A STUDY OF THE PROTEIN QUALITY OF SHRIMP MEAL AND KRILL MEAL IN DIETS FED TO RAINBOW TROUT

by

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ABSTRACT

Rainbow trout of approximately 2.5 grams were fed diets in which herring meal was the major protein source. Shrimp meal, freeze-dried krill meal, cooked krill meal and soybean were incorporated into the diets at two levels of replacement - 11% and 22% of total protein. The total protein content of these diets was 28%. An additional series of diets containing 36% protein was fed where 17% of the protein was replaced by test ingredients.

The diets containing shrimp meal and freeze-dried krill meal produced the best growth responses and showed most efficient feed conversion, protein utilization and energy utilization. Soybean meal and cooked krill meal produced favourable results at the low level of inclusion but at the higher level growth response and feed utilization were slightly depressed.

The possibility that chitin can be used to spare protein and energy is also discussed.

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INTRODUCTION

Krill, or euphausiids, (Euphausia sp.) are members of the Class Crustacea and comprise a significant portion of the diet of some salmonids in the wild. As such, they must provide a complete nutritional profile for these fish. Euphausiids are, indeed, an excellent source of protein and are also high in lipids - more so at certain times of year than others. In addition they are sources of carotenoid pigments and vitamins essential to the fish.

Because these organisms are such a valuable food source for salmonids, it is natural to assume that fish contain the enzymes chitinase and N-acetyl glucosaminidase (NAGase) necessary to break down the chitinous exoskeleton thus leaving the body contents available for digestion and absorption.

One nutrient type which is not found in great abundance in krill is any form of carbohydrate. In most terrestrial animals carbohydrates are a major source of dietary energy. Thus fish must have another source of energy in their diet. It is a well known fact that fish, especially carnivorous species, preferentially utilize protein as an energy source. This is one of the reasons why costs are elevated in the culture of fish as compared to terrestrial animals. Protein is a more expensive ingredient than carbohydrate.

One of the objectives in the field of fish culture at the present time is the formulation of diets that are economical for the fish culturist to use and yet will contain a sufficient amount of high quality protein such that superior growth

rates will be attained. If this objective is to be accomplished one of three events must occur. Either 1) strains of salmonids must be developed which can utilize carbohydrates efficiently; 2) an optimum protein or balance of proteins must be attained which would also incorporate an amount of carbohydrate that the fish could utilize; 3) further studies must be carried out on other components in the natural salmonid diet from which they may derive some benefit.

One material of considerable interest in this regard is chitin. Chitin is the main constituent in the exoskeletons of the Class Crustacea. It provides these animals with a tough exterior shell, thus rendering them impermeable to water, as well as potentially hazardous elements in their environment. In addition, it provides a means of protection from predators.

Chitin is a polymer of repeating N-acetylglucosamine units joined by β1-4 glycosidic linkages. Because of its structure this compound is extremely resistant to degradation and specific enzymes are required for breakdown. Thus chitin can only be utilized by animals in whose gut the necessary enzymes are present. The chitin may then be available as a form of energy. The end result would be that the chitin would have a protein sparing effect, thus the protein that may have been used as an energy source could now be utilized to furnish amino acids for protein synthesis. In addition, the amino groups made available by chitin hydrolysis may further enhance its protein sparing capacity as some of these may also be available for protein metabolism.

Information is limited as to the utilization of chitin by fish but the presence of certain chitinolytic enzymes in chitin ingesting species has been verified a number of times. The chitinase activity detected in the fish examined was produced either by the fish itself or by micro-organisms present in the gut. In some cases though it appears that this enzyme activity is a combination of both these sources. This study was conducted primarily to assess the overall protein quality of the test ingredients containing chitin. In addition, the possibility that chitin is utilized by the rainbow trout was investigated.

LITERATURE REVIEW

History of Aquaculture

For numerous centuries fish have been cultured for food and bred in captivity. The Egyptians have cultured tilapia since before 2000 B.C. and various Asian cultures have maintained populations of carp, milkfish, tilapia, barb and other species for generations (Calaprice, 1976). To date, milkfish are the largest pond-cultured fish in the world and account for 175,000 hectares of ponds in Asia (Slinger and Cho, 1978). Monoculture is prevalent in most areas, but Southeast Asian nations also practise polyculture to a certain extent. According to habit, certain species are divided into bottom, mid-water and surface dwellers, and advantage is thus taken of space, available food and certain beneficial action occurring in the ecology of the species involved (Lam, 1982). Other methods of culture such as heteroculture or co-culturing vertebrates with invertebrates (e.g. milkfish with prawn), and integrated farming or co-culturing fish, livestock and crops are also commonly practised (Lam, 1982). Thus aquaculture is a very firmly based tradition in the lifestyle of the populations of these countries and the potential for fulfilling a large portion of the protein requirement of the population can be realized. It is estimated that already approximately one third of the world's animal protein supply for human consumption comes from fish (Slinger and Cho, 1976). Only one tenth of this total is accounted for by cultural techniques, but the Food and Agriculture Organization (FAO) suggests that the contribution of farming to fish protein production will exceed seven million tonnes by the year 2000 (Slinger and Cho, 1978).

With the realization that the present stocks of wild fish cannot sustain the growing demands of the population, many western nations are also expressing an interest in aquaculture. Overexploitation and pollution are causing a decrease in harvestable stock while the requirement of the growing population increases. Thus, when commercial fishing reaches a plateau, and perhaps the plateau has already been reached, the burden of supplying fish protein will necessarily turn to fish farming.

European countries such as Denmark and Norway have had considerable success raising Atlantic salmon (Salmo salar) and rainbow trout (Salmo gairdneri). Scotland also produces salmon and Munich produces carp in sewage fish ponds. North America has recently begun to express some interest in fish culture, but in terms of development, the industry is proceeding less rapidly than other food sources (NRC, 1978). Nevertheless, catfish farming is practised on an increasing scale in the southern United States. Trout are raised in many of the northern states and interest in both salmon and rainbow trout culture is increasing in the northwestern states.

In Canada, the culture of fish is somewhat more limited because of climate, which places restrictions on the species amenable to culture and the growth rate of these species. Nevertheless, while not occurring on a large commercial scale, a great deal of progress is being made and the industry is expanding continuously.

The maritime provinces are mainly involved in raising Atlantic salmon, as well as 'tuna finishing' in which tuna are fed mackerel and trash fish until a certain weight is attained. They are subsequently exported (Slinger and Cho, 1978). Manitoba and Saskatchewan produce a significant amount of rainbow trout in the many lakes throughout these two provinces. The lakes are stocked with fingerlings soon after the spring thaw. Throughout the summer and fall they feed on zooplankton and other available nutrients. Just prior to winter freeze-up the trout are harvested having attained 'pan-size' - approximately 312-340 grams. In addition, arctic char (Salvelinus alpinus) has proven to be amenable to culture in Manitoba and current studies of this species may lead to large scale production (Tabachek, personal communication). Lake stocking also occurs to a certain extent in British Columbia, but much of the emphasis on fish culture in this province at the present time is directed towards raising Pacific salmon. Again, the commercial aspect remains on a small scale but has proven itself to be an economically feasible operation.

Increased public awareness and a steady decline of readily available fish protein are perhaps two major factors necessary to encourage the development of aquaculture enterprises (Larkin, 1982). Until this happens however, work must be continued on development of efficient techniques so that when supply is required, demand can be met.

Requirements for Salmonid Culture

In order for an aquaculture operation to be economically feasible, a number of criteria must be met. Growth rate, for example is a major factor to be considered when investigating suitable species for culture. There is a certain amount of variation among species but equally important are elements which control growth. Water temperature, photoperiod, nutrition and dissolved oxygen levels are a few of the conditions which can be manipulated to enhance growth (Novotny, 1975).

Disease resistance of fish is a very critical aspect of fish culture. The high density conditions in aquaculture make the environment very favourable to pathogens, which may enhance infectious disease incidence (Kennedy, 1978). Large scale mortality has occurred due to lack of knowledge in this area. Although pathogens are often present, if the conditions are not overcrowded and the fish are healthy they will remain resistant and may develop a certain degree of immunity. If the fish become stressed - whether this is a result of nutritional inadequacies, water conditions or transfer to sea-water in the case of Pacific salmon - their resistance may be reduced and the disease will manifest itself.

Nutrition is one of the most important aspects of fish culture. The type of diet and amount fed to fish determines growth rate, feed conversion efficiency, disease resistance and economic feasibility of a culture operation. Thus the aim of a fish culture project is to attain the maximum growth rate of the fish, on a

diet which fulfills the nutritional requirements of the animal, for as little cost as possible. Considerable research has been conducted regarding the nutritional requirements of salmonids with the result that values for essential amino acids, essential fatty acids, vitamins and minerals have been compiled (NRC, 1981). Progress is also being made in improving the quality of certain feeds with respect to increased availability of nutrients and reduction of enzyme inhibitors and toxins which may be present. Improvements of this sort may then result in better palatability and increased intake. In addition, greater knowledge on the metabolism of fish, their digestive physiology and hormonal control, will aid in the development of optimum diets.

Other factors are also involved in a culture operation such as environmental conditions, ease of handling of the species being considered, type of enclosure and achievable market price, but as the main topic of this thesis pertains to nutrition it will suffice to mention them only briefly.

Nutritional Requirements of Salmonids

The nutrient requirements of salmonid fish are qualitatively similar to those in higher animals in that they require in their diet certain levels of protein and lipid as well as various vitamins, minerals and a certain amount of energy. When dealing with actual amounts of these nutrients in the diet, however, quantities vary with respect to such environmental factors as water temperature

and chemistry, age of fish and other physiological factors (Hastings, 1976). With the expansion of fish culture, and increased demand for dietary information a number of guidelines have been established as well as various 'open formula' diets (NRC, 1981).

Protein Requirements

Because all salmonid species are carnivores, their gross protein requirement is somewhat higher than that of omnivorous and herbivorous fish. The amount of protein required throughout the life cycle generally ranges from 35% - 50% of the diet (NRC, 1981). The upper range of these values reflects the digestible protein required by the initial-feeding fry for maximum growth. Upon reaching six to eight weeks of age this requirement decreases to 40% of the diet and by the time they are yearlings the protein required is further reduced to 35% (NRC, 1981). The gross protein requirement has also been found to vary with temperature in some species. The above values were determined based on fish raised at standard environmental temperature. However, DeLong et al. (1958) performed a study on chinook salmon (Oncorhyncus tshawytscha) and the protein requirement of these fish was found to be 50% at 15°C but only 40% when the fish were raised at 7°C. Conversely though, rainbow trout showed no difference in growth when raised on protein levels of 35, 40 and 45 per cent and at water temperatures of 9, 12, 15 and 18°C (Slinger et al., 1977). Nevertheless, in the experiment by Slinger, fish at 18°C did consume greater quantities of the lower protein diets thus increasing their overall protein intake to satisfy requirements.

Maximum protein levels in the diet must be monitored carefully for the reason that if excess amounts are provided the fish will begin to metabolize protein as an energy source. Although this process imposes no stress on the metabolism of the fish, it is economically inefficient as protein is the most expensive dietary component.

In general, protein utilization is most efficient when fish consume diets of reduced protein content providing a sufficient energy source is included (Halver, 1976).

Amino Acid Requirements

All species of fish studied to date require the same ten indispensable amino acids, which in turn are the same as those required by other animals (Ketola, 1982). These ten amino acids are as follows: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. It is believed that tyrosine spares part of the phenylalanine requirement and cystine spares methionine (NRC, 1981). Quantitative requirements for essential amino acids appear to vary between species but by and large, amongst the salmonid species these requirements are the same (Ketola, 1982). Data for both chinook salmon and coho salmon (O. kisutch) are as indicated below. In order to provide some basis for comparison essential amino acid requirements for the rat have also been included. Note that optimum protein levels in the diet for chinook salmon are 40% and for the rat are 13.2% (Cowey and Sargent, 1979).

	Fish	Rat
Arginine	6.0[a]	1.0
Histidine	1.8	2.1
Isoleucine	2.2	3.9
Leucine	3.9	4.5
Lysine	5.0	5.4
Methionine	4.0	3.0
Phenylalanine	5.1	5.3
Threonine	2.2	3.1
Tryptophan	0.5	1.0
Valine	3.2	3.1

(Mertz, 1972)

[a] Values are expressed as percent of protein.

These values are in the same range as those expressed by Ketola (1982).

When considering the essential amino acid requirements one must also allow for the availability of these amino acids in the feed. As mentioned previously, phenylalanine and methionine can be spared by tyrosine and cystine respectively. Availability of lysine often poses a problem after processing of feeds, as processing may result in a reaction between the ε-amino group of lysine and the aldehyde groups in sugars present in the feedstuff, thus rendering the lysine biologically unavailable . In many plant feedstuffs lysine is often the first limiting amino acid. Tryptophan is also easily destroyed if acidic conditions prevail in the storage of dietary ingredients (Halver, 1976).

Amino acid balance is yet another factor involved when discussing amino acid requirements. For example maximum growth is obtained when the ratio of isoleucine to leucine is 1:2, however growth is inhibited once this ratio exceeds 1:3 (NRC, 1973). Decreased growth was also found to occur when leucine exceeded isoleucine by a three to one ratio (Halver, 1976).

Salmonids appear able to utilize free amino acids with various degrees of efficiency thus a protein source deficient in certain amino acids can be supplemented to the extent that optimum growth rates will be attainable.

