietary treatments and codes.

Dietary protein source	Formulated protein concentration	Dietary treatment code
Protein-free	0	PF
Freeze-dried pollock muscle & freeze-dried euphausids	7	FPE-7
	17	FPE-17
	27	FPE-27
	37	FPE-37
	47	FPE-47
Freeze-dried raw whole herring (hexane-extracted)	17	FRH-17
	27	FRH-27
	37	FRH-37
Low temperature dried whole herring meal (hexane-extracted)	17	LTH-17
	27	LTH-27
	37	LTH-37
High temperature dried whole herring meal (hexane-extracted)	17	HTH-17
	27	HTH-27
	37	HTH-37
Casein-gelatin mix, plus L-arginine & DL-methionine	17	CS-17
	27	CS-27
	37	CS-37
Oregon moist pellets(#2) (complete diet)		OMP

3.2.4 Protocol

Chinook salmon fry were obtained in March, 1981 from Qualicum Hatchery, B.C. A population of 1900 fish was selected for uniform size with a mean weight of 1.03g ± 0.24. Fish from the selected population were randomly distributed into groups of 150 in each of 38 tanks. Each row of tanks constituted a block in the experimental design. The 19 treatments were alloted at random to the tanks within each row.

During a 14-day acclimation period the fish were fed to excess with a diet similar in composition to FPE-47 (Table 3) except that protein comprised 50% and lipid 10% of the diet dry matter. Mean weight of fish at the start of test feeding was 1.56 ± 0.024g(SEM). During the experimental period fish were fed by hand three times per day until satiated and a daily record of feed intake was maintained for each group. The point of satiation was determined when "active" feeding ceased over a feeding period of one hour. The interval between the first and second, and second and third feedings was two and one half hours in each case. Food particle size was adjusted to suit fish size according to Fowler and Burrows (1971).

3.2.5 Measurement of growth

On day 0, 21 and 42 of the feeding trial, following 16 hrs of food deprivation, 60 fish were randomly removed from each tank, anaesthesized in 0.5 ml 2-phenoxyethanol/l and placed on an absorbent towel. Individual fish weights (to 0.01 g) were recorded.

3.2.6 Chemical analysis of fish and diets

Four samples containing approximately 25g of fish each, common to all treatment groups, were taken at day 0. At the end of the trial, following 48 hrs of starvation, samples of 12 fish were taken from each group. The fish were killed in 2 ml of 2-phenoxyethanol/l, blotted dry on a towel and stored for analysis at -20°C in heat-sealed bags.

Prior to chemical analysis partially thawed fish samples were homogenized in a blender. Aliquots of the homogenate were used to determine moisture and ash (AOAC, 1975), lipid (Bligh and Dyer, 1959) and total nitrogen (Technicon Instrument Co. Ltd. industrial methods 369-75 A/A and 334-74 W/B). Percent nitrogen was multiplied by 6.25 to estimate protein content. Diets were analyzed employing similar procedures.

3.2.7 Available lysine

Available lysine in the protein sources was determined by Carpenter's (1960) method as modified by Booth (1971). The values were reported in Table 2 as a percentage of the meal and as a percentage of crude protein, i.e. g/16g N.

3.2.8 Data analysis

The body weight data were subjected to analysis of variance (ANOVA). The analysis of the randomized block tank means was conducted as a mixed model two-way ANOVA without replication. The dietary treatments were not replicated within rows. Protein source and dietary protein concentration were assumed to be fixed effects and row (block) effects were random. The

appropriate interaction mean square was used to test for significant differences due to the fixed effects. According to Zar (1974) there is no correct term available to test for block effects in a non-replicated model. Nevertheless, the residual mean squares derived from the variance between individual fish is inclued in the ANOVA. However, no major inference was drawn. From a strict viewpoint the individual fish body weights within tanks were not replicates. The approach taken in this study was conservative to assure that the probability of a Type 1 error is reduced. The treatment means were then subjected to Duncan's (1955) New Multiple Range Test (DMR)(P = 0.05).

The data were transformed to log body weight and subjected to an analysis of covariance with day as the covariate. Specific growth rate (GR%)(Higgs et al., 1979) was derived from the covariate slope; GR% = (eslope- 1) x 100. GR% expresses percent increase in body weight per day (Brett, 1979). The covariate slopes were subjected to Scheffé's (1959) test (P = 0.05) to detect significant differences. These analyses were performed by computer using a general least squares analysis of variance program (U.B.C. GENLIN). Regression equations of body weight gain against protein intake were computed by means of a general linear models procedure (SAS, 1982). The slopes were compared by DMR test (P = 0.05).

Food intake data were used to calculate gross food conversion (GFC),(dry weight gain(g) x 100 ÷ dry food intake (g)) and gross energy utilization (GEU),(gross energy gain

(kcal) x 100 ÷ gross energy intake (kcal)). Protein efficiency ratio (PER), net protein ratio (NPR), and slope ratio (SR),(for weight gain) were calculated on a dry body weight basis (Higgs et al., 1979) according to the formulas described in the previous chapter. From fish carcass composition, protein productive value (PPV), net protein utilization and SR (for protein gain) were calculated. Net protein utilization was calculated both by the Bender and Miller (1953) method, designated (NPU-1), and by the method of Ogino et al.(1980)(NPU-2). The procedure for these methods is described in the previous chapter.

The above indices were subjected to ANOVA. As described above a two-way randomized block model without replication was assumed. The analyses are tabulated in the appendices. As an estimate of sample variance the standard error of the mean (SE) was derived from the respective error mean square of the ANOVA. These are shown on each table with the first value they describe. Superscripts were used to designate significant differences derived from multiple comparison tests. These are shown with each table.

3.3 RESULTS

3.3.1 The effect of dietary protein source and level on body weight gain

The changes in body weight of fish fed the various diets are shown in Figs. 3A, 3B, and 3C. The growth response over the 42 day period was generally linear for fish fed diets containing 27% and 37% protein. The exceptions were high temperature dried herring meals. Fish fed these diets, and those containing 7 and 17% protein showed a decline in the rate of growth from day 21 to 42 compared to day 0 to 21 (Fig. 3A). Predictably, fish fed the PF diet lost body weight during the entire period.

The growth response of the experimental fish to the various dietary treatments as depicted in Figs. 3A, 3B, and 3C are in the classical nutritional form showing body weight gains over time. Table 6 and Fig. 4 on the other hand summarize the responses to the dietary treatments in terms of the specific growth rates elicited in response to dietary concentration of protein from the different sources.

Although the diets were formulated to contain exact levels of nutrients, proximate analysis showed that the compositions of the diets were not all equivalent at each prescribed protein level (Table 4). However, over the range of protein levels fed to groups of fish with FPE as the sole source of protein the increase in growth rate diminished with each increment of dietary protein concentration (Fig. 4). The pattern of diminishing returns was not as clear for the other protein

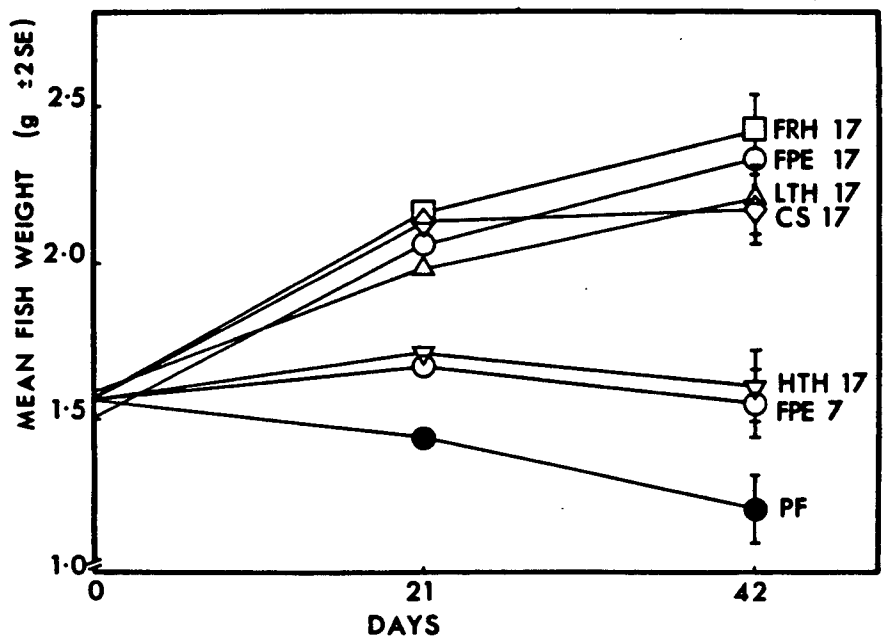


Fig. 3A. Growth of chinook salmon fed the various protein sources in diets containing approximately 17% protein. Growth of fish fed FPE-7 and protein free (PF) diets are also shown. Values plotted are mean wet fish weights, ± 2 standard errors (SE).

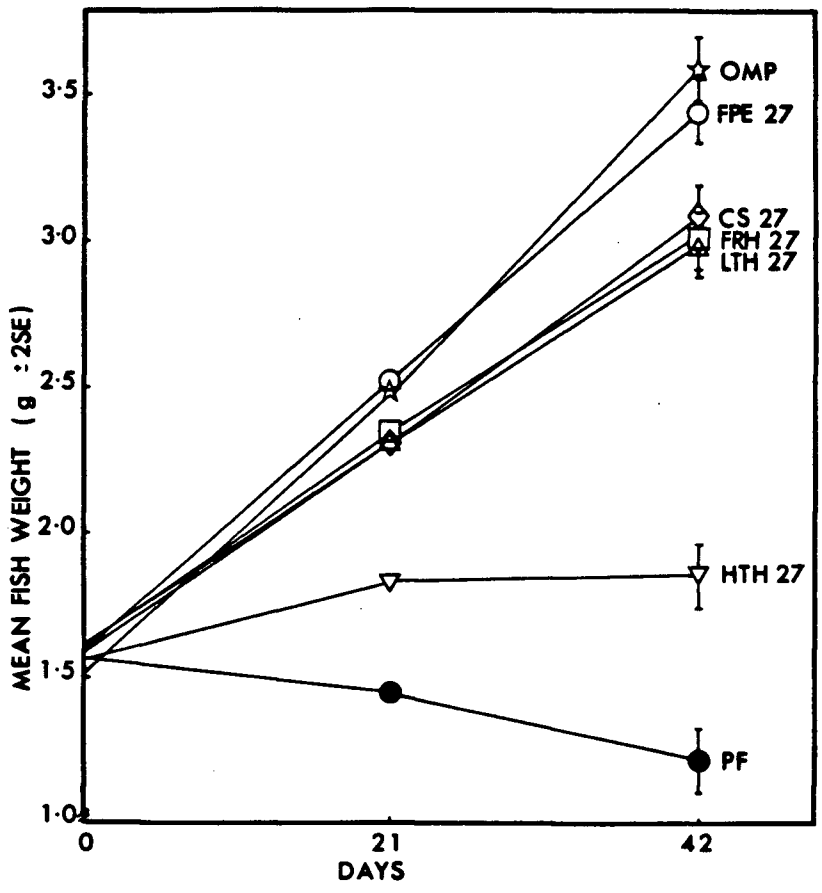


Fig. 3B. Growth of chinook salmon fed the various protein sources in diets containing approximately 27% protein. Growth of fish fed OMP and protein free (PF) diets are also shown. Values plotted are mean wet fish weights, ± 2 standard errors (SE).

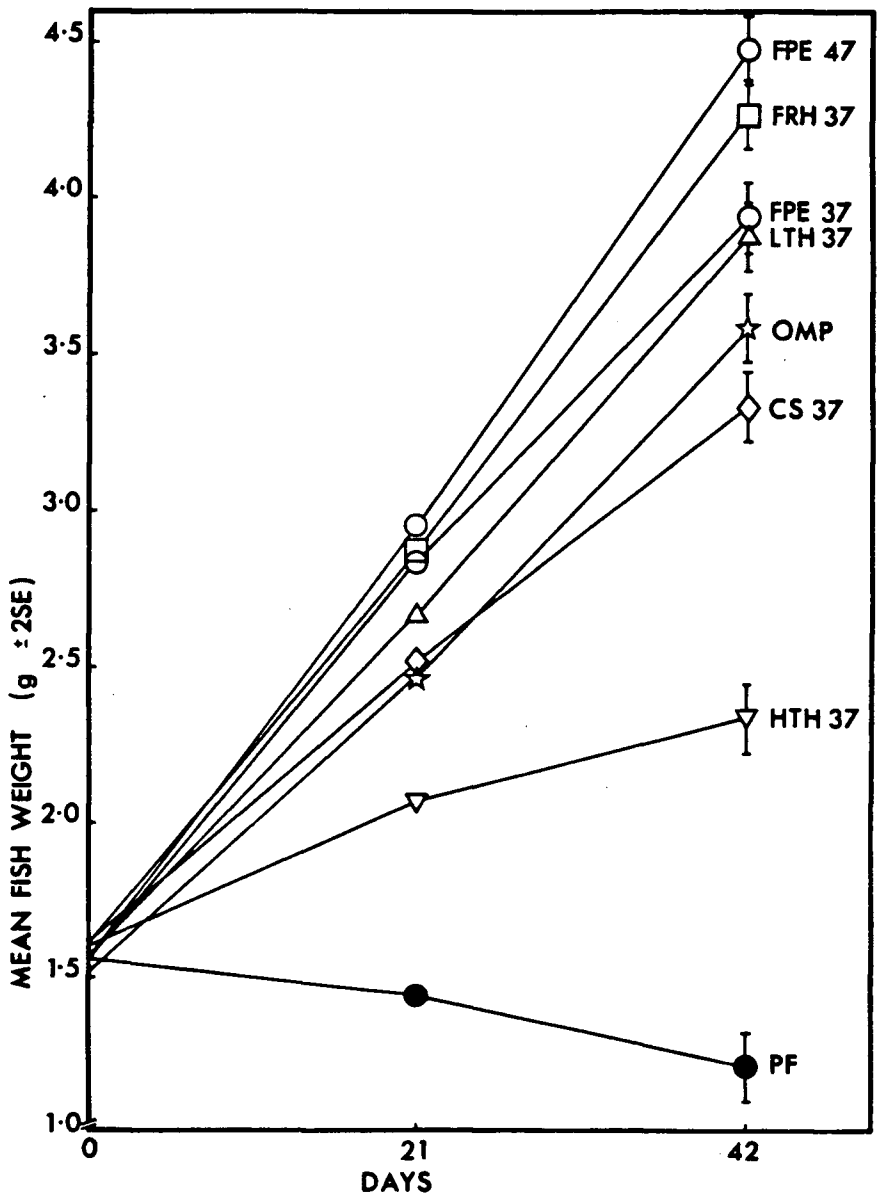


Fig. 3C. Growth of chinook salmon fed the various protein sources in diets containing approximately 37% protein. Growth of fish fed FPE-47, OMP, and protein free (PF) diets are also shown. Values plotted are mean wet fish weights, ± 2 standard errors (SE).

Table 6. Final (day 42) mean wet body weights (g) and specific growth rates (G.R.%; percent body weight per day) of fish fed the various protein sources at each dietary protein concentration.

Protein Source		% Protein in Diet 17	27	37	Mean
FPE	Final wt. (±SE)	^{cl}2.33 ±0.06	^{e}3.45	^{fg}3.94	^{x}3.24 ±0.03
	G.R. (%) (±SE)	^{CI}1.04 ±0.06	^{DE}1.86	^{EF}2.26	^{Y}1.72 ±0.03
FRH	Final wt.	^{c}2.43	^{de}3.02	^{g}4.25	^{x}3.23
	G.R. (%)	^{C}1.04	^{D}1.52	^{F}2.38	^{Y}1.64
LTH	Final wt.	^{bc}2.16	^{d}2.99	^{f}3.90	^{w}3.02
	G.R. (%)	^{BC}0.74	^{D}1.52	^{EF}2.20	^{X}1.48
HTH	Final wt.	^{a}1.59	^{ab}1.85	^{c}2.34	^{v}1.92
	G.R. (%)	^{A}0.06	^{AB}0.39	^{C}0.89	^{V}0.44
CS	Final wt.	^{bc}2.16	^{d}3.06	^{de}3.34	^{w}2.85
	G.R. (%)	^{BC}0.70	^{D}1.52	^{D}1.76	^{W}1.33
Mean	Final wt.	^{P}2.13 ±0.02	^{q}2.87	^{r}3.55	
	G.R. (%)	^{P}0.71 ±0.03	^{Q}1.36	^{R}1.90	
OMP[2]	Final wt.				3.59
	G.R.(%)				2.08

1. Values with the same superscript for each parameter with respect to the source x level (a - f), protein source (v - y), and protein level (p - r) effects do not differ significantly. Superscripts for G.R.(%) are capitalized (DMR test for body weight, P = 0.05); Scheffe's test for G.R. (%), P = 0.05).
2. OMP data was not analyzed statistically.

sources as was noted for fish fed FPE. Clearly, HTH was an inadequate protein source, demonstrating the effect of severe drying temperature on herring meal. The performance of fish fed OMP was found to be comparable to that of fish fed FPE, FRH and LTH at the 37% level of dietary protein. OMP is a popular commercial salmon feed containing 49% protein.

The performance of fish fed the test diets containing the various protein sources at three protein concentrations was compared by a two-way randomized block factorial analyses of variance. At day 42 marked differences in body weight due to protein source ($P < 0.001$) and protein level ($P < 0.01$) were found (Table 6). Similarly, a factorial analysis of covariance of $\log_e$ body weights also indicated a significant effect due to protein source ($P < 0.01$) and dietary protein level ($P < 0.05$). The specific growth rates (derived from the slopes) of groups of fish fed each protein source at all levels were compared (Table 6). Both the mean final body weights and specific growth rates showed that FPE and FRH promoted the fastest growth, followed by LTH, CS and lastly, HTH. The above results showed that differences in performance of fish fed proteins of varying quality were manifested at widely different dietary concentrations.

Since protein intake may have been restricted due to poor palatibility of particular diets, weight gain was regressed against protein intake for each protein source (Table 7). The slopes of the regression equations indicate the rate of weight gain relative to protein intake. These are depicted graphically

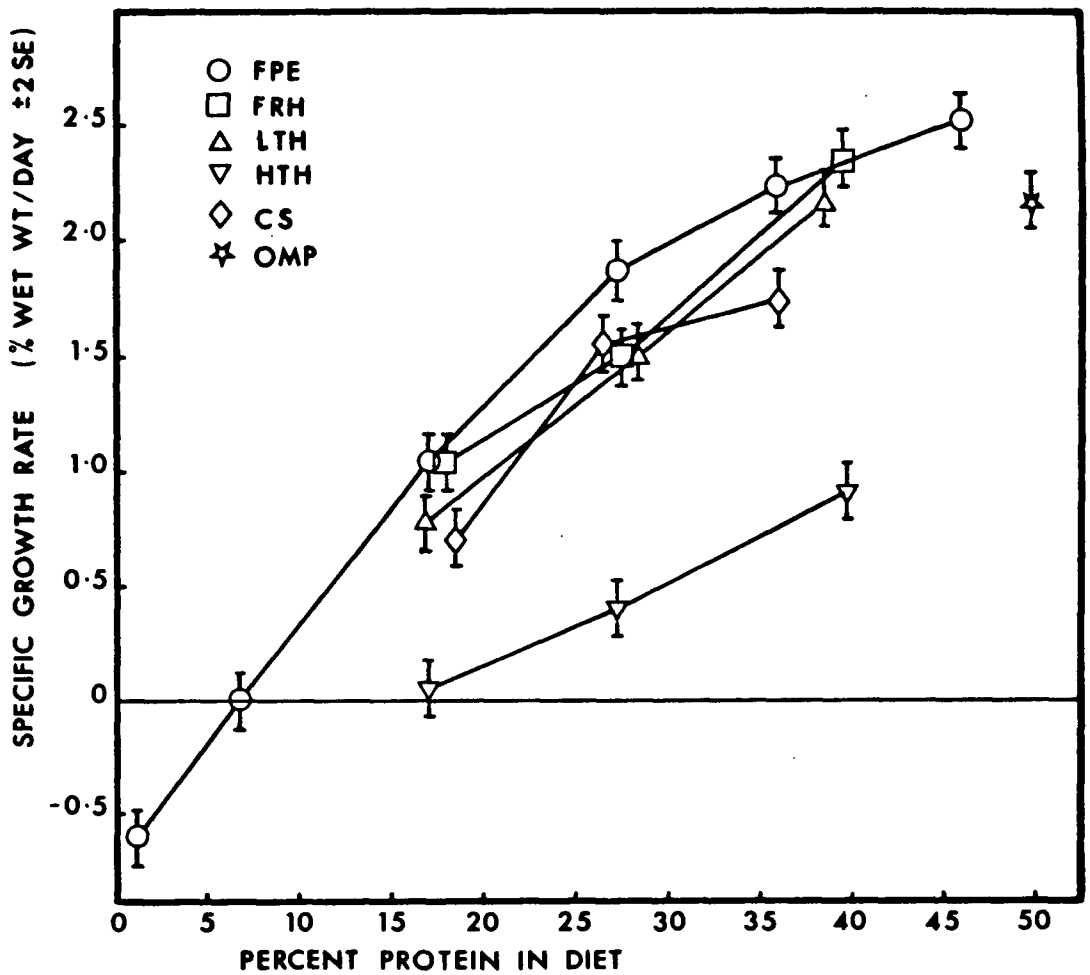


Fig. 4. Specific growth rates of chinook salmon fed various sources of protein at different dietary concentration. Values plotted are specific growth rate (% wet body weights/day), $\pm$ 2 standard errors (SE).

Table 7. Slopes of body weight gain against protein intake for the various protein sources.

Protein source	Equation	SE of slope
FPE	$y = -0.263 + 3.33x^{a}$	0.28
	(n=8, r=0.9969, P<0.01)	
FRH	$y = -0.2483 + 3.23x^{a}$	0.28
	(n=8, r=0.9957, P<0.01)	
LTH	$y = -0.1522 + 2.56x^{b}$	0.27
	(n=8, r=0.9843, P<0.01)	
HTH	$y = -0.3842 + 1.78x^{c}$	0.33
	(n=8, r=0.9966, P<0.01)	
CS	$y = -0.4271 + 3.52x^{a}$	0.24
	(n=8, r=0.9848, P<0.01)	

1. Slopes with the same superscript do not differ significantly (DMR test, P = 0.05).

(Fig. 5). The analysis indicated that the effects of the protein source and the covariate (protein intake) were significant ($P < 0.001$). The intercepts did not differ significantly ($P > 0.05$) since the relationships between weight gain and protein intake were linear ($P < 0.001$) and the data for the protein-free diet fed groups was common for all protein sources. Relative to protein intake the casein-based diets (CS) supported the highest rate of weight gain. This contrasts the growth results above where intake was not considered. This suggests that food intake was restricted in fish fed the CS diets. Similar slopes were obtained with the freeze-dried meals. The slope obtained with the low temperature dried meal (LTH) was lower than that obtained with the freeze-dried meals. The lowest slope was obtained with the high temperature dried meal (HTH).

3.3.2 The effect of dietary protein source and level on food intake and gross food conversion efficiency

Food intake of the groups of fish fed by hand to satiety at each feeding are shown both on a per 100 fish basis (TFI), and on a dry body weight basis (DFI)(Higgs et al. 1979, 1982) (Table 8). The latter may be considered more meaningful since it corrects for fish size and moisture content. Generally, DFI decreased with increasing body weight and dietary protein concentration. The trend was most evident for fish fed diets containing FPE over a wide range of dietary protein levels.

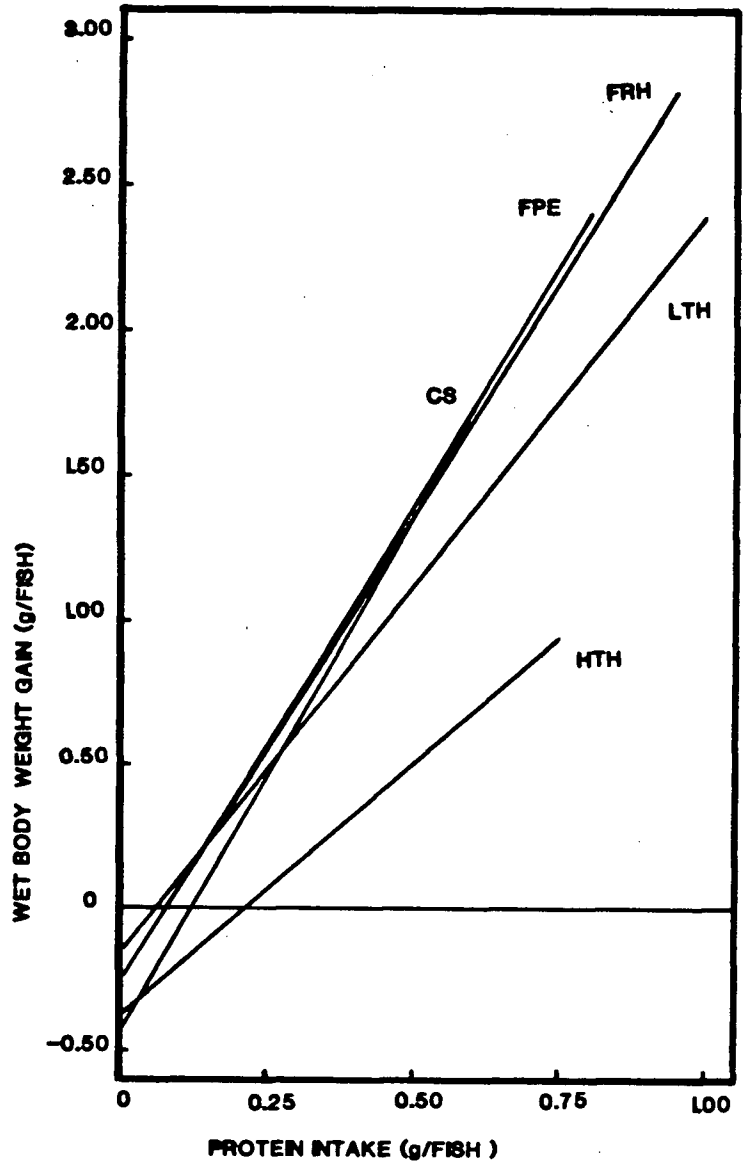


Fig. 5. Slopes of weight gain against protein intake of chinook salmon fed diets containing the test protein sources.

