UTILIZATION OF PACIFIC HAKE OFFAL MEALS OF DIFFERENT ASH CONTENT AS PROTEIN SOURCES FOR CHINOOK SALMON IN SEA WATER

by

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B. Sc., University of British Columbia, 1982

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Department of Animal Science)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1992

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Date October 15, 1992

ABSTRACT

Present demands for fish meal by the aquaculture industry in British Columbia exceed domestic supplies. The development of fisheries and onshore processing for domestically underutilized fish species have been identified as means of increasing the supply of fish waste for reduction into fish meal (DPA Group, 1988; Silver and Macleod, 1991). Pacific hake (*Merluccius productus*), with its large biomass and recent changes in quota allocations, is considered the most promising species for domestic exploitation (Silver and Macleod, 1991; Simpson, 1992). The following study was conducted to a) identify what impact high levels of ash in hake offal press cake meal would have on growth and various physiological parameters, and b) to compare press cake meals produced from non-deboned and deboned roe herring (*Culpea harengus*) and hake offals as protein sources for sea water-adapted chinook salmon (*Oncorhynchus tshawytscha*).

In Experiment 1, chinook salmon from the Robertson Creek strain were fed to satiation on diets containing either 13.4, 18.1, or 23.1% ash. Hake offal press cake meal was the sole protein source and all diets were formulated to be isonitrogenous (protein = 48%) and isocaloric (37.4 kcal of estimated metabolizable energy/kg dry diet). During the 42-day trial, there were no significant differences in fish growth, appetite or feed efficiency. The concentrations of supplementary minerals in the diet were sufficient to maintain normal growth and development in the fish.

Dietary ash levels had no impact on the terminal plasma concentrations of calcitonin or teleocalcin, gastrointestinal pH values, or gross morphology. Attempts to correlate hormonal levels to the amounts of feed in the gastrointestinal tract were unsuccessful. Elevated levels of calcitonin seemed to be related to the maturation state of some of the fish.

In Experiment 2, chinook salmon in sea water were fed to satiation on diets containing press cake meals made from deboned or non-deboned hake offal or roe herring. The diets were formulated to be isocaloric (36.8 kcal of estimated metabolizable

energy/ kg dry diet) and to contain either 35 or 48% protein which originated solely from one of the four test meals. A high protein diet (48% protein) based on freeze dried hake fillets was used as a positive control.

High protein diets supported better growth and feed consumption than the low protein diets. By the end of the experiment, fish fed the low protein diets had higher percentages of carcass lipid and correspondingly lower percentages of carcass protein than fish fed the high protein diets. Feeding behavior differed between fish consuming the low and high protein diets. In the former case, the fish would stop feeding sooner and would actively pursue, capture, and mouth the pellets before expelling them. Very little of the mouthing behavior was displayed by fish on the high protein diets..

Amongst the fish fed the high protein diets, deboning of roe herring offal tended to improve growth, feed consumption, feed efficiency, protein efficiency ratio, body protein content, productive protein value, instantaneous protein gain, and instantaneous lipid gain, whereas the opposite was true for those receiving the deboned hake offal. Examination of the performance data suggested that the amount and availability of dietary arginine, rather than the level of ash, was the limiting factor for growth of fish receiving diets based on deboned hake offal.

Fish fed the low protein diets containing deboned hake or herring meal exhibited different trends of performance to those described above for fish ingesting the high protein diets. In this case, deboning had no effect on the performance of chinook salmon fed the hake meal or herring meal diets. Despite the concentrations of arginine in the low protein hake meal diets not meeting estimated requirements and the arginine concentrations in the herring meal diets meeting the requirements, the fish consuming the hake diets tended to have the better growth performance and feed efficiency. However, the trend was not supported by the productive protein value. It can be concluded, therefore, that for the fish eating the low protein diets the hake and herring offal meals were of equal nutritional value.

This study indicates that deboning the hake offal was unnecessary and that chinook salmon seemed to be able to utilize hake offal press cake meal as effectively as the herring offal press cake meal.

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ACKNOWLEDGMENTS

This research was supported through a Department of Fisheries and Oceans grant to Dr. D. A. Higgs. Additional financial support was provided by B.C. Packers Ltd.

I would like to thank Dr. B. E. March and Dr. D. A. Higgs for their patience, dedication, support, and encouragement they provided. I would also like to thank Bakhshish Dosanjh for the laboratory expertise and general support he provided throughout the study. The generous assistance of Drs. R. C. Fargher, G . F. Wagner, and L. J. Deftos in determining the plasma calcitonin and stanniocalcin values for this study was greatly appreciated.

I would like to thank Carol Mazur and Jacquie Jacob for the assistance they provided at various times during the study. I would also like to thank the faculty, students and staff at U.B.C. as well as the staff and summer students at the Department of Fisheries and Oceans West Vancouver Lab for their assistance, and the friendship they extended throughout my studies.

1.0 INTRODUCTION

Salmonids, unlike most other domesticated animals, require diets containing up to 50% protein on a dry matter basis in order to achieve maximal growth. Kittens and rats, in comparison, only require 30% and 22% protein, respectively (Bowen, 1987; MacDonald et al., 1984; Pandian and Vivekanandan, 1985). Although salmonids and many domestic animals must consume the same amount of protein for a given amount of protein gain (3.0 - 4.6 g dietary protein/g protein gain), the energy cost of the gain is less for salmonids (Lovell, 1989). The lower energy costs occur in part because salmonids, as poikilotherms, do not use energy to maintain body temperature and they do not use energy converting nitrogenous wastes into urea or uric acid for excretion (Cowey, 1980; Randall and Wright, 1989; Van Waarde, 1988; McDonald et al., 1989).

Protein quality affects the growth of fish more than most other domestic animals (Cowey, 1975). The quality of marine protein sources is affected by freshness of the raw material, processing temperatures, presence of other compounds, essential amino acid profile, palatability, digestibility, and changes in pH (Finley, 1989; McCallum and Higgs, 1989; Phillips, 1989; Gulbrandsen, 1986; Stuart and Hung, 1989). McCallum and Higgs (1989), for instance, found that high temperature-dried herring meal (120-150^{o} C) was lower in nutritive value than low temperature-dried herring meal for juvenile chinook salmon.

Researchers have attempted to partially or totally replace expensive fish meal in salmonid diets with less costly alternative animal and plant protein sources. Results have been mixed depending upon the protein source, the species, the processing of the protein, and the level of replacement of fish meal in the diet. Some of the sources show future commercial promise, but work is still needed to determine optimal processing conditions, nutritional supplements, and degree of fish meal replacement possible for the different salmonid species (Watanabe and Pongmaneerat, 1991; Pongmaneerat and Watanabe, 1991; Pike et al., 1990; Alexis et al., 1985; Fowler, 1982; van den Ingh et al., 1991;

Dabrowski et al., 1989). For now, fish meal remains the main protein source in salmonid diets (Watanabe and Pongmaneerat, 1991).

Present levels of feed production for the B.C. salmon farming industry (est. 53,500 tonnes) already necessitate importation of fish meal to meet the industry's requirements (est. 26,750 tonnes). Domestic fish meal supplies are partly channeled into the poultry industry and the volume of fish waste available for processing into meal is insufficient. Foreign fish meal supplies can meet the long term demand, but they may be negatively impacted by economic and biological factors. (DPA Group, 1988)

The development of fisheries and onshore processing for domestically underutilized fish species have been identified as means of increasing the supply of waste for fish meal production (DPA Group, 1988; Silver and Macleod, 1991). Pacific hake (*Merluccius productus*) is considered the most promising of the West Coast species because of the large biomass of the stock and recent increases in the quota allocation for land based fish processing plants (Silver and Macleod, 1991; Simpson, 1992). For the first time, land based processors were given a quota of 30,000 tonnes (compared to 6,000 tonnes in 1991). (Simpson, 1992) This would translate into a maximum of 18,600 tonnes of offal or 4,000 tonnes of fish meal (Crappo et al., 1988; DPA Group, 1988).

Without mechanical deboning, fish meals produced from hake offal contain high concentrations of ash which may negatively affect growth and morphology of fish in fresh water (Romestand et al., 1986; Hicks et al., 1984). Little is known, however, about the influence of dietary ash content on the performance of salmonids in sea water. Hence, the current study was undertaken with sea water-adapted chinook salmon (*Oncorhynchus tshawytscha*) to: 1) evaluate the effect of graded concentrations of ash on growth, digestion, and physiology and 2) compare the quality of non-deboned and deboned hake offal to non-deboned and deboned roe herring (*Clupea harengus*) offal as protein sources.