Lipid Requirements

Dietary lipids are required in the diet of animals both as a source of metabolic energy and to provide the fatty acids essential to maintain structure and integrity of cellular membranes. In addition, they serve as carriers of the fat soluble vitamins and other lipid soluble materials (e.g. pigments).

In the wild, salmonid diets can contain as little as two to three per cent fat or as high as 20 per cent depending on the season (NRC, 1973). In the formulation of diets for cultured salmonids there have been no stringent values described such as those for protein. Workers in this area do agree, however, that sufficient amounts of lipids in the diet can minimize the use of the more costly protein as an energy source (NRC, 1981). This is particularly applicable when considering the carnivorous salmonids, since their ability to utilize carbohydrates as an energy source is rather limited. Studies have demonstrated that when diets are fed with a lipid content ranging from 15-20 per cent of the diet and protein content of 35 per cent an optimum balance is attained, however lipid levels below 15 percent resulted in decreased weight gain (Takeuchi et al., 1978). This study also revealed that all levels of lipid above 10 per cent resulted in some degree of greater protein utilization. A further advantage of most lipids incorporated into fish diets is that they are 85-90 per cent digestible (Hastings, 1976).

Lipids also serve to fulfill the essential fatty acid requirements of all animals. Because salmonid fish encounter colder water temperatures the degree of unsaturation of their fatty acids increases. The lower melting point of the longer chain polyunsaturated fatty acids serves to maintain the fluidity of the membranes and cellular structures (NRC, 1981). Thus the dietary requirement for fatty acids, and in turn, the fatty acid profile of the carcass of the fish differs quite markedly from that of terrestrial animals. The fatty acids of terrestrial animals are primarily of shorter chain length and have a higher degree of saturation. They are largely of the oleic (ω9) family or linoleic (ω6) family. In fish, the linoleic series is replaced by the linolenic (ω3) acids although smaller quantities of ω6 polyunsaturated fatty acids are present. If sufficient amounts of linolenic acid (18:3ω3) are present in the diet the fish are able to chain elongate to higher polyunsaturated fatty acids and thus are successful in preventing signs of fatty acid deficiency. These signs include low feed efficiency, high mortality and swollen, pale livers. Conversely dietary linoleic acid (18:2ω6) supplementation has been unable to prevent these signs from manifesting themselves (Cowey and Sargent, 1979). Ability to chain elongate and desaturate varies between freshwater carnivores and marine carnivores (Cowey and Sargent, 1979). This difference is apparently a reflection of the type of diet normally consumed by the fish in the wild. Freshwater fish (e.g. trout species), consume more food that is of terrestrial origin than marine fish. As a result they ingest greater quantities of shorter chain fatty acids - both of the linoleic and linolenic series. In order to fulfill their requirement for long chain polyunsaturates their metabolism is more efficient at desaturating and elongating than that of marine fish (e.g. salmon), which consume large amounts of

marine zooplankton. The predominant polyunsaturated fatty acids of zooplankton are 20:5ω3 and 22:6ω3 (Sargent et al., 1979) and this profile, in turn, is reflected in the lipid composition of salmon, herring and other marine fish which consume copious quantities of zooplankton (Cowey and Sargent, 1972).

Carbohydrates

The carnivorous salmonids have few sources of carbohydrates in their natural diet and, as such, are rather inefficient users of this inexpensive form of energy. Nevertheless, recent studies on rainbow trout indicate that there is a limited capacity to adapt to increased levels of carbohydrate in the diet and thereby spare dietary protein (Hilton and Atkinson, 1982).

Carbohydrate metabolism in salmonids resembles that of a diabetic animal. Studies have shown that these fish lack the ability to control blood glucose concentration closely. This is partially due to lack of glucose phosphorylating capacity in the liver when glucose intake is increased (Cowey et al., 1977a). In addition, studies by Thorpe and Ince (1976) have inferred that insulin release in the trout is controlled by amino acid levels and not blood glucose levels as in mammals. Hence, a diet high in carbohydrates would not stimulate the release of insulin and consequently blood glucose levels would remain abnormally high leading to detrimental effects on the fish. Conversely, a high protein diet would induce the release of insulin and normal blood glucose levels would also be maintained (Cowey et al., 1977a).

Carbohydrate digesting enzymes are produced, and simple sugars follow the same metabolic pathways in fish as in other animals, but carnivorous fish lack the ability to mobilize liver glycogen at a rapid rate, and during starvation the oxidation of substrates other than glucose takes precedence over the mobilization of glycogen (Cowey and Sargent, 1979; Hickling, 1982). It has also been noted that when glucose intake of rainbow trout is increased there is no corresponding increase in the activity of glucose phosphorylating capacity in the liver. Peripheral tissues also fail to respond by enhanced glucose uptake which normally occurs in other animals when glucose levels are elevated.

Thus, no carbohydrate requirement has been established for salmonids largely because they do not have a need for this type of nutrient. But with a suitable balance of protein and carbohydrate in the diet, a certain amount can be utilized. Studies have indicated that levels of dextrin up to 25% of the diet are effective as an energy source but higher levels result in decreased growth and feed conversion (NRC, 1981).

Vitamins and Minerals

To discuss the requirement for each individual vitamin and mineral required by salmonids is not necessary for the context of this thesis. However, when discussing nutrient requirements of salmonids these must be included.

Eleven water-soluble vitamins and four fat-soluble vitamins are known to be required by salmonids (Halver, 1982). The water-soluble vitamins are grouped into the eight B-complex vitamins, and the macrovitamins L-ascorbic acid, myo-inositol and choline. The fat-soluble group includes vitamins A, D, E and K.

As with some of the other nutrients mentioned, quantitative requirements for vitamins may vary with respect to the age of the fish, water conditions, stocking density and dietary conditions under which the animals are being raised (Halver, 1976). The dietary ingredients must be carefully considered in order to prevent the tying up of these nutrients thus rendering them biologically unavailable to the fish. In addition, because of the intensive conditions under which the fish are reared, and the low level of natural food, the importance of fulfilling these dietary requirements is amplified.

The minerals required by salmonids are somewhat more difficult to ascertain. Most minerals are required only in small amounts, and often these can be obtained via exchange across the gill membrane or absorption from the gut. For example, it is known that the requirement for calcium, cobalt, iron, magnesium, potassium, sodium and zinc can be partially fulfilled directly from the water (NRC, 1981). The degree of fulfillment depends on the mineral content of the water and the efficiency with which the fish can absorb the nutrients. In spite of this ability certain minerals must be provided in the diet although to date only seven dietary minerals have been shown to be required or utilized by salmonids (NRC, 1981). These minerals are: calcium, phosphorus (as phosphate), iron, iodine, selenium, magnesium and zinc.

Care must also be taken to ascertain that mineral ratios are kept in balance. For example sodium : potassium balance is important especially in marine fish and in anadromous salmonid species (Halver, 1976). Calcium and phosphorus are often considered together because the metabolism of both of these minerals is quite closely connected. In general a calcium : phosphorus ratio of 1:1 is considered optimum. Other ratios which must be taken into consideration are those involving the divalent cations such as Ca, Zn, Mg. Excessive levels of any one of these in the diet may inhibit the absorption of others and could result in signs of deficiency. Salmonid diets, in general, include some form of vitamin/mineral premix as a supplement. Thus if one of the feedstuffs being included in the diet is deficient in a certain vitamin or mineral this is usually compensated for in the pre-mix.

Energy

The last nutritional requirement to be considered in this brief compilation is energy. This is, indeed, the major requirement for all animals and most components of the diet contribute some amount of energy to the animal -with the exception of minerals, fibre and water. Energy is required for both growth and maintenance, and the major objective of the fish culturist is to divert as much energy as possible into growth. Protein, fat and carbohydrate each contribute different amounts of energy to the fish, but perhaps the major factor in determining the overall energy contributed by a certain diet is the digestibility of the individual ingredients. In contrast though, the proportion in which the feedstuffs occur in the diet will influence the energy available due to the

level and type of lipid and the biological value of the total protein (Cho et al., 1982). Certain feed ingredients result in 80 per cent of dietary energy being lost in the feces. These materials are usually high in fiber but have a low lipid content. For fish, feeds which are most efficient in providing energy are those which are high in protein and lipid. As mentioned previously these are the two types of nutrients which fish utilize most readily as energy sources. In general foods which contain less the 3000 kcal/kg are considered low in gross feed efficiency. A guideline in determining energy content sufficient for a diet is to allow eight kcal/g protein in the diet (Halver, 1976). If this value is attained and an adequate balance of nutrients is maintained there should be sufficient energy available to the fish to spare the protein for tissue synthesis. Another concept often utilized in determining the gross energy requirements of fish is to determine the number of kilocalories required to produce one kilogram of fish flesh. If the conversion of energy to flesh is two or greater it is generally agreed that the energy requirements are being met.

The ratio of protein to energy is an important consideration because of the preference of these animals to utilize protein for energy. An increase in the ratio of digestible energy : protein leads to an increase in lipid deposition, and protein efficiency ratio (BW gain/protein intake) has proven to be negatively correlated with the ratio of dietary protein : energy (Cowey and Sargent, 1979). Thus this ratio must be taken into account when determining optimum energy levels in the salmonid diet.

Protein Sources

When formulating a salmonid diet the most important factor in terms of economics and growth rate of the fish is the nature of the protein source, or sources. The protein supplements incorporated must be such that they fulfill the amino acid requirements of the fish. Since amino acid profiles will vary with different types of protein the biological value will also vary. Thus supplements must be combined in such a manner that amino acid deficiencies in one ingredient will be compensated for by another.

The high biological value of fish meal has led to heavy dependence on it as a protein source to date. But as a result of its high cost, insufficient supply, and variable quality, research has recently begun to place greater emphasis on the substitution of fish meal with more economical and more readily available animal and plant protein supplements. Numerous attempts have resulted in drastic declines in growth rate but optimum levels of inclusion of alternate protein sources are gradually being developed and there is an ever growing number of protein sources presently being used in fish feeds. Soybean meal, cottonseed meal and various animal by-products are typical examples. Most of these protein supplements have certain disadvantages. However if they are not incorporated into the diet in excessive amounts, maximum growth rates can still be achieved. One example of such a diet is the Abernathy diet which utilizes fish meal as its main protein source but also incorporates dried milk products,

wheat germ meal and cottonseed meal into the formula (Fowler and Burrows, 1971). The Oregon Moist Pellet diet - another commonly used diet on the Pacific coast incorporates tuna viscera, cottonseed meal, dried whey and wheat germ meal (Higgs et al., 1982).

Spinelli (1979) examined several unconventional feedstuffs categorizing them into vegetable sources, animal sources and potential sources which have not yet been commercialized. The general conclusion of this study was that many of the animal sources, e.g. fish silage, shrimp and crab meal, poultry by-products, were utilizable although there was some question as to the availability of some of the amino acids. The vegetable sources tended to have higher amounts of fiber and carbohydrates as well as certain natural toxins such as phytates and glucosinolates. Nevertheless, with better processing technology many of these ingredients are being utilized in commercial aquaculture diets. Some of the potential protein substitutes range from single cell proteins and zooplankton to recycled wastes and vegetable silage. Of the 'unconventional' feedstuffs he considered, Spinelli's conclusion was that krill are the most potentially exploitable protein source as a result of their great abundance and their role in the natural diet of salmonids.

Krill

Ecology

Members of the Order Euphausiacea, or krill as they are commonly called, are distributed throughout the oceans of the world. This crustacean order is comprised of 85 known species, the majority of which inhabit the epipelagic and

mesopelagic regions of the oceans. Their habit of congregating in vast quantities at the water surface and their diurnal vertical migration facilitates their harvest by fisheries and renders them invaluable as a food source to numerous marine animals. For example, euphausiids are widely recognized as the major source of nutrients for baleen whales (Mauchline, 1969). They are also the staple of such species as herring (Clupea harengus) (Sargent et al., 1979), holocephalans (Fange et al., 1979) as well as salmonids, and recently a surge of interest has arisen into the prospect of utilizing these organisms as a human protein source.

Because euphausiids lack sufficient swimming ability to combat the oceanic currents their distribution is largely a function of these currents as well as upwelling areas. The main centres of distribution tend to be contained in a specific water mass which can often be identified by coastal region, temperature or certain hydrographic conditions (Mauchline and Fisher, 1969). Some species are distributed circumpolarly but in the case of Euphausia pacifica, one of our abundant local species, the latitudinal range is from 35^{o}-50^{o}N with most occurring in latitudes greater than 40^{o}N. Georgia Strait is particularly abundant in marine zooplankton, including euphausiids, largely because of the frontal zones and upwelling plumes which make it very rich in nutrients. This high nutrient supply also contributes to the development of high standing stock on the outer coast of Vancouver Island (Mackas et al., 1980).

The abundance of zooplankton does vary seasonally as a result of their ontogenetic migration as well as in response to seasonal variation in phytoplankton and inorganic nutrients. Overall zooplankton biomass in the north

Pacific is somewhat different from that in other oceanic regions however, in that seasonal variation is not dependent on abundance of phytoplankton. Although the total biomass of zooplankton reaches a peak in the summer months (Parsons et al., 1977), when euphausiids in particular were observed, biomass variation was found to be very small and there was little or no seasonal pattern (Mauchline, 1980). Thus in terms of availability as a feed source for local aquaculture operations euphausiids are abundant year round although fishery regulations do limit the harvest to certain times of year.