Table 8. Total dry food intake (TFI)(g/100 fish), mean daily food intake (DFI)(g/100g mean dry body weight/day) and gross food conversion (GFC)(Percent) of the various diets during the experimental period.

Protein Source		% Crude Protein in Diets					
		0	7	17	27	37	47
FPE	TFI	123	162	188	234	233	247
	DFI	11.00	12.13	10.91	9.89	8.86	8.21
	GFC (±SE)		0.44 ±0.74	11.50	21.15	27.11	29.86
FRH	TFI			196	180	233	
	DFI			11.01	8.49	8.04	
	GFC			12.68	20.69	31.12	
LTH	TFI			173	187	271	
	DFI			10.18	8.85	10.48	
	GFC			9.79	19.84	22.67	
HTH	TFI			133	140	156	
	DFI			9.83	9.54	8.88	
	GFC			1.26	4.99	12.60	
CS	TFI			178	186	176	
	DFI			10.98	8.95	7.77	
	GFC			7.19	19.67	24.72	
OMP	TFI						203
	DFI						8.70
	GFC						25.28

Gross food conversion efficiency (GFC) was calculated as percent conversion to dry body weight (Brett, 1971; Higgs et al., 1982). A rapid increase in GFC was found as dietary protein level was increased from 7% to 37% (Table 8). The increase in GFC between fish groups fed FPE in diets containing 37% and 47% protein was relatively small.

The GFC data for fish fed the test diets containing protein sources at levels of 17%, 27% and 37% were analyzed as a two way randomized block factorial experiment. The effects of protein source and protein level were significant ($P < 0.001$ and $P < 0.05$). The average GFC values obtained for each source, across all levels of protein, were used as a means of evaluating protein quality. In this regard, relative GFC and the ranking of the proteins is shown in Table (9). Values for fish fed diets with FRH and FPE were highest, followed by those for fish receiving LTH and CS (Table 9). The GFC of fish ingesting diets with HTH relative to that of the other proteins shows that the HTH diets were extremely poorly utilized.

3.3.3 The effect of dietary protein source and level on energy intake and gross energy utilization

Food intake on a dietary energy basis by the various groups of fish was compared on both a gross (GEI) and metabolizable (MEI) basis (Table 10). Food energy intake was reported on a dry fish body weight basis in an attempt to correct for fish size and moisture content. Food energy intake decreased as the dietary protein level increased. This trend was noted to be

Table 9. Gross food conversion calculated on a dry body weight basis (GFC); relative GFC (in parenthesis) and ranking of different protein sources at different dietary concentration.

Protein Source		% Crude Protein in Diets 17	27	37	Mean
		[1]			
FPE	GFC (±SE)	cd 11.50 ±1.55	ef 21.15	h 27.11	wx 19.92 ±0.89
	Relative GFC	(100)	(100)	(100)	(100)
	Ranking	2	1	2	2
FRH	GFC	d 12.68	ef 20.69	j 31.12	x 21.50
	Relative GFC	(110)	(98)	(115)	(108)
	Ranking	1	2	1	1
LTH	GFC	c 9.79	e 19.84	fg 22.67	w 17.43
	Relative GFC	(85)	(94)	(84)	(88)
	Ranking	3	3	4	3
HTH	GFC	a 1.26	b 4.99	d 12.60	v 6.28
	Relative GFC	(11)	(24)	(46)	(32)
	Ranking	5	5	5	5
CS	GFC	b 7.19	e 19.67	gh 24.72	w 17.19
	Relative GFC	(63)	(93)	(91)	(86)
	Ranking	4	4	3	4
Mean	GFC	p 8.48 ±0.70	q 17.27	r 23.64	

1. Values with the same superscript with respect to source x level (a - i) and protein source (v - x) effects did not differ significantly (DMR test P = 0.05).

lower for GEI than for MEI. Energy intake was similar between fish fed OMP and fish fed FPE-47 which contained a similar protein and gross energy concentration (Table 10). However, OMP supported a lower gross energy utilization (GEU) than FPE-47.

Gross energy utilization of the diets was noted to be directly related to dietary protein level (Table 10). At dietary protein levels above 37% no further increase in energy utilization was noted for fish fed diets containing FPE. GEU was also employed to compare the quality of the protein sources. The trend in GEU of fish fed the various proteins is shown in Table (11). Fish fed diets containing the freeze-dried meals (FPE, FRH) and the low-temperature dried meal (LTH) utilized dietary energy most effectively. The casein based diet (CS) produced similar GEU to LTH. The GEU of the HTH diets was extremely poor.

3.3.4 The estimation of endogenous nitrogen loss (maintenance requirements) by juvenile chinook salmon

Two diets were employed to measure endogenous nitrogen loss by chinook salmon fry. One was a protein-free diet (fed to satiation) in which all of the dietary energy that could possibly be used originated from carbohydrate and lipid. Therefore, tissue protein would be the only source of nitrogen available for maintenance purposes. The second diet was designed to provide sufficient metabolizable energy to satisfy maintenance requirements under more normal physiological conditions than allowed by the protein-free diet. Exactly

Table 10. Mean daily gross energy intake (GEI) (kcal/100g mean dry body weight/day), mean daily metabolizable energy intake (MEI) (kcal/100g mean dry body weight/day) and gross energy utilization (GEU) of the various diets during the experimental period.

Protein Source		% Crude Protein in Diets 0	7	17	27	37	47
FPE	GEI	45.87	49.73	45.28	42.33	37.83	37.11
	MEI	45.65	48.76	43.09	39.56	35.71	32.51
	GEU (±SE)		-0.29 ±1.37	16.41	31.86	39.66	41.71
FRH	GEI			46.46	39.93	36.34	
	MEI			44.04	34.13	35.79	
	GEU			17.08	29.43	40.93	
LTH	GEI			43.06	37.70	46.32	
	MEI			41.03	34.69	41.50	
	GEU			14.42	29.11	32.14	
HTH	GEI			40.40	41.12	39.52	
	MEI			38.34	38.06	35.25	
	GEU			0.96	6.38	15.54	
CS	GEI			45.68	37.68	33.02	
	MEI			43.26	34.82	29.60	
	GEU			8.20	26.09	33.49	
OMP	GEI						38.80
	MEI						32.36
	GEU						33.88

Table 11. Gross energy utilization (GEU), relative GEU (in parenthesis) and ranking of different protein sources at different dietary concentrations.

Protein Source		% Crude Protein in Diets 17	27	37	Mean
		[1]			
FPE	GEU (±SE)	$16.41^{c} \pm 1.48$	31.86^{e}	39.66^{f}	$29.31^{x} \pm 0.85$
	Relative GEU	(100)	(100)	(100)	(100)
	Ranking	2	1	2	1
FRH	GEU	17.08^{c}	29.43^{de}	40.93^{f}	29.15^{x}
	Relative GEU	(104)	(92)	(103)	(99)
	Ranking	1	2	1	2
LTH	GEU	14.42^{c}	39.11^{de}	32.14^{e}	25.22^{wx}
	Relative GEU	(88)	(91)	(81)	(86)
	Ranking	3	3	4	3
HTH	GEU	0.96^{a}	6.38^{b}	15.54^{c}	7.62^{v}
	Relative GEU	(6)	(20)	(39)	(26)
	Ranking	5	5	5	5
CS	GEU	8.20^{b}	26.09^{d}	33.49^{e}	22.59^{w}
	Relative GEU	(50)	(82)	(84)	(77)
	Ranking	4	4	3	4
Mean	GEU	$11.41^{p} \pm 0.66$	24.57^{q}	32.35^{r}	

1. Values with the same superscript with respect to source x level (a - f), protein source (v - x), and protein level (p - r) effects did not differ significantly (DMR test P = 0.05).

sufficient protein energy was provided so that when the fish were fed to satiation, over an extended period of time (more than 42 days), the fish would maintain their body weight.

In preliminary studies FPE was shown to be highly digestible (97%) by chinook salmon fed diets varying in levels of protein and energy. Therefore, it may be reasonably assumed that the protein in diet FPE-7 was almost totally absorbed by the fish. It was postulated that the protein intake required to support zero growth and exact nitrogen balance represented the maintenance requirement. As mentioned previously, this corresponds to the endogenous protein-nitrogen loss of a group of fish maintained on a protein-free diet during the experimental feeding period.

Body weight data of fish fed the protein-free and FPE-7 diets for 63 days (Table 12) show the effects of severe protein deprivation. From day 0 to 42 the fish offered the protein-free diet lost an average of 22.5% of their initial body weight. From day 42 to 63 there was no further reduction in body weight. Fish fed FPE-7 containing 7% protein showed a slight increase in body weight from day 0 to day 21. By day 42, however, there was no further gain in weight and by day 63 the groups were observed to have maintained their initial weight. It is noteworthy that fish fed the protein-free diet and the 7% protein diet maintained their respective day 42 body weights to day 63 on rations of similar metabolizable energy content.

The data employed and the estimates observed for endogenous nitrogen loss are shown in Table 13. Endogenous nitrogen loss

Table 12. Wet body weight (BW) and percent of initial body weight (%BW) of fish fed protein free (PF) and a maintenance diet (FPE-7).

Diet		Day 0	21	42	63
PF	BW g/fish (±SE)	1.56 (±0.04)	1.44	1.21	1.21
	% BW		91.93	77.51	77.80
FPE-7	BW g/fish (±SE)	1.56 (±0.04)	1.67	1.55	1.52
	% BW		07.04	99.33	97.09

Table 13. Estimation of mean daily endogenouse nitrogen loss by carcass analysis of fish fed a protein-free (PF) and a low protein (FPE-7) diet for the 42 day experimental period.

Diet	Nitrogen gain	Nitrogen intake	Endogenous nitrogen loss	(SE)
	(Calculated on a wet body weight basis (mg/100gBW/day))			
PF	-17.09	3.63	20.73	(2.77)
FPE-7	-1.15	26.72	25.52	(0.18)
	(Calculated on a dry body weight basis (mg/100gDBW/day))			
PF	-88.48	18.80	107.28	(15.60)
FPE-7	6.16	130.64	124.96	(0.16)

was calculated by adding nitrogen intake to nitrogen loss. The low nitrogen content of the protein-free diet was assumed to be derived from protein and was therefore included in the determination. Endogenous nitrogen loss was calculated to be 20.73 mg nitrogen (N)/100g body weight (BW)/day (SE = 2.77) from the fish fed the protein-free diet and 25.52 mg N/100g BW/day (SE = 0.18) from the fish fed the maintenance protein level diet (FPE-7). Because fish fed the protein-free diet were found to have an elevated moisture content, endogenous nitrogen loss was also calculated on a dry body weight basis. In this case, endogenous nitrogen loss was estimated to be 107.28 mg N/100g dry BW/day (SE = 15.60) from the groups fed the zero protein diet and 124.96 mg N/100g dry BW/day (SE = 0.16) for the FPE-7 diet group. The above values show that the protein intake required for maintenance approximates the protein loss that occurred when fish were fed a non-protein diet.

The values obtained from fish fed the 7% protein diet (Table 13) can be employed to correct protein quality estimates for endogenous nitrogen loss (ENL) or protein loss (EPL)(ENL x 6.25). Since, endogenous nitrogen loss changes in direct proportion to fish body weight (size) (Brett and Groves, 1979) the method of Ogino (1980) was adapted as follows:

$$\text{ENL (g)} = \frac{W_1 + W_2}{2} \times 25.52 \times 10^{-5} \times d$$

$$\text{EPL (g)} = \frac{W_1 + W_2}{2} \times 159.5 \times 10^{-5} \times d$$

$$ENL\ (g) = \frac{DW_1 + DW_2}{2} \times 124.96 \times 10^{-5} \times d$$

$$EPL\ (g) = \frac{DW_1 + DW_2}{2} \times 781.0 \times 10^{-5} \times d$$

where : ENL = endogenous nitrogen loss

EPL = endogenous protein loss

W_1= initial body weight (g)

W_2= final body weight (g)

DW_1= initial dry body weight (g)

DW_2= final dry body weight (g)

d = days of feeding

3.3.5 The relationship between protein intake and protein utilization

To illustrate the relationship of nitrogen balance and protein quality, the way in which the groups of fish utilized the various dietary protein sources was investigated. Protein intake was partitioned into the amounts of protein utilized for maintenance and growth of body tissues and the amount lost through exogenous fecal and metabolic excretion (Table 14). In this discussion, exogenous excretions refer to the fraction of protein intake that was not digested plus the amount used for energy (Fig.1). This was obtained by the difference between protein intake and, endogenous protein loss (equal to maintenance, Fig. 1) plus body protein gain. The quantities were expressed in mg per 100g wet body weight per day

Table 14. The utilization of dietary protein on a daily basis by fish fed the various experimental diets during the 42 day period.

Diet	Protein Intake (mg/100gBW/d)	Amount of Protein Utilized by 100g fish for: (mg/100gBW/d)			Percent of Protein Intake used for:		
		Maintenance	Growth	Excretion	Maintenance	Growth	Excretion
FPE-7	167.0	160	7.1	0	96.0	4.3	0
FPE-17	395.3	160	146.6	88.7	40.5	37.0	22.5
FPE-27	604.1	160	298.2	145.9	26.6	49.4	24.1
FPE-37	728.9	160	351.4	217.5	22.0	48.2	29.9
FPE-47	898.6	160	378.8	359.8	17.6	42.2	40.3
OMP	941.4	160	312.7	468.7	17.0	32.3	49.8
FRH-17	422.8	160	154.0	108.9	38.3	40.5	21.3
FRH-27	511.8	160	227.5	111.7	31.3	44.5	24.2
FRH-37	749.9	160	321.4	268.6	21.3	41.8	36.9
LTH-17	366.4	160	131.0	75.5	43.7	35.8	20.5
LTH-27	554.5	160	232.6	161.9	28.9	42.0	29.1
LTH-27	914.3	160	340.5	413.8	17.5	37.3	45.2
HTH-17	341.7	160	-16.2	197.8	46.9	-4.1	57.2
HTH-27	534.8	160	53.3	321.6	30.0	10.0	60.1
HTH-37	754.8	160	121.9	473.0	21.2	16.2	62.6
CS-17	417.7	160	88.6	169.2	38.4	21.4	40.2
CS-27	500.9	160	238.6	102.5	32.0	43.1	25.0
CS-37	609.6	160	257.6	191.9	26.3	42.3	31.5

(mg/100g BW/day). Next, the utilization of protein for maintenance, growth and the quantity excreted, was expressed as a percentage of the protein fed (Table 14) and has been depicted graphically (Figs. 6A - 6E).

In regard to fish ingesting FPE, the level of protein intake for maintenance was 160mg/100g BW/day. At this level practically all of the protein was absorbed and utilized for maintenance and none was wasted or available for growth (Fig. 6A). Between maintenance and optimal protein utilization (Op), the percentage of protein intake used for growth rose sharply, whereas the proportion employed for maintenance dropped rapidly (Fig. 6A). Additional protein intake above the optimum resulted in a decreased rate of utilization for growth because of an increase in the percentage of protein lost in excreta. FPE protein was most efficiently utilized for growth at a protein intake of approximately 600mg/100g BW/day. In contrast to fish receiving diets containing FPE, the fish fed OMP utilized protein less effectively for growth largely because of increased excretory losses (Fig. 6A).

The method of herring meal processing markedly influenced the way protein was utilized (Fig. 6B, 6C, 6D). In comparison to all other groups, chinook fed diets containing HTH utilized protein poorly. Only 16.5% of the protein fed was utilized for growth in diets with a protein concentration of 37% (Table 14). A higher proportion of protein intake was required by the fish fed diets with HTH relative to those fed diets containing FRH or LTH to meet their maintenance

Fig. 6A - E. Percent utilization of protein fed for maintenance (○) and growth (△), and percent excreted (□) when fish fed the test protein sources at various levels of protein intake. (Op) stands for the protein intake that promotes optimal utilization of protein for growth. It is noteworthy that percent utilization for growth (△) is the same as protein productive value (PPV) (see Fig. 1). The protein utilization of fish fed OMP is shown in dark symbols for comparison with each of the test proteins. The dashed portions of the curves were extrapolated.

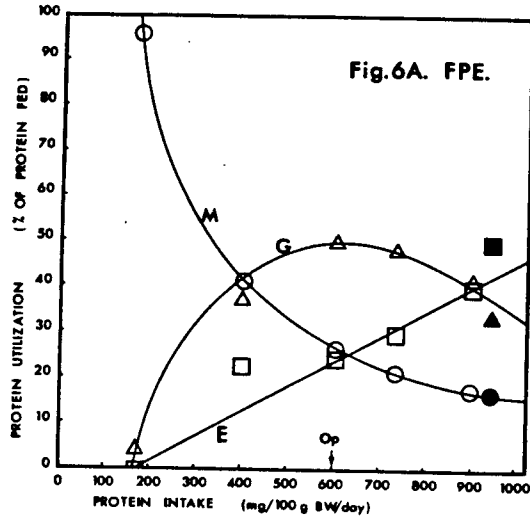


Fig.6A. FPE.
PROTEIN UTILIZATION (% OF PROTEIN FED)
PROTEIN INTAKE (mg/100 g BW/day)
M
G
E
Op

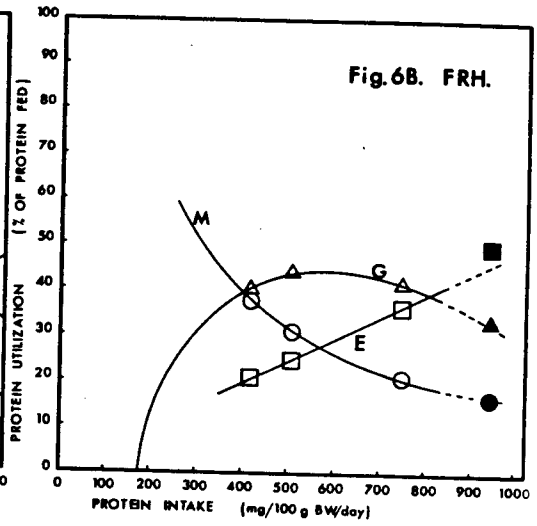


Fig.6B. FRH.
PROTEIN UTILIZATION (% OF PROTEIN FED)
PROTEIN INTAKE (mg/100 g BW/day)
M
G
E

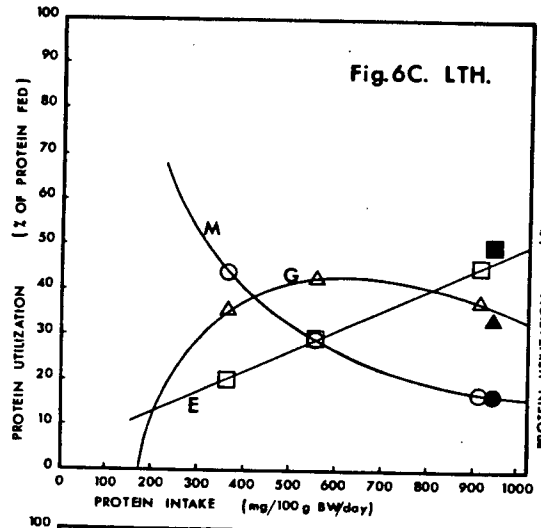


Fig.6C. LTH.
PROTEIN UTILIZATION (% OF PROTEIN FED)
PROTEIN INTAKE (mg/100 g BW/day)
M
G
E

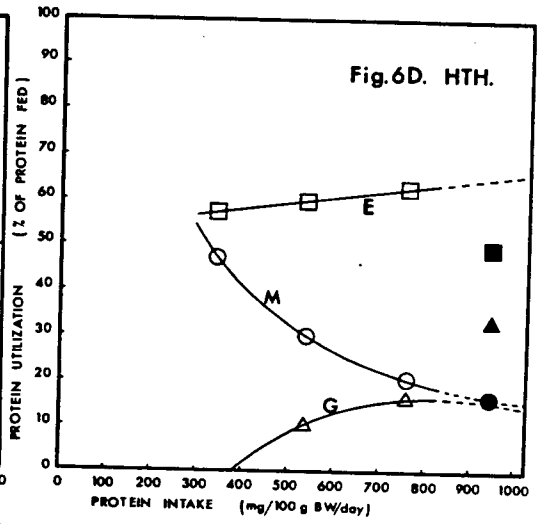


Fig.6D. HTH.
PROTEIN UTILIZATION (% OF PROTEIN FED)
PROTEIN INTAKE (mg/100 g BW/day)
E
M
G

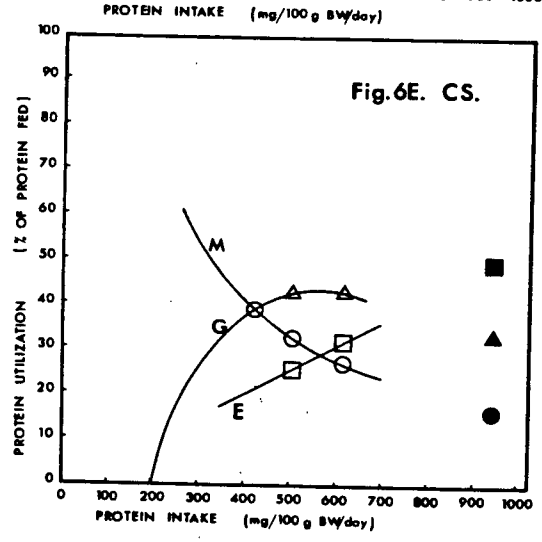


Fig.6E. CS.
PROTEIN UTILIZATION (% OF PROTEIN FED)
PROTEIN INTAKE (mg/100 g BW/day)
M
G
E

requirements for protein. Further, at the highest level of protein intake over 60% of the protein fed was excreted (Fig. 6D).

Because of an inconsistency in the performance of fish fed CS at low levels, and a palatability problem noted in fish ingesting high dietary levels of CS, the pattern of protein utilization was difficult to construct and interpret for the CS groups (Fig. 6E). The data indicated that CS was a good source of protein for meeting the protein requirements of chinook salmon in terms of protein utilization.

3.3.6 The measurement of protein quality by protein efficiency ratio and net protein ratio

Protein efficiency ratio (PER)(Osborne et al., 1919) and net protein ratio (NPR)(Bender and Doell, 1957) are commonly used to assess protein quality. Figure 7 shows the relationship between dietary protein concentration and PER in fish fed FPE diets. Predictably it followed a classical trend similar to that reported for rats and plaice by other investigators. The noteworthy differences among species are the protein concentrations at which maximum PER occurred. Figure 8 shows the extent to which PER was dependent on protein intake. The effect of low protein intake was partially overcome once an allowance was made for maintenance by the NPR method. The difference between the PER and NPR curve at different protein

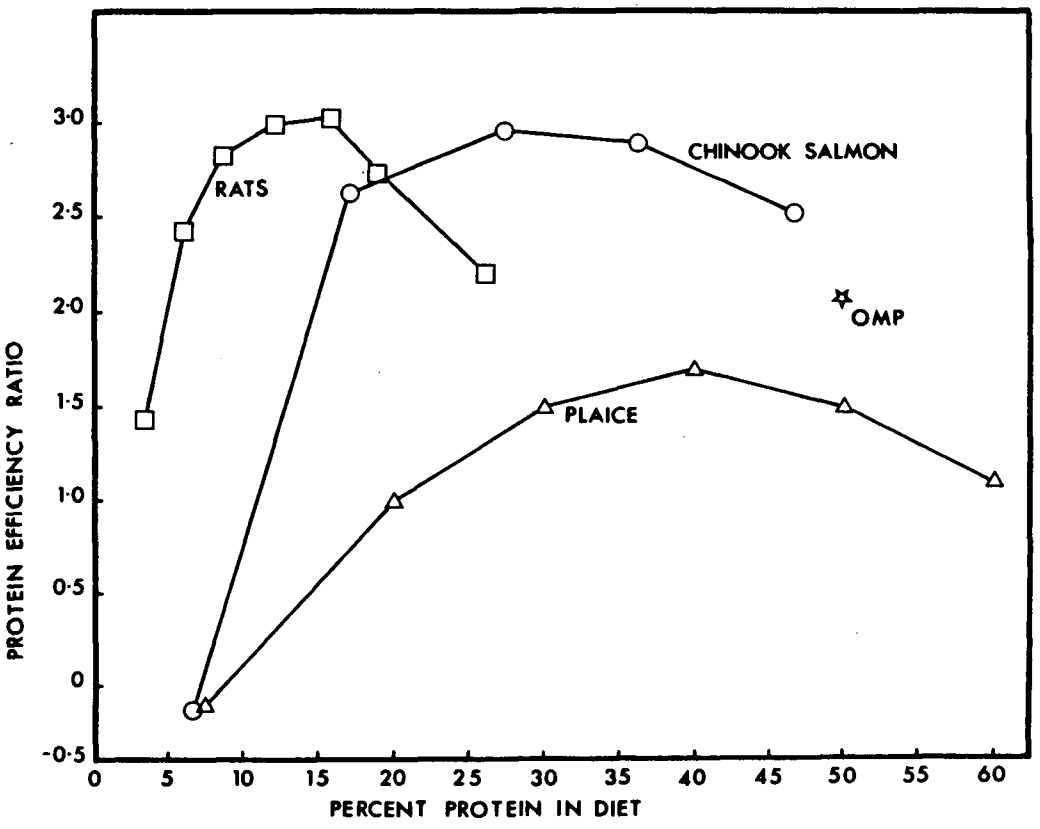


Fig. 7. Effect of dietary protein level on protein efficiency ratio (PER) calculated on a wet body weight basis for chinook salmon fry fed FPE, for plaice fed freeze-dried cod muscle (from Cowey et al., 1972) and for rats fed casein (from Hegsted and Chang, 1965) containing diets.