2.0 LITERATURE REVIEW

2.1 Pacific Hake (*Merluccius productus*) - The Resource

Hake are divided into 12 species and they can be found on both sides of the Atlantic, in the Eastern Pacific, and off of southern New Zealand. They are usually found on the continental shelf or the upper part of the continental slope. Hake can be located migrating poleward in inshore waters in the spring and summer, and migrating equatorially offshore in the fall and winter. All species grow rapidly for about 3 years and then they undergo slow growth as they sexually mature (Inada, 1986). Slow growth is thought to be a consequence of dietary energy being diverted into reproduction and migration (Beamish and McFarlane, 1986).

Pacific hake (*Merluccius productus*) is the most abundant species caught by Canada's West Coast ground fish fleet (Richards and Saunders, 1990). It can be divided into four breeding stocks (offshore, dwarf, Puget Sound, Georgia Strait) two of which, the offshore and Georgia Strait stocks, are exploitable by the Canadian fishery (Inada, 1986; Bailey et al., 1982, ; Shaw et al., 1990; Stauffer, 1985; Saunders et al., 1990).

The abundance of hake make them an attractive potential protein source for B.C.'s aquaculture industry. Hake has already been used for feeding salmonids in the early 1970's when some of the stocks were exploited as a reduction fishery by the USA. Hake stocks are now used solely for human consumption (Pedersen, 1985). The offal, however, still has potential for use in salmonid feeds.

The offshore stock is the largest with an estimated biomass ranging from 1 to 2 million tonnes with 1.8 million being a likely yearly average (Stauffer, 1985 ; Bailey, 1982). Since 1987, the total yearly catch for the stock has ranged from 234,000 to 309,000 tonnes of which Canadians caught 73,000 - 98,000 tonnes. Exact Canadian allocations of the harvest are currently under dispute with the Americans. Since there is no formal agreement setting catch limits, Canadian allocations up to 1991 were based upon the traditional level of 30% of the total harvest. Americans are now recommending

that the Canadian portion of the harvest be set at 20% while Canadians are insisting on a minimum of 28% (Saunders et al., 1990; Richards and Saunders, 1990).

In 1992, onshore processors have been given a quota to process 30,000 tonnes of hake which would produce a maximum of 18,600 tonnes of offal (Crappo et al., 1988; DPA Group, 1988; Simpson, 1992). Previously, domestic processing of the offshore stock ranged from 0 to 4,000 metric tonnes. Even though all of the hake is caught domestically, whatever is not processed onshore is sold to foreign factory ships and the resulting fillets, meals and/or offal are not imported back into Canada (Saunders et al., 1990).

The biomass of hake available to Canadians is ultimately determined by the strength of specific year classes (Beamish and McFarlane, 1986; Stauffer, 1985; Bailey and Francis, 1986). Since recruitment rather than adult mortality determines year class strength, any small changes in larval survival will have a large impact. Strong year classes are closely associated with warm water in the California current and isotherms running parallel to the shore. These two factors may improve larval survival by increasing growth rates, altering the location of spawning by adults, reducing advection of eggs and larvae, and increasing food availability (zooplankton). One theory is that warm water increases the growth rate of larvae which decreases the time when they are small and vulnerable to predators (Bailey and Francis, 1986).

The Canadian offshore fishery and its quotas are dependent upon and determined by the level of hake migration into Canadian waters. The offshore stock is highly migratory compared to the other stocks with yearly migrations from Baja California to as far north as Queen Charlotte Sound. The migration's northern limit is thought to be set by colder water temperatures and competition from walleye pollock which has similar feeding habits (Inada, 1986). The pattern of migration, poleward inshore and equatorially offshore, is like that seen in other hake species (Saunders et al., 1990; Stauffer, 1985; Inada, 1986; Bailey, 1982; Richards and Saunders, 1990).

As the fish age, the degree of migration increases (Bailey et al., 1982). Most fish will be at least 5 to 6 years old before entering Canadian waters. Although fish as old as 20 years have been caught, 87 to 97% are between 5 and 12 years old (Beamish and McFarlane, 1986). The fish in Canadian waters are further segregated by sex with 70% (60-82%) of the fish being female (Beamish and McFarlane, 1985). The effect of selectively harvesting older females on the entire fishery is unknown, but reduced quotas may be needed to lessen the impact. Migration into Canadian waters may be further enhanced by anomalous strongly northward currents caused by El Nino. The resulting biomass will contain an increased proportion of smaller younger fish (Smith et al., 1990).

The main body of offshore hake remain in Canadian waters from May until November. The fishery begins some time in early July to early August and ends in mid-to-late October. Hake range in size from 48.5 to 53.6 cm for males and from 50.7 to 56.9 cm for females. Females have a mean weight of 1056 g compared to 843 g for males (Beamish and McFarlane, 1985). The main diet is euphausiids and other euphausiid predators, but pelagic fishes such as herring can be an important food source. The impact of hake on the herring roe fishery is unknown (Livingston and Bailey, 1985; Beamish and McFarlane, 1986). Because of their size, hake in Canadian waters are less preyed upon by fish than elsewhere in their range. Although the degree of predation is uncertain, hake are known to be preyed upon by killer whales, seals, and other marine mammals (Livingston and Bailey, 1985).

The Georgia Strait stock is distinct from the offshore hake and it is the single largest resident stock of fish in the strait. It is possible to break down the stock into discrete local stocks (McFarlane and Beamish, 1986; Saunders et al., 1990). Its biomass is estimated at 112,000 tonnes with a sustainable catch of 11,000 tonnes. Prior to 1992, the Georgia Strait stock had completely displaced the offshore stock for domestic use. About 83% of the commercial catch ranges in age from 4 to 11 years, but fish as old as 20 years have been caught (Shaw et al., 1990, Saunders et al, 1990).

Spawning takes place in the south central strait from March until May. At this time the fish can be found in 2 layers with females mostly in the upper layer. After spawning, most fish are found in the upper layer. By late fall they move out to the Johnston Strait and become dispersed. Adult hake range in size form 40 to 50 cm with the modal length for males being 44 cm and females 46 cm (McFarlane and Beamish, 1986).

The Georgia strait hake eat euphausiids when schooling, but they switch to a diet containing more pelagic and semi pelagic fish when they disperse. During April and May, hake may eat significant quantities of herring. Herring consumption has been estimated to be 4 to 8 thousand tonnes or roughly the equivalent of 1/3 the commercial harvest of 19 thousand tonnes (McFarlane and Beamish, 1985). Hake are prey for dogfish, walleye pollock, Pacific cod, and possibly marine mammals and birds (McFarlane and Beamish, 1986; Livingston and Bailey, 1985).

The lack of domestically available hake offal is directly related to the presence of the parasite *Kudoa paniformis* in the more abundant offshore hake (Tsuyuki et al., 1982). The parasite produces a proteolytic enzyme which at neutral pH breaks down the proteins involved with maintaining flesh texture during slow cooking. Infested fish have a texture which is unacceptable to the North American consumer. It is the lack of the parasite in the Georgia Strait stock which has allowed it to displace the offshore hake for domestic consumption (Kudo et al., 1987; Anderson, 1985; Nelson, 1985). Both offshore and Georgia Strait hake may be infected with a second Kudoa parasite, *K. thyrsitis*. This parasite also produces a proteolytic enzyme, which is active under conditions of acidic pH. The enzyme, however, does not appear to break down proteins which affect flesh texture. While the hake is alive, its flesh is protected from the enzymes by physiological processes which actively degrade and excrete them. Once the hake dies, the parasites continue to produce the enzymes which build up in the flesh (Kabata and Whitaker, 1985; Tsuyuki, 1982).

Initial Kudoa infestations stimulate the production of white pseudocysts, while older infestations tend to have black pseudocysts. The latter are considered to be a defense response to the infestations. Melanin is deposited around the cyst and eventually leads to the destruction of the parasite. Dark cysts may produce an appearance which is unacceptable to the North American consumer. The wider distribution of *K. thyrsitis* in both the Georgia Strait and offshore stocks suggest that this is an older parasite in balance with its host. *K. paniformis*, only found in the offshore stock, is probably newer to the West Coast and more virulent in its effects (Kabata and Whitaker, 1985). Offshore hake caught in Canadian waters have less severe Kudoa infestations than those captured in American waters and they also have a lower incidence of *K. paniformis* relative to *K. thyrsitis*. The lower incidence of this parasite may improve the potential for developing a domestic market for offshore hake.

2.2 Minerals

In the present study, the effects of high levels of dietary minerals and their interactions could not be predicted accurately since both dietary and waterborne minerals may be used by fish to meet their mineral requirements (Hilton, 1989). The ability of fish to use the waterborne and dietary minerals may be dependent upon environmental as well species differences. Tilapia (*Oreochromis mossambicus*), for instance, appear to obtain considerable magnesium from the water and hence they can, in contrast to other fish species, be maintained on diets with low magnesium content (Van Der Velden et. al., 1991). The uptake of waterborne zinc by freshwater trout (*Oncorhynchus mykiss*) is independent of dietary levels, but it can partially compensate for dietary deficiencies (Spry et. al., 1988). In chinook salmon, potassium can be sequestered directly from water, but, as with zinc in trout, it can only partially compensate for dietary deficiencies (Shearer, 1988).