Attempts to culture euphausiids from egg to adult have met with a rather limited success rate. The major difficulty exists with hatching and early larval stages (Ross, 1981). Once the larvae reach the furcilia stages (late larval period) survival through the juvenile stages to adulthood has been significantly increased.

As a result of the lack of study in this area to date, if euphausiids are to be utilized as a feedstuff in the foreseeable future the emphasis will lie in harvest from the wild. However with increasing interest in this fishery and poor success rates with previous fisheries we would do well to restrict the exploitation of this food source.

Krill as a Feedstuff

The biochemical composition of krill has been studied extensively with respect to oceanographic purposes as well as aims towards fulfilling nutritional requirements. In addition, numerous studies have been performed utilizing krill as a feedstuff in certain fish diets.

A certain amount of seasonal variation does occur in the biochemical composition of krill. Clarke (1980) analyzed individuals of the species Euphausia superba at various seasons and found lipid content to be the most variable fraction. This was mainly the result of gravid females whose ovaries comprise up to 60 per cent of the krill's total lipid. Comparison of spent females indicated that these animals lost 54 per cent of the lipid at spawning. Another factor to be considered though, is the accumulation of lipid in males and immature animals as winter approaches.

Most proximate analyses on euphausiid species state protein values in the range or 65-80 per cent of dry matter (Nakai, 1942; Suyama et al., 1965; Ikeda, 1972) with lipid contents ranging between 7 per cent and 25 per cent. The factors above, no doubt, play a role in this variation but in addition, lipid content has been found to vary in relation to latitude. Those euphausiids inhabiting arctic latitudes were found to contain a significantly greater amount of lipid than those in more temperate-boreal waters (Falk-Petersen, 1981). Thus the lipid composition is also a function of the particular species being analyzed.

Carbohydrate content is negligible and ash content ranges from 10-15 per cent. If a chitin analysis is performed the value is generally between two per cent and five per cent.

Yurkowski and Tabachek (1979) performed proximate analyses on numerous freshwater organisms, among them several species of zooplankton and determined that there was sufficient protein present to satisfy the salmonid require-

ment as well as abundant lipid for energy to spare the amino acids for protein synthesis.

Amino acid and fatty acid analyses have also been performed on euphausiids. Suyama and his colleagues (1965) determined the amino acid profile on whole Euphausia pacifica and when this is compared with the essential amino acid requirements of chinook salmon fingerlings one can observe that the amino acid content of these euphausiids easily satisfies the requirements.

	Chinook Salmon Fingerling	E. pacifica
Arginine	6.0*	5.95
Histidine	1.8	2.22
Isoleucine	2.2	5.16
Leucine	3.9	7.83
Lysine	5.0	7.84
Methionine	4.0	3.25
Phenylalanine	5.1	6.50
Threonine	2.2	4.83
Tryptophan	0.5	1.57
Valine	3.2	5.19
	(NRC, 1981)	(Suyama et al. 1965)

* Expressed as percent of protein.

As mentioned previously, the fatty acid composition of many animals often reflects the type of diet which they consume. Thus one can predict that because the natural food of many salmonids is euphausiids, the fatty acid profiles of euphausiids and salmonids will closely resemble each other. In fact this is partially true, but some differences do exist. For example, Ackman and his colleagues (1970) performed lipid and fatty acid analyses on two species of North Atlantic euphausiids, Meganyctiphanes norvegica and Thysanoessa inermis. They

found the phospholipid fatty acid composition to be generally similar to that of other marine species of fish and shellfish. However, investigation of the polyunsaturated fatty acid composition of these krill showed that they were relatively low in the essential C_{18} acids. However these euphausiids were rich in highly unsaturated fatty acids such as 20:5ω3 and 22:6ω3. These fatty acids are of the essential linolenic acid group (ω3) and are believed to be more effective than 18:3 3 in satisfying essential fatty acid requirements because fish have the ability to saturate. The presence of these compounds thus prevents essential fatty acid deficiency signs from being manifested.

A number of growth trials using krill, as well as other types of zooplankton have been performed with varying degrees of success. Brett (1971), using young sockeye salmon (Oncorhyncus nerka), fed a number of different diets at different levels, and included in the regime was a diet consisting solely of marine zooplankton plus a vitamin supplement. Low conversion efficiencies and growth rates were obtained on this diet. This was attributed to the frozen state of the organisms which had resulted in ice-puncturing of the exoskeletons and subsequent leaching of nutrients.

Other studies have been performed with a considerably greater degree of success. Euphausia superba, frozen in plates were fed to rainbow trout (Salmo gairdneri) and proved to sustain a better growth rate than a commercial trout pellet (Grave et al., 1979). Koops and his colleagues (1979) performed a series of experiments whereby a diet containing fishmeal and poultry by-product mixture

was fed to rainbow trout. When the fishmeal was replaced by krillmeal the krill-fed fish did not differ markedly from the control group. Using krillmeal as the sole protein source resulted in an initial growth retardation but by the end of the experiment (105 days) the efficiency of utilization had increased such that there was no difference in body weight or feed efficiency between the krill group and the control fish.

An organoleptic test was also performed at the termination of this series of experiments. The krill-fed fish were more intensely coloured than the control fish as a result of the carotenoid deposition and were found to be of excellent quality when rated for colour, taste, smell and consistency.

Soybean Meal

Numerous attempts have been made to replace animal proteins with plant proteins in fish diets as mentioned in previous sections of this review. Various results have been obtained depending on the species used and the level of replacement. Complete replacement of fishmeal with soybean meal in all species studied has resulted in marked growth depressions and low protein efficiency ratios (Nose, 1971; Koops et al., 1976; Fowler, 1980; Viola et al., 1981). A study involving carp (Viola et al., 1981) showed that supplements of methionine, lysine and fish oil were adequate to bring performance up to the control fishmeal diet. Rainbow trout fed soybean meal as the sole source of protein lost weight (Nose, 1971; Koops et al., 1976) and chinook and coho salmon experiments obtained similar results (Fowler, 1980).

Partial replacement of fishmeal with soybean meal has yielded somewhat more favourable results. For example, when soybean meal and herring meal each contributed 50 per cent of the total protein in a rainbow trout diet, no difference in performance was observed either in growth or digestibility (Cho et al., 1974). Koops et al. (1976) performed a similar experiment replacing 25 per cent of the fishmeal protein with soybean protein. Test diets contained protein levels of 47 per cent and 39 per cent and no significant differences were detected in either feed conversion or growth rate.

Higher protein efficiency ratios were obtained in the aforementioned carp study and this was attributed to higher amylase activity found in carp enabling them to meet a greater portion of their energy requirement with carbohydrate. This result emphasizes the difference between carnivorous and herbivorous/omnivorous species in ability to utilize carbohydrates.

Chitin

Chitin is a widely distributed compound in both plant and animal kingdoms. As discussed in a previous section it is a major component in the exoskeletons of crustaceans - specifically krill. Bearing this in mind its breakdown and possible role as a nutrient will be discussed in the following section.

Chitin is the major constituent in the carapace of krill, in fact it constitutes 60 to 80 per cent of the dry organic matter of the cuticle (Jeuniaux, 1978). The remainder is protein, some wax esters and a significant amount of $CaCO_3$, phosphorus and other minerals (Ole et al., 1978).

The structure and chemical properties of chitin are very similar to cellulose. Where cellulose is a polymer of repeating glucose units, chitin is composed of repeating N-acetyl-D-glucosamine units.

Both polymers are linear molecules with β,1-4 linkages between the individual units. Chitin is even more insoluble and unreactive than cellulose, but can be broken down enzymatically. Complete enzymatic hydrolysis of chitin results in molecules of N-acetylglucosamine. Two enzymes are involved in this, chitinase (endo-β -N-acetylglucosaminidase) and chitobiase (exo-N-acetyl-β -D-glucosaminidase or NAGase). The action of chitinase is to split the molecule into dimers and trimers. This process is followed by the chitobiase which further splits the molecule into monosaccharide units (Fange et al., 1979).

The presence of chitinolytic enzymes in vertebrates was not even suspected until 1961 when Jeuniaux performed a series of assays on various species of fish, reptiles and mammals. He discovered extensive chitinase activity in the digestive tracts and glands of all insectivorous or planktivorous species studied. Since that time a certain amount of work has been done regarding the origin of these chitinolytic enzymes.

Currently uncertainty exists as to the exact origin of this enzyme activity in fish. Studies have been performed to determine whether the action is due to symbiotic bacteria performing a role analogous to that of the cellulytic bacteria in ruminants, or whether chitin-ingesting animals are able to secrete their own

chitinolytic enzymes. Chitin-decomposing bacteria have been isolated from the digestive tracts of a number of marine vertebrates and invertebrates which ingest chitinous food. The presence of these organisms suggests that a symbiotic relationship exists with the bacteria aiding in the digestion of chitin (Okutani, 1977).

A study on Enophrys bison (buffalo sculpin) and Platichthys stellatus (starry flounder) indicated high levels of chitinase activity. Treatment with the antibiotic chloramphenicol, however, removed all signs of this enzymatic activity (Goodrich and Morita, 1977).

Other work has indicated that various areas of the lining of the digestive tract are capable of secreting chitinolytic enzymes. Studies on the Japanese sea-bass (Lateolabrax japonicus) showed that the chitinolytic enzyme was present in the stomach, liver, and spleen of these animals, but little activity was detected in the pyloric caeca (Okutani and Kimata, 1964c). A more recent study by Fange and his colleagues (1979) indicated strong chitinase activity in the gastric mucosa of various elasmobranchs and teleosts studied. In fact, the chitinolytic activity was found to be higher in the gastric tissue than in the gastric contents. Thus these workers felt that this strongly indicated glandular origin of this enzyme system. Nevertheless the presence of chitin-digesting bacteria in the digestive tract was not entirely excluded. Possibly two types of chitinolytic enzyme systems exist in these species of fish.

As mentioned previously, the action of chitinase and NAGase yields the final breakdown product of chitin, the amino sugar N-acetylglucosamine. Very little work however, has been done on the fate of this sugar in the digestive tract of fish. Studies using rat livers have shown clearly that the presence of normal concentrations of glucose effectively inhibits the phosphorylation of N-acetylglucosamine (Spiro, 1965). Conversely however, this same study showed that large amounts of acetylglucosamine present in the rat liver inhibit the phosphorylation of glucose.

Alliot (1967) studied the absorption of N-acetylglucosamine by the intestine of the spiny dogfish (Scylliorhinus canicula) and found that the rate and amount of absorption of this amino sugar were significantly greater than that of glucose. The possibility of utilization of N-acetylglucosamine was not explored, but it was hypothesized that the higher absorption rate may prove to be a nutritional advantage to the fish. Pérès et al. (1973) also found that N-acetylglucosamine was absorbed by the intestine of both the eel (Anguilla anguilla) and the scorpion fish (Scorpaena porcus).

In addition to being a study of protein quality of certain dietary ingredients this thesis was undertaken to provide further evidence for the utilization of the breakdown product of chitin by comparison of protein and energy utilization of trout fed diets containing varying amounts of chitin.

MATERIALS AND METHODS

Animals and Maintenance

On Monday, November 15, 1982 approximately 3000 rainbow trout (Salmo gairdneri) were delivered to the West Vancouver Laboratory (Department of Fisheries and Oceans) from Sun Valley Trout Farms, Mission, B.C. The fish were of domestic strain and weighed approximately 2.1 grams.

The fish were evenly distributed into three 1,100 litre fibreglass tanks. Water exchange was a 100 per cent flow-through system with a flow-rate of 4-6 litres/minute. Water temperature was maintained at 10-12^{o}C. The tanks were illuminated overhead with 40W fluorescent lights and timed to 9 hours daylight/15 hours darkness.

Diets

Fourteen different diets were formulated on an isonitrogenous basis (Table 1). Nine of the diets were calculated to be 28 per cent protein and the remaining five were 36 per cent protein. Herring meal was used as the main protein source in all diets. In two of the diets steam-dried herring meal was the sole source of protein aside from ground wheat which was kept constant throughout all diets. These diets (28 and 36 per cent protein) were used as controls. The remaining twelve diets incorporated four test protein ingredients. These were shrimp meal, two different preparations of krill meal and soybean meal.

In the 28 per cent protein diets the test ingredients were incorporated at two different levels. Although the actual amount of the test ingredient varied, the diets were formulated so that each test ingredient replaced 11 per cent of the total protein at the low level (series-1) and 22 per cent of the total protein at the high level (series-2). In the 36 per cent protein diets each test ingredient replaced 17 per cent of the protein (series-3). The differences in the amounts of the test ingredients to supply the necessary amounts of protein were made up with dextrin.

All ingredients were analyzed for protein, lipid, ash and moisture content. In addition the shrimp meal and krill meal were analyzed for chitin content using the method of Black and Schwartz (1950).