Table 15. Protein efficiency ratio calculated on a dry body weight basis (PER), relative PER (in parenthesis) and ranking of different protein sources at different dietary concentrations.

Protein Source		% Crude Protein in Diets 17	27	37	Mean
		[1]			
FPE	PER (±SE)	def 0.679 ±0.010	f 0.776	f 0.754	x 0.736 ±0.06
	Relative PER	(100)	(100)	(100)	(100)
	Ranking	2	1	2	2
FRH	PER	f 0.704	f 0.750	f 0.786	x 0.747
	Relative PER	(103)	(96)	(105)	(101)
	Ranking	1	2	1	1
LTH	PER	d 0.583	ef 0.697	de 0.590	w 0.623
	Relative PER	(85)	(90)	(79)	(84)
	Ranking	3	4	4	3
HTH	PER	a 0.074	b 0.182	c 0.315	v 0.191
	Relative PER	(10)	(23)	(43)	(26)
	Ranking	5	5	5	5
CS	PER	c 0.387	f 0.745	def 0.687	w 0.606
	Relative PER	(57)	(96)	(92)	(82)
	Ranking	4	3	3	4
Mean	PER	0.486 ±0.055	0.630	0.626	

1. Values with the same superscript with respect to source x level (a - f) and protein source (v - x) effects did not differ significantly (DMR test P = 0.05).

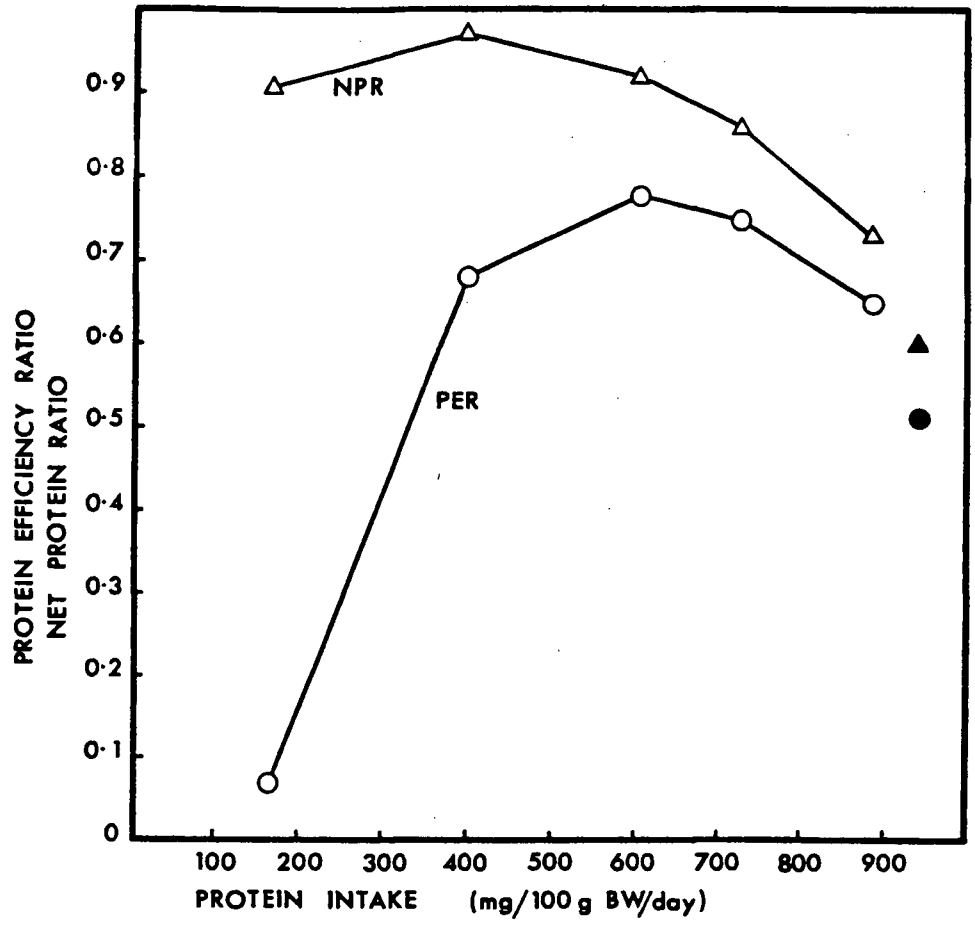


Fig. 8. The relationship between protein intake and protein efficiency ratio (PER)(○) and net protein ration (NPR)(△) of fish fed diets containing FPE. Values for OMP are shown in dark symbols for comparison.

intakes is due to the allowance made for maintenance by the latter method (Fig. 8).

Those diets formulated to have a protein concentration of 17, 27, and 37% were employed to evaluate protein quality. The effect of dietary protein source on PER and NPR was significant ($P < 0.005$). Despite the differences in PER and NPR both methods distinguished between the different protein sources in an almost identical manner (Tables 15 and 16). Inconsistant differences in relative PER and NPR values were noted for the proteins at different dietary concentrations. Excluding the CS diets, it was found that relative PER was lower for the poor quality protein (HTH) at low compared to high dietary protein concentrations. This effect was not noted for the freeze-dried meals (FPE, FRH). Low-temperature dried meal (LTH) show maximum PER at the intermediate protein concentration. The above observations may suggest that PER underestimates the protein quality of poor proteins when tested at low dietary levels. NPR on the other hand provided similar values at different dietary protein concentrations. However, an identical ranking of the proteins according to mean relative (FPE = 100) PER and NPR values was obtained. Comparisons with reports by other investigators (Table 17) were made by tabulating PER relative to CS (i.e. PER of CS = 100). The results indicated that HTH was an extremely poor quality protein whereas the other fishmeals were comparable to those reported in the literature.

Table 16. Net protein ratio calculated on a dry body weight basis (NPR), relative NPR (in parenthesis) and ranking of different protein sources at different dietary concentrations.

Protein Source		% Crude Protein in Diets 17	27	37	Mean
		[1]			
FPE	NPR (±SE)	ef 0.974 ±0.009	cdef 0.921	cd 0.864	y 0.920 ±0.005
	Relative NPR	(100)	(100)	(100)	(100)
	Ranking	2	3	2	2
FRH	NPR	f 0.993	def 0.934	cde 0.884	xy 0.937
	Relative NPR	(102)	(101)	(102)	(102)
	Ranking	1	1	1	1
LTH	NPR	cdef 0.901	cd 0.872	b 0.679	wx 0.817
	Relative NPR	(93)	(95)	(79)	(89)
	Ranking	3	4	4	3
HTH	NPR	a 0.481	a 0.422	a 0.464	v 0.456
	Relative NPR	(49)	(46)	(53)	(50)
	Ranking	5	5	5	5
CS	NPR	b 0.667	def 0.934	c 0.833	w 0.811
	Relative NPR	(69)	(101)	(97)	(88)
	Ranking	4	1	3	4
Mean	NPR	0.803 ±0.004	0.816	0.745	

1. Values with the same superscript with respect to source x level (a - f) and protein source (v - y) effects did not differ significantly (DMR test P = 0.05).

Table 17. Protein efficiency ratio (PER), relative PER, net protein utilization (NPU), and relative NPU for various proteins, when fed as the sole source of protein to different fish species held under dissimilar experimental conditions.

Protein Source of Diet	Species	Size (g)	Temperature degree C	Ration	Protein level % of dry diet	PER wet body weight basis	Relative PER (casein=100)	NPU[1,2]	Relative NPU (casein=100)	Reference
Freeze dried cod muscle	Plaice (*Pleuronectes platessa*)	13	15	satiation	50	1.78		42		Cowey et al. (1974)
Single cell protein plus methionine	"	14	15	"	50	1.23		38		
White fish meal	"	15	15	"	50	1.29		35		
Soy protein plus methionine	"	14	15	"	50	0.89		34		
Fish protein concentrate	"	13	15	"	50	0.60		23		
Lipid extracted herring muscle	"	13	15	"	50	0.84		30		
Lipid extracted sprats	"	14	15	"	50	0.98		33		
Leaf protein concentrate	Carp (*cyprinos carpio*)	2.5	25	not stated	41	1.78	68	34	74	Ogino et al. (1978)
Casein	"	2.5	25	"	43	2.60	100	46	100	
Leaf protein concentrate	"	2.5	25	"	30	2.09	59	38	72	
Casein	"	2.5	25	"	27	3.55	100	53	100	

cont'd.../2

Table 17. cont'd..(2)

Protein Source of Diet	Species	Size (g)	Temperature degree C	Ration	Protein level % of dry diet	PER wet body weight basis	Relative PER (casein=100)	NPU[1,2]	Relative NPU (casein=100)	Reference
Leaf protein concentrate	Rainbow trout (salmo gairdneri)	3.5	19	satiation	41	2.03	70	38	69	Ogino et al. (1978)
Casein	"	3.5	19	"	39	2.91	100	55	100	
Herring meal	Rainbow trout (salmo gairdneri)	30	12	"	30	1.91	97	38	95	Atack and Matty (1978)
Methanophilic bacteria	"	30	12	"	30	1.62	82	37	93	
Spirulina algae	"	30	12	"	30	1.33	68	32	80	
Petroyeast	"	30	12	"	30	2.01	102	42	105	
Soybean	"	30	12	"	30			18	45	
Brewers yeast	"	30	12	"	30	1.17	59	30	75	
Casein	"	30	12	"	30	1.97	100	40	100	
Herring meal	Carp (cyprinus carpio)	30	25	4% of body weight	25	2.82	114	64	131	Atack et al. (1979)
Methanophilic bacteria	"	30	25	"	35	2.54	102	49	100	
Spirulina algae	"	30	25	"	30	1.15	42	36	73	
Petroyeast	"	30	25	"	31	2.08	84	47	97	
Soybean	"	30	25	"	29	1.35	54	42	86	

cont'd.../3

Table 17. cont'd..(3)

Protein Source of Diet	Species	Size (g)	Temperature degree C	Ration	Protein level % of dry diet	PER wet body weight basis	Relative PER (casein=100)	NPU[1,2]	Relative NPU (casein=100)	Reference
Casein	Carp (cyprinus carpio)	30	25	4% of body weight	30	2.48	100	49	100	Atack et al (1979)
White fish meal	Rainbow trout (salmo gairdneri)	34	10.7	not stated	31	3.2	89	63	95	Watanabe et al. (1983)
Peruvian anchovy meal	"	34	10.7	"	31	3.7	103	61	92	
Sardine and pollock scrap meal	"	34	10.7	"	32	3.2	89	63	95	
Greenling meal	"	34	10.7	"	30	3.6	100	66	100	
Casein	"	34	10.7	"	33	3.6	100	66	100	
Egg yolk	Rainbow trout (salmo gairdneri)	2.0	17	not stated	30	3.8	109	60	107	Ogino and Nanri (1980)
Whole egg	"	2.0	17	"	30	3.8	109	61	109	
Egg albumin	"	2.0	17	"	30	3.9	111	62	111	
Trout muscle protein	"	2.0	17	"	30	3.8	109	62	111	
Squid muscle protein	"	2.0	17	"	30	3.9	111	62	111	
Casein	"	2.0	17	"	30	3.5	100	56	100	

cont'd.../4

Table 17. cont'd..(4)

Protein Source of Diet	Species	Size (g)	Temperature degree C	Ration	Protein level % of dry diet	PER wet body weight basis	Relative PER (casein=100)	NPU [1,2]	Relative NPU (casein=100)	Reference
Casein & squid muscle (1:1)	Rainbow trout (salmo gairdneri)	4.0	17	not stated	30	3.9	108	61	105	Ogino and Nanri (1980)
Casein & whole egg (1:1)	"	4.0	17	"	30	3.8	106	63	109	
Casein	"	4.0	17	"	30	3.6	100	58	100	
FPE	Chinook salmon (oncorhynchus tshawytscha)	1.50	11	satiation	27	2.94	98	79	103	Present study
FRH	"	1.50	11	"	28	2.87	96	78	101	
LTH	"	1.50	11	"	28	2.65	88	73	95	
HTH	"	1.50	11	"	27	0.76	25	40	52	
CS (casein-gelatin, + arginine & methionine)	"	1.50	11	"	26	3.00	100	77	100	

1. Determined by method of Bender and Miller (1953).
2. Determined by method of Ogino et al.(1980).

3.3.7 The measurement of protein quality by protein productive value and net protein utilization

Protein productive value (PPV) and net protein utilization (NPU) (Miller and Bender, 1955; Ogino et al., 1980) relate protein retention to protein intake. PPV and NPU are analogous to PER and NPR respectively. The latter methods, however, cannot differentiate between proteins that promote similar gains in body weight but different gains in body protein. Therefore, PPV and NPU would best measure the effectiveness of a protein to provide for muscle protein synthesis.

The values obtained for PPV are shown in Table (18) and are depicted graphically for the various protein sources in Fig.(6A - 6E). PPV is identical to the estimate of the percentage of protein fed that was retained. The relationship between this parameter and protein intake was previously described with respect to the mode of protein utilization for growth (section 3.3.5).

A progressive decrease in mean PPV was observed as the protein source in chinook salmon diets changed in the following order: FPE > FRH > LTH > CS > HTH (Table 18). As stated previously the quality of the freeze-dried meals was highest. Low temperature dried meal was found to have a lower (not statistically significant $P > 0.05$) quality than freeze-dried herring meal (FRH). The casein based protein source (CS) was of similar quality to LTH. An identical ranking (of mean values) for the protein sources was obtained by PPV and NPU (Tables 18, 19, 20). Although NPU was calculated by two methods, the

Table 18. Protein productive values (PPV), relative PPV (in parenthesis) and ranking of different protein sources at different dietary concentrations.

Protein Source		% Crude Protein in Diets 17	27	37	Mean
		[1]			
FPE	PPV (±SE)	d 36.87 ±3.23	f 49.55	ef 48.11	x 44.84 ±1.87
	Relative PPV	(100)	(100)	(100)	(100)
	Ranking	2	1	1	1
FRH	PPV	def 40.45	def 44.56	def 41.78	wx 42.26
	Relative PPV	(110)	(90)	(87)	(94)
	Ranking	1	2	3	2
LTH	PPV	d 34.23	def 42.08	de 37.53	wx 37.95
	Relative PPV	(93)	(85)	(78)	(85)
	Ranking	3	4	4	3
HTH	PPV	a -4.81	b 9.81	bc 16.23	v 7.07
	Relative PPV	(-13)	(20)	(34)	(16)
	Ranking	5	5	5	5
CS	PPV	c 21.50	def 43.06	def 42.23	w 35.59
	Relative PPV	(58)	(87)	(88)	(79)
	Ranking	4	3	2	4
Mean	PPV	25.65 ±1.45	37.81	37.18	

1. Values with the same superscript with respect to source x level (a - f) and protein source (v - x) effects did not differ significantly (DMR test P - 0.05).

Table 19. Net protein utilization calculated by the method of Bender and Miller (1953)(NPU-1), relative NPU-1 (in parenthesis) and ranking of different protein sources at different dietary concentrations.

Protein Source		% Crude Protein in Diets 17	27	37	Mean
		[1]			
FPE	NPU-1 (±SE)	de 56.88 ±0.90	e 59.31	de 55.65	w 57.32 ±0.52
	Relative NPU	(100)	(100)	(100)	(100)
	Ranking	2	1	1	1
FRH	NPU-1	e 60.02	de 57.00	bcd 48.42	w 55.14
	Relative NPU	(105)	(96)	(87)	(96)
	Ranking	1	2	3	2
LTH	NPU-1	de 55.74	de 53.86	bc 43.5	w 51.10
	Relative NPU	(98)	(91)	(78)	(89)
	Ranking	3	4	4	3
HTH	NPU-1	a 22.69	a 26.12	a 26.32	v 25.04
	Relative NPU	(40)	(44)	(47)	(44)
	Ranking	5	5	5	5
CS	NPU-1	b 40.41	de 55.78	cde 52.27	w 49.51
	Relative NPU	(71)	(94)	(94)	(86)
	Ranking	4	3	2	4
Mean	NPU-1	47.11 ±0.40	50.41	45.23	

1. Values with the same superscript with respect to source x level (a - e) and protein source (v - x) effects did not differ significantly (DMR test P = 0.05).

Table 20. Net protein utilization calculated by the method of Ogino et al.(1980)(NPU-2), relative NPU-2(in parenthesis) and ranking of different protein sources at different dietary concentrations.

Protein Source		% Crude Protein in Diets 17	27	37	Mean
		[1]			
FPE	NPU-2 (±SE)	ef 79.06 ±3.46	ef 78.69	def 72.56	x 76.74 ±2.00
	Relative NPU-2	(100)	(100)	(100)	(100)
	Ranking	3	1	1	1
FRH	NPU-2	f 84.05	ef 77.69	bcd 65.78	wx 75.84
	Relative NPU-2	(106)	(99)	(91)	(99)
	Ranking	1	2	3	2
LTH	NPU-2	ef 79.57	def 73.07	b 56.99	wx 69.88
	Relative NPU-2	(101)	(93)	(79)	(91)
	Ranking	2	4	4	3
HTH	NPU-2	a 41.85	a 39.70	a 38.24	v 39.93
	Relative NPU-2	(53)	(51)	(53)	(52)
	Ranking	5	5	5	5
CS	NPU-2	bc 59.82	def 76.56	cde 70.10	w 68.89
	Relative NPU-2	(76)	(97)	(97)	(90)
	Ranking	4	3	2	4
Mean	NPU-2	68.87 ±1.55	69.16	60.74	

1. Values with the same superscript with respect to source x level (a - f) and protein source (v - x) effects did not differ significantly (DMR test P = 0.05).

Table 21. The relationship between protein intake (x) and net protein utilization (NPU)(y) calculated by the Bender and Miller (1953) formula.

Chinook Salmon (present study)

Protein Source	Equation
FPE	$y = 65.01 - 0.0161x$ (n=10, r= -0.5611, NS)
FRH	$y = 73.81 - 0.0333x$ (n=6, r= -0.8848, $P<0.05$)
LTH	$y = 65.28 - 0.0233x$ (n=6, r= -0.8358, $P<0.05$)
HTH	$y = 20.38 - 0.0085x$ (n=6, r= 0.3732, NS)
CS	$y = 22.17 - 0.0536x$ (M=6, r= 0.6036, NS)

Table 22. The relationship between protein intake (x) and net protein utilization (NPU)(y) calculated by the method of Ogino et al. (1980)

Protein Source	Equation
Chinook Salmon (present study)	
FPE	$y = 104.76 - 0.0476x$ ($n=10$, $r= -0.9120$, $P<0.01$)
FRH	$y = 106.45 - 0.0545x$ ($n=6$, $r= -0.9594$, $P<0.01$)
LTH	$y = 95.69 - 0.0422x$ ($n=6$, $r= -0.8595$, $P<0.05$)
HTH	$y = 45.15 - 0.0096x$ ($N=6$, $r= -0.6599$, NS)
CS	$y = 47.93 + 0.0412x$ ($n=6$, $r= 0.4076$, NS)
Rainbow Trout (calculated from Ogino et al., 1980)	
Egg yolk	$y = 97.9 - 0.0458x$ ($n=5$, $r= -0.9664$, $P<0.01$)
Plaice (calculated from Cowey et al., 1972)	
Freeze-dried cod muscle	$y = 75.8 - 0.0579x$ ($n=6$, $r= -0.9823$, $P<0.01$)

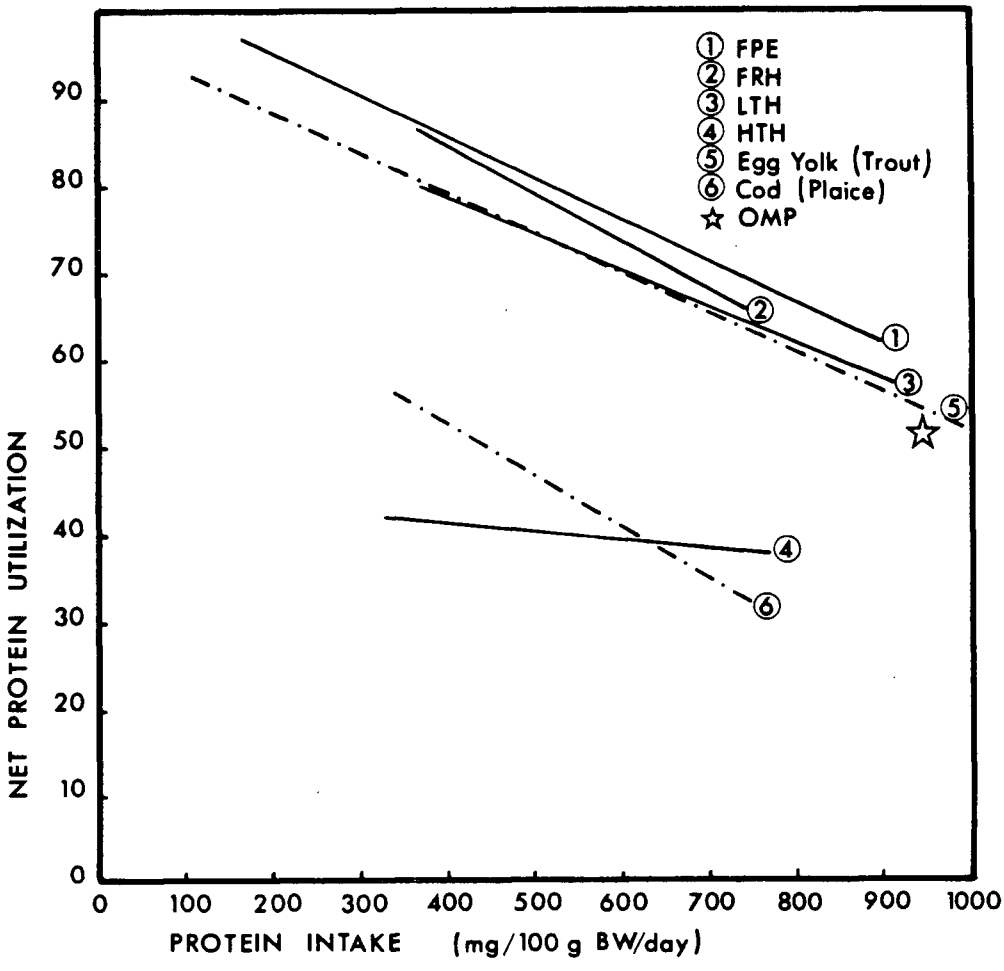


Fig. 9. The relationship between protein intake and net protein utilization (NPU) of the test protein sources determined by the method of Ogino et al. (1980). Slopes for rainbow trout fed egg yolk (taken from Ogino et al., 1980) and plaice fed cod muscle (taken from Cowey et al., 1972) containing diets are also shown for comparison. The NPU value for fish fed OMP is also shown.

Bender and Miller (1953) (NPU-1) and Ogino et al.(1980) (NPU-2) respectively, very close relative NPU (FPE = 100) values were obtained by both procedures. As outlined previously, NPU-2 allows for maintenance on a body weight basis. Relative NPU (CS = 100) values obtained in the present study are tabulated below those reported by other investigators (Table 17) for comparison.

The relationship between protein intake and NPU was described by regression equations (Tables 21, 22). When the method of Ogino et al.(1980) was employed a significant inverse dependence of NPU on protein intake was evident for FPE ($P < 0.01$) FRH ($P < 0.01$), and LTH ($P < 0.05$). However, when the method of Bender and Miller (1953) was used, the correlation between NPU and protein intake was considerably less (Table 21). Cowey and Sargent (1972) state that in the absence of standardized dietary concentrations for determining the nutritive value of protein sources, regression equations may provide the best relative measure of their quality for fish species. These equations are depicted (Fig. 9) for NPU determined by the method of Ogino et al.(1980). The line for CS was omitted because the positive slope obtained cannot be adequately explained.A greater inverse relationship of NPU on protein intake would have been expected for fish fed HTH than was found in this assay. The regression lines obtained in this study for FPE, FRH, and LTH compare well with one another and that obtained with rainbow trout fed egg yolk (Ogino et al., 1980)(Fig. 9).

3.3.8 The measurement of protein quality by slope ratio

The slope ratio (SR) assay is a multi-dose procedure which includes a non-protein diet and three or more dietary levels of protein (Hegsted and Chang, 1965). Alternatively, in some rat bioassays, the zero protein fed group has been omitted when calculating the slope (McLaughlan, 1974; Samonds and Hegsted, 1977). The assay is based on the response (slope) of body weight gain or body protein gain on protein intake. In the ideal SR assay, the slopes of the reference protein (FPE) and the test proteins are linear and meet at a common intercept.