Fish species also exhibit differences in their ability to utilize minerals from a common dietary source. For example, in experiments by Satoh et. al. (1987a, 1989,

1991) who examined the availability of manganese (Mn) from white fish meal in the diets of freshwater trout and carp (*Cyprinus carpio*), it was observed that each species required different supplemental levels of manganese in the diet (15 μg/g and 10 μg/g, respectively) to achieve normal growth. Satoh et. al. (1989) concluded that the inability of tricalcium phosphate to affect manganese absorption in carp, even though it affected zinc uptake in trout, was due to the absence of a stomach in the carp. Consequently, the carp were unable to dissolve tricalcium phosphate and this led to less interactions between Mn and calcium or phosphate.

The concentrations of individual minerals in the diet can impact the utilization of other dietary components including other minerals. Suboptimal levels of magnesium in carp diets increases hypercalcinosis and reduces growth (Dabrowska et al., 1991). In trout, copper and selenium appear to interact and modify the toxicity of each other. In the case of protein, minerals may complex with digested protein fragments rendering them unavailable, or the minerals, in a transitional form, may bind with protein fragments making the minerals more available (Wapnir, 1989).

2.3 Calcium Homeostasis

2.3.1 Plasma Calcium

Plasma calcium may be bound to protein, complexed with compounds like bicarbonate, or exist in ionized form. In healthy humans with plasma calcium concentrations of approximately 2.42 mmol/L, 45-50% of the calcium is ionized, 40-45% is bound and 5-10% is complexed (Driessens et al., 1989; Nordon, 1990). Freshwater trout plasma normally contains 2.33-2.60 mmol of calcium/L with 1.33-1.54 mmol/L (57-59% of total plasma calcium) of it being ionized (Andreasen, 1985). Overall calcium homeostasis is maintained by hormonal control of the ionized fraction and by the concentration of albumin, globulin, bicarbonate and organic acids with which the calcium can bind (Nordin, 1990; Andreasen, 1985).

The relative proportions of plasma calcium components remain constant as total calcium levels change. Even when there are high plasma calcium concentrations, theoretically saturable plasma components like albumin are well below their maximum calcium-carrying capacity (Nordin, 1990). The stability of plasma calcium in response to stress is unclear. Andreasen (1985) reported that plasma calcium concentrations of trout did not significantly change (2.33-2.60 mmol/L) during normal stress while Kostecki (1984) found significant changes (1.1-3 mEq/L) during osmotic and ion-osmotic stresses.

2.3.2 Calcium Uptake

As with other minerals, the relative importance of environmental and dietary calcium varies with the condition of the fish, the level of calcium in the diet, the water temperature, the adaptation of the fish to fresh water or sea water, and the level of calcium in the water (Berg, 1968; Romanenko et al., 1987; Shehadeh and Gordon, 1969, Hobe et al., 1984; Perry and Wood, 1985; Kostecki, 1984). In fresh water-adapted trout, the importance of calcium uptake from ingested water is limited because the fish do not drink. Conversely, salt water-adapted trout drink continuously and they can absorb 0.43 mmol of calcium/kg body weight/day from ingested sea water (Shehadeh and Gordon, 1969). Likewise, it has been estimated that Atlantic cod absorb 0.48 mmol of calcium/kg body weight/day or approximately 70% of the total calcium ingested (Bjornsson and Nilsson, 1985). Under conditions of starvation or dietary calcium deprivation, trout and goldfish (*Carassius auratus*) can compensate by using gill uptake to meet their physiological needs for calcium. Other species like tilapia (*Oreochromis niloticus*) may require both gill and dietary uptake to meet their calcium needs (Berg, 1968; Ichii and Mugiya, 1981; Takagi et al., 1989b). Studies on trout have indicated that, depending upon waterborne calcium levels, the gill can account for about half of the whole body calcium uptake (Perry and Wood, 1985; Perry and Flik, 1988; Fenwick, 1989).

For the influx of calcium across the gill to occur, fresh water-adapted fish must be able to absorb calcium from a hypocalcemic environment, relative to its body, and then

actively transport the calcium up a concentration gradient into the blood (Fontaine, 1964; Andreasen, 1985). The uptake of calcium in fresh water is thought to be a two stage transcellular process (Fenwick, 1989).

The first stage of uptake involves the passive movement of calcium from the water, at a concentration of 10^{-3} - 10^{-4} M, into the cytosol of the chloride cell, at a concentration of 10^{-7} M (Grodfraind-DeBecker and Godfraind, 1980; Racker, 1980; Rega, 1986a, b; Fenwick, 1989; Perry and Wood, 1985). Since the passive uptake appears to be saturable, the process likely involves a carrier-mediated mechanism. To protect chloride cell functions from changing calcium levels, the cell probably contains calcium binding protein(s) (Fenwick, 1989; Perry and Wood, 1985; Krause, K., 1991).

The final stage of calcium uptake involves the active transport of calcium across the basal-lateral membrane. In the membrane, a high-affinity Ca^{2+}-ATPase transport system is thought to be the Ca^{2+}-pump. (Fenwick, 1989; Flik et. al, 1984). Calcium efflux seems to occur by passive movement along a paracellular route. A small amount of calcium influx probably occurs along the same path (Fenwick, 1989).

Several authors have speculated that, if the high-affinity Ca^{2+}-ATPase transport system is not saturated under normal conditions, the rate of calcium uptake by the gill could quickly be adjusted by changing the permeability of the apical membrane. This would enable the fish to react to sudden changes in external calcium concentrations or to altered hormonal levels (Fenwick, 1989; Perry and Wood, 1985).

The general model for calcium uptake in the sea water gill is thought to be similar to that for the intestine of terrestrial animals. In terrestrial animals, dietary calcium uptake occurs by active and/or passive transport. The significance of each mechanism is determined by the their location in the gut and the calcium concentration. Active transport involves the movement of calcium into the cytosol by diffusion or with the assistance of an alkaline phosphatase-Ca^{2+} ATPase and related brush border protein, a vitamin D-dependent membrane calcium-binding protein, or calmodulin (Haussler et al.,

1970; DeJonge et al., 1981; Wasserman et al., 1977; Kowarski and Schachter, 1980; Thomasset et al., 1981; Nellans and Popovitch, 1981; Nemere et al., 1984; Bikle and Munson, 1985, 1986; Robertson, 1989). Once in the cytosol, calcium binding protein causes the calcium to bind to various cellular components or to be taken up by mitochondria (Bronner, 1987; Villereal and Palfrey, 1989). Calcium is then actively transported across the basal-lateral membrane by a Ca^{2+}-ATPase or a calcium release mechanism which requires sodium (De Jonge et al., 1981; Nellans and Popovitch, 1981; van Os et al., 1981; Ghijsen and van Os, 1982; Nellans and Popovitch, 1984; Robertson, 1989).

It is uncertain how closely the mechanisms for dietary calcium uptake in fish and terrestrial animals resemble each other. Nakamura (1985) found no evidence of active calcium absorption in the carp intestine. Sundell and Bjornsson (1988) working with coho salmon (*Oncorhynchus kisutch*) detected both a passive and saturable uptake of calcium with the latter accounting for 60% of the uptake. Chlorpromazine, an inhibitor of calcium-dependent ATP hydrolysis, was found to suppress the hormonal influences of vitamin D or its analogs on gut calcium uptake in goldfish, North American eels (*Anguilla rostrata*), and Atlantic cod (*Gadus morhua*) (Fenwick, 1984; Fenwick et al., 1984; Sundell and Bjornsson, 1988). All of these studies hint at, but do not prove, a mechanism for active transport of calcium across the gut. It is possible that hormonal influences alter the permeability of the mucosal membrane rather than modifying active transport mechanisms (Sundell and Bjornsson, 1990).

Even though the calcium concentration gradient across the gut favours influx, there is some evidence that marine fish may be able to increase calcium uptake in response to increased physiological demand. Atlantic cod, for instance, were found to increase dietary calcium uptake during sexual maturation (Sundell and Bjornsson, 1988; Sundell and Bjornsson, 1990).

2.3.3 Calcium Excretion

Calcium excretion along the intestine is affected by water temperature, amount of dietary calcium, and the level of environmental calcium (Dabrowski et al., 1986; Sundell and Bjornsson, 1988; Romanenko et al., 1987). Sundell and Bjornsson (1988) discovered that calcium efflux across the Atlantic cod's gut is a saturable process that accounts for 50% of the extrarenal excretion. Carp increase intestinal calcium excretion in response to increasing levels of environmental calcium. The same response is affected by water temperature (Romanenko et al., 1987).