All feedstuffs were commercial products except the shrimp meal and the krill meal. The shrimp meal was prepared from waste from a hand peeling shrimp operation. The material was dried overnight in a drying oven at 95°C to 3.5 per cent moisture. The krill (Euphausia pacifica) was obtained frozen. One preparation involved placing the krill in retort pouches and submerging them into rapidly boiling water for 15 minutes in order to coagulate the protein. The krill was then oven-dried overnight at 95°C to 3.2 per cent moisture. The other krill preparation was freeze-dried for approximately 48 hours in a Virtis 10-145 MR-BA freeze drier to 5.6 per cent moisture (for proximate analysis of test ingredients see Table 2). The krill and shrimp were then ground in order to produce the meal required. The soybean meal and wheat were ground in a Fitz Mill grinder to pass through a US 20 sieve (840 µm). The diets were blended in a

Hobart mixer for thirty minutes and then cold pelleted in a California model CL-type 2 laboratory pellet mill having a 2.38 mm die. The pellets were placed on a tray and put in a drier which cooled and hardened them. After pelleting and cooling, each diet was put through a crumbler and then sieved. Feed particles collected on a US 12 sieve were the appropriate size for fish used in the experiment. Salmon oil was used as a lipid source and in all diets half the amount required was incorporated when the other ingredients were mixed and the remainder was then sprayed on after pelleting.

Experimental Treatment

During the period prior to beginning the experiment (approximately one week) the fish were fed a chinook starter diet ad libitum. On November 22, 1982 the fish were anaesthetized in 0.5 ml 2-phenoxyethanol per liter water, and 800 fish were selected in the weight range of 2.4-3.1 grams. The fish were distributed at random (25 fish/tank) into 28 23-litre bucket tanks. In addition 35 fish from this weight range were selected for a fasting tank plus 37 fish of a larger weight range (3.5-3.6 grams) were selected for another fasting tank. Forty fish from the former weight range were individually weighed at zero time to determine initial weight.

Each of the experimental diets was fed to duplicate tanks of fish. The fish were fed to near satiation three times daily for 23 days. Records of daily food consumption and mortality were maintained.

At the end of the period the fish in each tank were distributed into two lots and each pooled lot was weighed. The fish were kept frozen at -18°C until the chemical analyses were performed.

Chemical Analysis

All fish were lyophilized prior to chemical analysis and then weighed in order to determine moisture content. The fish were then ground in a Kurzzeitbetrieb grinder for five minutes and stored in a desiccator throughout the time of analysis.

Carcass protein was determined by the macro-Kjeldahl procedure (AOAC, 1970) for nitrogen determination on a Buchi 430 Digestor and a Buchi 325 Nitrogen Distillation Unit. Total nitrogen was then multiplied by a factor of 6.25 to estimate crude protein. Lipid content was determined by Goldfisch extraction with diethyl ether. Following ether extraction samples underwent combustion in a muffle furnace for 8 hours at 600°C in order to determine ash content.

Proximate analysis of the diets was performed using these procedures as well.

Estimation of Energy Availability

Calculations were also carried out for determination of per cent energy availability. This was performed using the following equation:

$$\% \text{ Energy availability} = \frac{E\text{maintenance} + E\text{carcass}}{E\text{gross ingested} - E\text{ chitin}} \times 100\%$$

The denominator in this equation was based on the assumption that potentially all energy ingested was available except the chitin and crude fibre component. In this manner, if the chitin was being utilized it would be reflected in the figure obtained. Maintenance energy was calculated from the slope of a line obtained from the maintenance energy calculated from the fasting fish as well as from previous experiments (March, unpublished data).

Statistical Analysis

The design of the overall experiment was 2x2x5 factorial. Factor 'A' was protein level (28% vs 36%), factor 'B' was test ingredient level i.e. replacement of 11% and 22% of dietary protein and factor 'C' was source of test ingredient (herring meal, shrimp meal, cooked krill meal, freeze-dried krill meal or soybean meal).

The data were subjected to analysis of variance (ANOVA) and significant differences were identified by Duncan's Multiple Range Test (Steel and Torrie, 1960).

RESULTS

Series I Dietary Treatments

At the beginning of the three week feeding period the average body weight of fish in all groups was 2.69 ± .04 grams (SEM). At the end of the feeding trial the fish on series-I diets had body weights of 4.54 ± .13 grams (see Table 4). Although there were no significant differences in final body weight of fish fed these diets the energy gain of fish on the herring meal diet (HM-I) was significantly lower than that for the fish whose diet contained any of the test ingredients (Table 5). Lower protein and lipid gains would also be reflected in the energy figures. Fish fed shrimp meal and freeze-dried krill meal diets performed similarly in terms of protein, lipid and energy gains with values for fish fed the freeze-dried krill diet (FD-I) being slightly higher than the others. Inclusion of soybean meal (SBM-I) did not statistically influence performance.

In terms of feed efficiency ratios, again, there was generally no significant difference for either feed conversion efficiency or protein efficiency ratios between any of the diets, with one exception (Table 6). The ration containing shrimp meal (SM-I), however, had a PER value which was significantly greater than either of the groups fed the two krill diets. The value was the same as those obtained for fish ingesting HM-I and SBM-I. The protein gain/protein intake value for fish receiving SM-I was also higher than values for fish given the other diets. The values for energy gain/feed consumed for the fish receiving series-I diets (Table 6) showed similarity between fish on all diets except HM-I

which was significantly lower than the other values. Energy gain/energy intake values indicated that energy utilization in fish fed the freeze-dried krill diet (FD-1) was slightly better than values for fish fed the other diets. Determination of energy availability showed no significant differences between any of the diets (Table 7).

Series 2 Dietary Treatments

The average final body weight of trout on the series-2 diets was 4.87 ± .11 grams (Table 4). As with the fish on series-1 diets the weights were not significantly different among fish given diets containing test ingredients with the exception of fish receiving the herring meal diet which had depressed growth relative to the others. The values for protein and lipid gains (Table 5) were also not statistically different, although overall energy gain per fish was slightly higher for fish fed the shrimp meal diet (SM-2) and the soybean meal diet (SBM-2). Again, the herring meal control group was well below the other values for groups receiving test diets.

Feed efficiency ratios for fish presented series-2 diets showed somewhat more divergence than was observed for groups fed series-1 diets (Table 6). Protein efficiency ratio was higher in fish fed shrimp meal (SM-2) and freeze-dried krill (FD-2) relative to those fed the other three diets. The value for feed conversion efficiency for fish given diet FD-2 was also significantly higher than those given the other diets whereas the value for fish on diet SM-2 was higher but the difference was not significant. The values obtained for protein

utilization (Table 6) indicated that fish fed diets SM-2 and FD-2, as well as those on the soybean ration (SBM-2) were most efficient in regard to protein gain/protein intake. No significant differences were found between test groups in energy utilization. However fish fed diets SM-2, FD-2 and SBM-2 had significantly higher energy utilization than those fed the herring meal control diet. Energy availability was also similar from all the series-2 diets (Table 7).

Series 3 Dietary Treatments

The series-3 diets were those formulated to contain 36 per cent protein where 17 per cent of the total protein was replaced by test ingredient. The final body weights of trout fed these diets were in the range 5.12 ± .16 grams (Table 4). There were no significant differences in final body weights of the fish fed the different diets of this series. However, observation of energy, protein and lipid gains per fish did indicate some differences (Table 5). Fish on diets HM-3, FD-3 and SBM-3 showed the greatest energy gains although values for the latter two groups were not significantly different from those of fish on the other diets (SM-3, CK-3). Protein gains were similar with fish fed the FD-3 diet having the highest overall value. Lipid gains were not significantly different amongst the fish fed series-3 diets.

Feed conversion efficiency values for diets FD-3 and CK-3 were highest within the series-3 diets (Table 6). Values for fish receiving the herring meal and shrimp meal diets were similar, and the SBM-3 group proved to be much inferior in this respect. Protein efficiency ratios also indicated the superiority of the

FD-3 diet, however the values for fish given the cooked krill (CK-3) diet was reduced. Again, fish on the soybean meal diet did not perform well in this area. The ratio of protein gain/protein intake showed the value for the group fed the FD-3 diet to be slightly higher than those for groups receiving the other diets, and those fish fed SBM-3 diet were slightly poorer in this regard. Similar observations were made for values for energy gain/feed consumed and energy gain/energy intake although in the latter term the trout fed the shrimp meal diet (SM-3) had slightly improved energy utilization than those presented the other diets. As observed with the previous series the values for availability of dietary energy showed no significant differences (Table 7). It should be noted, however that the value for fish on SM-3 was slightly higher than the others whereas the fish given SBM-3 gave a value that was slightly lower.

Comparison Among Series

Comparison of fish performance between diet series gives an overall indication of the nutritive value of the test ingredients. Final body weights of trout used in this study (Figures 1 and 2) indicate similar gains in fish on series-2 and series-3 diets. In all aspects regarding efficiency of utilization of both energy and protein the shrimp meal and freeze-dried krill meal diets produced results indicating superior performance compared with the herring meal, cooked krill and soybean meal diets (Figures 3, 4, 5, 6). Although this was not always manifested statistically the trends are clearly observed. Similar trends are seen when protein production values (protein gain/protein intake) (Figures 7 and 8) and those for energy gain/feed consumed (Figures 9 and 10) are compared. Values

obtained for the ratio of energy gain/energy intake (Figures 11 and 12) are also comparable in this respect. These results appear to be more pronounced in fish fed series-2 and series-3 diets in which each test ingredient was included at higher levels. Possible reasons for this performance will be discussed in the following section.

Overall comparison of the values for energy availability among the three series revealed few significant differences (Table 7). Diets HM-1 and SBM-3 were significantly lower than the SM-3 diet. In general the figures for series-1 tended to be slightly lower than both series-2 and series-3 however this trend was not statistically significant.

It should be noted that any values concerned with energy and/or protein intake were corrected for the chitin content. This correction was achieved by converting chitin nitrogen into protein equivalents and subtracting this figure from the total protein present. Energy values were corrected by assumed 4 cal/g for chitin.

TABLE I

COMPOSITION OF EXPERIMENTAL DIETS (air-dry basis)

	Diets													
	HM-1 g	SM-1 g	CK-1 g	FD-1 g	SBM-1 g	SM-2 g	CK-2 g	FD-2 g	SBM-2 g	HM-3 g	SM-3 g	CK-3 g	FD-3 g	SBM-3 g
Ground wheat	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Herring meal	339.4	297.0	297.0	297.0	297.0	254.0	254.0	254.0	254.0	450.0	365.0	365.0	365.0	365.0
Shrimp meal	-	70.0	-	-	-	140.0	-	-	-	-	140.0	-	-	-
Cooked krill meal	-	-	44.7	-	-	-	89.4	-	-	-	-	89.4	-	-
Freeze-dried krill meal	-	-	-	44.7	-	-	-	89.4	-	-	-	-	89.4	-
Soybean meal	-	-	-	-	64.5	-	-	-	129.05	-	-	-	-	129.05
Dextrin	205.6	178.0	203.3	203.3	180.63	151.0	201.6	201.6	156.19	95.0	40.0	90.6	90.6	45.31
Salmon oil	120.0	120.0	120.0	120.0	122.87	120.0	120.0	120.0	125.76	120.0	120.0	120.0	120.0	125.64
Sodium chloride iodized	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Carboxymethyl cellulose	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin premix[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

[1] To supply 60 mg riboflavin, 164 mg calcium pantothenate, 300 mg niacin, 36 mg pyridioxine HCl, 10 mg folacin, 34 mg thiamin HCl, 3 mg biotin, 0.06 mg vitamin B_{12}, 80 mg menadione, 1728 mg choline chloride, 1200 mg ascorbic acid, 400 mg inositol, 600 IU vitamin E, 10,000 IU vitamin A, 2000 IU vitamin D_3.

TABLE 2

PROXIMATE COMPOSITION OF DIETARY INGREDIENTS (%)

	Herring Meal	Cooked Krill	Freeze-Dried Krill	Shrimp Meal	Soybean Meal	Ground Wheat
Crude Protein (N x 6.25)	71.90	68.54	68.54	43.79	47.51	12.0
Ether Extract	8.21	8.23	8.17	11.81	1.14	1.61
Ash	12.95	12.28	11.41	22.60	6.0	5.86
Moisture	6.77	3.20	5.58	3.49	6.36	5.39
Chitin	--	2.46	2.37	9.56	--	--
Total:	99.83	94.71	96.07	91.24	61.01	24.86

TABLE 3

PROXIMATE COMPOSITION OF DIETS (% on as fed basis)

	HM-1	SM-1	CK-1	FD-1	SBM-1
Crude protein (% N x 6.25)	27.56	26.91	30.49	30.51	26.88
% Protein (chitin-N corrected)	27.56	26.62	30.44	30.46	26.88
Crude lipid (Goldfisch)	12.85	14.02	11.59	11.69	12.23
Ash	5.03	5.88	5.15	5.03	4.93
Nitrogen-free extract	40.01	37.99	38.03	38.39	41.05
Chitin and/or crude fibre[a]	.72	1.39	.83	.83	.95
Moisture	13.83	13.81	13.91	13.55	13.96
Gross energy (kcal/g)[b]	4.42	4.44	4.39	4.42	4.37
Gross energy (chitin-corrected)	4.39	4.37	4.36	4.38	4.34

[a] Crude fibre content of diets was estimated.

[b] Coefficients used for estimating calorie content of diets were: 5.7 kcal/g crude protein; 9.5 kcal/g crude lipid; 4.0 kcal/g carbohydrate, 4.0 kcal/g chitin.