The slopes for the various proteins fed to chinook fry were compared by analysis of covariance of dry body weight gain (Table 23) and body protein gain (Table 24) with protein intake as the covariate. The slopes for data including the zero protein fed groups are depicted in Figs. 10, 11. The standard errors of the slopes were calculated as percentages (Hegsted and Chang, 1965)(Tables 23 and 24) and may be considered as estimates of precision of the assay. These were reduced considerably when the data for the protein free diet fed groups were included in the assay. The correlation coefficients obtained for each slope indicated that under the conditions of the present study, the relationship between body weight or protein gain and protein intake satisfied the requirements for linearity in a slope assay (Hegsted and Chang, 1965; McLaughlan, 1979). When the slopes of body protein (including the protein free diet fed groups) were compared, the assay distinguished FPE, LTH and HTH from one another (Table 24). With the

Table 23. Slope ratios of dry body weight gains on protein intake for fish fed the various protein sources. Calculated both by including and excluding the protein free diet (PF) fed groups.

Protein Source		Slope ratios of dry body weight gains excluding PF groups	Slope ratios of dry body weight gains including PF groups
FPE	Slope [1]	0.8048 [ab2]	0.8817 [BC]
	SE of slope	±0.0623	±0.0432
	SE of slope as %	7.74	4.90
	Intercept	-0.0329	-0.0852
	Correlation coefficient	0.9937	0.9957
	Relative slope	(100)	(100)
	Ranking	3	3
FRH	Slope	0.8382 [b]	0.8940 [C]
	SE of slope	±0.0517	±0.0405
	SE of slope as %	6.17	4.53
	Intercept	-0.0445	-0.0840
	Correlation coefficient	0.9973	0.9970
	Relative slope	(104)	(101)
	Ranking	2	1
LTH	Slope	0.5752 [a]	0.6769 [AB]
	SE of slope	±0.0431	±0.0361
	SE of slope as %	7.49	5.33
	Intercept	0.0275	-0.0520
	Correlation coefficient	0.9858	0.9827
	Relative slope	(71)	(77)
	Ranking	4	4
HTH	Slope	0.4618 [a]	0.4675 [A]
	SE of slope	±0.0826	±0.0611
	SE of slope as %	17.89	13.07
	Intercept	-0.0943	-0.0972
	Correlation coefficient	0.9873	0.9928
	Relative slope	(57)	(53)
	Ranking	5	5
CS	Slope	1.0202 [b]	0.8875 [BC]
	SE of slope	±0.1093	±0.0591
	SE of slope as %	10.71	6.66
	Intercept	-0.1859	-0.1168
	Correlation coefficient	0.9566	0.9835
	Relative slope	(127)	(101)
	Ranking	1	2

1. An analysis of covariance of dry body weights with the covariate (protein intake) having different slopes for different protein sources indicated $P > 0.001$, when calculated both by including and excluding PF groups.

2. Slopes with the same superscript do not differ significantly (Scheffés' test $P = 0.05$).

Table 24. Slope ratios of body protein gains on protein intake for fish fed the various protein sources. Calculated both by including and excluding the protein free (PF) fed groups.

Protein Source		Slope ratios of body protein gains excluding PF groups	Slope ratios of body protein gains including PF groups
FPE	Slope[1]	0.5588[b2]	0.5756[C]
	SE of slope	±0.0422	±0.0286
	SE of slope as %	±7.55	±4.97
	Intercept	-0.0541	-0.0652
	Correlation coefficient	0.9849	0.9943
	Relative slope	(100)	(100)
	Ranking	2	1
FRH	Slope	0.4255[ab]	0.4896[BC]
	SE of slope	±0.0350	±0.0268
	SE of slope as %	±8.23	±5.47
	Intercept	0.0000	-0.0454
	Correlation coefficient	0.9986	0.9887
	Relative slope	(76)	(85)
	Ranking	3	3
LTH	Slope	0.3785[ab]	0.4345[B]
	SE of slope	±0.0292	±0.0238
	SE of slope as %	±7.71	±5.48
	Intercept	-0.0029	-0.0405
	Correlation coefficient	0.9818	0.9839
	Relative slope	(71)	(76)
	Ranking	4	4
HTH	Slope	0.2807[a]	0.2711[A]
	SE of slope	±0.0560	±0.0404
	SE of slope as %	±19.95	±14.90
	Intercept	-0.0715	-0.0671
	Correlation coefficient	0.9807	0.9881
	Relative slope	(57)	(47)
	Ranking	5	5
CS	Slope	0.6489[b]	0.5483[BC]
	SE of slope	±0.0740	±0.0390
	SE of slope as %	±11.40	±7.11
	Intercept	-0.1327	-0.0790
	Correlation coefficient	0.9661	0.9838
	Relative slope	(116)	(95)
	Ranking	1	2

1. An analysis of covariance of body protein gains with the covariate (protein intake) having different slopes for different protein sources indicated $P > 0.001$, when calculated both by including and excluding PF groups.

2. Slopes with the same superscript do not differ significantly (Scheffés' test $P = 0.05$).

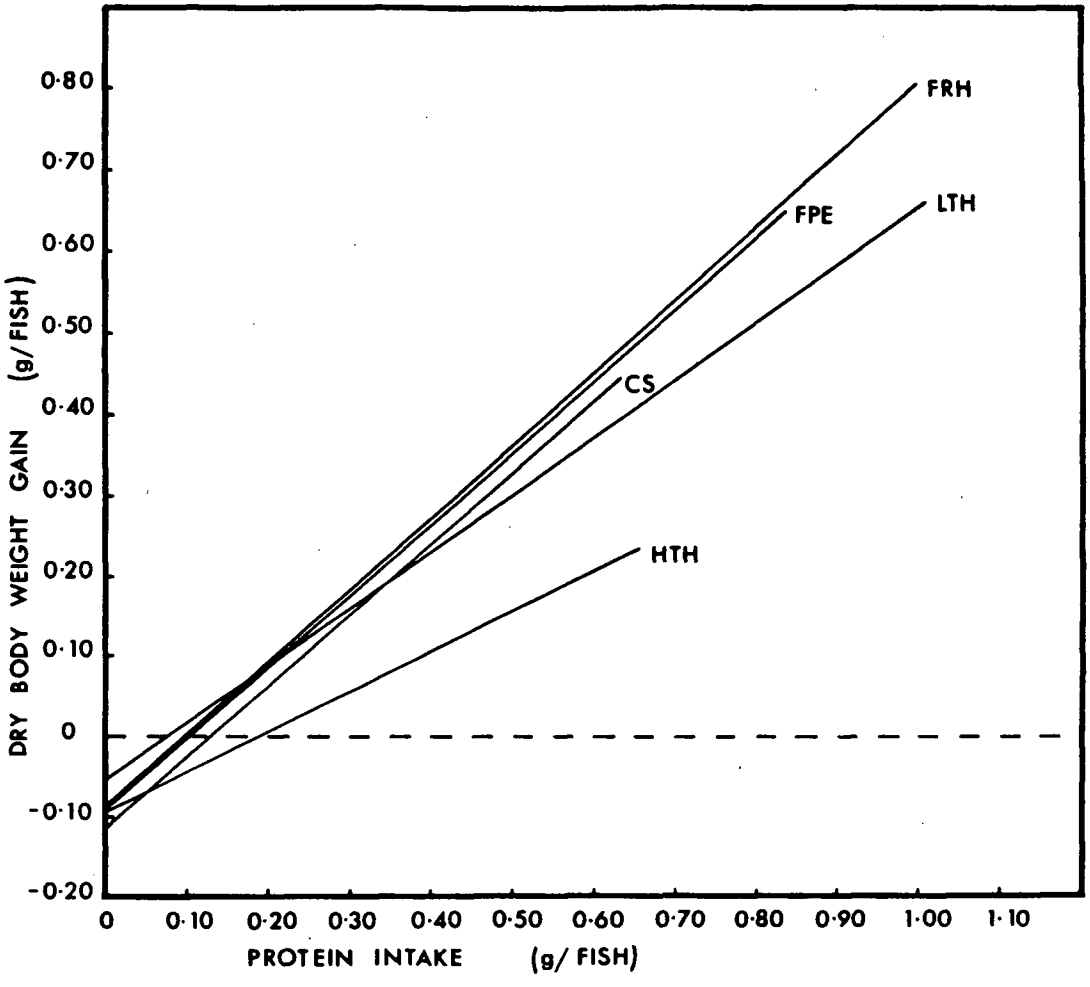


Fig. 10. Slopes of dry body weight gain on protein intake of chinook salmon fed diets containing the test protein sources. The slopes were calculated including points obtained from groups of fish fed the protein free diets (see Table 23).

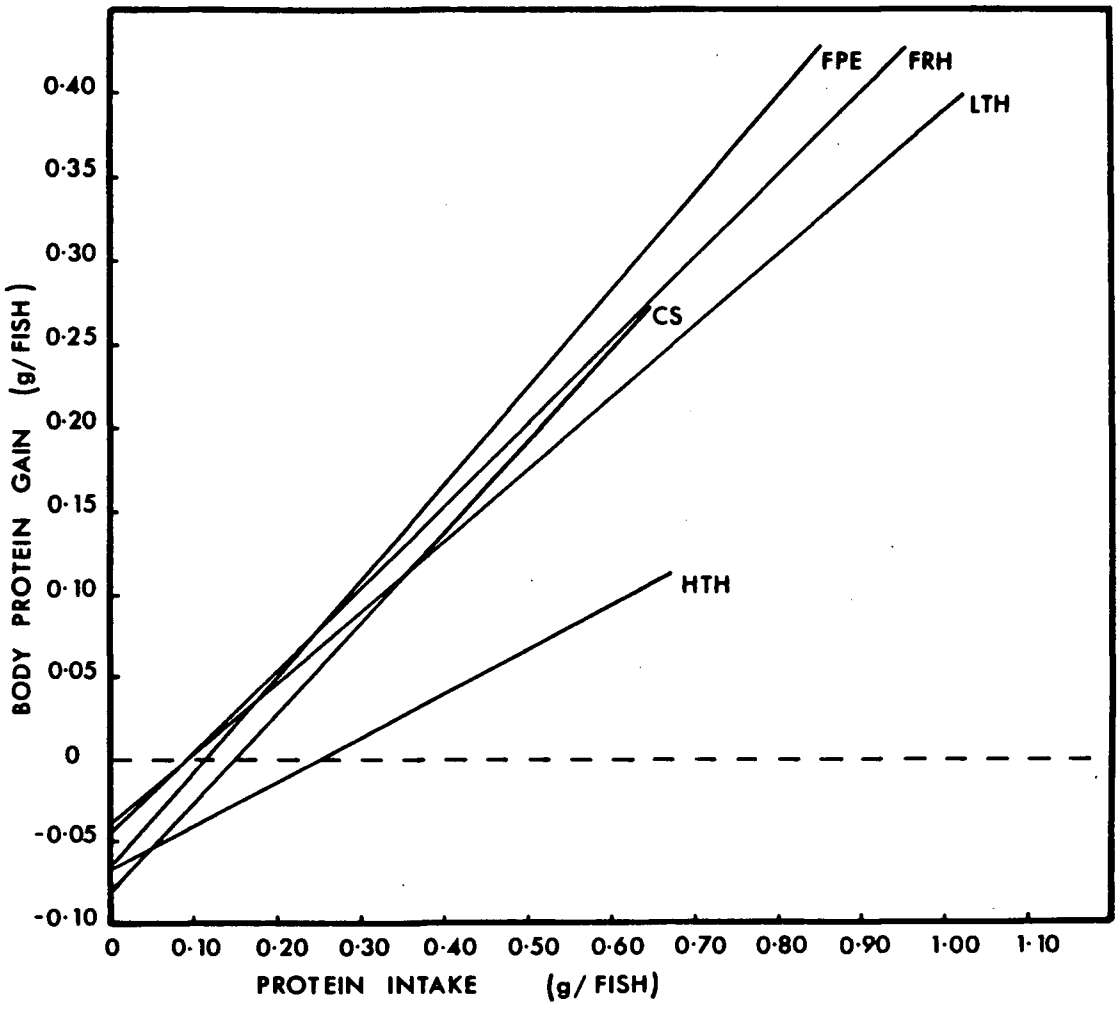


Fig. 11. Slopes of body protein gain on protein intake of chinook salmon fed diets containing the test protein sources. The slopes were calculated including points obtained from groups of fish fed the protein free diets (see Table 24).

exception of FRH similar relative slope values were obtained by either body weight or body protein gain.

3.3.9 Available lysine content of the protein sources

The available lysine content of the protein sources was determined in an attempt to evaluate the extent of protein damage in relation to the processing method used. The lysine values are shown in Table (2). They were used to calculate the available lysine content of the diets and are compared to the known lysine requirements of salmonids (Table 25). Halver et al.(1958) stated that the lysine requirement for chinook salmon is 5% of the dietary protein or 2% of the diet containing 40% protein. Ogino (1980) and Ketola (1982) showed that the minimum requirement of rainbow trout for lysine was 5.3% and 6.1% of dietary protein respectively.

Employing the lysine requirement value of 5% of dietary protein (Halver et al., 1958) only HTH failed to meet the requirement (Table 25). However, if the lysine requirement is based on fish egg composition, only FPE contained sufficient surplus available lysine relative to the lysine requirements stated by Ketola (1982) (Table 25). LTH and FRH contained almost sufficient available lysine to meet requirements (Ketola, 1982). Based on the available lysine content obtained for HTH coupled with the performance of fish fed HTH, the results may suggest that a certain amount of lysine was rendered unavailable in this protein source due to the severe heating during the drying of the meal.

Table 25. Available lysine, lysine requirements for chinook salmon and percentages of requirements supplied by each protein source (in parenthesis).

		% Crude Protein in Diets									
		7		17		27		37		47	
			% of Req		% of Req		% of Req		% of Req		% of Req
FPE											
Available lysine	% of dry diet	0.59		1.45		2.33		3.08		3.95	
Requirement:											
Halver et al.(1958)	5% of protein	0.34	(171)	0.85	(171)	1.36	(171)	1.80	(171)	2.31	(171)
Ketola (1982)	8.8% protein	0.59	(97)	1.49	(97)	2.40	(97)	3.16	(97)	4.06	(97)
FRH											
Available lysine	% of dry diet			0.99		1.51		2.17			
Requirement:											
Halver et al.(1958)	5% of protein			0.90	(109)	1.38	(109)	1.98	(109)		
Ketola (1982)	8.8% protein			1.58	(62)	2.43	(62)	3.48	(62)		
LTH											
Available lysine	% of dry diet			0.90		1.53		2.07			
Requirement:											
Halver et al.(1958)	5% of protein			0.84	(108)	1.42	(108)	1.92	(108)		
Ketola (1982)	8.8% protein			1.48	(61)	2.50	(61)	3.38	(61)		
HTH											
Available lysine	% of dry diet			0.77		1.25		1.79			
Requirement:											
Halver et al.(1958)	5% of protein			0.85	(91)	1.27	(91)	2.00	(91)		
Ketola (1982)	8.8% protein			1.50	(52)	2.41	(52)	3.51	(52)		
CS											
Available lysine	% of dry diet			1.37		1.95		2.65			
Requirement:											
Halver et al.(1958)	5% of protein			0.93	(148)	1.32	(148)	1.80	(148)		
Ketola (1982)	8.8% protein			1.63	(84)	2.32	(84)	3.17	(84)		

3.4 DISCUSSION

3.4.1 The effect of dietary protein source and level on body weight gain

An inadequate supply of protein would be expected to have a profound influence on growth. Various investigators in fish growth studies calculate growth rate by different methods. Zeitoun (1973) compared average daily gain, percent gain of initial weight and specific growth rate (% increase in body weight per day). He and other investigators (Brett et al., 1969) concluded that the latter provided the most useful way to compare growth of different size fish. The use of average daily gain in animal experiments has been critized because it does not accurately describe weight change at any specific age (Crampton and Lloyd, 1959). However, in the present study, similar inferences could be made by expressing growth by both final body weight and specific growth rate (Table 6).

The results of the growth study demonstrated the effect that protein source and level had on the performance of chinook salmon. With the exception of fish fed HTH body weight increase was approximately linear for fish fed dietary protein levels of 27% and higher. The decline in growth rate from day 21 to 42 compared to day 0 to 21 for fish fed the low protein diets or HTH (Figs. 3A, 3B, 3C) is likely due to the influence of body nutrient reserves accumulated during the acclimation period. Daily growth rates do not adjust instantly to dietary change. The existence of a lag effect in the metabolic adaptation to diets of differing nutrient composition has been well

established (Maynard and Loosli, 1969). This was recognized in fish nutrition by Steffens (1981) who stated that a reliable indication of protein utilization by means of growth rate tests would be reached only after a 1 to 2 week period of adaptation. The effect of the acclimation period diet, which was similar in composition to FPE-47, is the most likely reason that fish weight gain was not noted to increase exponentially for most of the dietary treatments. From the results of feeding trials with juvenile chinook salmon, Higgs et al.(1982, 1983) reported that weight gain over time was nearly exponential. In contrast to the present study, the growth rates reported by Higgs et al. (1982, 1983) were measured during a longer experimental period, which perhaps had the effect of masking the influence of the acclimation diet during the trial period.

Nevertheless, following a 42-day experimental period the growth response to increasing levels of protein supplied by FPE followed a smooth curve with a steadily decreasing rate of increase (Fig. 4). A similar observation was reported for plaice fed freeze-dried cod muscle (Cowey et al., 1972). A smooth curve was not obtained with the other protein sources tested. Since this was considered to be due to differing food intakes, the performance of the fish fed the various protein sources was compared by the slopes of weight gain to protein intake rather than to dietary protein concentration. Thus, although the casein based diets (CS) supported rapid growth per unit of protein intake, actual weight gain was not realized. It was conjectured that this was due to a palatability problem with

the CS diets. The nutritional quality of the various protein sources will be discussed later.

The growth rates of chinook fed OMP were similar to those of fish fed diets containing 37% protein supplied by freeze-dried or low temperature dried fish meals. OMP is the diet most used to rear juvenile chinook salmon at local hatcheries. The findings of this study suggest that the protein content of OMP could be reduced from the present 50%. However, OMP contains a variety of protein sources including some of plant origin, and lowering the protein level of OMP would perhaps impair the growth rate of fish and consequently ocean survival.

3.4.2 The effect of dietary protein source and level on food intake and gross food conversion efficiency

Food intake in fish is regulated by the caloric content of the diet (Lee and Putman, 1973), and fish size (Brett, 1971); assuming that all other biotic and abiotic factors (Brett, 1979) have been standardized. The results of this study agree with a report by Brett (1971) who showed that food intake of sockeye salmon was influenced by fish weight, the smaller fish consuming the largest relative amount. The influence of fish size on level of dietary intake is accounted for in hatchery feeding tables (Fowler and Burrows, 1971).

Food conversion is a parameter of particular interest to fish producers. The ratio of weight gain to food intake represents a major influence on the profit margin in an animal

production enterprise. The trend of a steadily decreasing rate of increase in GFC to increments in dietary protein concentration are consistent with the results of other investigators who have studied the protein requirements of salmonids. For example, Zeitoun (1973), Satia (1974), Gulbrandsen and Utne (1977) and Koshiishi (1980) obtained maximum GFC when the protein level of the diets lay between 35% and 45%. More precise comparisons with these reports cannot adequately be made because of widely differing experimental conditions. Comparison with an experiment by Higgs et al.(1982) is more valid because it was conducted on the same species and under similar experimental conditions as the present study. They obtained GFC values ranging between 20% and 25% for a series of test practical diets containing approximately 50% protein. Higher values were obtained in this study with diets containing substantially less protein. This suggests that the protein level in practical chinook salmon diets could be reduced without suppressing GFC, provided that the quality of dietary protein is high. The protein sources judged to be of highest value by GFC in the present study were the freeze-dried fish meals.

3.4.3 The effect of dietary protein source and level on energy intake and gross energy utilization

As mentioned previously, fish eat to satisfy their energy needs (Lee and Putman, 1973). In this study an attempt was made

to formulate the diets (except OMP) to be isocaloric on a metabolizable energy basis. The inaccuracy and inconsistency of this practice in fish growth and nutrition studies has recently been reviewed and harshly criticized by Jobling (1983). His main objections pertain to the unreliability of methods used to measure metabolizable energy, the dependency of estimates of metabolizable energy on level of feeding, and the use of inaccurate caloric conversion coefficients.

In this study dietary lipid was kept constant while protein was replaced by carbohydrate on a metabolizable energy basis (protein = 4.5 kcal/g; carbohydrate = 4 kcal/g). Since the fish were fed to satiety one may expect metabolizable energy intakes (MEI) to be similar at each protein level. The general trend noted, however, was a decreased energy intake by fish consuming the diets containing more protein and less carbohydrate. This suggests that either the energy value assigned to protein was too low or the value for carbohydrate was too high, causing an overestimation of MEI in fish receiving the high carbohydrate diets. Actually, the metabolizable energy of polysaccharides is dependent on their concentration in the diet since their digestibility in trout was shown to decrease progressively as their dietary concentration was increased (Singh and Nose, 1967). However, the absorption of monosaccharides was not found to depend on dietary level. In the present study equal portions of glucose monohydrate and partially hydrolyzed dextrin were employed. Thus an error in the present study may have been introduced by not correcting the ME value ascribed to

carbohydrate with consideration for its level in the diet. Higgs et al.(1983) encountered a similar dilemma which they ascribed to impaired digestibility of protein and energy due to the high fiber and dextrin contents of the low protein diets. They also suggested that dietary fiber (cellulose in the present study) depresses mineral bioavailability and increases the rate of transit of the digesta. It is interesting to note that the fish fed the protein-free diet also appeared to eat to satisfy their energy requirements.

Lee and Putnam (1973) showed that dietary energy utilization was influenced by the protein:calorie ratio of the diet. Similarly, in the present study, dietary protein level and source had an effect on GEU, suggesting that this parameter may be used as a criteria for evaluating the nutritive value of proteins for fish diets. Pieper and Pfeffer (1980) found GEU to be useful in comparing the relative utilization of various supplements of carbohydrates, fats, and proteins to a basal diet. The GEU of fish receiving HTH was extremely poor in comparison to values recorded for fish fed diets containing the other protein sources. Perhaps this was due not only to the inadequacy of HTH as a source of available energy from protein but also from lipid.

3.4.4 The estimation of maintenance requirements for protein

The relationship between nitrogen (N) intake and nitrogen retention states that when nitrogen intake is zero, nitrogen

retention is, and must be, equal to the total endogenous nitrogen excretion (Bressani, 1977). Conversely, when nitrogen retention is zero, nitrogen intake should equal endogenous nitrogen loss. Several studies discussed earlier (Gerking, 1955a; Birkett, 1969; Savitz, 1971; Brett and Groves, 1979) consider endogenous nitrogen loss to represent the protein-nitrogen requirement for maintenance in fish (see Fig. 1). The protein requirement for maintenance of chinook salmon fry was estimated to be 0.160g/100g BW/day for fish fed diets containing approximately 3950 kcal/kg dry food. The assumption was made that FPE was totally assimilated by the fish. This assumption is valid since a preliminary study found that FPE was 97% digested and is in agreement with reports on digestibility of raw cod by plaice (Cowey et al., 1974) and rainbow trout (Skrede et al., 1980; Opstvedt et al., 1984).

The maintenance requirement in the present study may be contrasted with estimates of daily endogenous nitrogen excretion in fishes (Brett and Groves, 1979). The protein requirement above corresponds to an endogenous nitrogen excretion rate of 25.5mg/100g BW/day. This value is in close agreement with that reported by Brett and Zala (1975) for sockeye salmon, namely 22.1mg N/100g BW/day, although their estimate was obtained using larger fish and at a higher water temperature than in the present study.

Gerking (1955a,b) was the first to study maintenance protein requirements of fish. Bluegill sunfish were starved for three days and then they were fed daily an amount of glucose via

stomach tube equivalent to the metabolic rate of the fish. Gerking (1955a) found that a 29.7g sunfish excreted 5.84mg of nitrogen daily (19.7mg N/100g BW). However, it is unlikely that this initial estimate based upon a three day fasted fish provided a good measure of endogenous nitrogen excretion since the fish were probably not fully depleted of protein reserves. When fish are placed on a protein-free diet there is an adaptive decline in the rate of nitrogen excretion over a period of time until a new equilibrium is established. During this period the protein reserve is depleted and urinary nitrogen excretion ensues at a minimal constant level. The higher the level of previous nutrition, the longer the period of time necessary to establish the minimum level of nitrogen excretion. Morgulis (1918) determined nitrogen balance in brook trout and found no stabilization of nitrogen excretion following a four week fast. Body weight data in this study (Table 12) suggest that stabilization had occurred by day 42 in fish fed the protein-free diet.

Gerking (1955b) regarded endogenous nitrogen excretion as being equivalent to the minimum amount of protein required to maintain the fish in nitrogen equilibrium. This was determined from the difference in body nitrogen content of fish sampled at the beginning and the end of a 30 day feeding period. The fish were fed mealworms at different rates of intake. The sunfish were shown to have absorbed 98% of the protein in the mealworms. Accordingly, Gerking (1955b) estimated that 7.2mg N/day was required to maintain a 29.7g sunfish in nitrogen

equilibrium at 25°C. Thus, the maintenance nitrogen requirement amounted to 24.24mg N/100g BW/day. Gerking (1955b) regarded this value to be in close agreement with that obtained from nitrogen determination of aquarium water following oral glucose administration. This value agrees with the estimate reported in this study for chinook salmon fry.