The kidney is an important organ for the excretion of water and metabolic waste. Interspecies differences in the kidney structure may significantly affect the excretion and reabsorption of calcium (Laverty and Clark, 1989; Yokota et al., 1985; Dantzler, 1989). In the Atlantic cod, the kidney accounts for 60% of the total calcium excretion and the rate of calcium excretion is determined by the rate of urine flow (Sundell and Bjornsson, 1988; Bjornsson and Nilsson, 1985). By contrast, Hickman (1968) found the southern flounder (*Paralichthys lethostigma*) kidney only accounts for 12% of total calcium excretion and Flik et al. (1985) found no substantial amount of calcium excreted by the tilapia kidney.

One kidney structure that might impact calcium excretion is the glomerulus. Differences in the degree and rate of filtration by glomeruli can modify the composition of the urine further down the nephron. The glomerular filtration rate (GFR) is dependent upon the net pressure gradient, glomerular plasma flow, the permeability of glomerular capillaries, capillary surface area, the number of glomeruli, and the activity of the glomeruli (Yokota et al., 1985, Dantzler, 1989).

A typical 100 g freshwater teleost has a GFR of 0.88 ml/hr, a predicted single nephron GFR of 0.89 nl/min, an average glomerular diameter of 88 micrometers, and a total of 16,500 glomeruli. A comparable marine teleost has a GFR of 0.12 ml/hr, a predicted single nephron GFR of 0.20 nl/min, an average glomerular diameter of 49 micrometers, and a total of 9,780 glomeruli (Kayser and Heusner, 1964; Nash, 1931;

Yokota et al., 1985). Fractional water reabsorption is commonly 61% in saltwater teleosts and 40% in freshwater teleosts (Kayeser and Heusner, 1964) . The differences in glomeruli between freshwater and saltwater fish are related to the need of freshwater fish to excrete water and saltwater fish to conserve it (Yokota et al., 1985).

Measured single nephron GFR's for trout were reported to differ from the predicted values of 0.89 nl/min for fresh water-adapted trout and 0.20 nl/min for sea water-adapted trout. Measured values were found to be 1.31 nl/min and 3.74 nl/min, respectively. The values may have been influenced by the heterogenicity of glomerular function and possible intermittent nephron function. In freshwater trout, 45% of the glomeruli actively filter while in saltwater trout the value is only 5% (Brown et al., 1978; Brown et al., 1980; Yokota et al., 1985; Curtis and Wood, 1991).

Excluding the glomeruli, the remainder of the freshwater teleost nephron is composed of a ciliated neck, a two segment proximal tubule, an intermediate segment, and distal tubule which empties into a collecting duct. This contrasts with the marine teleost where the collecting tubule replaces the proximate tubule, a third segment in the proximal tubule may be present, and an intermediate segment may occur between the first and second proximal tubules. The euryhaline teleosts have the same structure as the freshwater teleost, but lack the intermediate segment (Dantzler, 1989).

In salt water-adapted euryhaline and marine teleosts there is a net efflux of calcium across the renal tubules (Hickman and Trump, 1969). The rate of secretion is determined by the level of plasma calcium and controlled by both a fast and slow mechanism. The latter is saturable, ATP-dependent, sodium dependent, and partially inhibited by magnesium (Renfro et al., 1982; Renfro, 1978; Dantzler, 1989).

Unlike the other teleosts, the renal tubules of fresh water-adapted euryhaline and freshwater teleosts absorb calcium and other electrolytes (Hickman and Trump, 1969; Butler and Youson, 1988). So far, the mechanism(s) for absorption across the tubules is unknown (Dantzler, 1989).

Curtis and Wood (1991) noted that freshwater rainbow trout urinated about once every 25 minutes. During the residency time in the bladder there was a significant reabsorption of sodium and chloride, but not calcium.

2.3.4 Calcium Reserves

In the goldfish and tilapia, most of whole body calcium is found in the scales (approx. 20%) and the bones (approx 75%) (Berg, 1968; Takagi et al., 1989a). Takagi et al. (1989a) found the influx of calcium into isolated scales seems to be a passive process which is dependent upon the condition of the fish, but independent of calcemic hormones. Wendelaar Bonga and Lammers (1982) reported that scale growth could be stimulated by 9 days of continuous calcitonin infusion. The calcitonin, however, did not affect calcium levels in the scale matrix. Calcium uptake by bone is an active process that can be affected by hormones and the level of plasma calcium (Wendelaar Bonga et al., 1982; Flik et al., 1986, Wendelaar Bonga et al., 1983).

Under conditions of increased physiological demand, calcium from both scales and bone can be utilized to maintain plasma calcium homeostasis (Carragher and Sumpter, 1991; Takagi et al. 1989b; Parenti, 1986). The relative contribution of each is not clear. Carragher and Sumpter (1991), after injecting estradiol into rainbow trout, noted that scale calcium was significantly reduced but rib and vertebrae calcium were not. Takagi et al. (1989) found indications that hypercalcemia found in descaled tilapia was caused by the mobilization of bone calcium.

The availability of bone calcium may be affected by the bone type(s) present. Acellular bone types are composed of matrices which lack lacunar spaces, canaliculi or encapsulated osteocytes while cellular bone types are composed of matrices which encompass osteoblasts and osteocytes (Parenti, 1986; Wendelaar Bonga et al. 1983). Because there is no active bone degradation by osteoblasts, calcium from acellular bone is only available when plasma calcium levels are low enough to create a concentration gradient from the bone to the plasma (Wendelaar Bonga et al. 1983). Conversely,

calcium from cellular bone can become actively available when hormonal signals stimulate increased osteocyte bone degradation (Lopez et al., 1977).

Acellular bone is associated with higher teleosts and cellular bone with lower teleosts. The skeletons of some fish species, such as those in the family Salmonidae, incorporate both bone types (Parenti, 1986).

2.4 Hormonal Control of Plasma Calcium

2.4.1 Calcitonin

Calcitonin has been noted for its ability to lower plasma calcium (Copp et al., 1962). It is a 32 amino acid peptide with regularly spaced hydrophobic amino acids in the central region, a 1-7 disulfide bridge at the amino end, and a proline amide at the carboxyl end. This composition allows at least part of the hormone to form an amphipathic helix in which one face of the helix is hydrophobic and the other hydrophilic. The helix, along with other conformational properties, contributes to the biological activity of the hormone (McDermott and Kidd, 1987; Epand and Epand, 1986).

Thyroid C cells are the main source of calcitonin in mammals, but calcitonin production has been noted in the pituitary, cerebrospinal fluid, lung, liver, gut, bladder, and thymus (McDermott and Kidd, 1987). While calcitonin has been studied extensively in mammals, the exact physiological role of the hormone is uncertain. Calcitonin is thought to be involved in chronic regulation of skeletal homeostasis, calcium balance and 1,25-dihydroxycholecalciferol antagonism (McDermott and Kidd, 1987; Stevenson, 1980). Calcitonin may also play a role in gastrointestinal activities and vasoconstriction.

In fish the hormone is produced in the ultimobranchial bodies (Copp and Parkes, 1967). Its function, however, is much less understood in fish than in mammals. Some studies on salmonids have found plasma calcitonin elevation associated with hypocalcemia, as in mammals, while others have linked the hormone to either hypercalcemia or no response (Foucheereau-Peron et al., 1987; Foucheereau-Peron et al.,

1986; Pang and Grant, unpublished data, c.f., Pang, 1971). Recent evidence suggests that calcitonin may have no immediate impact on short-term hypercalcemia in salmonids. Bjornsson et al. (1989) have proposed that the changes in plasma calcium and calcitonin concentrations seen in fish studies may be purely coincidental since mutual physiological occurrence does not mean the events are linked.

Changes in plasma calcitonin concentrations may be related to the processes of sexual development and smoltification. Positive correlations have been found between increases in calcitonin and sexual maturation in sockeye, desmoltification of coho, and early smoltification of coho (Bjornsson et al., 1989; Watts et al., 1975). In trout, increases in plasma calcitonin concentrations have been noted to be directly related to the gonadosomatic index. The same study also found a positive correlation between levels of plasma calcitonin, ultimobranchial calcitonin, and plasma calcium in females. The increased plasma calcium concentration was caused by the movement of calcium to the ovaries for uptake by the developing oocytes (Pang, 1973; Van Bohemen and Lambert, 1982; Fouchereau-Peron, 1990). Data in the literature, however, do not reveal any mechanism or causative relationship between calcitonin and maturation. It is not apparent, for example, whether increased calcitonin production affects maturation or whether maturation affects calcitonin (Fouchereau-Peron, 1990). In mammals calcitonin production has been found to be stimulated by gonadal hormones (Fouchereau-Peron, 1990; Garel and Julienne, 1977; Toverud et al., 1978).