Table 3 Cont'd

	SM-2	CK-2	FD-2	SBM-2
Crude protein (% N x 6.25)	28.92	31.09	30.55	29.77
Protein (chitin-N corrected)	28.34	30.99	30.14	29.77
Crude lipid (Goldfisch)	14.34	12.39	13.82	11.26
Ash	7.19	5.24	5.22	4.86
Nitrogen free extract	34.81	38.05	37.88	39.00
Chitin and/or crude fibre[a]	2.07	.94	.94	1.17
Moisture	12.67	12.29	12.00	13.94
Gross energy (kcal/g)[b]	4.49	4.51	4.58	4.37
Gross energy (chitin-corrected)	4.37	4.46	4.57	5.32

[a]Crude fibre content of diets was estimated.

[b] Coefficients used for estimating caloric content of diets were: 5.7 kcal/g crude protein; 9.5 kcal/g crude lipid; 4.0 kcal/g carbohydrate, 4.0 kcal/g chitin.

Table 3 Cont'd

	HM-3	SM-3	CK-3	FD-3	SBM-3
Crude protein (% N x 6.25)	36.23	35.23	37.96	35.30	36.29
Protein (chitin-N corrected)	36.23	34.65	37.87	35.21	36.29
Crude lipid (Goldfisch)	13.67	15.94	14.19	14.47	14.17
Ash	6.30	8.58	6.49	6.39	6.10
Nitrogen-free extract	30.10	25.20	27.71	30.01	27.23
Chitin and/or crude fibre[a]	.72	2.07	.94	.94	1.17
Moisture	12.89	12.98	12.71	12.89	15.04
Gross energy (kcal/g)[b]	4.60	4.61	4.66	4.88	4.55
Gross energy (chitin-corrected)	4.57	4.50	4.62	4.58	4.50

[a] Crude fibre content of diets was estimated.

[b] Coefficients used for estimating caloric content of diets were: 5.7 kcal/g crude protein; 9.5 kcal/g crude lipid; 4.0 kcal/g carbohydrate, 4.0 kcal/g chitin.

TABLE 4

BODY COMPOSITION OF TROUT IN RESPONSE TO DIFFERENT DIETARY TREATMENTS (± SEM)

Dietary Treatment[1]	Wet Weight (g/fish)	Protein (mg/fish)	Lipid (mg/fish)	Ash (mg/fish)	Energy[2] (cal/fish)
HM-1	4.34[d][3] ±.23	536.5 ± 7.9	330.2 ± 1.7	80.15 ± 1.15	6943.1 ± 612.6
SM-1	4.47[cd] ±.02	577.15 ±5.05	370.35 ±8.95	88.7 ±3.0	6808.1 ± 56.3
CK-1	4.61[cd] ±.05	580.3 ± 0.9	389.5 ± 7.7	89.25 ±1.35	7008.0 ± 68.1
FD-1	4.66[cd] ±.06	590.85 ±5.35	395.5 ±11.6	91.35 ±3.05	7125.1 ±140.7
SBM-1	4.63[cd] ±.12	584.4 ± 3.7	390.75 ±21.25	91.9 ±3.1	7043.2 ±223.0
SM-2	4.89[abc] ±.14	620.0 ±12.6	435.95 ±19.95	93.6 ±1.6	7675.6 ±261.4
CK-2	4.73[bcd] ±.17	589.4 ±19.7	393.2 ±29.9	91.35 ±2.05	7095.0 ±396.4
FD-2	4.90[abc] ±.04	618.55 ±5.95	412.05 ±3.95	92.7 ±2.0	7440.2 ±3.6
SBM-2	4.98[abc] ±.07	626.2 ±7.8	433.9 ± 0.3	98.85 ±1.75	7691.4 ±47.3
HM-3	5.32[a] ±.01	666.65 ±10.05	480.2 ±0.7	109.75 ±.95	8378.9 ±50.6
SM-3	4.93[abc] ±.14	631.05 ±4.05	455.95 ±13.35	101.5 ±6.1	7928.5 ±149.9
CK-3	4.98[abc] ±.18	639.25 ±21.65	437.7 ±28.3	93.0 ±4.8	7801.9 ±392.3
FD-3	5.20[ab] ±.04	670.0 ±1.1	446.65 ±6.65	102.0 ±2.2	8062.2 ±56.9
SBM-3	5.19[ab] ±.27	667.05 ±22.15	461.45 ±23.25	103.4 ±1.1	8186.0 ±347.2

[1] The following abbreviations will be utilized throughout this thesis and will denote the test ingredient in a particular ration: HM - herring meal (control diet); SM - shrimp meal; CK - cooked krill; FD - freeze-dried krill; SBM - soybean meal.
Numbers following diet codes are: 1 - 28% protein, low level of test ingredient; 2 -28% protein, high level of test ingredient; 3 - 36% protein, high level of test ingredient.

[2] Coefficients used for energy calculations were: 5.7 k cal/g protein and 9.5 k cal/g lipid.

[3] Means in each column followed by different superscripts were found to be significantly different by Duncans Multiple-Range Test ($p < .05$).

TABLE 5

BODY COMPOSITION GAINS OF TROUT IN RESPONSE TO DIFFERENT DIETARY TREATMENTS (± SEM)

Dietary Treatment	Wet Weight (g/fish)	Protein (mg/fish)	Lipid (mg/fish)	Ash (mg/fish)	Energy (cal/fish)
HM-1	1.72 ±.22	161.75^{f} ±6.55	205.45^{f} ±1.25	20.5 ±1.0	2873.5^{g} ±48.9
SM-1	1.81 ±.03	195.85^{ef} ±5.85	243.40^{ef} ±8.7	27.9 ±2.9	3428.6^{f} ±49.3
CK-1	1.91 ±.04	195.15^{ef} ±1.65	261.25^{de} ±7.45	27.85 ±1.45	3594.2^{f} ±61.4
FD-1	1.95 ±.07	203.35^{e} ±7.05	266.45^{cde} ±12.15	29.55 ±3.35	3690.4^{ef} ±155.6
SBM-1	1.96 ±.08	203.95^{e} ±1.25	264.10^{de} ±19.6	31.25 ±2.35	3671.5^{ef} ±179.2
SM-2	2.19 ±.16	233.05^{cde} ±15.15	307.1^{abcd} ±20.8	31.9 ±1.2	4245.9^{cd} ±284.0
CK-2	2.05 ±.13	207.45^{de} ±14.85	266.0^{cde} ±28.3	30.45 ±2.85	3709.4^{ef} ±353.5
FD-2	2.18 ±.07	229.45^{cde} ±9.35	282.5^{bcde} ±2.8	30.65 ±2.55	3991.6^{de} ±26.7
SBM-2	2.22 ±.05	232.15^{cde} ±5.85	302.65^{abcd} ±.35	36.05 ±2.05	4198.4^{cd} ±30.0
HM-3	2.60 ±.06	277.55^{ab} ±16.75	352.45^{a} ±1.55	47.7 ±2.0	4930.3^{a} ±110.2
SM-3	2.24 ±.11	246.25^{bcd} ±.15	327.80^{ab} ±12.1	40.15 ±5.45	4517.7^{bc} ±115.8
CK-3	2.31 ±.17	257.20^{abc} ±19.30	310.50^{abcd} ±27.5	32.1 ±4.4	4415.8^{bc} ±371.3
FD-3	2.55 ±.07	291.60^{a} ±5.90	320.65^{abc} ±5.05	41.65 ±2.95	4708.4^{ab} ±14.4
SBM-3	2.49 ±.27	281.25^{ab} ±22.75	332.95^{ab} ±23.45	41.90 ±1.0	4766.2^{ab} ±352.5

TABLE 6

FEED EFFICIENCY RATIOS FOR TROUT IN
RESPONSE TO DIFFERENT DIETARY TREATMENTS (± SEM)

Dietary Treatments	BW gain / Feed consumed	BW gain / Protein intake (PER)	Protein gain / Protein intake	Energy gain / Feed consumed	Energy gain / Energy intake
HM-1	$.651^{f}$ ±.000	2.429^{bcd} ±.067	$.232^{bcd}$ ±.026	1.106^{d} ±1.21	$.251^{d}$ ±.028
SM-1	$.707^{def}$ ±.001	2.655^{ab} ±.022	$.288^{a}$ ±.005	1.343^{bc} ±.039	$.303^{abcd}$ ±.009
CK-1	$.681^{ef}$ ±.053	2.237^{cde} ±.175	$.229^{cd}$ ±.025	1.282^{c} ±.105	$.293^{abcd}$ ±.025
FD-1	$.722^{cdef}$ ±.031	2.369^{cde} ±.101	$.248^{abcd}$ ±.011	1.369^{abc} ±.070	$.310^{abc}$ ±.016
SBM-1	$.701^{def}$ ±.032	2.607^{abc} ±.117	$.272^{abc}$ ±.025	1.311^{c} ±.048	$.300^{abcd}$ ±.011
SM-2	$.765^{bcde}$ ±.025	2.698^{a} ±.080	$.288^{a}$ ±.010	1.487^{abc} ±.036	$.331^{abc}$ ±.008
CK-2	$.700^{def}$ ±.025	2.257^{cde} ±.080	$.228^{cd}$ ±.010	1.265^{cd} ±.085	$.281^{cd}$ ±.019
FD-2	$.803^{abc}$ ±.001	2.678^{ab} ±.003	$.282^{abc}$ ±.003	1.477^{abc} ±.033	$.323^{abc}$ ±.008
SBM-2	$.706^{def}$ ±.009	2.371^{cde} ±.031	$.248^{abcd}$ ±.004	1.335^{bc} ±.003	$.306^{abc}$ ±.001
HM-3	$.774^{bcd}$ ±.005	2.136^{e} ±.012	$.228^{cd}$ ±.010	1.470^{abc} ±.010	$.320^{abc}$ ±.003
SM-3	$.777^{bcd}$ ±.006	2.243^{cde} ±.017	$.247^{abcd}$ ±.010	1.570^{ab} ±.025	$.341^{a}$ ±.006
CK-3	$.821^{ab}$ ±.041	2.169^{de} ±.109	$.242^{abcd}$ ±.011	1.571^{ab} ±.059	$.338^{ab}$ ±.013
FD-3	$.876^{a}$ ±.016	2.487^{abc} ±.044	$.284^{ab}$ ±.003	1.617^{a} ±.021	$.332^{abc}$ ±.005
SBM-3	$.675^{f}$ ±.011	1.858^{f} ±.029	$.211^{d}$ ±.009	1.296^{c} ±.065	$.285^{bcd}$ ±.014

TABLE 7

ENERGY AVAILABILITY VALUES FOR TROUT FROM THE DIFFERENT EXPERIMENTAL DIETS (± SEM)

	% Energy Availability
HM-1	36.54 ± 5.24^{c}
SM-1	42.60 ± 1.44^{abc}
CK-1	41.27 ± 5.70^{abc}
FD-1	42.95 ± 2.47^{abc}
SBM-1	41.53 ± 2.52^{abc}
SM-2	$45.26 \pm .73^{ab}$
CK-2	38.98 ± 2.76^{abc}
FD-2	44.35 ± 1.48^{ab}
SBM-2	$41.63 \pm .05^{abc}$
HM-3	$42.30 \pm .02^{abc}$
SM-3	$45.76 \pm .99^{a}$
CK-3	45.32 ± 3.24^{ab}
FD-3	$44.11 \pm .85^{abc}$
SBM-3	37.85 ± 3.10^{bc}

TABLE 8

FOOD INTAKE VALUES FOR TROUT FED DIFFERENT EXPERIMENTAL DIETS

Dietary Treatment	Total Food Intake (g/fish)	Average Daily Food Intake (mg/fish)
HM-1	2.63	114.3
SM-1	2.55	110.9
CK-1	2.83	123.0
FD-1	2.70	117.4
SBM-1	2.81	122.2
SM-2	2.88	125.2
CK-2	2.82	122.6
FD-2	2.91	126.5
SBM-2	3.70	160.9
HM-3	3.35	145.7
SM-3	2.85	123.9
CK-3	2.93	127.4
FD-3	2.70	117.4
SBM-3	3.15	137.0