Ogino et al.(1980) analysed rainbow trout carcasses and excreta after feeding them a protein-free diet for periods of 14 to 19 days. They estimated nitrogen lost endogenously by carcass analysis and by total nitrogen excreted to be 8.89 and 9.96mg/100g BW/day, respectively. These values are considerably lower than the one obtained for chinook salmon in this study. Other estimates of maintenance protein requirements in fishes, obtained from experiments where fish were fed either maintenance protein diets, or estimated from feeding a series of low protein diets and conducting carcass analysis, are shown in Table 26.

In contrast to the present estimate for chinook salmon fed a diet containing 7% protein from FPE, Zeitoun et al.(1973,1974) found that the maintenance dietary protein requirement for coho salmon and rainbow trout, fed casein-gelatin based diets was 23.5% and 15% respectively. Discounting the effects of low salinity of the water in their study, it would appear that either casein-gelatin was poorly assimilated, or more likely, their method of extrapolation of percentage dietary protein at zero growth was inaccurate.

In a study with young rats, Henry (1965) found that the maintenance protein level was about 2% for egg albumin and

Table 26. Estimated absorbed nitrogen requirement for maintenance of fish, determined by feeding and carcass analysis experiments.

Species	Size (grams)	Water Temperature (degrees C)	Diet	Requirement (mg/100gBW/d)	Reference
Bluegill sunfish (Lepomis macrochirus)	30	25	mealworms	24.24	Gerking (1955b)
Yearling plaice (Pleuronectes platessa)	11 - 34	17	lugworms	20.3	Birkett (1969)
Yearling sole (Solea vulgaris)	0.2 - 1.8	17	whiteworms	24.4	Birkett (1969)
Yearling sole (Solea vulgaris)	5 - 53	17	lugworms	38.1	Birkett (1969)
Perch (Perca fluvialis)	100 - 150	17	earthworms	17.3	Birkett (1969)
Carp (Carassius auratus)	2.5 - 4.5	20	chironomid larvae	22	Iwata (1970)
Rainbow trout (Salmo gairdneri)	0.9 - 26	12 - 19	protein-free	8.9	Ogino (1980)
Carp (Carassius auratus)	1.5 - 12	19 - 25	protein-free	13.9	Ogino (1980)
Chinook salmon (Oncorhynchus tshawytscha)	1.56	11	protein-free	20.7	Present study
Chinook salmon (Oncorhynchus tshawytscha)	1.56	11	FPE-7	25.5	Present study

herring meal and between 2% and 3% for casein. The requirement increased when poor quality proteins such as gluten and zein were fed. Similarly, it was observed that the dietary protein level for maintenance varied with protein source. Whereas a dietary protein intake of approximately 180mg/100g BW/day was required for fish fed diets containing FRH and LTH, double the amount of protein was required when HTH was employed (Fig. 6).

A final note should be added with respect to the interpretation of maintenance requirements for protein in growing fish. These values (Table 26) are based on total body nitrogen turnover by fish. Maintenance nitrogen balance does not necessarily reflect maintenance of a steady state level of individual tissue protein metabolism or the nutritional status of a particular organ within the body. The practical application of maintenance protein requirements to fish culture is dangerous since they do not consider the physiological requirements of the growing animal. Rather they serve to aid in the partitioning of protein intake according to the scheme described by Fig. 1.

3.4.5 The effect of dietary protein source on protein utilization

The results of this study demonstrated the magnitude of the effect that proteins of different nutritive value had on the utilization of dietary protein in growing salmon. Since, protein utilization is also a function of protein intake, the

protein sources were compared at different dietary concentrations.

The relationship between protein intake and the utilization of protein for maintenance and growth and amount excreted described in section (3.3.5) followed a similar pattern to that described by Ogino et al. (1973) for carp. As the amount of protein consumed by carp increased above that needed for maximum efficiency, the protein used for growth decreased gradually as did the proportion used for maintenance. Exogenous loss, however, concomitantly increased. Apart from representing an economic loss, nitrogenous waste accumulation in the form of ammonia and decomposing feces pose a hazard to confined fish.

The utilization of dietary protein was found to vary considerably between protein sources of excellent and poor nutritional value. That FPE supported excellent growth and the most desired mode of protein utilization is not surprising since it is entirely made up of teleostean skeletal muscle proteins. The amino acid composition of freeze-dried pollock muscle compares favourably with the stated amino acid requirements for chinook salmon and the composition of fish eggs (Table 1). It has been amply demonstrated that supplemental amino acids patterned after the composition of fish eggs or whole fry produced the highest gain in fish fed a basal diet (Arai, 1981; Ketola, 1982). FPE was processed by freeze-drying which is not known to cause any detrimental effects on amino acid availability. Experiments conducted by Cowey et al.(1971) with plaice and Miller and Bender (1953) with rats have shown

excellent protein utilization with vacuum-dried cod muscle. Cowey et al.(1972) reported that freeze-dried cod muscle protein was completely digested by plaice at lower dietary levels and over 90% digested at the highest level of inclusion in the diet.

FRH was utilized almost as efficiently as FPE. Although both meals were freeze-dried, FRH was prepared from whole fish and some of the integumental proteins may not have been completely assimilated. Recently, Watanabe et al. (1983) compared three kinds of brown meals made from greenling, sardine and anchovy to white fish meal in rainbow trout diets. They reported comparable protein utilization in trout regardless of the meal source. The results obtained with FPE and FRH, a white fishmeal and a brown meal respectively, support the observations of Watanabe et al.(1983).

Cowey et al.(1971) observed that although freeze-dried and low temperature dried (30°C) cod meal were almost indistinguishable in their essential amino acid content, they supported different growth rates. They presumed that the different drying conditions for the cod meal had to some extent affected the biological availability of certain essential amino acids. The findings in this study would corroborate the same presumption although the effect of a mild drying temperature was minimal.

LTH was processed in a laboratory facility to simulate an ideal commercial herring meal. Considering past and present depletion of the herring stocks in British Columbia, every attempt should be made towards improving the utilization of the

rendered product. The performance of fish fed LTH demonstrates that whole herring can be cooked, pressed and dried to produce a product suitable for chinook salmon fry diets with little adverse effect on protein quality. It is noteworthy that the herring meal included in OMP tested in this study appears likely to have been of similar quality to LTH. The slightly lower utilization of protein in OMP compared to protein from LTH is probably due to the other ingredients present in this diet.

It is well established that excessive heat during drying can severely impair protein digestibility and biological value (Tarr and Biely, 1972). This was amply demonstrated by the extremely low rate of utilization of HTH for growth and high digestive and metabolic loss of this protein in the present study. Tarr et al. (1954) observed a reduction of digestibility and biological value of menhaden meal subjected to flame drying as compared to steam dried meal. During processing the damage to HTH may have resulted from the destruction of amino acids by oxidation, modification of some of the linkages between the amino acids so that their release was delayed during digestion, and, the formation of linkages that are not hydrolyzed during digestion (Bender, 1972). Certainly, as evidenced by odor and dark color, some charring of HTH had occurred. Although a relatively minor destruction of available lysine due to temperature (Table 1) was observed, other amino acids may have suffered more damage. For example, in heated herring meal Carpenter et al. (1962) found that tryptophan, arginine, methionine and lysine were not totally destroyed but rendered somewhat less available. Opstvedt

et al. (1984) reported a depression in digestibility in trout of pollock muscle subjected to various heat treatments.These investigators noted that the depression in digestibility due to heat (above 95°C) coincided with the formation of disulfide bonds. Cross-linkages via lysine residues were not found to affect protein digestibility in their study.

Several investigators have attempted to relate protein quality measured biologically to the chemical determination of available lysine. Hence, both the lysine requirement of the animal and the available lysine content of the diet have to be considered. Ketola (1982) published the amino acid composition of chinook salmon eggs and proposed that the fish egg amino acid pattern should be used as a guideline for formulating fish foods. He reported that the lysine content of chinook eggs is 8.8% of protein which agrees with the value of 8.65% obtained from the analysis of eyed chinook eggs in this study (Table 1). Based on a feeding trial, Halver et al. (1958) stated that the lysine requirement was 5% of protein. Table 25 shows that FPE and CS contained lysine in excess of most stated requirements. FRH and LTH appeared to be protein sources that would not warrant lysine supplementation in practical diets for juvenile chinook salmon according to the lysine requirement proposed by Halver et al. (1958).

March et al.(1966) reported that although available lysine values were not found to be significantly correlated with the biological assays, supplementation of the fish meals with lysine in combination with arginine and methionine significantly

increased chick weight gains. Cowey et al.(1971) observed that the total lysine content in freeze-dried cod muscle and low temperature dried cod were almost indistinguishable. But, the available lysine content of the latter was lower than in the former. The low temperature air-dried product also supported a somewhat (sic) reduced growth rate of plaice. However, both products contained available lysine in excess of the requirements for plaice (Cowey et al., 1971). March et al.(1961) concluded that estimates of protein quality of fish meals based upon laboratory analyses for the availability of individual amino acids were useful in detecting meals of distinctly inferior quality. However, the estimates failed to differentiate among meals of average or improved quality for poultry. Similarly, in this study, it may be concluded that the determination of available lysine in the various protein sources cannot be relied upon to predict the nutritive value of proteins for inclusion in diets for juvenile chinook salmon.

The utilization of protein by fish fed diets containing HTH may also have been impaired by damage done to other nutrients in herring meal. Tarr et al.(1954) conducted a number of feeding trials to determine the effects of heat on the nutritive value of herring meal in chick diets. They compared presscake dried at 43°C to portions of this meal heated for 1, 2, or 3 hours at 149°C in a rotating drum. They noted that heat-labile vitamins were destroyed after heating for 1 hour, whereas the impairment in nutritive value attributed to availability of essential amino acids occurred after prolonged heating (Bisset and Tarr, 1954).

By comparing hexane-extracted and unextracted meals subjected to heat damage, Biely et al.(1955) found no differences in chick growth that could be attributed to lipid quality except when heating was excessive. Chick performance was improved by hexane extraction of meals heated for 2 hours at 149°C. However, prolonged heating severely impaired the quality of both extracted and complete meals. This was not improved by the addition of fresh herring oil, suggesting that severe damage to protein had occurred. Similarly, in this study an attempt was made to extract the lipids in the test herring meals with hexane. However, extraction was not complete and at higher levels of HTH in the diets some impairment of growth and protein utilization due to lipid damage may have occurred.

Casein has been used extensively as a protein source in test diets for fish. However, casein alone has been shown to be deficient in certain amino acids (Ogino and Nanri, 1980; Rumsey and Ketola, 1975). In the test diet for salmonids, Halver et al.(1958) employed gelatin to correct for an arginine deficiency in casein. The casein-gelatin mix employed in this study was supplemented with arginine and methionine. The results indicated that although CS supported an efficient rate of protein synthesis in fish tissues, food intake was depressed at a higher level of CS in the diet. The major problem encountered with CS in this study was its apparent low palatability for chinook salmon relative to high quality fish meal protein. Consequently it did not support a growth rate comparable to FPE. These findings concur with reports by other investigators

who concluded that protein sources of marine origin were preferable to casein to meet the genetic potential for growth in fish, and to determine the dietary protein requirement of various fish species (Cowey et al., 1970,1972; Pfeffer et al., 1980; Winfree and Stickney, 1981; Watanabe et al., 1983).

3.4.6 The determination of protein quality by bioassay

The methodology for protein quality evaluation has been discussed frequently with respect to human, animal and bird applications (Allison, 1959; Samonds and Hegsted, 1977; McLaughlan, 1979). Although it has been generally agreed that there is an urgent need for adequate measures of protein quality so that diets or ingredients which vary in protein quality as well as quantity can be compared, there has been no consensus on the most appropriate method to use. The same problem exists in fish nutrition (Cowey and Sargent, 1972, 1979) and very few investigations with fish have attempted to evaluate protein quality by more than one method.

There were three main points to consider in the application of the protein quality estimates determined in this study. First, the primary function of dietary protein is to provide a mixture of essential amino acids in the right proportions for the synthesis and maintenance of tissue proteins. In this respect fish are no different than other animals. In salmonids and other fish species, proteins are also major sources of energy (Walton and Cowey, 1982). This contrasts with omnivorous

mammals where protein catabolism is of less significance in supplying energy. The quality of a protein, as determined by a measurable biological response, is dependent on the protein satisfying the required levels and balance of essential amino acids, for growth and maintenance, as well as supplying an unknown quantity of protein for energy. Rat studies have shown that the amino acid needs for maintenance differ both quantitatively and qualitatively from those for rapid growth (Maynard and Loosli, 1969). Therefore, it would be expected that protein sources would differ in their ability to support growth and provide for maintenance. Methods which determine protein quality at different levels of protein intake have the advantage of giving a more complete picture of protein utilization than would be obtained at single levels of protein intake (Samonds and Hegsted, 1977; McLaughlan, 1979). On the other hand, as protein is replaced by carbohydrate on an estimated metabolizable energy basis, the effect of carbohydrate intake in excess of that believed to be tolerable by salmonids (Phillips, 1969; Hilton et al., 1982) on protein quality estimates is not fully understood.

The second aspect concerns the purpose of the protein quality assay in fish studies. This was to classify biologically the suitability of the essential amino acid balance of a protein as the sole source of protein in a diet. It is most desirable to make this classification in relation to a well identified standard. In this study protein sources were classified relative to FPE (Table 27). Ultimately, it was

Table 27. Summary of relative (FPE = 100) estimates of the nutritive value of the test protein sources employed in this study determined by different methods.

Protein source	GR	GFC	GEU	PER	NPR	PPV	NPU-1	NPU-2	Slope (weight gain)	Slope (protein gain)
FPE	100	100	100	100	100	100	100	100	100	100
FRH	95	108	99	101	102	94	96	99	101	85
LTH	86	88	86	84	89	85	89	91	77	76
HTH	26	32	26	26	50	16	44	52	53	47
CS	77	86	77	82	88	79	86	90	101	95

1. NPU-1 and NPU-2 refers to the methods of Bender and Miller (1953) and Ogino et al. (1980) respectively employed to calculate this parameter.

desired to identify the proteins as good, intermediate or poor in quality, just as the chemical determination of protein content indicates the amount of the ingredient that is to be included in the diet. In this sense, the results of a bioassay served to confirm the suitability of the essential amino acid content of the protein for growth of fish. For example, Hegsted (1972) states that what is required by human dieticians is the measure of some factor (f), such that the protein content of the feedstuff x f = protein available to the animal. Such a factor should vary from 0 to 1.0 and be proportional to the true quality. Therefore, this scheme would enable the fish nutritionist to formulate diets based on the biological value of protein available to the fish rather than on protein content alone.

Thus, the biological evaluation of protein in salmonids has several potential applications which are as follows:

i) The measurement of the effectiveness of a protein source to meet specific performance requirements of cultured fish (Cowey and Sargent, 1972).

ii) The monitoring of processing methods that are used in the manufacture of fish food ingredients (i.e. fishmeal) and fish feed.

iii) The determination of the minimum requirements for amino acids and proteins (Zeitoun, 1973).

iv) The comparison of results obtained with novel protein sources by other investigators when expressed on a relative basis.

v) The prediction of the nutritive value of dietary proteins for the other animals, particularly carnivorous species.

The third aspect that should be recognized is that all the protein quality parameters determined in this study are interrelated and are affected by the same factors. For example, the level of protein intake affects in the same manner PER, NPR, PPV, NPU and slope assay. Feeding trial methods with rats are usually subdivided into those based on weight gain and those based upon body nitrogen retention (McLaughlan, 1979). In the present study, protein gain was highly correlated with weight gain (correlation coefficient = 0.9888) and the former could be predicted from the latter by the following equation:

Protein gain = 0.6172 x dry weight gain - 0.0096.

Zeitoun et al. (1976) also reported that percent weight gain and protein retention were highly correlated. The important point is whether the extra work in determining the protein content of the carcass is worth the effort. Clearly it was for the slope assay. Under the experimental conditions of the present study disagreement was found in the relative values and ranking of the good protein sources tested at different levels. This shortcoming in terms of sensitivity and precision suggests that modifications are necessary to the experimental protocol. The mean values for PER, NPR, PPV, and NPU respectively (Table 27) were taken from the values obtained at all dietary protein concentrations for each protein source. In the slope assays this problem was partly overcome since in the analysis of linear regression all values were considered. Although there is

disagreement with respect to the use of a protein-free diet fed group in protein quality assays (Samonds and Hegsted, 1977) it was noted to be advantageous in the present study. In spite of the shortcomings in estimating protein quality it was found to be clear that proteins were measurably different in their abilities to support growth and meet maintenance needs. The inference is in that a poor quality protein such as HTH could suffice if fed at a sufficiently high level. If HTH has a nutritive value 50% of the best quality protein (FPE) based on NPR, NPU, and slope estimates (Table 27), then double the amount of that protein would be needed in fish diets. The value will be quadrupled if the relative value of HTH lies in the region of 25% of FPE as estimated by PER, and PPV. In fact similar performances in juvenile chinook salmon in terms of growth, feed conversion and dietary energy utilization were obtained with the good quality protein sources (FPE, FRH, LTH and CS) and a poor quality protein (HTH) when the protein content of the diet with the latter source was approximately doubled (Table 5, 7, 9). Ultimately, the usefulness of a protein source lies in its cost effectiveness in the diet as a whole for growth and feed efficiency. In the case of farmed fish raised for food, one should consider the quality and yield of the final product. For hatchery-reared fish consideration should be given to the effectiveness of the diet to promote ocean survival.

If the above statements are accepted, then it is difficult to state categorically which protein quality assay method should be recommended for routine feeding trials with salmonids. When

the relative scores for the various proteins were compared (Table 27), there was a general agreement between the various biological assays for evaluating protein quality and growth rate, gross food conversion efficiency and gross energy utilization. Henry and Toothill (1962), Johnston and Coon (1979) and McLaughlan (1979) found that PER values in rats and chicks were correlated with those for NPU and NPR for a variety of proteins. Henry (1965) obtained numerically similar relative values (casein = 100) for high quality proteins by PER, NPU and NPR. For poor quality proteins, PER yields much lower values than the other methods. The results of this study confirm both these findings. Since PER and PPV on one hand, and NPR and NPU on the other, are essentially the same assay (i.e. weight gain and protein gain are related), agreement between these two methods was excellent (Table 28).

The values for PER were calculated on a dry fish weight basis (Higgs et al., 1979) to facilitate comparison with values obtained by Higgs et al.(1982) and Plotnikoff et al.(1983) with chinook salmon fry. Comparisons between the results reported herein (Table 15) and those of other investigators should be interpreted cautiously because of differing experimental conditions. Morrison and Campbell (1960) stated that PER varied not only with sex, age, genetics, the quantity and quality of the protein, and other dietary components, but also with the duration of the test. The PER values for a commercial dry diet (Abernathy) and various modifications of this diet with protein from rapeseed byproducts (Higgs et al., 1982) were determined

Table 28. Correlation coefficients and level of significance between the different parameters used to estimate the nutritive value of diets containing the various protein sources.

GFC	0.9668 P = 0.000							
GEU	0.9658 P = 0.000	0.9936 P = 0.000						
PER	0.7172 P = 0.000	0.4635 P = 0.002	0.8096 P = 0.000					
NPR	0.2360 P = 0.083	0.2790 P = 0.50	0.3126 P = 0.032	0.6765 P = 0.000				
PPV	0.7391 P = 0.000	0.8056 P = 0.000	0.8308 P = 0.000	0.9765 P = 0.000	0.6519 P = 0.000			
NPU-1	0.2525 P = 0.069	0.2863 P = 0.045	0.3271 P = 0.026	0.6319 P = 0.000	0.9620 P = 0.000	0.6669 P = 0.000		
NPU-2	0.0213 P = 0.451	0.0396 P = 0.409	0.0727 P = 0.337	0.4037 P = 0.007	0.8896 P = 0.000	0.4374 P = 0.004	0.9266 P = 0.000	
LYS	0.8503 P = 0.000	0.8410 P = 0.000	0.8379 P = 0.000	0.5349 P = 0.001	0.0497 P = 0.390	0.5776 P = 0.000	0.0955 P = 0.295	-0.1220 P = 0.246
	GR	GFC	GEU	PER	NPR	PPV	NPU-1	NPU-2

under similar experimental conditions to those of the present study except that a 69 day trial period was employed. The value obtained for a commercial diet (OMP) (Table 15) is of the same order as the PER reported by Higgs et al.(1982) for various practical diets. However, in the present study a higher PER was obtained with FPE at a similar protein level to all of the above diets. It may be suggested therefore that if a premium protein source such as FPE is employed as a control in practical diet evaluation, a more meaningful comparison may be made with a group of fish performing closer to their genetic potential for growth.

Higgs et al.(1982) and Plotnikoff et al.(1983) also evaluated practical diets, including OMP under hatchery management conditions in a different locality (Robertson Creek Hatchery) to the present study. They reported PER values considerably higher than those determined at the West Vancouver laboratory. This is probably due to differences between the chinook stocks in genetic constitution and to elevated water temperatures and different feeding strategy at the hatchery. It may be postulated that if FPE were used as a sole protein source, or only premium quality herring meal were employed in diets fed to Robertson Creek chinook salmon, outstanding PER values may be obtained.

Higgs et al.(1983) showed that dietary protein content significantly influenced protein utilization as determined by PER with chinook fry. The protein levels employed in the present study for diets containing FPE covered a wider range

than those of Higgs et al. (1983). The downward trend in PER in diets containing more than 27% protein (Table 15, Fig. 7) is consistent with that found with chinook salmon (Higgs et al., 1983), carp (Ogino and Saito, 1970), juvenile rainbow trout (Takeuchi et al, 1978), juvenile chum salmon (Koshiishi, 1980) and grass carp fry (Dabrowski and Kosak, 1979). Over a wider range of protein levels the relationship with PER followed a similar pattern to that observed in plaice fed diets containing freeze-dried cod muscle (Cowey et al., 1972) and rats fed casein diets (Hegsted and Chang, 1965) (Fig. 7). The noteworthy difference between these curves is the level of dietary protein at which maximum PER was obtained. Maximum PER with the respective proteins occurred at dietary protein levels of 16% in rats, 27% in chinook, and 40% in plaice. Differences between the dietary protein level for maximum PER in this study (27%) and other studies may be due to species differences, although confounded by the previously mentioned factors affecting the determination of PER.

In the United States and Canada, PER is used to measure protein quality, particularly for regulatory purposes, and is an official method of the Association of Official Analytical Chemists. Tests are performed with rats. It is customary to feed casein as a control protein. Casein is used because it is a readily obtainable pure protein. PER estimates relative to that determined for casein are reported in an attempt to facilitate comparisons in PER measurements among investigators. For example, when the PER value for casein was set at 100, for

each assay, the relative PER for fish meal was 109, defatted beef 88, soy 78, pea protein 23 and wheat gluten 6 (McLaughlan, 1979). Similarly, the values obtained in this study can be cautiously compared with those of other investigators; bearing in mind that casein was supplemented with gelatin, arginine and methionine in the present study (Table 17). Generally, relative PER values obtained with fish fed animal proteins (fish meals, egg proteins) by other investigators were of a similar order to those determined for FPE, FRH, and LTH in this study. The PER values for proteins of plant origin are consistently lower than those of animal origin, probably because plant proteins have lower digestibility and a poorer amino acid balance relative to animal proteins. Also, each plant protein source contains one or more anti-nutritional factors (Higgs et al., 1983). Single cell protein sources such as petroyeast and methanophilic bacteria look promising for inclusion into chinook salmon diets while algal proteins do not (Table 17).

Several studies in fish report the efficiency of protein deposition to measure the nutritive value of a feedstuff (Ogino and Saito, 1970; Zeitoun et al., 1973; De la Higuera et al., 1977; Higgs et al., 1979; Pfeffer, 1982; Clarke et al., 1982). In the present discussion this is termed protein productive value (PPV). Since PPV and PER are essentially the same assay (the former relates body protein gain and the latter body weight gain to protein intake) the results obtained by the two methods were highly correlated (Table 28).

Under similar experimental conditions to the present study, Higgs et al.(1982) reported an equivalent PPV (32%) for another commercial diet (Abernathy). At higher water temperatures and with a different stock of chinook fry (Robertson Creek) Higgs et al.(1982) and Plotnikoff et al.(1983) reported a value for PPV of approximately 40% with fish fed OMP and various test practical dry diets. Higher estimates for PPV have been reported for chinook salmon in the above investigations and in the present study compared to those for coho salmon (Zeitoun et al., 1973; Higgs et al., 1979; Clarke et al., 1982) and rainbow trout (Zeitoun et al., 1973; Pfeffer, 1982). This may suggest that chinook salmon can utilize dietary protein more effectively than coho salmon for body protein synthesis and therefore the former species may be more preferable than the latter for fish farming.

In fish nutrition studies two methods have been adopted for measuring NPU (Table 17). Although NPU was calculated by the Bender and Miller (1953) and Ogino et al. (1980) methods, very close relative NPU (FPE = 100) values were obtained by both procedures. The main difference between the two methods is that the latter corrects for maintenance requirements on a body weight basis. Theoretically this is more accurate since it avoids overestimating the quality of protein sources measured in fish achieving a higher mean body weight. With the method of Ogino et al.(1980) a NPU of 100% was obtained with a dietary protein intake that exactly met maintenance requirements (Fig. 9).