2.4.2 Stanniocalcin

A relationship between the Corpuscles of Stannius (CS) and plasma calcium homeostasis was noted in early studies on stanniectomized European eels (*Anguilla anguilla*) (Fontaine, 1964). Fontaine (1964) found that the removal of CS caused the plasma calcium concentration to increase by 141%. Similar studies conducted on North American eels found that plasma calcium titres rose by 75% (Butler, 1969).

Experiments on eels have pointed to the loss of an anti-hypercalcemic factor (hormone) in the CS as the cause of increased plasma calcium titres (Milet et al., 1979; So and Fenwick, 1979; Lopez, 1970; Perry et al., 1989; Hanssen et al., 1989). The hormone, previously known as teleocalcin or hypocalcin, has recently been named stanniocalcin.

The duration of the antihypercalcemic effect of stanniocalcin is unclear. Fenwick and Brasseur (1991) reported that after an acute calcium challenge, the effect of stanniocalcin on eels disappeared once normocalcemia was achieved 6 hours later. A short duration effect would reduce the chances of the hormone causing hypocalcemia. Hanssen et al. (1989) discovered a different response in hypercalcemic stanniectomized eels. A single injection of stanniocalcin continuously reduced plasma calcium concentration for 24 hours and the action of stanniocalcin continued for over 48 hours. Moreover, these researchers reported that they were unaware of any other protein hormone which could produce effects of similar duration.

Extrinsic stanniocalcin efficacy seems to be influenced by cyclical changes of plasma calcium concentration in trout, sockeye and coho (Wagner et al., 1985; Wagner et al., 1986; Milliken et al., 1990). Stanniocalcin administered by injection, for instance, was not shown to have any effect at the low points in the cycles, but at the peaks they reduced calcium uptake. High levels of endogenous stanniocalcin at the cycles' low points might act to limit the impact of exogenous hormone (Milliken et al., 1990). Some of the cycling in the plasma calcium concentrations may be due to changing levels in stanniocalcin. Alternatively, the calcium cycling and efficacy of stanniocalcin could be affected by other hormones like prolactin (Wagner et al., 1988).

The target tissue of stanniocalcin is the gill where it inhibits transbranchial calcium uptake but not calcium efflux (Lafeber et al., 1988; Perry et al., 1989; Wagner et al., 1988; Fenwick and Brasseur, 1991). The level of calcium in the external environment, therefore, determines the degree of post-stanniectomy hypercalcemia (Pang

et al., 1973; Fenwick, 1974). Lafeber et al. (1988) suggested that the hormone acts by modulating calcium channels in the apical membranes of the transporting cells (chloride cells).

The gill uptake of calcium may be tonically inhibited by basal levels of stanniocalcin (Perry et al., 1989). Such action would allow the uptake to be increased or decreased in response to environmental or physiological conditions.

There is some evidence that the intestine might also be a target tissue for stanniocalcin (Takagi et al., 1985). Stanniectomized trout adapted to a mixture of 2/3 fresh water and 1/3 salt water had significant calcium uptake across the anterior portion of the gut. Although the results do not prove a direct link to stanniocalcin, they do point to an anti-hypercalcemic factor in the CS causing the effect.

The CS, unique to bony fish, are located in the anterior head and cardinal vein walls of the mesonephric kidney (Butler, 1969). Two cell types (I and II) are though to be within the CS, but it is possible that they are different forms of a single cell type (Kaneko et al., 1988). Although both cell types contain stanniocalcin, Type I cells with their large secretory granules are its primary source (Kaneko et al., 1988; Wagner et al., 1988).

Originally, the size and structure of the hormone was unclear. Various authors reported molecular weights in salmonids of 54 kDA (Lafeber et al., 1988), 39 kDA (Wagner et al 1986), 56 kDa (Flik et al., 1990) and 3 kDA (Copp and Ma, 1981). Most evidence in salmonids points to the hormone having a molecular weight of around 54 to 56 kDA and a pro-form of 60 to 64 kDA (Flik et al. 1989; Wagner et al., 1989; Wagner and Friesen, 1989; Flik et al. 1990). In the eel, the hormone is likely 2 kDA heavier in both the native and pro form (Flik et al., 1989).

Stanniocalcin is comprised of two identical glycosylated polypeptide chains which are linked by disulfide bridges - a homodimer (Wagner and Friesen, 1989; Flik et al., 1990). Only one other hormone, Mullerian inhibiting substance, is known to be a

homodimer (Wagner et al., 1989). Each peptide in the pro-form of eel stanniocalcin is composed of 263 amino acids which can be broken down into a 17 amino acid signal peptide, a prosegment of 15 residues, and a 231 amino acid mature hormone (Butkus et al., 1989). The first 40 amino acids from the N-terminal of trout and eel stanniocalcin are 80% homologous while within the salmonids there is greater than 90% homology (Wagner and Friesen, 1989; Butkus et al., 1989). Stanniocalcin shows no homology with any other known hormone (Wagner et al., 1988; Milliken et al., 1990).

Stanniocalcin N-terminal fragments containing the first 20 amino acids seem to reduce calcium uptake in trout (Milliken et al., 1990). This portion of the molecule is likely involved with the hormonal receptor binding site. C-terminal fragments containing the final amino acids (202-231) in the hormone actually stimulate calcium uptake. The latter probably interfere with receptor binding sites. This, in turn, would limit the inhibitory effect of stanniocalcin on the gill.

2.4.3 Prolactin

Prolactin is a globular protein from the pituitary gland which contains 177-200 amino acids (Wallis, 1978; Jibson and Li, 1979; Nicoll et al., 1986; Hirano et al., 1987). In two dimensional form, all prolactins have a small loop of amino acids at the carboxyl end of the protein and a second larger loop comprising about 70% of the molecule. Mammalian prolactins differ from those of teleosts due to the presence of a small amino acid loop at the protein's amino terminus (Nicoll et al. 1986; Kawauchi et al., 1983). In three dimensions, the protein contains alpha helixes which form tertiary structures composed of coils (Rubinshtein and Pankov, 1989).

Two forms of prolactin have been identified in the Japanese eel, chum salmon, carp and tilapia. Within each species, the forms are highly similar with homologies of 70% in tilapia and 97% in chum salmon. Between teleost species there is also a high degree of homology. Prolactin homologies of 77% have been found between carp and salmon and 60-80% between Japanese eel and other teleosts. These values contrast with

homologies of 36% between carp and mammals and 20-30% between Japanese eels and mammals (Suzuki et al., 1991; Yasuda et al., 1986; Yamaguchi et al., 1988; Yasuda et al., 1987). There may, however, be some species differences in prolactins. Suzuki et al. (1991) noted that both chum prolactin and a homogenate of prolactin producing cells (rostral pars distalis) from the Japanese eels, unlike ovine prolactin, produced no naturemia in tilapia.

Prolactin is known to have at least 82 different functions in vertebrates. These can be classified as being involved with either reproduction, osmoregulation, growth and development, changes in ectodermal structures, or synergism with steroid hormones (Nicoll and Bern, 1972; Hirano et al., 1987).

In teleosts, the primary role of prolactin is believed to be related to the maintenance of water and mineral balance in fresh water (Bern, 1975; Grau et al., 1984). Indirect evidence of this can be seen by changes in plasma prolactin levels, receptor binding affinity, and release of prolactin from pituitary cells in response to shifts in osmotic pressure. The relative importance of the hormone appears to vary with species. Some fish cannot survive without prolactin in fresh water while others possess this ability (Hirano et al., 1987; Hirano et al., 1986; Prunet et al. 1985, Helms et al., 1991; Dauder et al., 1990, Young et al., 1989; Yada et al., 1991, Hasegawa et al., 1987). As with other vertebrates, teleostean prolactin may also play a role in other physiological functions such as stress response, reproduction, growth, and metabolism (Hirano, 1986; Avella et al., 1991).

Prolactin is thought to be a hypercalcemic hormone in teleosts. For example, ovine prolactin has been found to induce hypercalcemia in tilapia (Flik et al., 1986), killifish (*Fundulus hereroclitus*) (Pang, 1981), and the American eel (Flik, 1984). Also, the Japanese eel (*Anguilla japonica*) (Hasegawa et al., 1986) and coho salmon (Fargher and McKeown, 1989) were noted to develop hypercalcemia in response to chum salmon

(*Oncorhynchus keta*) prolactin. Moreover, Fargher and McKeown (1989) demonstrated that they could decrease prolactin levels by injecting coho with calcium chloride.

Prolactin appears to maintain water and mineral balance by acting on osmoregulatory surfaces such as the gill, gut, kidney and urinary bladder to reduce their permeability to water and ions and increase the activity of ionic pumps. The exact mechanism may vary among species. Why some species cannot survive in fresh water without prolactin while others can remains unclear (Hirano, 1986; Hirano et al., 1987; Flik et al, 1984; Suzuki, 1991). The prolactin-induced hypercalcemia may be a result of the same mechanism(s). Flik et al. (1984), working with the American eel, reported that ovine prolactin was able to stimulate high-affinity Ca^{2+}-ATPase, which is the ionic calcium pump in the gill.