FIGURE 1. BODY WEIGHTS

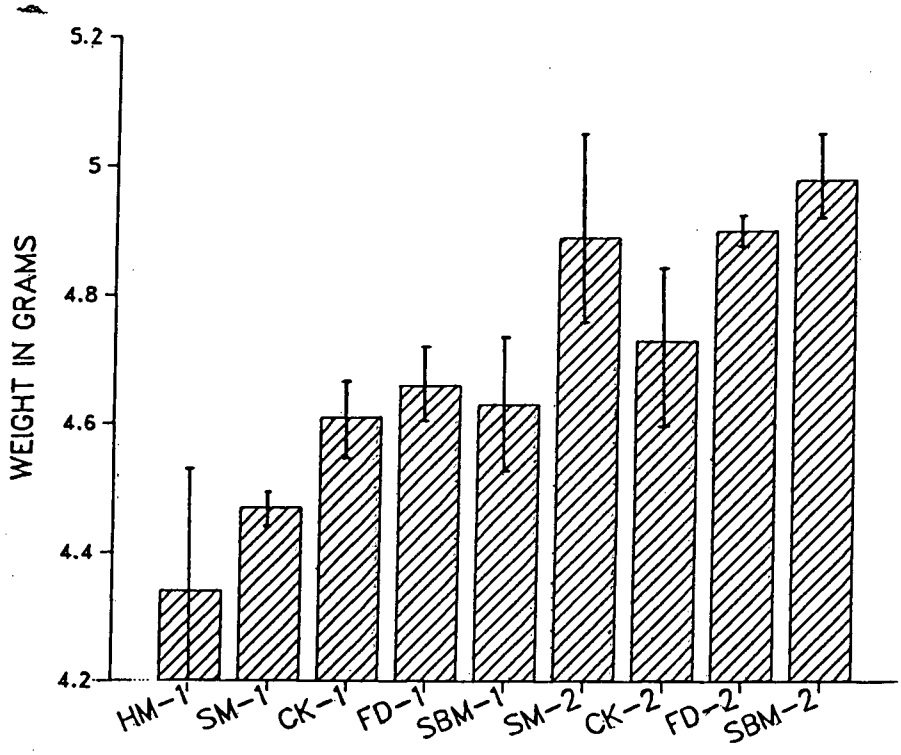


FIGURE 2. BODY WEIGHTS

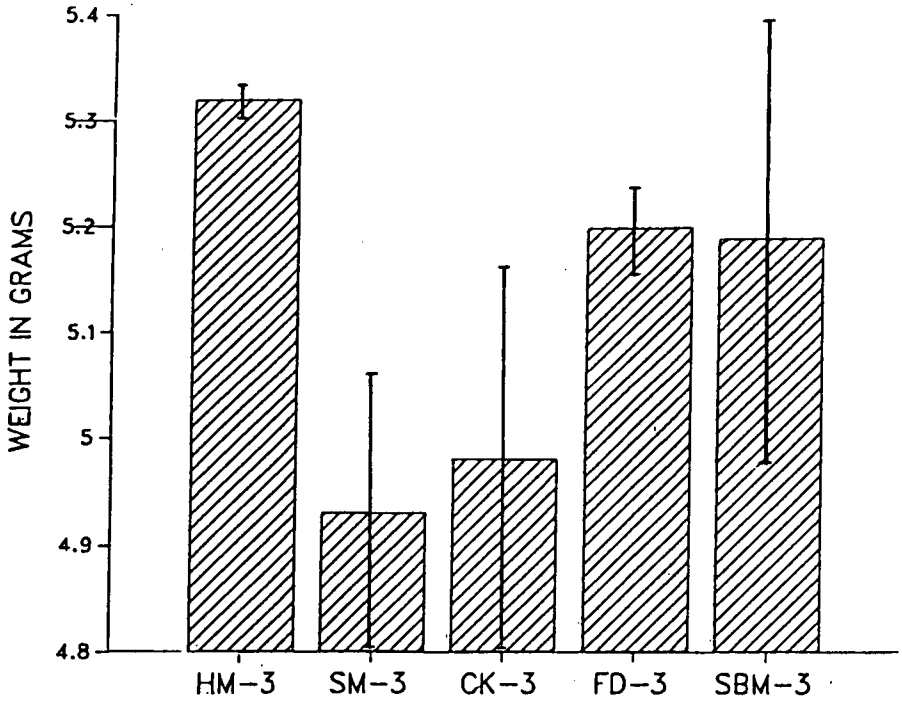


FIGURE 3. FEED EFFICIENCY RATIO

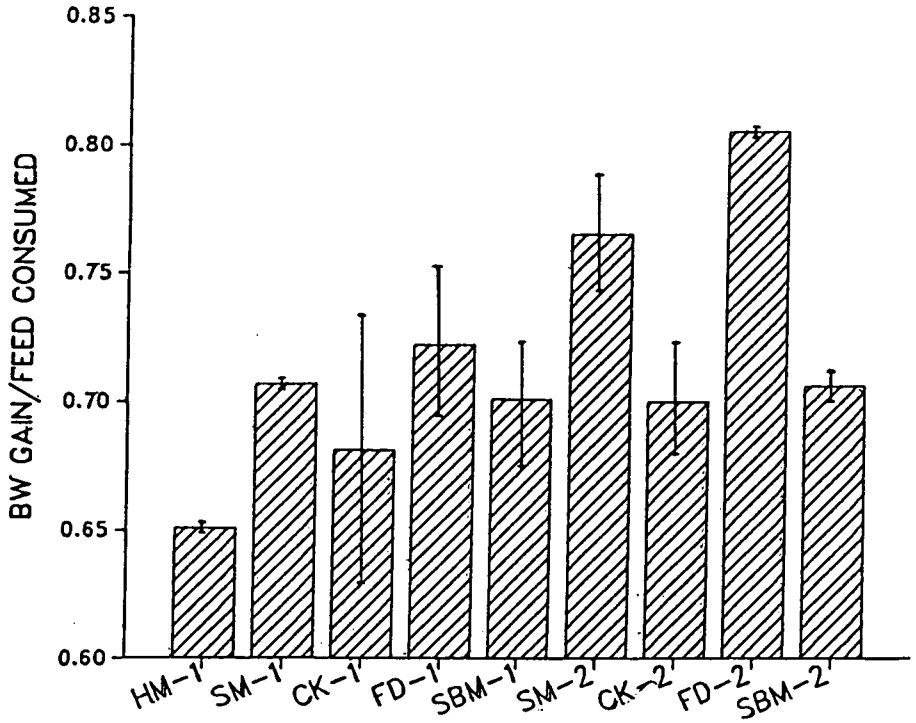


FIGURE 4. FEED EFFICIENCY RATIO

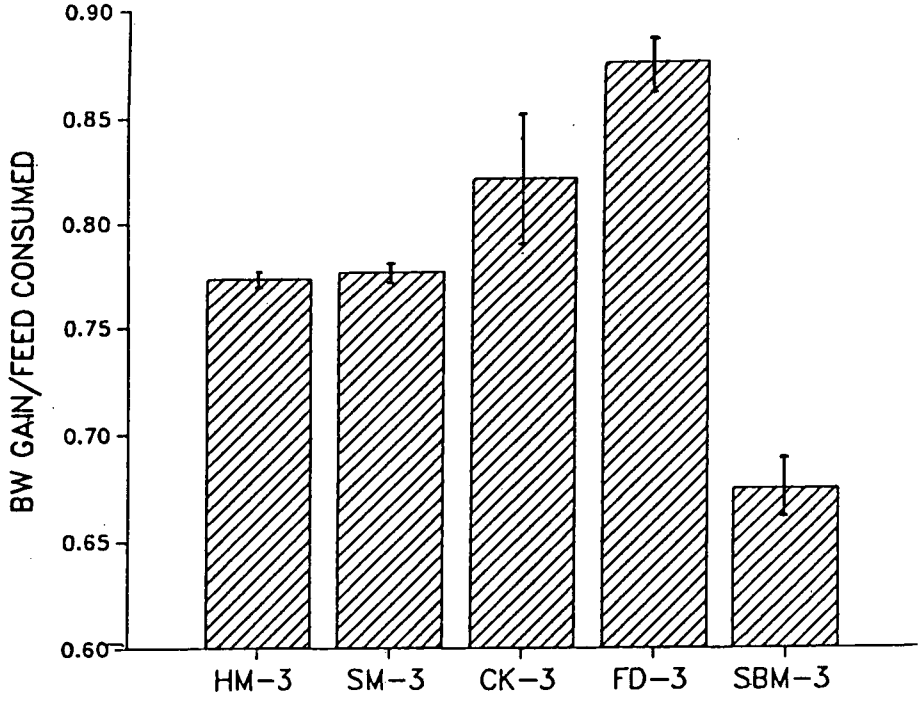


FIGURE 5. PROTEIN EFFICIENCY RATIO

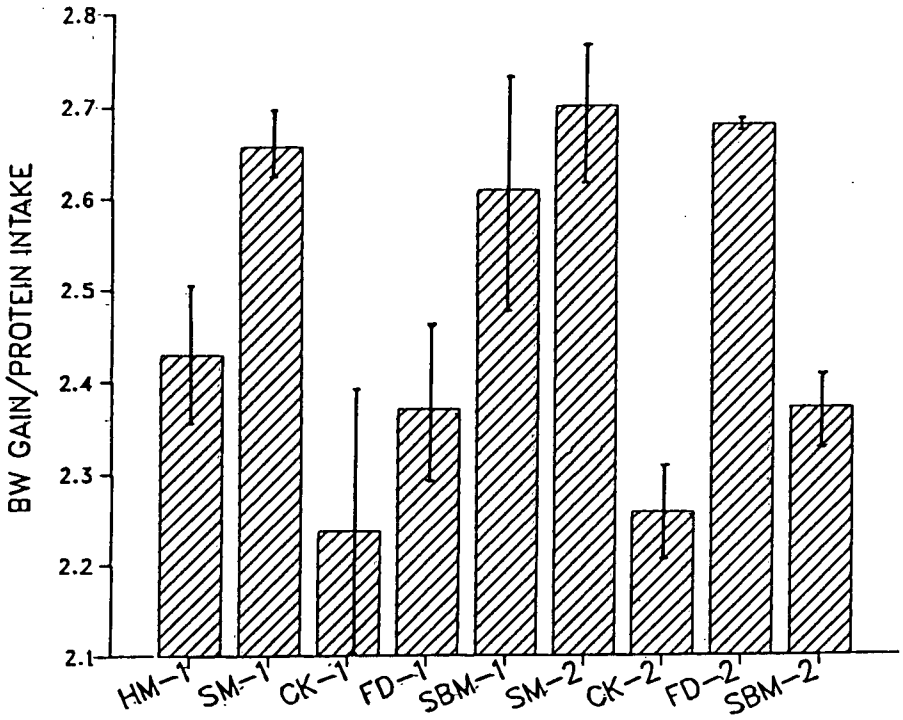


FIGURE 6. PROTEIN EFFICIENCY RATIO

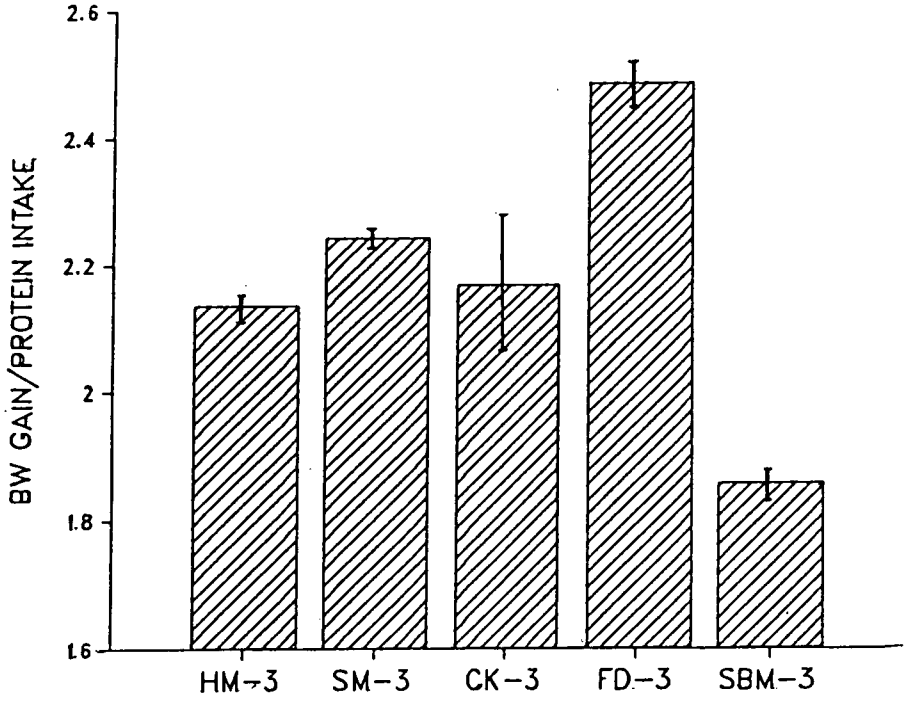


FIGURE 7. PROTEIN GAIN/PROTEIN INTAKE

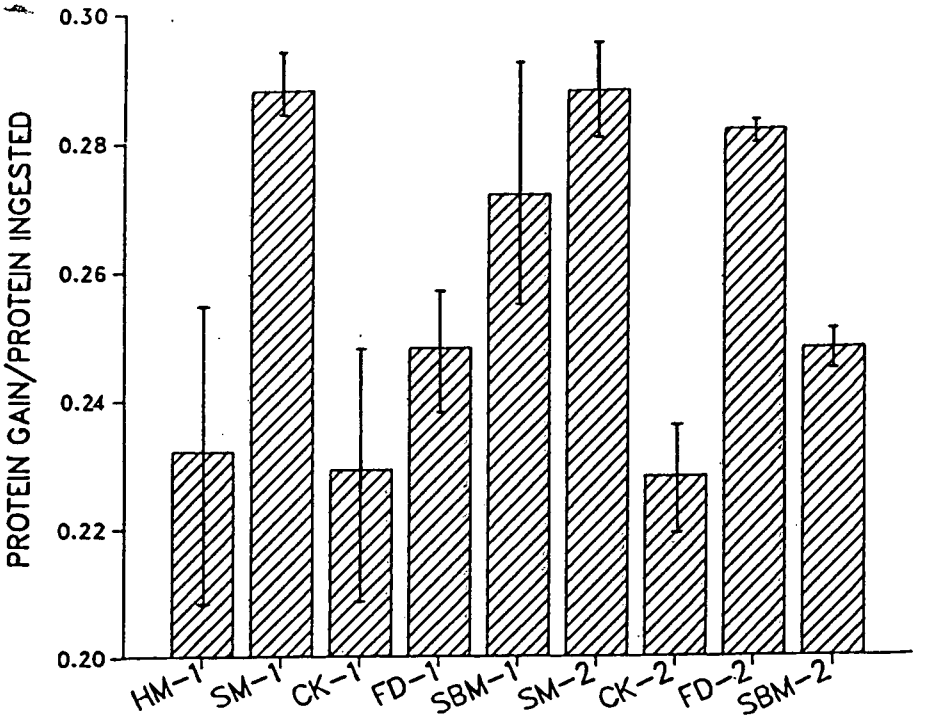


FIGURE 8. PROTEIN GAIN/PROTEIN INTAKE

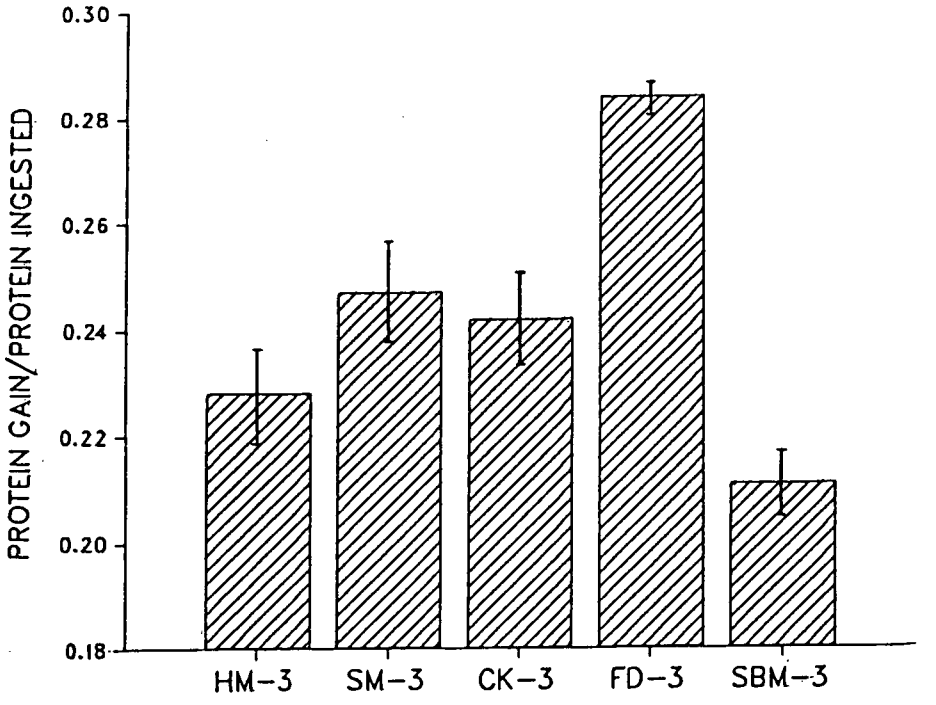


FIGURE 9. ENERGY GAIN/FEED CONSUMED

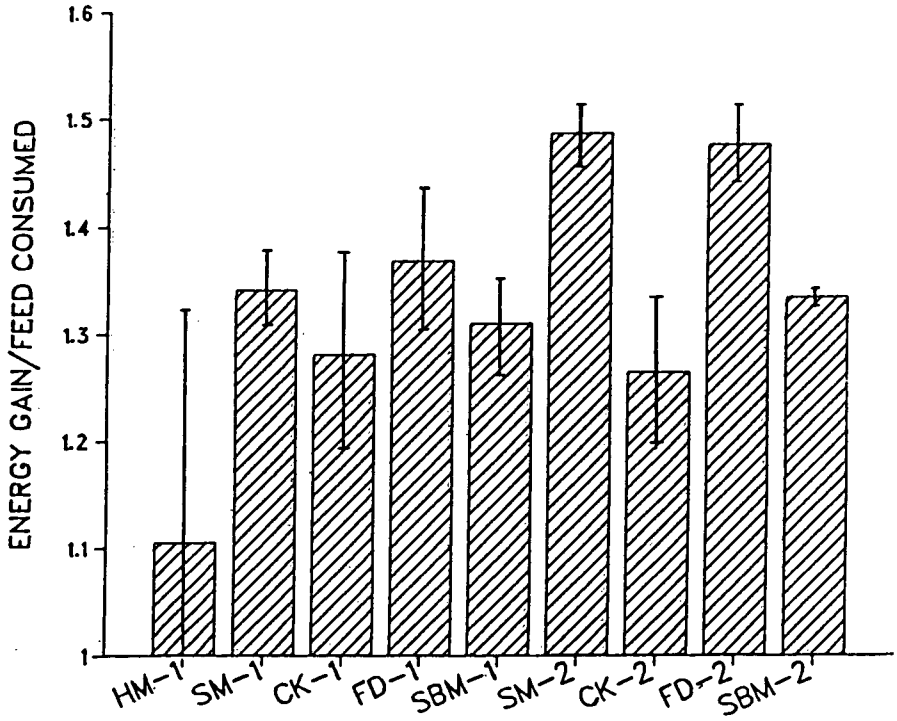


FIGURE 10. ENERGY GAIN/FEED CONSUMED

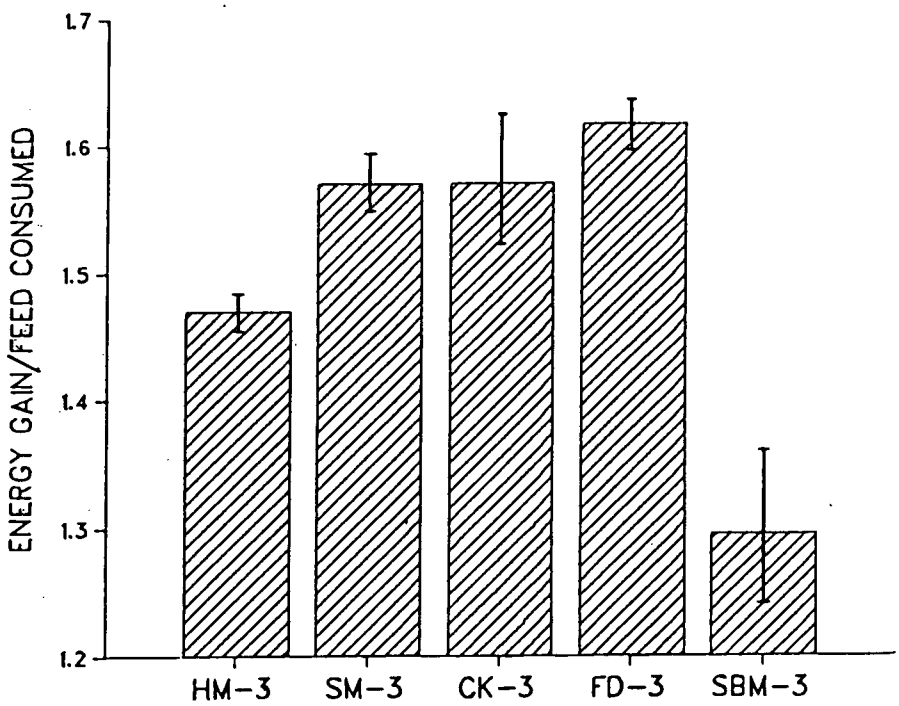


FIGURE 11. ENERGY GAIN/ENERGY CONSUMED

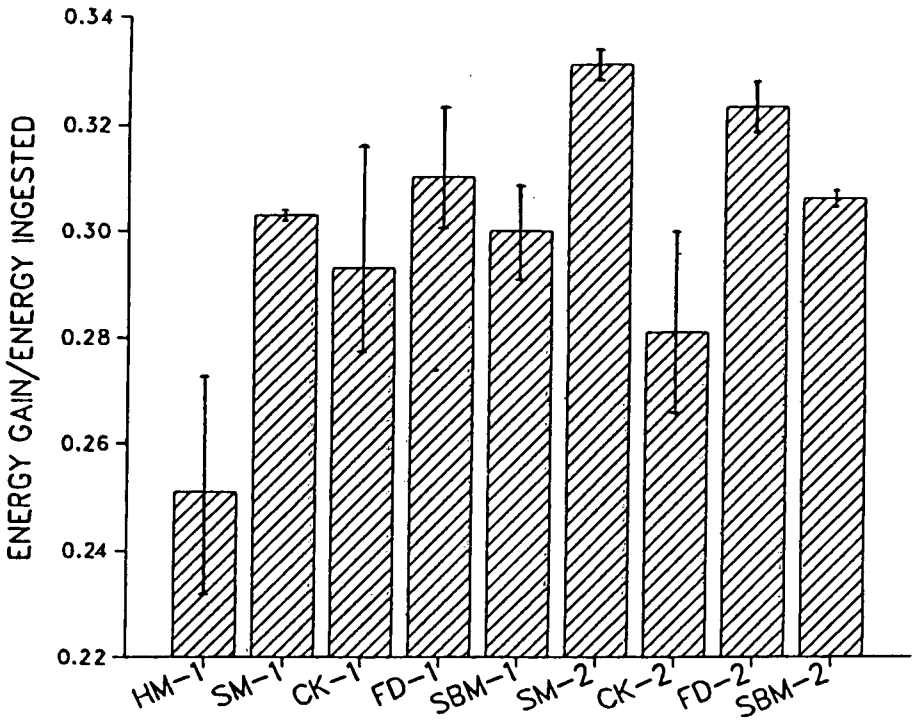
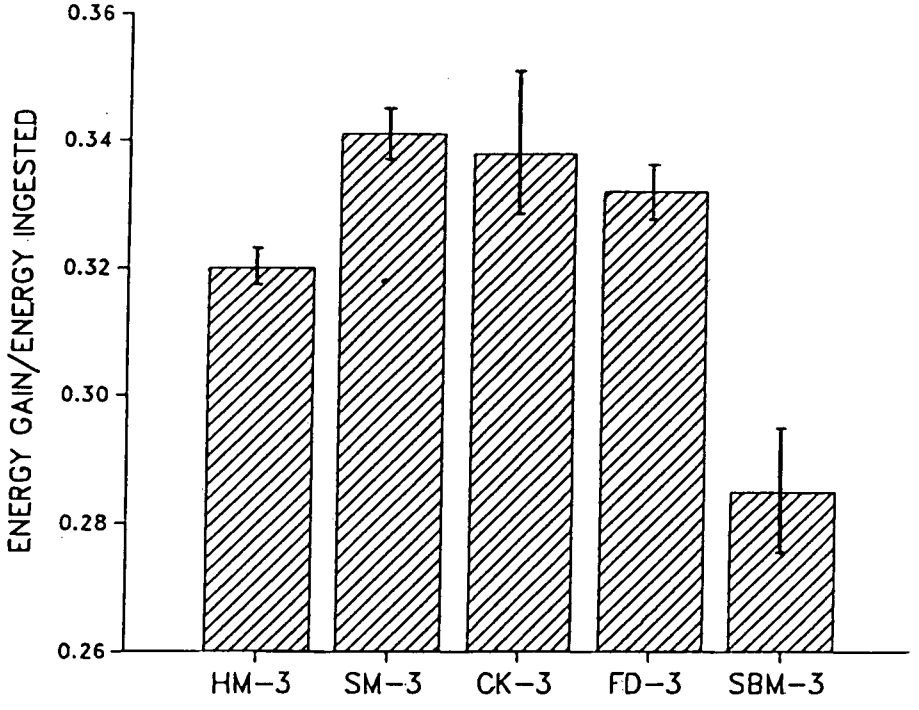


FIGURE 12. ENERGY GAIN/ENERGY CONSUMED



DISCUSSION

Protein Quality

Results of this study strongly indicate the excellent quality of shrimp meal and freeze-dried krill meal preparations as protein supplements, replacing up to 22 per cent of fishmeal protein. This is apparent from observation of growth rates as well as feed conversion and protein utilization values. The replacement of 22 per cent of dietary protein in a 28 per cent protein diet with these ingredients resulted in better body weight gains than for fish fed diets in which 11 per cent of dietary protein was replaced. Feed efficiency values were also an indication of the degree to which the shrimp meal and freeze-dried krill meal diets were utilized. It is also interesting to note in comparing the response of fish to the series-2 and series-3 diets that where higher protein levels would be expected to reduce feed conversion and protein efficiency ratio, the similarity of these values in SM-2, SM-3 