In this study NPU was determined at several levels of dietary protein. This avoided the problem encountered by Atack et al.(1979) who had difficulty comparing NPU values obtained with carp for methanophilic bacteria and herring meal because their diets contained 35% and 25% protein, respectively (Table 17). The level of dietary protein for evaluating protein sources for fish has not been standardized at one arbitrary level as it has been for methods employing rats. However, since the evaluation of protein quality is dose dependent, an estimate at one level of intake may not be proportional to true protein quality (Samonds and Hegsted, 1977). As mentioned previously, standardization at one level of dietary protein penalized both high quality and low quality proteins (McLaughlan, 1979). A dependence of NPU on protein intake has also been reported for carp fed casein (Ogino and Saito, 1970), trout fed casein (Watanabe et al., 1978) and plaice fed freeze-dried cod muscle (Cowey et al., 1972)(Fig. 9).

The range of NPU values reported for juvenile chinook in this study are comparable to those obtained with rainbow trout for similar types of protein by a similar method (Takeuchi et al., 1978; Ogino, 1980; Ogino and Nanri, 1980; Watanabe et al., 1983). The differences in values between the studies (Table 17) are most likely due to differences in species, strain, fish size, temperature, feeding regimen and other ingredients in the diets. Comparison of results obtained by other investigators can only be meaningful if relative NPU (casein = 100) values are used as they were for PER (Table 17). However, since casein was

not used as the sole source of protein in the present study, a meaningful comparison with other studies is highly subjective.

Because few investigators have attempted to measure protein quality at more than one level of protein intake, the slope ratio method (Hegsted and Chang, 1965a) has not been adopted *per se* to evaluate protein quality in fish. Several studies were discussed earlier where, in reporting observations on nitrogen balance, body protein gain was regressed against protein intake (Iwata, 1970; Gerking, 1971; Nose, 1971; Rychly, 1980). Up to the level of protein intake where the minimum requirement of protein for growth was met (see Chap. 4) a linear relationship between intake and response (weight gain or protein gain) was assumed (De Long et al., 1958; Hegsted and Chang, 1965; Gerking, 1971). The question of linearity in the slope assay has often been raised with respect to the use of a protein-free diet fed group of animals (McLaughlan, 1977). Objections to the employment of a zero-protein fed group in rat assays is due to reports of excessive downward curvature of the slopes at very low intakes, which is encountered with some proteins. Specifically, lysine-deficient proteins such as wheat gluten have caused this problem (Yanez and McLaughlan, 1979). The rate of catabolism of lysine in rats is dependent upon the availability of that amino acid (Bodwell, 1977). At low lysine intakes, catabolism of lysine is retarded, resulting in the conservation and re-utilization of this amino acid. Threonine deficient proteins, on the other hand, show upward curvature at low intakes as this amino acid appears to have no conservation

mechanism (McLaughlan and Keith, 1977). Hegsted et al.(1968) and McLaughlan (1979) concluded that the slope ratio assay which includes the protein-free diet fed group was a more suitable method than the one excluding this group. This is because the former situation tended to reduce parallelism between lines which led to erroneous values. In this study the intercepts for the dose response lines were brought closer together when the data for the protein-free diet fed groups was included. At the same time the correlation coefficients were high.

Hegsted et al.(1968) found that the precision of the slope ratio assay depended primarily on the number of rats used. When the number of animals fed each diet was reduced from six to three, the standard error of the slopes increased by approximately 30%. In the present study, duplicate groups were employed and undoubtedly both precision and sensitivity could have been improved if additional groups of fish had been fed each diet. When the slopes for body protein gain including the zero protein diet fed groups were analyzed (Table 24), meaningful comparisons were found. By this method the assay was able to distinguish between the test protein sources.

3.5 Summary of Experiment 1

In this experiment the relationship between protein source in the diet of chinook salmon fry, and performance in terms of growth, food conversion and protein utilization was investigated. The fish were fed the experimental diets to measured satiation for 42 days. The results showed that the processing conditions of fish meal had a pronounced effect on growth and the utilization of protein as measured by various parameters. Rapid growth and best protein utilization was achieved by freeze-drying the raw material. Cooking followed by low temperature (75°C) drying of herring meal resulted in slightly inferior performance. High temperature (150°C) drying had a severe adverse effect on the protein quality of herring meal. In addition a casein-gelatin based diet, although apparently adequate in terms of protein quality, gave inconsistent results because of poor palability. Comparisons between fish fed the test diets and a commercial diet (OMP) revealed that the protein level of the latter could probably be lowered.

An attempt was made to compare various biological assay techniques for evaluating protein quality. The results were generally found to be more reliable when protein quality was measured at more than one level of dietary protein. In this respect slope ratios may have provided the best means of direct comparison between the proteins. Also, the measurement of protein gain rather than weight gain was considered to be biologically more valid. The maintenance requirements for

protein were estimated by feeding fish a protein-free and a low protein diet. This enabled the estimation of protein quality by methods which correct for the endogenous loss of nitrogen from the body.

Protein intake was partitioned into the amounts utilized for maintenance and growth, and the amount of exogenous excretion. This provided a clear depiction of how the various protein sources compared as the level of protein intake increased. Thus the level of protein intake for maxmimum efficiency with a particular protein source could be interpreted.

Lastly, the nutritive value of the test proteins was evaluated in terms of their available lysine content. Although the processing conditions of the meals affected protein quality as assessed by bioassay, the effect could not have been adequately predicted by the chemical determination of available lysine.

In conclusion, certain procedures were judged to be preferable to others, and substantial improvements in experimental protocol may provide a more sensitive and precise method to measure the nutritive value of proteins in diets for fish.

Chapter 4

EXPERIMENT 2

4.0 Protein requirements of juvenile chinook salmon in relation to dietary energy content

4.1 Introduction

The previous experiment showed that a blend of freeze-dried pollock muscle with freeze-dried whole euphausids (FPE) provided a more palatable and higher quality protein source than a combination of casein and gelatin, supplemented with arginine and methionine, for inclusion in juvenile chinook salmon diets. The gross protein requirements of juvenile chinook salmon were established (NRC 1973,1981) using casein-gelatin based diets (De Long et al., 1958). The requirements of juvenile chinook salmon for protein have not, however been determined with protein derived from fishery products with a demonstrated high biological value. Moreover, the basal diet employed by De Long et al.,(1958) had a high content of omega-6 type fatty acids from corn oil which, in light of more recent information (Yu and Sinnhuber, 1979), is known to depress growth rate of salmonids when fed at dietary concentrations in excess of 1% . It therefore seemed worthwhile to investigate the protein requirements of juvenile chinook salmon using FPE as the sole source of protein in diets containing two levels of dietary lipid supplied by marine oil. This approach also enabled determination of the optimal ratio of protein to energy in diets for juvenile chinook salmon.

In regard to the latter aim, Lee and Putnam (1973) conducted one of the few experiments on salmonids in which dietary levels of both protein and energy were varied. Their methodology provided estimation of an acceptable ratio of protein energy:total energy (PE:TE) in practical diets for rainbow trout. Similar estimates have not, however, been obtained for Pacific salmon diets.

4.2 Materials and methods

4.2.1 Protocol

Whereas Experiment 1 was conducted for 43 days, Experiment 2 was continued for 105 days. This period approximates the freshwater residency period of juvenile chinook salmon. The same stock of chinook fry were employed in Experiment 2 that was used in Experiment 1. The fish were distributed at the same time and the same manner as described in the previous experiment.

The experiment was designed as a 4 x 2 randomized block factorial without replication (Zar, 1974). Four concentrations of dietary protein and two levels of dietary energy were the factors tested. Each of the eight dietary treatments was randomly assigned in each of the two rows. As in Experiment 1, each row of tanks constituted a block.

4.2.2 Diets

The diets were formulated to contain 17%, 27%, 37% or 47% of dry matter as crude protein from FPE at each of two levels of

available dietary energy, 3150 and 3950 kcal/kg dry diet (Table 29). At each level of metabolizable energy, lipid levels were kept constant at 6% or 13% respectively. Carbohydrate, in the form of equal proportions of dextrin and glucose, was substituted for protein on an equal metabolizable energy basis as described in the previous experiment. The proximate analysis of the diets is shown in Table 30, together with the calculated metabolizable and gross energy concentration of the diets.

4.2.3 Data analysis

The data for fish body weight, feed consumption, feed analysis and fish carcass analysis were treated as described in Experiment 1. The parameters were subjected to a two-way randomized factorial ANOVA. A mixed effects model was employed similar to that described for Experiment 1. Dietary protein and energy concentration were assumed to be fixed effects and row a random effect. Treatment means were then subjected to Duncan's New Multiple Range Test (1955)(DMR) ($P = 0.05$). Data on fish fed OMP was not included in the statistical analysis but are included in the tables and figures for comparison.

An analysis of covariance of $\log_e$ body weights with day as the covariate was also conducted as described in Experiment 1. Specific growth rate was selected as the principal response criterion on which protein requirements were based. Linear and polynominal regression equations were derived (Zar, 1974) from growth rate data and interpreted according to Cowey et al.

Table 29. Composition of diets containing various levels of protein and energy (Experiment 2).

% protein in diet	17		27		37		47	
Metabolizable energy (kcal/kg)	3150	3950	3150	3950	3150	3950	315	3950
Freeze-dried pollock and euphausid	192.20	192.20	305.26	305.26	418.32	418.32	531.37	531.37
Herring oil	54.1	173.27	50.1	119.32	46.2	115.36	42.2	111.40
Dextrin	225.0	250.5	169.0	194.5	112.5	138.0	56.5	82.0
Glucose	225.0	250.5	169.0	194.5	112.5	138.0	56.5	82.0
Ground cellulose	224.5	85.5	235.0	127.52	248.15	142.15	259.95	116.73
Carboxy methyl cellulose	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Mineral mix	71.1	71.1	62.7	62.7	54.3	54.3	45.7	45.7
Vitamin mix	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Choline Chloride (50%)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5

1,2,3. See Table (3).

Table 30. Proximate composition and calculated energy contents of the diets employed in Experiment 2.

% protein in diet	17		27		37		47		
Metabolizable energy (kcal/kg)	3150	3950	3150	3950	3150	3950	3150	3950	OMP
Crude protein (N x 6.25)	18.05	16.94	27.36	28.24	36.68	35.96	45.81	46.15	49.40
Total crude lipid	6.09	12.40	5.70	12.31	6.32	11.79	6.49	12.95	14.53
Ash	7.47	7.15	7.19	7.37	7.34	7.61	7.39	8.30	11.92
Digestible carbohydrate	45.0	50.1	33.8	38.9	22.5	27.6	11.3	16.4	16.60
Moisture	8.31	7.89	6.95	7.72	8.37	6.72	5.80	6.03	24.62
Energy (kcal/kg)									
Protein									
ME (4.5 kcal/g)	812	762	1231	1126	1651	1618	2062	2077	2075
GE (5.7 kcal/g)	1029	966	1560	1553	2091	2050	2611	2631	2816
Lipid									
ME & GE (9.5kcal/g)	579	1179	542	1169	600	1120	617	1230	1280
Carbohydrate									
ME & GE (4.0kcal/g)	1800	2004	1352	1556	800	1104	452	655	266
Total ME	3191	3945	3125	3951	3151	3842	3130	3963	3721
Total GE	3408	4145	3453	4278	3591	4274	3680	4517	4462
mg protein/kcal ME	56.6	42.9	87.6	68.9	116.4	93.6	146.4	116.5	132.8
Protein energy (ME): total energy (ME)	0.25	0.19	0.38	0.28	0.52	0.42	0.66	0.52	0.56

1. - 6. See Table (4).

(1972) and Zeitoun et al. (1973,1976) to estimate protein requirements as discussed in the text. Statistical analyses were performed by computer through a general least squares analysis of variance program (GENLIN). A general linear models (GLM)(SAS, 1982) procedure was used to fit a regression model to growth rate.

4.3 Results

4.3.1 Estimation of the protein requirement of juvenile chinook salmon from growth data

The mean body weights of fish fed the various experimental diets and OMP increased exponentially during the experimental period (Fig. 12). Differences in wet fish body weight due to the different dietary concentrations of protein and energy were apparent early in the experiment. An analysis of variance indicated that by day 42 there were significant differences ($P < 0.001$) in body weight associated with dietary protein concentration (Table 31). The mean differences of fish body weights were significant (Duncan's new multiple range test, $P = 0.05$) with each increment in dietary protein level. At day 105, significant differences ($P < 0.001$) due to dietary protein level were still evident. However, in contrast to day 42, at day 105 the differences in final weights between groups receiving diets containing 37% and 47% protein respectively were no longer significant ($P > 0.05$)(Table 31). This suggests that the protein requirement for chinook salmon fry is higher during the first half of their growing period in fresh water than during the second.

A three-way analysis of covariance of $\log_e$ wet weights with time (day) as the covariate indicated a significant difference ($P < 0.001$) in slope (specific growth rate) due to dietary protein concentration. However, the slopes obtained with fish fed diets containing 27% protein were similar to those with

fish fed diets containing 37 and 47% protein (Table 31).

The lowest level of protein at which fish attain maximum weight gain is commonly considered to be the minimum requirement level for growth. The quantitative protein requirements for fish have been determined from "broken line" type plots (De Long et al.,1958; Satia, 1974; Zeitoun et al., 1973, 1974; Ogata et al., 1983; Anderson et al., 1981). A similar method was applied to the data in the present study. Following the procedure of Zeitoun et al.,(1973) for the growing fish, a regression line was fitted to the ascending portion of the response line of specific growth rate to dietary protein level. By averaging the highest observed means which did not differ by more than two standard errors of the mean within treatments, a horizontal line establishing maximum growth rate was drawn (Fig. 13). The intersection point of these two lines was taken as the minimum protein level, as a percent of dry diet, required to support maximum growth in juvenile chinook salmon. Employing the above method, the protein requirement for growth in this study was found to be approximately 35%, regardless of the dietary energy level tested (Fig. 13).

Other investigators studying the nutrient requirements of fish (Cowey et al., 1972; Zeitoun et al., 1976; Dabrowski, 1977; Murai et al., 1979) have employed non-linear growth curves to estimate requirements. Therefore, the growth rate data were fitted to a second degree polynominal by the procedure of Cowey et al. (1972). The fitted equation for fish fed diets

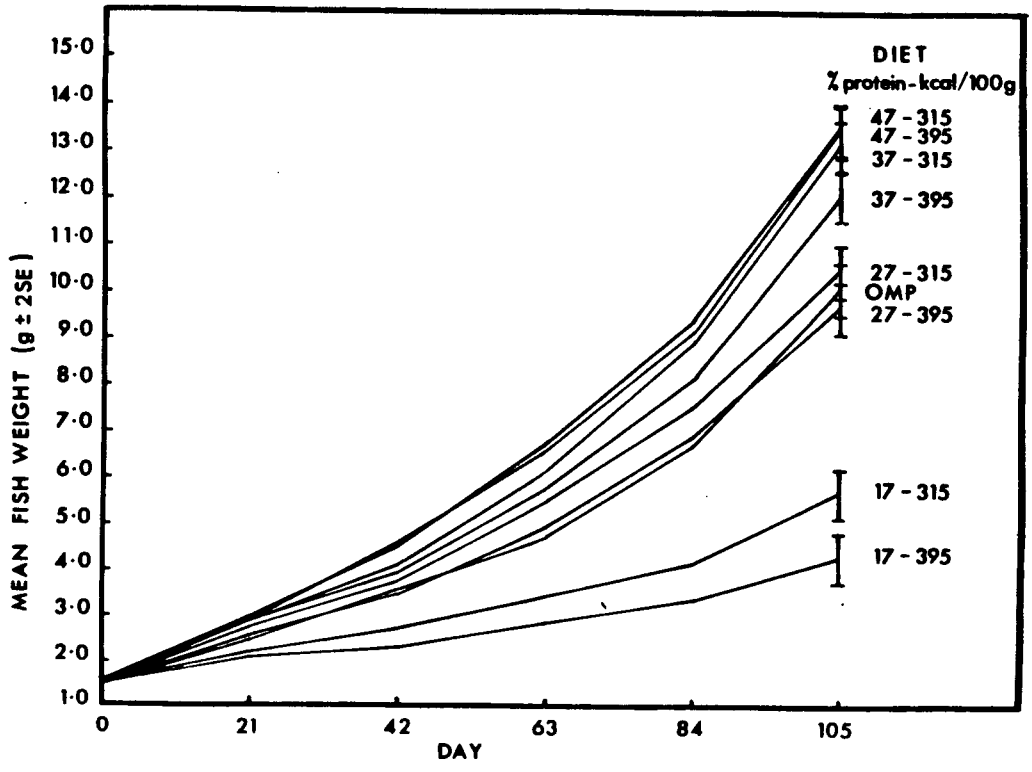


Fig. 12. Weight gain (± 2 SE) of chinook salmon from the fry- to smolt-stage fed diets containing various levels of protein and energy, and fed OMP.

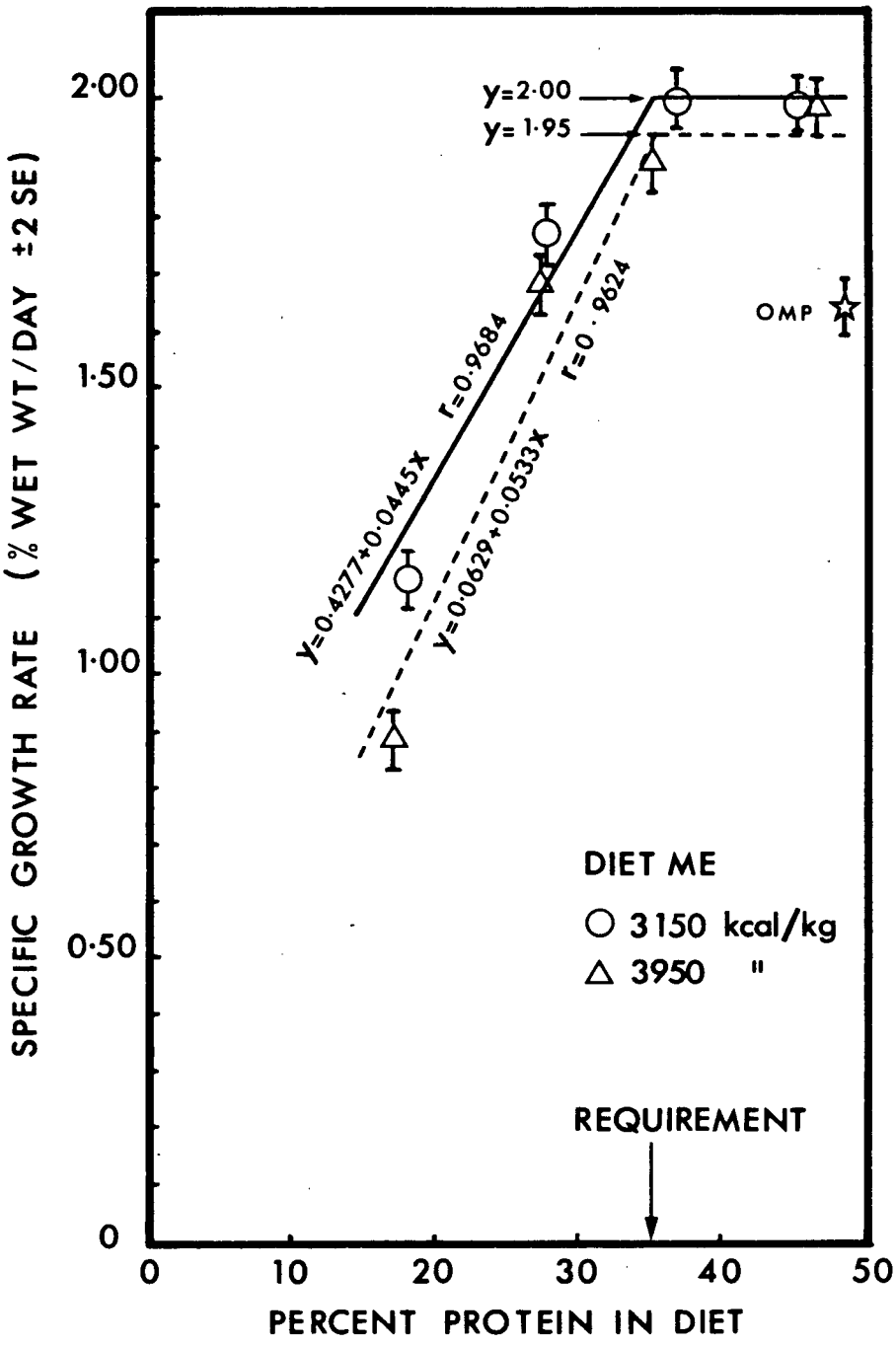
Table 31. Wet fish body weight (mean ± SE) at day 43 and day 105, and specific growth rates of diets containing different levels of protein and energy and OMP.

Metabolizable energy content of diet (kcal/kg)	Percent Protein in Diet 17	27	37	47	Mean
Mean body weight (+ SE)					
Day 42					
3150	2.71 ± 0.068	3.77	4.10	4.57	3.79 ± 0.034
3950	2.33	3.45	3.94	4.48	3.55
Mean	2.52[p1] ± 0.048	3.61[q]	4.02[r]	4.52[s]	
OMP				3.59 ± 0.025	
Day 105					
3150	5.66 ± 0.23	10.47	13.25	13.49	10.72 ± 0.12
3950	4.23	9.55	12.13	13.45	9.84
Mean	4.94[p1] ± 0.16	10.01[q]	12.69[r]	13.47[r]	
OMP				10.06 ± 0.46	
Specific Growth Rate (% wet wt/day)					
3150	1.17 ± 0.024	1.77	2.00	1.99	1.73 ± 0.012
3950	0.89	1.68	1.89	2.00	1.61
Mean	1.03[p2] ± 0.017	1.73[r]	1.95[r]	2.00[r]	
OMP				1.74 ± 0.06	

1. Values with the same superscript for each parameter with respect to protein level (p - r) do not differ significantly (DMR test P = 0.05).

2. Slopes with the same superscript with respect to protein level (p - r) effects do not differ significantly (Scheffé's test P = 0.05).

Fig. 13. Specific growth rate (percent wet body weight per day) (± 2 SE) of juvenile chinook salmon fed diets containing different levels of protein and energy. A regression line was fitted to the data over the ascending portion of the growth response to protein concentration at both levels of dietary energy. A straight line parallel to the abscissa was drawn by averaging the highest observed means which did not differ from each other by more than two standard errors of the means. The value obtained for OMP is included for comparison.


SPECIFIC GROWTH RATE (% WET WT/DAY ±2 SE)
2·00
1·50
1·00
0·50
0
y=2·00
y= 1·95
y=0·4277+0·0445x
r=0·9684
y=0·0629+0·0533x
r=0·9624
OMP
DIET ME
○ 3150 kcal/kg
△ 3950 "
REQUIREMENT
0
10
20
30
40
50
PERCENT PROTEIN IN DIET

containing 3150 kcal/kg was:

$$y = -0.81225 + 0.14212x - 0.00177x^2,$$

$$(n = 4, r = 0.9991)$$

and 3950 kcal/kg was:

$$y = -1.07943 + 0.14671x - 0.00174x^2,$$

$$(n = 4, r = 0.9956)$$

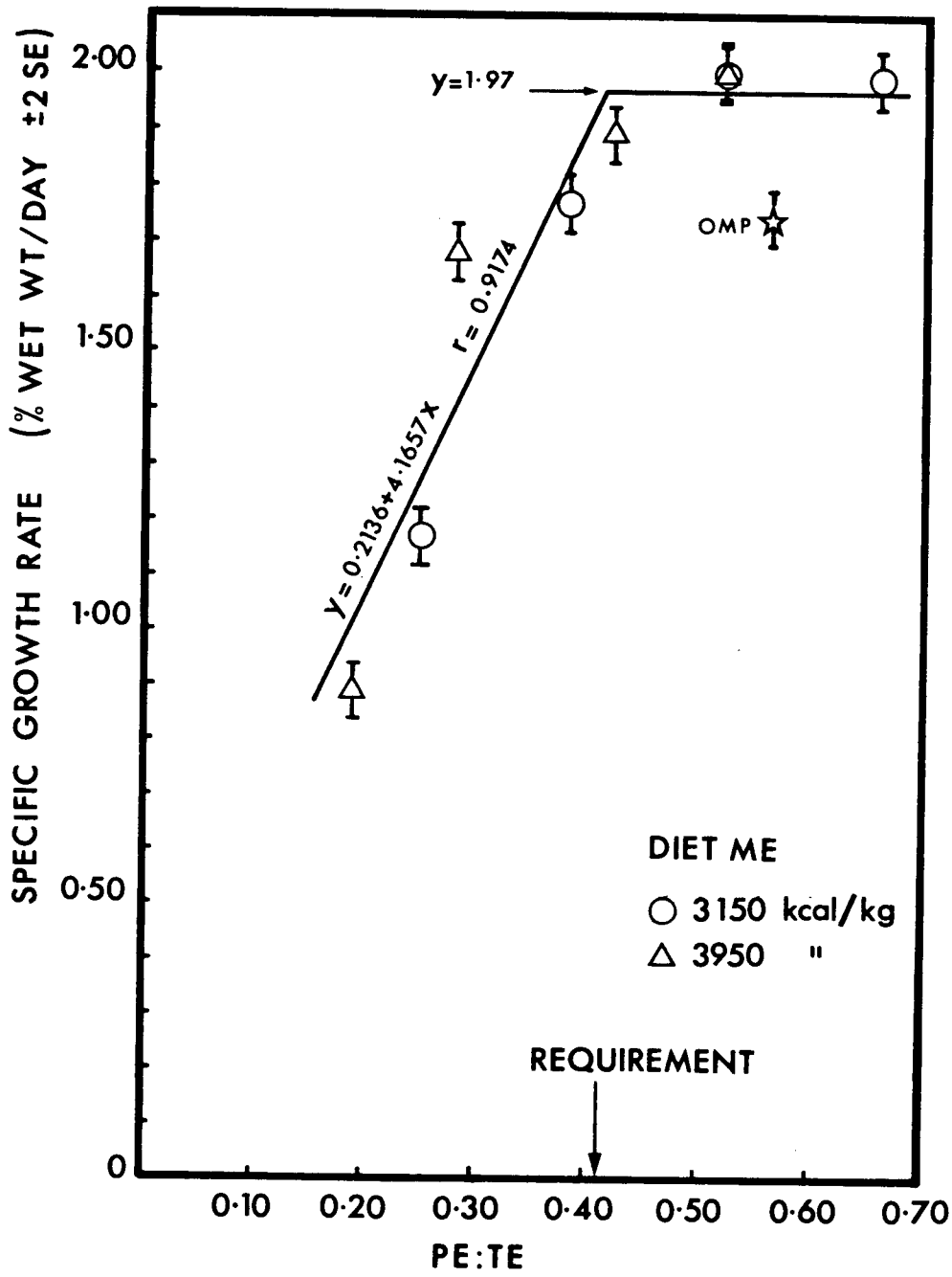
where y = specific growth rate,

x = percent protein in the diet.