2.4.4 Cholecalciferol

Teleosts require dietary cholecalciferol to achieve normal growth (Halver et al., 1982). In trout, cholecalciferol is stored in the liver as a fatty acid ester of cholecalciferol (10.9 ng/g liver) with smaller amounts of cholecalciferol (3.6 ng/g). Even smaller amounts are stored as 25-hydroxycholecalciferol (0.7 ng/g) (Hayes et al., 1986; Fraser, 1979; Bills, 1927; Sundell and Bjornsson, 1990).

Studies on the metabolism of cholecalciferol in teleosts have yielded contradictory results. Hayes et al. (1986) discovered that 25-hydroxycholecalciferol is converted in the liver into 1,25-dihydroxycholecalciferol and 25,26-dihydroxycholecalciferol in fresh water- and salt water-adapted rainbow trout. The former metabolite was produced when trout were held under hypocalcemic conditions while the latter was observed when they were held under hypercalcemic conditions (Hayes et al., 1986). These findings, however, conflict with those of Barnett et al. (1979, 1982) who observed that in rainbow trout cholecalciferol was not converted into any other metabolite.

In both cellular and acellular bone, 24,25-dihydroxycholcalciferol stimulates osteoblasts to increase the production of new bone (Lopez et al., 1977; Wendelaar Bonga et al., 1983), whereas 1,25-dihydroxycholcalciferol demineralizes both bone types by different mechanism(s). In cellular bone the latter metabolite acts directly by stimulating osteoclasts to actively osteolyse the bone (Lopez et al., 1977). In acellular bone it acts indirectly by reducing plasma calcium levels (Wendelaar Bonga et al., 1983).

Intestinal calcium absorption is stimulated by cholecalciferol in goldfish and by cholecalciferol and 1,25-dihydroxycholcalciferol in the American eel (Fenwick, 1984, Fenwick et al., 1984). In these fish, cholecalciferol and its derivative produced hypercalcemia only when a dietary calcium source was present. This finding suggests that the intestine was the only site for stimulated calcium uptake. Ergocalciferol and 24,25-dihydroxycholecalciferol had no effect.

The results in the marine teleost, Atlantic cod, differ from those found in freshwater teleosts (Sundell and Bjornsson, 1990). In this case 25-hydroxycholecalciferol was found to increase intestinal calcium uptake by 65% while 24,25-dihydroxycholecalciferol decreased uptake by 36%. Cholecalciferol and 1,25-dihydroxycholecalciferol had no effect on calcium uptake. These findings suggest that the activities of cholecalciferol metabolites are different for freshwater and marine teleosts.

2.5 Protein

2.5.1 Protein Requirements

The dietary protein requirements for growing fish seem to be subject to different interpretations. On a percentage of the diet basis, they resemble those for terrestrial carnivores rather than omnivores. Fish generally require diets with 35-55% protein to achieve maximal growth rates (Bowen, 1987). In contrast, the requirements for kittens (carnivore) and rats (omnivore) are 30% and 22%, respectively (Bowen, 1987, MacDonald et al., 1984). On this basis, it could be concluded that fish have a higher

dietary protein requirement than most domestic monogastric animals (Stuart and Hung, 1989).

When protein requirements of growing fish are viewed in relation to dietary energy, the conclusions drawn can be different. The grams of dietary protein per gram protein gain for the catfish (*Ictalurus punctatus)* (3.2) is approximately the same as for the pig (4.6) and broiler chicken (3.0). The dietary energy needed per gram of protein gain, though, is significantly lower in the catfish (21 kcal) compared to the pig (83 kcal) and broiler chicken (43 kcal) (Lovell, 1989). Kim et al. (1991), feeding a purified diet with 40% protein to trout, reported that 60% of the protein was used for indispensable amino acid requirements and the remainder was catabolized for energy needs.

Although the protein-sparing effects of lipids and carbohydrates are limited in some fish species, their protein requirements for maintenance are still less than those for domestic monogastric animals (Kaushik and Gomes, 1988; Lie et al., 1988). Fish do not need to maintain a constant body temperature and, compared to terrestrial animals, they use less energy to excrete nitrogenous wastes and counteract gravity (Lovell, 1989). In this context, the higher dietary protein levels could be interpreted as resulting, in part, from less dilution by purely caloric components (e.g. carbohydrates) found in the diets of domestic monogastric animals (Lovell, 1989, 1991; Bowen, 1987).

Protein requirements can be affected by a variety of factors. Feeding frequency, other energy sources in the diet, the relative levels of protein to lipid in the feed, the amount of available oxygen in the environment, the total energy level of the diet, and environmental stresses have all been found to alter the protein requirements of fish (Kaushik and de Oliva Teles, 1985; Kim et al., 1991; Silver et al., 1992; Legrow and Beamish, 1986; Pedersen, 1987; Kaushik and Gomes, 1988)

2.5.2 Protein Digestion

Generally, the digestion of protein is initiated by acid and pepsin in the stomach. Thereafter, protein is further digested by endopeptidases, such as trypsin and

chymotrypsin, secreted by the pancreas and intestinal wall into the intestinal lumen. Finally, the protein is broken down into amino acids and small peptides by enzymes in the brush border of the intestine (Reichenbach-Klinke, 1972; Madge, 1975).

The mucosa of the fish intestine contains a single layer of columnar cells thrown into a series of longitudinal ridges. These are lined by a brush border (Ferraris and Ahearn, 1984). The length of the gut and degree of intestinal epithelial folding seems to be related to the type of diet naturally consumed by the species. Using the ratio of gut length to total body length, Reifel and Travill (1978) and Kapoor et al. (1975) found a tendency for carnivores to have the shortest intestines whereas omnivores had intermediate lengths and herbivores had the longest intestines. Carnivores also seem to have greater intestinal epithelial folding than herbivores, possibly to increase the absorptive surface area to compensate for the shorter intestine (Ferraris and Ahearn, 1984).

Even though some families of fish lack stomachs and the resulting acid/pepsin activity, their ability to digest protein appears to be roughly equivalent to fish with stomachs (Steffens, 1981; Grabner and Hofer, 1989; Reichenbach-Klinke, 1972). Food digestion in the trout stomach enhances the hydrolysis of soluble proteins into smaller peptides, but does not result in the release any free amino acids. For some species of fish, stomach digestion is also associated with absorptive capabilities in the pyloric ceca and shorter intestinal length (Grabner and Hofer, 1989; Buddington and Diamond, 1987; Buddington et al., 1987).

The pyloric cecae, when present, appear to participate in protein and lipid digestion and absorption. By sieving out larger particles, trout cecae contain chyme which is rich in partly fragmented and solubilized digesta plus digestive secretions (Buddington and Diamond, 1987). Free amino acids may be produced in the cecal region since the brush border contains dipeptidases (Ash, 1980). The pyloric cacae may also provide a way to increase intestinal absorptive surface area without increasing

intestine length or thickness. In trout and tuna, the cecae account for 70% and 90%, respectively, of the total gut surface area (Buddington and Diamond, 1987).

Fish are able to adjust the digestive process in response to diet and the environment (Brannon, 1990, Kawai and Ideda, 1972; Kawai and Ikeda, 1973; Reichenbach-Klinke, 1972; Nagata, 1989; Kuz'mina, 1989; Hofer, 1979; Hofer et al., 1975). The activity of digestive enzymes, gastric evacuation, and total intake can change in response to temperature (Kuz'mina, 1989; Hofer, 1979; Nagata, 1989). Atlantic salmon (*Salmo salar*), rainbow trout, lake trout (*Salvelinus namaycush*), and coho salmon can moisten their digesta if they are consuming dry diets (Hughes and Barrows, 1990). Increases in dietary protein content enhance the ratio of pancreatic enzymes relative to other digestive enzymes in the gastrointestinal tract of rainbow trout (Kawai and Ikeda, 1973; Stevens and McLeese, 1988) and carp. Rainbow trout, however, cannot adjust the amount of trypsin secreted in relation to the size of the meal (Stevens and McLeese, 1988). Exogenous enzymes from consumed organisms can play a significant role in the digestion process of some species (Reichenbach-Klinke, 1972).

2.5.3 Uptake of Protein Across the Intestine

Ferraris and Ahearn (1984) speculated that, in fish, the lower intestinal affinities for the uptake of amino acids in herbivores and for carbohydrates in carnivores were adaptations to lower physiological concentrations of each substrate in the respective intestines. Buddington et al. (1987), who examined the uptake of proline and glucose, came to similar conclusions about the rate of uptake of those substrates. The authors argued, however, that the gut's summed uptake capacity or uptake along the entire length of the intestine, is the measure of physiological significance. Based upon summed uptake they found that herbivores, omnivores, and carnivores differed in their capacity for the uptake of carbohydrates, but not amino acids. They also found that catfish develop a greater capacity for carbohydrate uptake as they grow.