and FD-2, FD-3 indicates that the feed ingredients were being utilized to a full extent in these diets. The high protein gains and high ratios of protein gain/protein intake are also an indication that protein is being spared as an energy source and instead, is being used more beneficially for protein synthesis. In addition, scrutiny of the energy values, both gains and ratios, emphasize the high nutritive value of fish fed the shrimp meal and freeze-dried krill meal preparations. The high lipid gain shows that a considerable fraction of the energy ingested was being deposited as lipid rather than being utilized as an energy source. Observation of values for the ratio energy gain/energy intake illustrates this point more clearly.

The hypothesis arises that perhaps there is an alternate source of energy available to the fish which they are utilizing in addition to that supplied by the lipid, protein and carbohydrate in the diet.

It is well-known that fish are rather inefficient in utilizing most forms of carbohydrate as energy sources (Cowey et al., 1977a; NRC, 1981, Garin, 1977) although studies have indicated that they do have a limited ability to adapt to increased level of carbohydrate in the diet (Hilton and Atkinson, 1982). Nevertheless, glucose tolerance in the rainbow trout and other carnivorous fish remains low, and as a consequence, dietary carbohydrate levels above a certain tolerance level result in detrimental effects to these fish. Still, when investigating the effects of the diets formulated in this experiment it is apparent that the only test ingredient that contained an appreciable amount of carbohydrate was soybean meal. Indeed, the two superior test ingredients, shrimp meal, and freeze-dried krill meal are extremely low in carbohydrate. This leads one to consider chitin as a possible energy source for the fish.