The data fit the above equations with probability levels of $P < 0.05$ and $P < 0.10$ for diets containing 3150 and 3950 kcal/kg respectively. The maximum of the response curves occurred at a protein level of 40% and 42% for diets containing 3150 and 3950 kcals/kg respectively. According to the criteria of Cowey et al. (1972) these values would be the protein requirements of chinook salmon for maximum growth.

The analyses of variance conducted on fish body weights indicated that the metabolizable energy concentration of the diets was not a factor affecting growth of juvenile chinook salmon (Table 31). Dietary energy was provided by protein and non-protein sources (Table 30). Since protein is utilized both for tissue synthesis and as an energy source, it is the relationship between protein energy and non-protein energy in the diet that is important in the nitrogen balance scheme described in Fig. 1. The observations made above support the case made by Cowey and Sargent (1979) for stating the protein requirement of a fish diet in terms of the proportion of energy it contributes, relative to total dietary energy. When the

Fig. 14. Specific growth rate (percent wet body weight per day) (± 2 SE) of juvenile chinook salmon fed diets containing different protein energy:total energy ratios (PE:TE). The protein requirement was obtained by a similar method to that described in Fig. (13). The value obtained for OMP is included for comparison.


SPECIFIC GROWTH RATE (% WET WT/DAY ±2 SE)
2·00
1·50
1·00
0·50
0
y=1·97
y= 0·2136+4·1657x
r= 0·9174
OMP
DIET ME
○ 3150 kcal/kg
△ 3950 "
REQUIREMENT
0·10
0·20
0·30
0·40
0·50
0·60
0·70
PE:TE

protein requirement was estimated relative to that of total dietary energy, the minimum PE:TE ratio for growth of chinook salmon was found to be 0.41 by the broken line method (Fig. 14).

The protein requirement expressed as PE:TE was also quantified by applying a second order polynomial equation. The quadratic equation derived was:

$$y = -0.50699 + 9.25398x - 8.37872x^2 ,$$

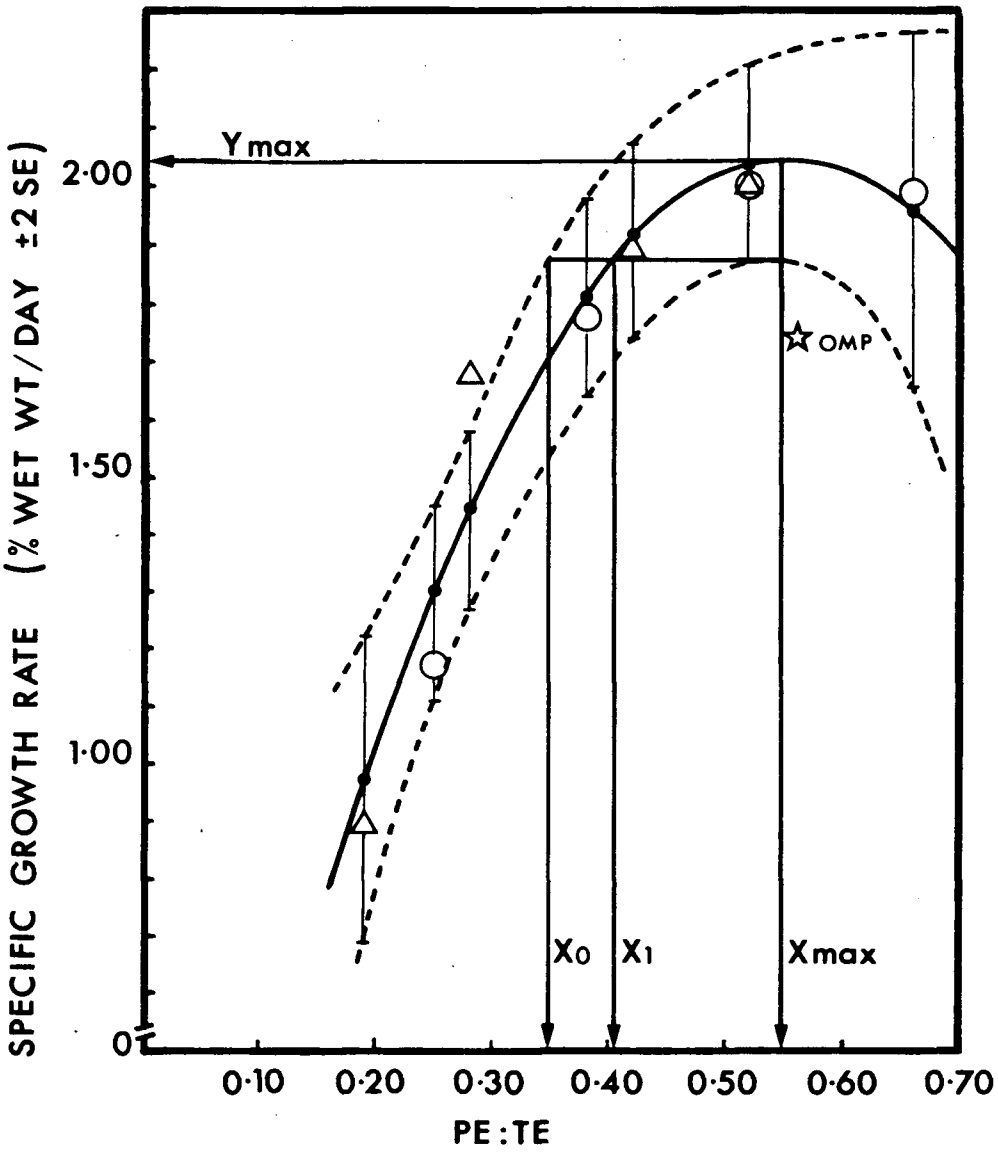
$$(n = 8,\ r = 0.9647),$$

where y = specific growth rate,

x = PE:TE ratio

The curve obtained (Fig. 15) fit the data adequately ($P < 0.005$), both linear and quadratic terms being significant ($P < 0.05$). Cowey et al. (1972) defined the dietary protein requirement as the level that produced the highest point on the curve. In the present study, maximum growth of the fish was achieved at a PE:TE ratio of 0.55 (x max., Fig. 15). This value corresponds to the PE:TE ratio in OMP, a popular commercial salmon feed (Table 30). However, Zeitoun et al. (1976) pointed out that x max on the quadratic curve relating growth to dose "does not reflect the practically insignificant differences in percentage gain below and beyond the maximum point, nor does it consider the ability of the animal to adapt to a range of dietary protein levels." They applied a statistical approach to determine the nutrient level which could provide a response that lay within a certain confidence range of the maximum response. In the present study, upper and lower confidence limits of 95% of the means were plotted, and a straight line was drawn

Fig. 15. The second order polynomial curve (solid curved line) fitted to specific growth rate (± 95% confidence limits of the estimated means) of juvenile chinook salmon fed diets with different PE:TE ratios. Dashed lines represent the 95% confidence limits of the relationship. x max represents the PE:TE ratio for maximum growth. x0 and x1, represent the range of dietary PE:TE that would predictably result in relatively minor decreases from maximum growth rate (y max). The concomitant reduction in the dietary PE:TE ratio would be relatively large. The value obtained with fish fed OMP is included for comparison.


Ymax
2·00
1·50
1·00
0
SPECIFIC GROWTH RATE (% WET WT/DAY ±2 SE)
OMP
X0
X1
Xmax
0·10
0·20
0·30
0·40
0·50
0·60
0·70
PE:TE

parallel to the abssissa and passing through the maximum level of the lower confidence limit (Fig. 15). This line crossed the polynomial curve at x1 and intersects the upper confidence limit at x0. Statistically, the confidence limits of the response expected with a diet having a PE:TE ratio of 0.55 (x max) include the mean response obtained with a diet having a PE:TE ratio of 0.41 (x1) and the upper limit response with a PE:TE ratio of 0.35 (x0). According to the criterion of Cowey et al. (1982) the PE:TE requirement of chinook salmon was 0.55. Other investigators (Robbins et al., 1979) would interpret the data differently and select the minimum PE:TE ratio that corresponds to the lower limit of the maximum response (x1, Fig. 15). The PE:TE requirement by this method was found to be 0.41 in the present experiment. Other interpretations of the curve will be discussed later.

4.3.2 The effect of dietary protein and energy level on food conversion and gross energy utilization.

Gross food conversion efficiency (GFC) was noted to increase as both the protein level and the energy level of the diets were raised (Table 32). Differences in GFC between fish fed 3150 and 3950 kcal/kg were not significant ($P > 0.05$) in fish fed diets containing low levels of dietary protein. At high dietary protein concentration food conversion increased as the energy level of the diet was increased.

Gross energy utilization (GEU) was influenced by both the level of dietary protein and the metabolizable energy content of the diet (Table 32). GEU was found to increase as the dietary protein level increased until approximately the point at which the protein requirement of the fish was reached. The efficiency of energy utilization in fish as indicated by GEU follows the laws of diminishing returns. At all levels of protein, increasing the energy content of the diet by 800 kcal/kg caused a reduction of GEU in the same order of magnitude. This observation suggests that the total energy content of diets employed in this study was not limiting. This was certainly the case for the diets containing 3950 kcal/kg (Table 32).

4.3.3. The effect of dietary protein and energy level on protein utilization

The efficiency of protein utilization, as indicated by PER and PPV, gives an indication of the extent to which protein was used for growth. The relationship between the protein concentration of the diet and the utilization of dietary protein followed a similar pattern to that described in the previous chapter. As the dietary protein concentration was increased, efficiency increased, and reached a maximum at a lower dietary concentration than the minimum requirement level for growth (Table 33). At higher protein levels PER and PPV decreased. Because protein utilization for maintenance is accounted for, NPU-2 values are not lower in fish fed diets containing protein

Table 32. Gross food conversion efficiency (GFC)and gross energy utilization (GEU) of diets containing different levels of protein and energy.

Metabolizable energy content of diet (kcal/kg)	% Crude Protein in Diet 17	27	37	47	Mean
GFC					
3150	11.56^{a}[1] ± 0.89	19.31^{b}	23.48^{c}	24.70^{cd}	19.76^{w} ± 0.45
3950	11.26^{a}	21.58^{b}	27.53^{de}	30.06^{e}	22.61^{v}
Mean	11.41^{p} ± 0.63	20.44^{q}	25.51^{r}	27.38^{r}	
OMP				24.40 ± 0.20	
GEU					
3150	20.49^{a} ± 1.59	36.71^{bc}	44.01^{d}	45.77^{d}	36.74^{w} ± 0.79
3950	16.41^{a}	31.25^{b}	39.86^{cd}	41.20^{cd}	32.18^{v}
Mean	18.45^{p} ± 1.12	33.98^{q}	41.94^{r}	43.48^{r}	
OMP				32.03 ± 1.54	

1. Values with the same superscript for each parameter with respect to protein level x energy level (a - c), protein level (p - s) and energy level (v,w) effects do not differ significantly (DMR test, P = 0.05).

levels below minimum requirements. Protein utilization by all three indicators was improved when the energy content of diets was increased from 3150 to 3950 kcal/kg. The higher energy content of the diet had a sparing effect on protein, allowing more dietary protein to be utilized for synthesis of tissue protein.

4.3.4 The effect of dietary protein and energy level on the proximate body composition of fish

A pooled sample of whole fish taken at day 0 contained 79.88% moisture; and on a dry matter basis 9.99% ash, 16.6% lipid and 76.54% protein. The gross composition of chinook salmon sampled at day 42 and 105 of the experiment varied with dietary energy concentration (Table 34 and 35). Overall, the moisture concentration of fish decreased slightly from samples taken at day 0, 42, and 105 respectively. The moisture concentration of the fish at day 42 and 105 was found to decrease as the dietary energy level was raised. A trend of decreasing moisture as dietary protein concentration increased was also noted, which may have been due to concomittant increases in fish size.

As one would expect, increasing the dietary energy level resulted in an increase in body lipid. An inverse relationship between dietary energy level and body protein was observed. Dietary protein concentration was not found to have an effect on body ash, lipid or protein at day 42. At day 105 differences in

Table 33. Protein efficiency ration (PER), protein productive value (PPV), and net protein utilization (NPU-2) of diets containing different levels of protein and energy.

Metabolizable energy content of diet (kcal/kg)	% Crude Protein in Diet 17	27	37	47	Mean
PER[1]					
3150	0.64^{ab}[2] ± 0.025	0.71^{bcd}	0.64^{ab}	0.54^{a}	0.63^{w} ± 0.013
3950	0.66^{bc}	0.79^{d}	0.77^{cd}	0.65^{ab}	0.72^{v}
Mean	0.65^{pq} ± 0.18	0.75^{r}	0.72^{q}	0.60^{p}	
OMP				0.49 ± 0.02	
PPV[1]					
3150	42.79^{abc} ± 1.69	44.73^{abc}	43.27^{abc}	38.37^{a}	42.29^{w} ± 0.86
3950	42.77^{abc}	48.87^{c}	46.48^{bc}	41.20^{ab}	44.83^{v}
Mean	42.78^{pq} ± 1.20	46.80^{qr}	44.87^{q}	39.78^{p}	
OMP				31.42 ± 1.05	

Table 33. cont'd...(2)

Metabolizable energy content of diet (kcal/kg)	% Crude Protein in Diet 17	27	37	47	Mean
NPU-2					
3150	60.60^{bc} ± 2.20	59.60^{bc}	56.08^{abc}	49.24^{a}	56.38^{w} ± 1.10
3950	63.63^{bc}	65.91^{c}	61.89^{bc}	54.10^{ab}	61.38^{v}
Mean	62.11^{q} ± 1.55	62.75^{q}	58.99^{q}	51.67^{p}	
OMP				54.80 ± 1.35	

1. A two-way randomized ANOVA indicated significant differences in PER, PPV and NPU-2 due to protein level ($P > 0.005$) and energy level ($P > 0.05$). Differences due to protein level x energy level and block effects were not significant ($P < 0.05$).

2. Values with the same superscript for each parameter with respect to protein level x energy level (a - d), protein level (p - r) and energy level (v,w) effects do not differ significantly (DMR test, $P = 0.05$).

Table 34. Whole body proximate composition at day 42 of the diets containing different levels of protein and energy, and OMP.

Metabolizable energy content of diet (kcal/kg)	Percent Protein in Diet 17	27	37	47	Mean
	% Moisture (± SE)				
3150	78.09^{a}[1] ± 0.27	77.54^{ab}	72.09^{abc}	77.80^{a}	77.68^{w} ± 0.14
3950	77.80^{a}	76.53^{bc}	76.11^{c}	76.18^{c}	76.65^{v}
Mean	77.94^{p} ± 0.19	77.04^{q}	76.60^{q}	76.99^{q}	
OMP				77.38 ± 0.27	
	% Ash (Dry Basis)				
3150	10.41^{a} ± 0.26	9.78^{ab}	9.74^{ab}	9.55^{ab}	9.87^{w} ± 0.13
3950	9.59^{ab}	9.04^{b}	9.38^{b}	8.91^{b}	9.23^{v}
Mean	10.00^{p} ± 0.18	9.41^{pq}	9.56^{pq}	9.23^{q}	
OMP				9.86 ± 0.07	
	% Lipid (Dry Basis) (± SE)				
3150	17.89^{c} ± 1.12	17.18^{c}	19.75^{bc}	17.15^{c}	17.99^{w} ± 0.56
3950	22.05^{ab}	24.52^{a}	23.94^{a}	24.81^{a}	23.83^{v}
Mean	19.97 ± 0.79	20.85	21.85	20.98	
OMP				20.96 ± 0.78	
	% Protein (Dry Basis) (± SE)				
3150	72.98 ± 2.27	73.02	67.47	71.02	71.12^{w} ± 1.14
3950	65.57	67.71	67.06	68.69	67.25^{v}
Mean	69.27 ± 1.61	70.36	67.26	69.85	
OMP				68.67 ± 1.94	

1. Values with the same superscript for each proximate component with respect to protein level x energy level (a - c), protein level (p,q) and energy level (v,w) effects do not differ significatnly (DMR test, P = 0.05).

Table 35. Whole body proximate composition at day 105 of the diets containing different levels of protein and energy, and OMP.

Metabolizable energy content of diet (kcal/kg)	Percent Protein in Diet 17	27	37	47	Mean
	% Moisture (± SE)				
3150	78.60^{a}[1] ± 0.27	76.12^{cd}	76.73^{bc}	77.52^{b}	77.24^{w} ± 0.13
3950	77.38^{b}	75.43^{d}	75.23^{d}	75.40^{d}	75.86^{v}
Mean	77.99^{p} ± 0.19	75.77^{r}	75.98^{q}	76.46^{q}	
OMP				76.05 ± 0.15	
	% Ash (Dry Basis)				
3150	11.70^{a} ± 0.72	10.53^{ab}	9.52^{ab}	9.97^{ab}	10.43 ± 0.36
3950	10.18^{ab}	9.65^{ab}	10.06^{ab}	9.57^{b}	9.57
Mean	10.94 ± 0.51	10.09	9.79	9.19	
OMP				8.89 ± 0.43	
	% Lipid (Dry Basis) (± SE)				
3150	18.70^{de} ± 0.85	23.42^{bc}	21.08^{cd}	18.17^{e}	20.34^{w} ± 0.42
3950	22.38^{c}	26.59^{a}	27.48^{a}	25.21^{ab}	25.41^{v}
Mean	20.54^{q} ± 0.60	25.01^{p}	24.28^{p}	21.69^{q}	
OMP				23.23 ± 0.68	
	% Protein (Dry Basis) (± SE)				
3150	66.61^{bc} ± 1.39	62.43^{cde}	68.20^{b}	74.91^{a}	68.04^{w} ± 0.70
3950	60.12^{de}	58.58^{e}	58.32^{e}	64.23^{bcd}	60.31^{v}
Mean	63.37^{q} ± 0.98	60.50^{q}	63.26^{q}	69.57^{p}	
OMP				63.58 ± 0.41	

1. Values with the same superscript for each proximate component with respect to protein level x energy level (a - c), protein level (p,q) and energy level (v,w) effects do not differ significatnly (DMR test, P = 0.05).

body lipid and protein due to dietary protein concentration were found but no clear trends were noted (Table 35).

4.4 Discussion

4.4.1 The dietary protein requirement of juvenile chinook salmon

The results of the growth trial suggested that the chinook salmon may require diets containing a higher concentration of protein during the fry stage than during the latter stage of freshwater rearing. This is in agreement with a report by Satia (1974) who found that the protein requirement for rainbow trout dropped down from 50 to 40% as the fish grew from the fry to fingerling stage. There is a general trend of lower protein requirements, as a percent of diet, in older and larger fish of several species (Ogino and Saito, 1970; Nose and Arai, 1972; Page and Andrews, 1971). Gulbrandsen and Utne (1977) on the other hand, found no change in the protein requirements of rainbow trout ranging in size from 5 to 70g. In this study, the experimental period covered almost the entire period of fresh water residency for juvenile chinook salmon. It is likely that the higher protein requirement of salmonids is indicated for during the fry stage only (Fowler, 1980). During this period it may be recommended to feed diets with a protein concentration to support maximum growth (x max, Fig. 15). Diets for fry should contain a minimum PE:TE ratio of 0.55.

The empirical requirement for a nutrient depends, not only on the physiological and environmental conditions of the experiment, but also on the method used to estimate the requirement (Robbins et al., 1979). When expressed as a percentage of dry diet the minimum dietary protein concentration for maximum growth was found to be 35% of the diet by the "broken line" method. This value is lower than that stated for chinook salmon, obtained by a similar method, reared in 7°C water (40%) and in 15°C water (50%)(Delong et al., 1958). In the present study the fish were reared at 10.5°C. Alternatively, other investigators have used continuous growth curves to arrive at the protein requirements for plaice (Cowey et al., 1972), rainbow trout (Zeitoun et al., 1976), grass carp (Dabrowski, 1977) and shad (Murai et al., 1979). They stated that the relationship of growth to dose did not exhibit the abrupt change from linearity implied by the "broken line" method. Also, the right-hand section of the response line was assumed to be horizontal and representative of maximum average growth after the minimum protein requirement level is reached. Cowey et al. (1972) and Zeitoun et al. (1976) found that very high dietary protein levels depressed growth in plaice and rainbow trout respectively. These authors found that their data was best described by a second degree polynominal.

In the present study,dietary concentrations of protein tested did not exceed 47%. However, Delong et al. (1958) reported a decline in growth of chinook salmon with diets containing more than 60% protein. The dietary protein

concentration providing for maximum growth rate from a second degree polynomial equation was found to be 40 and 42% for diets containing 3150 and 3950 kcals/kg respectively. These requirements are approximately 10% lower than the requirement arrived at by a similar method for rainbow trout using diets containing casein and gelatin as a protein source (Zeitoun et al., 1976).

At this point in the discussion, it must be emphasized that in this study the fish were fed to satiation. Feed intake by fish, as by other animals, is governed by the energy content of the diet (Lee and Putnam, 1973; Page and Andrews, 1973). The protein requirement, based on growth rate, stated above was found to be the same at both levels of dietary energy. This implies that protein intake alone was responsible for the differences in the growth rate. The effects due to dietary energy level and the interaction of protein level and energy level were minimal. The metabolizable energy content of the diets was not a factor limiting the growth of the fish.

In fish fed diets containing low protein levels, increasing the dietary energy level had the effect of depressing growth rate due to a reduction in protein intake. Growth rate was not depressed by increasing the dietary energy level in diets containing 47% protein. Protein intake by these groups of fish was not restricted by the energy content of their diet. It is interesting to note that growth rates of fish were similar when the diets fed contained the same proportion of total energy as protein energy even though the composition of the diets differed

(i.e., diets with 37% protein and 3150 kcal/kg and 47% protein and 3950 kcal/kg respectively). Ogino et al. (1976) found that the dietary protein concentration required for maximum growth in trout was 30 to 35% when non-protein dietary energy was provided by lipid (1:1, soybean and cod liver oil) but the requirement shifted to approximately 40% with carbohydrate (starch and dextrin). In contrast, in the present study the source of non-protein energy was not a major factor affecting protein requirements for growth. This aspect will be discussed in more detail later.

Furthermore, the diets also differed markedly in their content of ground cellulose (Table 29). Buhler and Halver (1961) reported depressed growth rates of chinook salmon fed diets with increasing levels of cellulose. Hilton et al. (1983) concluded that dietary fiber levels for rainbow trout should be less than 10% of the diet because their natural diet contains little fiber. Higgs et al. (1983) suggested that fiber may depress mineral uptake, adversely affect transit time of digesta and decrease the digestibility of nutrients. In the present study, however, it would seem unlikely that cellulose interacted with other nutrients. Rather, fish fed the low energy diets were able to attain a similar growth rate to those fed high energy diets by increasing feed intake, and hence, as noted above, protein intake by alteration of gastric evacuation and stomach distention (Hilton et al., 1983). This is in agreement with the conclusions made by Dupree and Sneed (1966) and Lee and Putnam (1973) in channel catfish and rainbow trout respectively.

Discrepancies with the other studies may be due to effects of temperature (Brett and Higgs, 1970), feeding frequency (Adron et al., 1973) and other environmental factors (Brett, 1979).

The observations made above support the case made by Cowey and Sargent (1979) for stating protein requirement in terms of the proportion of energy contributed. By the broken line method (Fig. 14) the PE:TE ratio for maximum growth rate was found to be 0.41. This value is lower than that previously reported for chinook salmon, PE:TE of 0.50 (Combes et al., 1962; Fowler et al., 1964). Discrepancies between the values likely resulted from several sources including dietary ingredients, different fish stocks, water temperature and caloric values assigned to the nutrients. Recently Fowler (1980) found that the best PE:TE ratio in the diet for newly hatched chinook fry was 0.51 to 0.55. However, the protein was provided by several ingredients, some of which may not have been digested by the very small fish. The PE:TE of 0.41 found in this study agrees with that reported for rainbow trout, 0.37 - 0.41 (Gulbrandsen and Utne, 1977) and arctic char, 0.35 - 0.45 (Jobling and Wandsvik, 1983).