The amount of and capacity for amino acid uptake seems to decrease along the length of the gut from the proximal to distal end (Marcotte and De La Noue, 1984; Buddington and Diamond, 1987; Dabrowski and Dabrowska, 1981; Dabrowski, 1983a, 1983b; Shcherbina, 1984). In trout, most of the amino acids are absorbed in the anterior to middle part of the gut including the pyloric cecae (Marcotte and De La Noue, 1984; Buddington and Diamond, 1987; Dabrowski and Dabrowska, 1981). Buddington and Diamond (1987) found that, depending on the amino acid, the cecae accounted for 67% - 81% of the total intestinal uptake of the amino acid. Bell et al. (1987) working with Atlantic salmon found that rate of glutamine uptake in the proximal portion of the intestine (including the cecal region) was more adaptable to dietary glutamine levels than the distal portion.

The uptake of amino acids across the intestine requires them to traverse the apical membrane, basolateral membrane, and cytosol of enterocytes. Absorption across the apical membrane involves active transport, facilitated diffusion, or simple diffusion. Once in the enterocyte, the amino acids can be used for structural proteins or moved by active transport or facilitated diffusion into the portal blood (Argiles and Lopez-Soriano, 1990). In the membranes, specific transport systems have been characterized by the amino acids they transport (small neutral, large neutral, basic, acidic, and imino) and by their dependence or independence on the presence of Na^+ (Nassar, 1989; Argiles and Lopez-Soriano, 1990).

The regulation of amino acid uptake may involve several processes. In mammals there is a direct correlation between protein digestion and increased brush border transport activity. Increased activity results from changes in the Vmax, rather than transporter affinity, and the response may take up to 2 weeks to complete. Control of the transport systems, although somewhat independent from each other, may be influenced by factors other than the substrate being transported (Kasarov et al., 1983, 1987; Kasarov

and Diamond, 1983, 1987). Bell et al. (1987) found that increased dietary glutamine potentially repressed carriers for proline and methionine.

Peptides may be taken up by enterocytes or they may pass by paracellular routes directly into the portal blood. Those peptides taken up by enterocytes are mostly degraded into amino acids, but some may cross intact into the portal blood (Argiles and Lopez-Soriano, 1990; Gardner, 1984, 1987; Reshkin and Ahearn, 1991; McLean et al., 1990). The uptake of intact proteins is of limited nutritional value, but if the intact or partially digested proteins have biological activity they may have physiological or pathological significance (Gardner, 1987; McLean et al, 1991).

Peptides transported into enterocytes do not use the same mechanisms as those for amino acids (Argiles and Lopez-Soriano, 1990; Reshkin and Ahearn, 1991). Most of the peptides are composed of 2 - 4 amino acid residues and they tend to contain glycine or proline residues (Adibi and Mercer, 1973). In mammals, the uptake of amino acids as di and tripeptides may be greater than as free amino acids (Mathews, 1975). Reshkin and Ahearn (1991) working with tilapia found that the dipeptide glycyl-L-phenylalanine and its transporter contributed significantly to total phenylalanine uptake. The authors suggested that amino acid absorption may be a scavenger activity after the bulk of nitrogenous digestive products have already been absorbed as peptides.

2.5.4 Protein Metabolism

Amino acids from dietary protein are used for energy, protein synthesis, and as precursors to nitrogen containing compounds (Young and Marchini, 1990, Lloyd et al., 1978). The activity of the various pathways is determined by, among other factors, the level of dietary protein (Kim et al., 1991, Lupianez et al., 1989), the energy content of the diet (Henken et al. 1986), the amino acid profile of the diet (Nose and Murai, 1990; Moyano et al., 1991), and the ambient temperature (Henken et al., 1986).

Fish preferentially use protein over carbohydrates as an energy source (Cho and Kaushik, 1985; Moyano et al., 1991; Van den Thillart, 1986). The main energy pathway

involves the deamination of amino acids and the subsequent use of the carbon skeletons in the Krebs cycle (Walton, 1985; Van den Thillart, 1986; Lupianez et al., 1989). An alternate pathway involves using the carbon skeletons for gluconeogenesis and the resultant glucose can be used as a fuel for red blood cells, nervous tissue, gonads, and for the production of mucopolysaccharides (Suarez and Mommsen, 1987; Walton, 1985).

The deamination of alanine into pyruvate, glutamate into ketoglutarate, and aspartate into oxaloacetate are thought to be how amino acid carbon skeletons enter the Krebs cycle (Moyano et al., 1991; Chandrasena and Hird, 1978; Cornish et al., 1978) . In catfish, Dean et al. (1986) found that the activity of enzymes involved with deamination, i.e. glutamate dehydrogenase (GDH), aspartate aminotransferase (GOT) and alanine aminotransferase (GPT), increased as dietary protein increased but GOT activity decreased and GDH increased as protein quality declined. Similar enzymatic responses to protein quantity were found in trout by Lupianez et al.(1989), but Moyano et al. (1991) found that only GDH activity increased as protein quality decreased.

The increase in GDH activity in trout as protein quality declined was probably in response to limiting amino acids reducing the rate of protein synthesis. As protein synthesis declined more amino acids would have become available for energy production causing GDH activity to increase (Moyano et al., 1991; Dean et al., 1986).

In fish, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are essential amino acids (Nose, 1979). In coho salmon, carp, rainbow trout and channel catfish, cystine and tyrosine can be used to partially meet the needs for the sulfur amino and aromatic amino acids, respectively (Nose and Murai, 1990; Akiyama et al., 1985; Walton et al., 1984a, 1984b; Kim et al., 1987; Kim et. al., 1992). Cowey and Luquet (1983) suggested that the essential amino acid requirements of fish species should be similar because the amino acid composition of muscle, the main product being formed, varies little among species.

While excess amino acids are generally used for energy production, excessive dietary levels of some amino acids can alter the requirements for others or cause toxicity in some species. Although rainbow trout and carp can suffer from methionine toxicity, carp are apparently much more sensitive to dietary levels of this amino acid (Nose, 1974, 1979; Murai et al., 1986). Choo et al. (1991) found that 20% of rainbow trout fed a diet containing 13.4% leucine developed gross lesions and there was attendant scale loss and scoliosis.

In growing fish, muscle protein is the main form of protein synthesized with fibre hypertrophy occurring during periods of rapid growth and fibre recruitment during slower growth (Houlihan and Laurent, 1987; Kiessling et al., 1991). Increased dietary protein causes increased protein synthesis, retention and degradation (Houlihan et al., 1988, 1989).

During periods of reduced ration, the amount of mRNA for protein and myosin heavy chain synthesis both decline in muscle, but the mRNA for the myosin heavy chain decreases at a slower rate. This is reflected in the relatively higher level of myosin heavy chain relative to total muscle protein. Myosin heavy chain and the corresponding mRNA are likely conserved so that fish can maintain physical activity and restore rapid growth once dietary conditions improve. The speed with which the mRNA can respond to improved dietary conditions, however, is unknown (Von Der Decken, 1989; Von Der Decken and Lied, 1989; Houlahin et al., 1989).

Work on trout has indicated that specific dynamic action (SDA) or heat increment is mainly related to deamination of excess amino acids for energy rather than using the amino acids for the synthesis of new body protein. SDA also includes the metabolic costs associated with lipid and carbohydrate synthesis in tissues, the uptake of dietary substrates, and their interconversions. The SDA may also reflect the efficiency of protein utilization (Legrow and Beamish, 1986).

Amino acids can be metabolized to produce non-protein nitrogen containing compounds. The polyamines putrescine, spermidine and spermine are produced via pathways that start with ornithine decarboxlyase (Corti et al., 1988). Imidazole dipeptides act as biological buffers and are involved in the transport of copper and zinc. Trimethylamine oxide is a result of the detoxification of trimethylamine. The trimethylamine oxide can be used for osmoregulation by marine fish (Van Waarde, 1988).

Waste nitrogen is transported in the blood as ammonia to the gill epithelia. Most ammonia moves across the gill by a nonionic diffusion process. The diffusion may be enhanced by an external boundary layer of water and mucous. The layer, kept acidic by CO_2 diffusion, helps to maintain an NH_3 gradient by converting diffused NH_3 into NH_4^+. Some ammonia may move across the gill by a Na^+-NH_4^+ exchange or by paracellular routes (Randall and Wright, 1989; Van Waarde, 1988; McDonald et al., 1989).

2.6 Protein Sources

Protein quality affects the growth of fish more than it does other domestic monogastric animals (Cowey, 1975). Palatability, essential amino acid composition, digestibility, and the presence of antinutritional compounds all play a role in determining the quality of a protein source (Stuart and Hung, 1989). Antinutritional effects may be caused by the presence of compounds such as tannins, alkaloids and glucosides (Mehansho et. al., 1987; Bondi and Alumont, 1987). There may be species differences in sensitivity to antinutritional compounds. Carp, for example, may be less sensitive than other fish species to the appetite-suppressing factor(s) in soybean (Huisman, 1990; Bondi and Alumont, 1989; Murai et. al., 1986).