Previous studies have reported that chitinolytic enzyme systems are present in certain species of chitin-ingesting fish (King et al., 1977; Fange et al., 1979; Okutani and Kimata, 1964a, 1964b, 1964c; Jeuniaux, 1961; Goodrich and Morita, 1977). Although analysis has not been carried out with rainbow trout per se, there is good indication that chitinase activity would be present in their gut. In fact, because they ingest euphausiids in large quantities this may be the case with many of the salmonid species.

Nutritional implications have also been considered to a certain extent in the study of chitin, however the literature is very sparse in studies considering the actual utilization of breakdown products and whether these materials have any sparing effect on energy or even protein for the fish.

Although the results of this study did not indicate any significant differences between groups ingesting varying amounts of chitin, definite trends could be observed. The favourable overall performance of the shrimp meal diet, for example, with its significant chitin content is an indication that some protein and energy sparing action was occurring.

This theory is supported by a study involving the feeding of krill shells to rats (Kuhl et al., 1978). Analytical data concerning N-balance in these krill-fed rats indicated that there was partial utilization of chitin-N in the protein metabolism. In addition, Alliot (1967) performed an experiment with the dogfish (Scylliorhinus canicula) investigating the absorption of N-acetylglucosamine - a breakdown product of chitin. Results showed that absorption of this compound was superior to glucose absorption in this fish thus implying that benefits may be obtained as an energy source.

Further studies involving greater amounts of shrimp meal replacing fishmeal would be of interest in order to determine more clearly the degree to which chitin can be utilized to spare energy and protein in fish diets.

Krill Meal

Numerous studies have been carried out on various species of fish utilizing krill as a protein replacement (Grave et al., 1979; Koops et al., 1979). Varying degrees of success have been obtained largely as a result of the means of processing the material before incorporation into the diet. Previous results have proven that the use of frozen products results in growth rates well below control diets (Brett, 1971). The main reason for this is the rapid rate of nutrient leaching once the material is placed into water.

The two methods of preparation in the present study produced distinctly different results. Freeze-dried krill proved to be of far superior quality to the cooked krill preparation, yielding better results in terms of growth rates, and efficiency ratios. The poor growth response obtained from the cooked krill preparation may be an indication that even moderate heating causes nutrient damage, e.g. heat damage to protein.

Both of these preparations were expected to produce favourable results; the hypothesis being that both freeze-drying and coagulating the protein by cooking would decrease the amount of leaching of the soluble material. Indeed Grabner et al. (1982) found that plankton which had been freeze-dried and pelleted had still retained 85 per cent of enzyme activity (e.g. proteases, dehydrogenases)after ten minutes in water, whereas only 27 per cent remained after ten minutes with frozen material indicating a significant decrease in the amount of leaching of water-soluble material including water soluble nutrients.

The poor results from the cooked krill were unexpected for reasons just discussed. As can be observed the results obtained were well below most of the other diets. A possible explanation for this would be that a certain degree of heat damage occurred during the processing resulting in destruction of certain amino acids. Thus the fish on diet CK-2 had higher PER values for the reason that since there was less protein available to them, more of the amino acids would have been channeled into protein synthesis than would be expected in a lower protein diet. No literature could be found on studies which had previously utilized cooked krill as a protein replacement for fish thus the results could not be compared to any other values.

Soybean Meal

Previous experiments concerning replacement of fish meal with soybean meal as a protein source have given various degrees of success depending on the level of replacement, species used and method of processing. Inadequacies have been found with the use of full-fat soybeans fed to chinook and coho salmon (Fowler, 1980). In contrast, Rumsey and Ketola (1975) fed rainbow trout soybean meal as the sole protein source but supplemented the diet with the necessary levels of essential amino acids to simulate the levels in fish eggs. They found growth to be slow but significantly improved over the growth rate attained with a non-supplemented diet. Feed conversion efficiency was improved and mortality decreased with amino acid supplementation of the diet.

In the present study, although the growth rates were not depressed both feed conversion efficiency and protein utilization ratios in fish fed diet SBM-3 were poorer than for the HM-3 control fish. In contrast, the response to both SBM-1 and SBM-2 was significantly greater than their common control diet (HM-1). Koops et al. (1976) also found feed conversion and growth rates of trout fed diets containing soybean meal to be equal to the responses of the fish on a herring meal control diet. It is interesting to note in the present study that although the control diet (HM-1) was equalled by SBM-1 and SBM-2, none of these diets were as comparable to the diets containing shrimp meal or freeze-dried krill meal.

The results of feeding the soybean meal diets serve to emphasize the limited capacity of the rainbow trout to utilize plant protein sources due to high carbohydrate content as well as the presence of anti-nutritional factors. Nevertheless, as Hilton and Atkinson (1982) showed in their experiments, rainbow trout are able to adapt to and utilize increased levels of carbohydrate in the diet. The results also indicate that although soybean meal may not be a desirable protein replacement as a sole source its performance is favourable for use as a protein supplement. It may be noted that the soybean meal diets were highly palatable to the trout.

SUMMARY

Comparison of shrimp meal and krill meal diets fed to rainbow trout fingerlings showed that not only are high levels of chitin not detrimental to these fish, but appear to be of benefit to them. The growth and feed efficiency results also indicate that the protein quality of shrimp meal and krillmeal is excellent, and in general, these feedstuffs proved to be very palatable to the fish. In conclusion one can assume that the potential of these ingredients as a replacement for more expensive sources of animal protein is very promising.

Comparison of protein and energy utilization , as well as feed conversion and digestibility indicate that chitinous substances in the diet may provide an added source of nutrients to the fish. The results obtained from this study lead us to hypothesize that the chitin nitrogen may be providing some nitrogen which can be utilized in protein metabolism. In addition, the energy efficiency values of this study coupled with evidence from previous studies (Alliot 1967; Pérès et al., 1973) indicate that N-acetylglucosamine may be an alternate source of energy to the fish. Should this prove to be statistically significant at higher levels in the diet the addition of chitinous substances to fish rations will remove some of the emphasis for large amounts of high protein ingredients. The saving of these costly ingredients will prove to be immensely cost efficient to the commercial fish farmer, as at present the exoskeletons from shrimp and krill peeling operations are waste material.

It is notable that even moderate heat-treatment in the preparation of meal from krill reduced its nutritive quality.

In addition it was shown that although soybean meal did not perform as efficiently as the chitin-containing diets its performance was above that of the control diets indicating its usefulness in trout diets. Previous studies have indicated a similar belief.

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APPENDIX TABLE I

STATISTICAL ANALYSIS OF BODY WEIGHTS OF TROUT IN RESPONSE TO DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Series (Ser.) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	3.19848	27			
Series	1.72706	2	.8635	18.957	$< .001^{**}$
Source	.30677	4	.0767	1.684	$< .213^{o}$
S x Ser	.57247	8	.0716	1.571	$< .225^{o}$
Error	.59218	13	.0456		

Duncan's Multiple Range Test $p \leq .05$

		Mean (g/fish)
Series	1	4.542^{c}
	2	4.768^{b}
	3	5.124^{a}

APPENDIX TABLE 2

STATISTICAL ANALYSIS OF BW GAIN/FEED CONSUMED IN RESPONSE TO DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Series (Ser) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	.13843	27			
Series (Ser)	.04327	2	.02164	17.039	< .001**
Source	.04814	4	.01204	9.480	< .001**
S x L	.03045	8	.00381	3.000	< .038*
Error	.01657	13	.00127		

Duncan's Multiple Range Test $p < .05$

		Mean
Series	1	$.692^{b}$
	2	$.725^{b}$
	3	$.785^{a}$

		Mean
Source	HM	$.692^{c}$
	SM	$.750^{b}$
	CK	$.734^{bc}$
	FD	$.800^{a}$
	SBM	$.694^{c}$

APPENDIX TABLE 3

STATISTICAL ANALYSIS OF PROTEIN EFFICIENCY RATIO (PER) IN RESPONSE TO DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Series (Ser) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	1.60005	27			
Series (Ser)	.54595	2	.27298	22.997	< .001**
Source	.52137	4	.13034	10.981	<.0004**
S x L	.37839	8	.04730	3.985	< .013**
Error	.15434	13	.01187		

Duncan's Multiple Range Test $p < .05$

		Mean
Series	1	2.459^{a}
	2	2.487^{a}
	3	2.179^{b}

		Mean
Source	HM	2.331^{b}
	SM	2.532^{a}
	CK	2.221^{b}
	FD	2.511^{a}
	SBM	2.279^{b}

APPENDIX TABLE 4

STATISTICAL ANALYSIS OF PROTEIN GAIN/PROTEIN CONSUMED IN RESPONSE TO DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Level (L) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	.02514	27			
Level	.00122	2	.00061	2.458	<.124°
Source	.01066	4	.00267	5.369	<.009**
S x L	.00681	8	.00085	1.713	<.187°
Error	.00645	13	.00050		

Duncan's Multiple Range Test $p < .05$

		Mean
Source	HM	$.231^{b}$
	SM	$.274^{a}$
	CK	$.233^{b}$
	FD	$.271^{a}$
	SBM	$.244^{ab}$

APPENDIX TABLE 5

STATISTICAL ANALYSIS OF ENERGY GAINS IN RESPONSE TO DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Series (Ser) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	12,729,573.89	27			
Series (Ser)	7,830,054.525	2	3,915,027.263	45.256	< .001**
Source	1,594,979.072	4	398,744.768	4.609	< .015**
S x L	2,179,942.608	8	272,492.826	3.150	< .03*
Error	1,124,597.685	13	86,507.514		

Duncan's Multiple Range Test $p < .05$

		Mean (cal/fish)
Series	1	3451.6^{c}
	2	3803.8^{b}
	3	4667.7^{a}

		Mean (cal/fish)
Source	HM	3559.1^{b}
	SM	4064.1^{a}
	CK	3906.5^{ab}
	FD	4130.1^{a}
	SBM	4212.0^{a}

APPENDIX TABLE 6

STATISTICAL ANALYSIS OF PROTEIN GAINS IN RESPONSE TO DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Series (Ser) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	47,795.202	27			
Series (Ser)	33,327.051	2	16,663.526	55.512	< .001**
Source	6,621.357	4	1,655.393	5.515	< .008**
S x L	5,944.489	8	743.061	2.475	< .07^{o}
Error	3,902.305	13	300.177		

Duncan's Multiple Range Test $p < .05$

		Mean (mg/fish)
Series	1	192.01^{c}
	2	212.77^{b}
	3	270.77^{a}

		Mean (mg/fish)
Source	HM	200.35^{b}
	SM	225.05^{a}
	CK	219.93^{ab}
	FD	241.47^{a}
	SBM	239.12^{a}

APPENDIX TABLE 7

STATISTICAL ANALYSIS OF ENERGY GAIN/FEED CONSUMED IN RESPONSE TO DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Series (Ser) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	.82518	27			
Series (Ser)	.27142	2	.13571	13.099	$< .001^{**}$
Source	.27918	4	.06979	6.736	$< .004^{**}$
S x L	.13991	8	.01749	1.688	$< .193^{o}$
Error	.13467	13	.01036		

Duncan's Multiple Range Test $p < .05$

		Mean
Series	1	1.282^{b}
	2	1.334^{b}
	3	1.505^{a}

		Mean
Source	HM	1.227^{c}
	SM	1.467^{a}
	CK	1.373^{ab}
	FD	1.488^{a}
	SBM	1.314^{bc}

APPENDIX TABLE 8

STATISTICAL ANALYSIS OF ENERGY GAIN/ENERGY CONSUMED IN RESPONSE TO DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Series (Ser) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	.02959	27			
Series (Ser)	.00601	2	.00301	6.076	< .010**
Source	.01074	4	.00269	5.430	< .010**
S x L	.00641	8	.00080	1.615	< .212^{o}
Error	.00644	13	.00050		

Duncan's Multiple Range Test p < .05

		Mean
Series	1	.291^{b}
	2	.298^{b}
	3	.323^{a}

		Mean
Source	HM	.274^{b}
	SM	.325^{a}
	CK	.304^{a}
	FD	.322^{a}
	SBM	.297^{ab}

APPENDIX TABLE 9

STATISTICAL ANALYSIS OF ENERGY AVAILABILITY OF DIFFERENT DIETARY TREATMENTS

Analysis of Variance

Series (Ser) = 28% protein, 11% test ingredient (1) versus 28% protein, 22% test ingredient (2) versus 36% protein, 17% test ingredient (3).

Source (S) = herring meal (HM) versus shrimp meal (SM) versus cooked krill meal (CK) versus freeze-dried krill meal (FD) versus soybean meal (SBM).

	SS	df	MS	F value	F prob
Total	401.51199	27			
Series (Ser)	24.72915	2	12.3646	1.196	$< .334^{o}$
Source	149.42716	4	37.3568	3.613	$< .03^{*}$
S x L	92.942181	8	11.6178	1.214	$< .409^{o}$
Error	134.41350	13	10.3395		

Duncan's Multiple Range Test p. .05

		Mean (%)
Source	HM	38.46^{b}
	SM	43.33^{a}
	CK	41.85^{ab}
	FD	43.82^{a}
	SBM	40.33^{ab}