Based on the finding that the growth response to the protein concentration of the diets was not influenced by changes in the proportion of metabolizable energy supplied by carbohydrate and lipid, a single second order polynomial curve was fitted to the data (Fig. 15). With a polynomial curve such as the one described above, one is confronted with the task of recommending a PE:TE requirement. While the PE:TE value of 0.55 (x max) may be physiologically correct, this estimate is unsatisfactory for

practical use because it ignores economic considerations. An identical dilemma exists in the estimation of nutrient requirements from growth data with other livestsock. For example, when Robbins et al. (1979) compared non-linear models to linear models to re-evaluate the amino acid and vitamin requirements of the growing chick, they arbitrarily chose the dose at which the response reached 95% of the total response. Despite the subjectivity in defining the "requirement" these authors concluded that the non-linear models were preferable, although the estimated requirements by both methods were nearly the same. An identical conclusion may be drawn from the results in the present study as the PE:TE ratio at x1 is identical to that determined by the broken-line model, namely PE:TE = 0.41 (Figs. 14,15).

From a strict statistical point of view one cannot establish the protein requirement at x0 or x1 on the basis that the growth response at these levels is not statistically significantly different from that at x max (Zeitoun et al, 1976). Also, one must bear in mind that increasing the number of observations in the regression analysis may have had the effect of narrowing the confidence limits and hence shift x0 and x1 to the right. Zeitoun et al. (1976) very succinctly point out that "statistical significance is somewhat irrelevant for regressions with significant parameters. What is relevant is a difference which has a practical importance." Economically, the difference in PE:TE of the diets could be important since protein is one of the most expensive components of a fish diet. The implication

is that the protein requirement could be based on the cost of each dietary protein increment relative to the corresponding monetary value assigned to the growth rate of the fish. Thus the curve shown in Fig. 15 could be adapted to cost benefit analysis in a fish production enterprise when information on the cost of feed, growth of the fish and selling price of the product is available.

Feed conversion was used by Satia (1974) as the principal criteria for determining protein requirements of rainbow trout. In this study, GFC could be used as an indicator of protein requirements only when isocaloric diets are considered. Hence, maximum food conversion was obtained at a dietary protein level of approximately 37% at both levels of total dietary energy (Table 32). Predictably, the efficiency of dietary energy utilization increased in a similar manner to GFC as dietary protein concentration was raised to 37% of the diet. The trend noted of increased GEU as the PE:TE ratio of the diets was increased may support the point that, for fish, protein is the preferred source of energy (Walton and Cowey, 1982). The higher GEU by fish fed diets containing a lower energy density may be due to increased lipid digestibility. Takeuchi et al. (1978) on the other hand, reported that lipid digestibility in trout was not affected by dietary lipid concentration. The results obtained for GFC and GEU served to support the protein requirement estimate made previously.

4.4.2 The effect of dietary energy on the efficiency of protein utilization

The replacement of protein energy with non-protein energy to furnish the metabolizable energy requirements, is a potential means for reducing the cost of rearing fish. This protein sparing effect has been observed by several investigators (Lee and Putnam, 1973; Page and Andrews, 1973; Takeda et al., 1975; Adron et al., 1976; Garling and Wilson, 1976; Gulbrandsen and Utne, 1977; Takeuchi et al., 1978; Shimeno et al., 1980). For example, Takeuchi et al. (1978) fed trout diets containing levels of protein ranging from 16 to 48% and levels of lipid ranging from 5 to 25%. They found that best weight gains were obtained when diets contained 18% lipid. At this level of dietary lipid, the protein content of the diet could be reduced from 48 to 35% with no loss in weight gain. Buhler and Halver (1961) fed chinook salmon a series of diets in which the protein level decreased from 71 to 40% by increasing levels of dextrin up to 43% of the diet. They showed that protein efficiency ratios were increased by substituting dextrin for protein although growth rate remained similar among dietary treatments. In the present study, protein in the diets was replaced by both carbohydrate and lipid on a metabolizable energy basis. The highest values of protein utilization for growth as measured by PER and PPV occurred when PE:TE ratio ranged from 0.28 to 0.42. Protein utilization was significantly ($P < 0.05$) lower when the PE:TE increased from 0.42 to 0.66 (Table 33). Therefore increasing the PE:TE ratio of the diet beyond the minimum

protein requirement level (i.e: PE:TE = 0.41) has the effect of reducing the efficiency of protein utilization without promoting any increase in the growth rate. In fact, the growth rate of fish fed a diet with a PE:TE ratio of 0.42 was slightly lower, although not significantly lower ($P > 0.05$), than that of fish fed diets with a PE:TE ratio of 0.52 (Table 31).

Several studies (reviewed in Chapter 2) have demonstrated the sparing of dietary protein by lipid and carbohydrate. Increased use of lipids in fish diets can be disadvantageous because of their high cost and susceptibility to oxidative rancidity. On the other hand, the use of carbohydrates is limited by the digestibility of starch and the amount of glucose carnivourous fish can tolerate. In the present study, identical high growth rates were achieved by fish fed either of two diets with a PE:TE ratio of 0.52 (Table 31). One contained 22.5% and 6.32%, and the other 16.4% and 12.95% digestible carbohydrate and lipid respectively. With both diets dietary protein was spared equally (Table 33). Dietary protein was further spared by fish fed the diet with a PE:TE ratio of 0.42 that contained 27.6% digestible carbohydrate and 11.79% lipid. Still further savings may be achieved by feeding fish the diet with a PE:TE ratio of 0.38. This diet promoted growth at only a slightly lower rate than did the diet with a PE:TE ratio of 0.42 and similar to that of fish fed OMP (Table 33). However, it contained 33.8% digestible carbohydrate, and only 5.70% lipid and 27.4% protein, which contrasts markedly with the commercial diet (OMP, Table 30).

The above observations support the findings of Buhler and Halver (1961) who found that the optimal level of dietary carbohydrate was approximately 20% although chinook salmon could tolerate diets containing higher levels. However, this is at variance with reports by Phillips (1969) and Hilton and Atkinson (1982) who claim that digestible carbohydrate levels in trout diets should not exceed 12 to 14%. It would seem doubtful that major differences exist between related species and considering that rainbow trout are a more domestic species of salmonid than were the chinook salmon employed in the present study. Subsequently, Hilton et al., (1982) suggested that the optimum levels of inclusion of digestible carbohydrate may well be variable depending on the overall balance of protein, fat and carbohydrate as well as the total energy content of the diet. These authors also reported that the digestibility of glucose was uniformly high (96 to 99%) and was not affected by the level of inclusion in the diet. The digestibility of raw starch and to a lesser extent, cooked starch, and dextrin decreases as the level in the diet of rainbow trout is increased (Singh and Nose, 1967). Therefore, since the results of present study indicate that juvenile chinook salmon are able to utilize absorbed carbohydrate to a greater extent than previously thought (Phillips, 1969), it may be recommended that starch-containing ingredients be processed prior to inclusion in practical diets for this species. This may lessen the reliance on high lipid levels as a source of energy in fish diets. This point has been amply demonstrated (Smith, 1976; Pieper and Pfeffer, 1980;

Hilton et al., 1981) yet does not appear to have been widely adopted by fish feed manufacturers.

Although high carbohydrate diets may be advantageous when applied to fish farming, fish fed these diets develop enlarged livers and increased liver glycogen levels (Phillips, 1969; Lee and Putnam, 1973). This could be hazardous to the health of fish destined for release since it has been shown that abnormal liver size and liver glycogen content in trout reduce the tolerance of these fish to waterborne toxicants and impair liver function (Hilton, 1982).

4.4.3 The effect of the source and level of dietary energy on the proximate body composition of fish

The results of this study are in agreement with those of Groves (1970) who observed that the rate of increase in body protein relative to body water is rapid during early growth but by the time the sockeye salmon reached smolt size further change was not noted. However, throughout the life of salmonids gross compositional changes do occur as a result of variations in body lipid relative to the non-lipid fraction of the fish (Buckley and Groves, 1979). This was also observed in the present study with chinook salmon and in a recent study with steelhead trout (Fagerlund et al., 1984) where both experimental and commercial diets were employed.

Imposed on the above observations is the effect of diet composition. Increasing the energy content of the diet with

herring oil had the effect of increasing body lipid. This has also been noted by other investigators (Cowey and Sargent, 1979; Watanabe, 1982). The concomitant decrease in body moisture, protein and ash is essentially independent of dietary lipid level when allowances are made for changes in body lipid. Higgs et al., (1983) cite several studies that report an inverse relationship between protein level, or protein to caloric ratio of the diet, and body lipid content. Lee and Putnam (1973) on the other hand observed that higher protein to calorie ratios were positively correlated with percentage body fat. Over the range of protein levels tested in the present study body lipid content tended to rise and subsequently fall regardless of age of the fish or total energy content of the diets (Table 32, 35).

Comparison of results between studies is complicated because of differences in species, dietary ingredients and environmental factors. Comparison of proximate constituents in the present study between fish fed diets with identical PE:TE ratios but differing diet composition revealed differences in body composition. Clearly, the moisture content and the amount of lipid and protein deposited in fish is also a function of the source of dietary non-protein energy. These changes in composition would have a direct bearing on the quality characteristics of the flesh. Current human dietary trends suggest that lean flesh is more desirable to consumers. On the other hand, high lipid retention may be advantageous to the production of hatchery-reared fish where survival in the wild environment may depend on lipid reserves (Buckley and Groves,

1979). The results of the present study demonstrate that the energy sources as well as energy concentration of the salmonid diet may be manipulated to alter the body composition of fish with respect to protein, lipid and glycogen. As mentioned previously, high liver glycogen reserves in fish destined for release could be a factor that adversely affects ocean survival.

4.5 Summary of Experiment 2

Determining the gross protein requirement for juvenile chinook salmon presents a compromise between the objectives of maximum growth rate and protein utilization efficiency. A similar dilemma was discussed by Zeitoun et al., (1976) with reference to rainbow trout who suggested the concept of an "economic protein requirement." Their method involved the application of a second order polynomial equation. It was modified and successfully applied to the results of the present study. The modification involved expressing protein requirements in terms of metabolizable energy rather than as a percentage of the dry diet. Depending on the objectives of the fish culturist, the PE:TE ratio of fish diets may be adjusted to suit a particular need. Thus producing chinook salmon smolts for release with an enhanced adaptability to a wild environment may require a diet which meets the physiological requirement for protein. Although, a successful commercial diet (OMP) contained a PE:TE ratio similar to that required for maximum growth (PE:TE = 0.55); results on growth, dietary energy retention and the efficiency of protein utilization indicate that major improvements can be made in the selection of ingredients and manufacture of commercial fish feeds. The fish farmer producing table fish may select a PE:TE ratio that maximizes returns over input costs. Where this information is not available it may be safe to select an optimal PE:TE ratio of 0.41. This value lies just within the range of optimal protein utilization efficiency.

The above PE:TE relationship was not found to be affected by

the source of non-protein energy in the diet. When the proportions of carbohydrate (supplied by glucose and hydrolyzed dextrin) and lipid (herring oil) were varied in diets of similar PE:TE ratios, growth and the efficiency of dietary energy and protein utilization were similar. However, body composition of the fish reflected their diet, the higher lipid diet fed fish having higher content of body lipid. Experiment 2 showed that by manipulating the PE:TE ratio and the proportions of carbohydrate and lipid in the diet of juvenile chinook salmon, fish can be reared to a prescribed performance and body composition.

CHAPTER 5

5.0 Conclusions

The subject of protein nutrition is very wide in scope and involves several factors. An attempt was made in this thesis to draw attention to the two most relevant aspects concerning the protein content of successful fish diets. These are the effects of quality and quantity of dietary protein on growth and efficiency of protein utilization. Along with the findings of other investigators, the conclusions drawn from this study may serve to aid in the formulation of diets for the rearing of juvenile chinook salmon. The present protein requirements for salmonids have been calculated largely from experiments in which deficient diets have been supplemented with pure proteins and amino acids to determine the level of nutrient giving maximum growth.

Experiment 1 demonstrated the extent to which growth, feed conversion and protein utilization efficiency may differ in response to both source and concentration of dietary protein. The proteins tested included a pure protein source (i.e. a mixture of vitamin-free casein and gelatin supplemented with amino acids to satisfy the known amino acid requirements for chinook salmon) and protein sources derived from the commercial fishery. The responses obtained with the latter were dependent on the nature of the raw material and processing conditions employed. In Experiment 2 the protein requirements for juvenile chinook salmon were re-evaluated

employing a freeze-dried pollock and euphausid mix (9:1), the protein source found to have the highest quality in Experiment 1. In both experiments comparisons were made between groups fed the test diets and a popular commercial salmonid diet (OMP). The results showed that there is ample scope for improvement in commercial fish feeds. The results also indicated that the casein-gelatin based protein source may not be satisfactory as a reference standard for protein quality evaluation.

The best responses in terms of growth and protein utilization were obtained by groups fed a mix (9:1) of freeze-dried pollock and euphausid. Therefore it may be concluded that the pattern of available amino acids present in this mix (FPE) is optimal for chinook salmon and preferred over that present in the purified diet. This conclusion would only be valid if the assumption were made that no other growth enhancing factors were present in FPE.

The results of the bioassays for protein quality showed that the availability to the fish of amino acids or loss of some amino acids from herring meal was drastically impaired by high drying temperatures (150°C). Mild drying temperature (75°C) was found to cause a slight reduction in protein quality compared to that of a freeze-dried meal made from the same lot of raw material. This product may be considered to be similar to that which can be manufactured by local plants equipped with steam jacketed fish meal dryers. It would seem doubtful that production of a freeze-dried meal would be commercially viable.

Definitive conclusions regarding the best methodology for protein quality bioassays can not be drawn from the results of this study. Nevertheless, methods involving regression of protein gain on protein intake are considered to be more meaningful than estimations made at a single level of protein intake. When considered along with body weight gain or body protein gain the various protein sources may be adequately compared. It is also concluded that partitioning protein intake into that used for maintenance, growth and the amount lost through exogenous excretions provides an interesting means of depicting protein utilization at different intakes of the test protein. The determination of available lysine was not found to be an accurate measure of heat damage nor a reliable predictor of the nutritive value of fishmeal. In conclusion, the use of a bioassay to define the presence of a limiting amino acid in a fish diet requires improvements in methodology. The procedures described in this thesis for biological testing of protein quality were all non-specific in that the proportions and available quantities of all essential amino acids were tested for simultaneously.

The results of Experiment 2 provided convincing evidence that the protein requirements for fish should be stated in terms of the proportion of dietary energy supplied by protein. Since the fish in this study were fed to satisfy their total energy needs, protein intake was governed by the protein energy to total energy ratio of the diet. Although the varying proportions of carbohydrate and lipid energy in the diets could

have confounded the above observations, no indication of this effect was detected under the experimental conditions of this study. Therefore, it may be concluded that juvenile chinook salmon are able to adapt to either carbohydrate or lipid as a source of metabolizable energy, within the limits of practical diet formation. This conclusion would advance the suitability of chinook salmon as a species for domestication.

It is also concluded that the empirical gross protein requirements of chinook salmon depend, not only on the physiological requirement of the fish, but also on the objectives and economic considerations of the fish culturist. This study described the use of a polynomial curve relating a growth response to dietary protein energy level, which, with the establishment of suitable confidence limits, would provide the fish nutritionist with a basis for formulating fish feeds. The range for the quantitative protein requirements for juvenile chinook salmon found in this study agrees with those stated by other investigators for carnivorous fish species.

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Table 1. ANOVA of day 42 body weights in Experiment 1.

Source	df	Mean squares	Prob.>F	Test term
Protein (PS)	4	106.33	0.0002	PSxRow
Level (PL)	2	301.82	0.005	PLxRow
PSxPL	8	9.16	0.006	PSxPLxRow
Row	1	0.0914	0.62	Residual
PSxRow	4	0.774	0.078	Residual
PLxRow	2	1.730	0.009	Residual
PSxPLxRow	8	1.309	0.0004	Residual
Residual	1770	0.3688		
Total	1799			

Table 2. Analysis of variance and covariance for slope (GR%) test of $\log_e$ body weights in Experiment 1.

Source	df	Mean squares	Prob.>F	Test term
Protein (PS)	4	12.5495	0.001	PSxRow
Level (PL)	2	29.0717	0.01	PLxRow
PSxPL	8	0.4654	0.1	PSxPLxRow
Row	1	0.02686	0.5	Residual
PSxRow	4	0.2592	0.0000	Residual
PLxRow	2	0.4192	0.0000	Residual
PSxPLxRow	8	0.2122	0.0000	Residual
Residual	5369	0.00517		
DayxPS	4	8.3211	0.05	DayxPSxPLxRow
DayxPL	2	18.2168	0.01	DayxPSxPLxRow
DayxPSxPL	14	5.1357	0.01	DayxPSxPLxRow
DayxRow	1	0.00140	0.8	Residual
DayxPSxPLxRow	29	2.5032	0.0000	Residual
Residual	5349	0.03840		
Total	5399			

Table 3. Analysis of covariance of body weight gain against protein intake (Experiment 1).

Source	df	Mean squares	Prob.>F
Protein (PS)	4	1.3426	0.0001
Row	1	0.0053	0.63
PSxRow	4	0.006	0.89
Prot. intake (PI)	1	31.5088	0.0001
PIxPS	4	0.2593	0.0001
PIxRow	1	0.0006	0.88
Error	24	0.0219	
Total	39		
Model	15	2.5297	0.0001
Error	24	0.0219	
Total	39		

Table 4. ANOVA of GFC and GEU of diets fed in Experiment 1.

Parameter		GFC		GEU	
Source	df	Mean squares	Prob.>F	Mean squares	Prob.>F
Protein (PS)	4	213.62	0.0001	478.34	0.0016
Level (PL)	2	579.18	0.014	1120.04	0.015
PSxPL	8	9.26	0.0002	17.70	0.0076
Row	1	0.584	0.25	0.66	0.63
PSxRow	4	2.26	0.017	6.90	0.11
PLxRow	2	2.73	0.017	6.25	0.15
PSxPLxRow	8	0.388		2.63	
Total	29				

Table 5. ANOVA of PER and NPR of diets fed in Experiment 1.

Parameter		PER		NPR	
Source	df	Mean squares	Prob.>F	Mean squares	Prob.>F
Protein (PS)	4	0.3097	0.0013	0.2267	0.0019
Level (PL)	2	0.0679	0.077	0.0144	0.064
PSxPL	8	0.0129	0.0004	0.0162	0.0001
Row	1	0.00348	0.057	0.00864	0.0049
PSxRow	4	0.00364	0.025	0.00373	0.0133
PLxRow	2	0.00508	0.017	0.000836	0.2921
PSxPLxRow	8	0.000715		0.000579	
Total	29				

Table 6. ANOVA of PPV of diets fed in Experiment 1.

Parameter		PPV	
Source	df	Mean squares	Prob.>F
Protein (PS)	4	1391.96	0.002
Level (PL)	2	468.87	0.097
PSxPL	8	49.61	0.04
Row	1	97.64	0.03
PSxRow	4	22.91	0.24
PLxRow	2	46.76	0.08
PSxPLxRow	8	13.45	
Total	29		

Table 7. ANOVA of NPU-1 and NPU-2 of diets fed in Experiment 1.

Parameter		NPU-1		NPU-2	
Source	df	Mean squares	Prob.>F	Mean squares	Prob.>F
Protein (PS)	4	1011.84	0.003	1372.41	0.002
Level (PL)	2	69.70	0.17	228.83	0.25
PSxPL	8	58.52	0.02	98.00	0.003
Row	1	1.03	0.77	164.39	0.004
PSxRow	4	24.54	0.20	24.04	0.15
PLxRow	2	13.81	0.39	77.75	0.016
PSxPLxRow	8	12.95		10.56	
Total	29				

Table 8. Summary of statistical analysis for the slopes of dry body weight and body protein gain. Slopes were analyzed both including and excluding data for the protein-free diet (PF)(Experiment 1).

	Treatment df	Error df	Treatment MS	Error MS	Prob.>F
Dry weight gain					
Slope (exc. PF)	4	15	0.00967	0.00109	0.0007
Slope (inc. PF)	4	25	0.01901	0.00149	0.0000
Protein gain					
Slope (exc. PF)	4	15	0.00362	0.00050	0.0019
Slope (inc. PF)	4	25	0.00786	0.00082	0.0000

Table 9. ANOVA of day 42 body weights in Experiment 2.

Source	df	Mean squares	Prob.>F	Test term
Protein (PL)	3	174.32	0.0002	PLxRow
Energy (EL)	1	13.52	0.32	ELxRow
PLxEL	3	1.142	0.80	PLxELxRow
Row	1	0.527	0.33	Residual
PLxRow	3	0.334	0.62	Residual
ELxRow	1	4.021	0.0076	Residual
PLxELxRow	3	3.317	0.0005	Residual
Residual	944	0.561		
Total	959			

Table 10. ANOVA of day 105 body weights in Experiment 2.

Source	df	Mean squares	Prob.>F	Test term
Protein (PL)	3	3561.3	0.0004	PLxRow
Energy (EL)	1	184.35	0.12	ELxRow
PLxEL	3	21.59	0.35	PLxELxRow
Row	1	58.44	0.003	Residual
PLxRow	3	13.83	0.09	Residual
ELxRow	1	6.286	0.32	Residual
PLxELxRow	3	13.07	0.11	Residual
Residual	944	6.46		
Total	959			

Table 11. Analysis of covariance for body weights in Exp. 2.

Source	df	Mean squares	Prob.>F	Test term
PL	3	3252.7	0.0005	PLxRow
EL	1	154.17	0.14	ELxRow
PLxEL	3	23.74	0.37	PLxELxRow
Row	1	57.14	0.0000	Residual
PLxRow	3	8.534	0.014	Residual
ELxRow	1	7.466	0.079	Residual
PLxELxRow	3	15.59	0.0002	Residual
Day	1	47510.	0.021	DayxRow
DayxPL	3	2336.1	0.0005	DayxPLxRow
DayxEL	1	107.29	0.106	DayxELxRow
DayxPLxEL	3	17.1	0.33	DayxPLxELxRow
DayxRow	1	50.49	0.0000	Residual
DayxPLxRow	3	10.40	0.0048	Residual
DayxELxRow	1	3.002	0.26	Residual
DayxPLxELxRow	3	9.976	0.006	Residual
Residual	5727	2.412		
Total	5758			

Table 12. Statistical analysis for the second order polynomial model to estimate protein requirements in Exp. 2.

Dietary energy		3150 kcal/kg		3950 kcal/kg	
Source	df	Mean square	Prob.>F	Mean square	Prob.>F
Model	2	0.22743	0.042	0.37356	0.093
Error	1	0.00081		0.00658	
Total	3				
Linear term	1	0.36332	0.030	0.63271	0.065
Quadratic term	1	0.09155	0.059	0.11441	0.150
Error	1	0.00081			
Total	3				

Table 13. Statistical analysis for the second order polynomial model to estimate the PE:TE requirements in Experiment 2.

Source	df	Mean square	Prob.>F
Model	2	0.57562	0.0013
Error	5	0.01715	
Total	7		
Linear term	1	0.90859	0.0008
Quadratic term	1	0.24265	0.013
Error	5	0.01715	
Total	7		

Table 14. ANOVA of GFC and GEU in Experiment 2.

Parameter	GFC		GEU	
Source	df	Mean squares	df	Mean squares
Protein (PL)	3	204.23**	3	525.22**
Energy (EL)	1	32.29	1	83.25
PLxEL	3	6.003	3	0.40
Row	1	0.360	1	1.438
PLxRow	3	0.708	3	2.451
ELxRow	1	1.183	1	2.495
PLxELxRow	3	1.596	3	5.033
Total	15		15	

Level of significance ** = 0.01.

Table 15. ANOVA of PER and PPV in Experiment 2.

Parameter	PER		PPV	
Source	df	Mean square	df	Mean square
Protein (PL)	3	0.0174*	3	36.136*
Energy (EL)	1	0.0302	1	25.702
PLxEL	3	0.00202	3	3.223
Row	1	0.000405	1	4.852
PLxRow	3	0.000769	3	1.925
ELxRow	1	0.00132	1	7.930
PLxELxRow	3	0.00127	3	5.738
Total	15		15	

Level of significance * = 0.05.

Table 16. ANOVA of NPU-2 in Experiment 2.

Parameter		NPU-2
Source	df	Mean square
Protein (PL)	3	103.36 **
Energy (EL)	1	100.11
PLxEL	3	2.089
Row	1	10.652
PLxRow	3	3.269
ELxRow	1	10.115
PLxELxRow	3	9.656
Total	15	

Level of significance ** = 0.01.

Table 17. Table of mean squares for fish body moisture, ash, lipid and protein at day 42 (Experiment 2).

Source	df	Mean squares			
		Moisture	Ash	Lipid	Protein
Protein (PL)	3	1.2915**	0.4367	2.355	7.368
Energy (EL)	1	3.8025**	1.6448	136.422**	59.714*
PLxEL	3	0.2934	0.0403	3.713	9.661
Row	1	0.4356	0.1278	0.483	50.730
PLxELxRow	7	0.1473	0.1325	2.527	10.331
Total	15				

Level of significance * = 0.05, ** = 0.01.

Table 18. Table of mean squares for fish body moisture, ash, lipid and protein at day 105 (Experiment 2).

Source	df	Mean squares			
		Moisture	Ash	Lipid	Protein
Protein (PL)	3	4.0058**	2.1109	17.8373**	58.782**
Energy (EL)	1	7.6591**	2.9241	102.8703**	238.780**
PLxEL	3	0.3493	0.9528	3.7150	9.959
Row	1	0.6931	0.3080	0.1871	7.798
PLxELxRow	7	0.1436	1.0499	1.4302	3.868
Total	15				

Level of significance ** = 0.01.