The inherent quality of a protein source can be affected by how the protein is processed. Extrusion, heating, changes in pH, the presence of other dietary components and storage can all affect protein quality (Finley, 1989; McCallum and Higgs, 1989;

Philips, 1989; Gulbrandsen, 1986). Processing, for instance, may be able to reduce or eliminate the effects of the antinutritional factors (Murai, 1989; Pike et. al., 1990; Mehansho et. al., 1987).

Both the freshness of the raw materials and drying temperatures used in meal production can have a significant impact on the nutritional quality of marine protein sources (Jensen, 1986; McCallum and Higgs, 1989; Pike et. al., 1990; Gulbrandsen, 1986). Jensen (1986) reported that at drying temperatures of 60°C, meals manufactured from fresh offal had higher digestibilities in rainbow trout than those produced from spoiled fish, but at drying temperatures of 140°C there were no significant differences in digestibility between meals. In chinook salmon, McCallum and Higgs (1989) found that the nutritive value of herring meal dried at 75°C was higher than that of meal dried at 120-150° C.

In carnivorous fish, proteins from marine sources have the advantage of being the best sources of essential amino acids. Moreover, they act as feed attractants and they are possibly sources for unidentified growth factors (Wijkstrom and New, 1989). Even in herbivorous fish, marine protein addition to the diet improves growth (Mohsen and Lovell, 1990; Degani et. al., 1989).

The production of fish meal from marine sources involves the following general steps:

1) the minced, chopped or whole fish are heated in a cooker
2) the cooked product enters a press where the liquids (solubles and lipid) and press cake are separated
3) press cake moisture, with or without the addition of condensed fish solubles (see 5 and 6), is reduced by a drier
4) dried press cake is ground and stabilized with antioxidant to produce the final meal
5) liquids are separated into oil and stickwater by a separator/decanter
6) optionally, stickwater is evaporated and the remaining solids added back to the press cake between steps 2 and 3.

Drying can either be direct using an open flame or indirect using steam or a vacuum-steam combination. Open flame drying causes more damage to the meal than the other

methods. The best quality meals are produced by the vacuum-steam combination since the meals are dried at a lower temperature, but the process has the highest costs and the rate of throughput is reduced (Theriault, 1989).

Non-marine protein sources such as poultry-byproduct meal, meat meal, and corn gluten meal have been investigated as alternate sources of protein for complete and/or partial replacement of marine proteins in fish diets. Pongmaneerat and Watanabe (1991) and Watanabe and Pongmaneerat (1991) reported that carp and trout fed meat meal, meat and bone meal, or corn gluten meal as the sole dietary protein source did not grow as well as those fed diets based on white fish meal. They attributed the results to poor amino acid profiles in the alternate protein sources. Fowler (1982) found that poultry by-product meal could replace up to 75% of the fish meal in chinook diets while Alexis et. al. (1985) found that a combination of soybean meal and poultry by-product fed to trout outperformed a fish meal-only diet. Findings such as those by Alexis et. al. (1985) indicate that plant proteins may be able to replace or at least reduce the level of marine protein sources in the diets of carnivorous fish.

3.0 EXPERIMENT 1

The Effects of Graded Dietary Ash Concentrations on the Growth, Feed Consumption, Gastrointestinal pH Values, Calcemic Hormones, and Gross Morphology of Sea Water-Adapted Chinook Salmon Fed Diets Containing Hake Offal Press Cake Meal as the Sole Protein Source.

3.1 OBJECTIVES

The experiment was undertaken to evaluate the effects of high dietary ash concentrations on the growth performance of sea water-adapted chinook salmon and to establish possible mechanisms for any negative effects on fish performance.

3.2 MATERIALS AND METHODS

3.2.1 Fish Meals

The test meals studied included hake offal press cake meal and two derivative meals in which the ash levels were reduced.

The hake offal press cake meal was produced from frozen blocks of Pacific Hake (*Merluccius productus*) obtained from the B.C. Packers fish processing plant in Steveston, British Columbia. The 10 kg blocks contained viscera, frames, heads, fins, and any residual skin or scales from the filleting line. Blocks were stored at -20° C until they were processed.

Blocks to be processed were removed from the freezer, placed in covered plastic containers, and allowed to partially thaw at room temperature. Next morning, the partially frozen blocks were pried apart and minced by a meat grinder. The minced fish were then processed into fish meal by a Pilot Fish Meal Manufacturing Machine (Chemical Research Organization, Denmark). The temperature for the cooker and dryer were set at 85° C and 75° C, respectively. Liquids from the press were screened (600 openings/micrometer) and discarded. Particulate matter caught by the screen was added to the fish meal as it entered the drier. After drying, the meal was spread onto metal screens and cooled to room temperature. The cooled meal was then placed into plastic bags which each held about 15-20 kilograms of meal. Thereafter, the plastic bags were

sealed with tape, put into paper feed bags, and stored at -20° C. Processing all of the blocks took approximately two and a half weeks.

Upon completion of meal production, ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethyl-quinoline) was added to the meal to stabilize the lipids. For better dispersal, ethoxyquin was added to a salmon oil carrier to produce a mixture of 0.2 grams ethoxyquin per 0.8 grams of oil. All the meal was placed in a Marion mixer and the ethoxyquin/oil mixture was blended in at a ratio of 1 gram mixture per kilogram meal. The meal and antioxidant were blended for 15 minutes. The blended meal was then reground using a Fitz hammer mill.

Two meals of reduced bone content were produced by passing portions of the original meal through a Kason vibrating screen separator. The separator was configured with 3 screens containing 10 openings/mm, 100 openings/mm, and 600 openings/ micrometer, respectively. The meal containing the intermediate concentration of bone was composed of the screenings from the second and third screens and the meal in which the most bone was removed contained only the product from the finest screen. It was determined visually that all screens, except the 600-mesh screen, were only removing bone.

3.2.2 Diets

The proximate composition of the meals and diets are given in Tables 1 and 3, respectively. Diets were formulated, as shown in Table 2, to be isonitrogenous (480 g/kg) and isocaloric (3714 kcal ME/kg with protein, lipid, and carbohydrates contributing 4.5, 8.5, and 3.8 kcal ME/g) on a dry matter basis. The mineral profiles of the meals and basal diet protein sources were determined by plasma emission spectroscopy at Quanta Trace Laboratories Inc. Burnaby, B.C. Using the mineral profiles of the protein sources, mineral mixes were made for each diet so that ratios between specific minerals were maintained within determined limits (See Tables 4 and 5 which show the mineral mix and total dietary concentrations for each diet)

All dietary ingredients, except vitamins and oil, were blended together for 15 minutes in a Hobart Commercial Mixer and then ground with a Fitz hammer mill. To reduce the amount of lipid sprayed on the final pellets, lipid and ethoxyquin were added to the mash until it contained 13 % oil and 150 ppm of antioxidant. Vitamins were then added and the final diets blended for 30 minutes in the Hobart Commercial Mixer.

The mashes were pelleted using a California Pellet Mill through a 3/16 inch die under reduced steam pressure. The pellets were placed onto metal trays and air dried in a forced air chamber for 10 minutes. The supplemental oil needed to produce isocaloric diets was then added to the pellets. The addition of the oil involved placing the pellets in a Monarch cement mixer and spraying oil with a electrically operated paint gun as the mixer rotated. To maximize oil uptake by the pellets, they were placed into plastic tubs and allowed to sit at room temperature for about 45 minutes. Finally, the pellets were placed into plastic bags which, in turn, were placed inside of paper feed bags. The plastic bags were sealed with tape and the feed bags were tied shut. All diets were stored at -20^{o} C.

3.2.3 Pre-experimental Treatment of Fish

On April 15, 1987, 2,000 chinook salmon (*Oncorhynchus tshawytscha*) of the Robertson Creek strain, averaging 3-4 grams, were brought to the Department of Fisheries and Oceans West Vancouver Laboratory. The fish were kept outdoors in a three metre diameter (7500 litre) tank which was supplied with aerated water and it was covered by a combination of tarpaulin and nylon mesh net. Fresh well water (11^{o} C) was supplied to the tank at a rate of 20 litres per minute until the end of May when the flow rate was increased to 35 litres per minute. All fish were dip-vaccinated against *Vibriosis* (*ordalli* and *anguillarum*) when the average fish weight was approximately 8 grams. The chinook were fed the Oregon moist pellet diet 3 times per day until early September when the feeding rate was reduced to 2 times per day.

The timing for transferring the fish to salt water was determined by the date when

Table 1 - Proximate analysis of dietary protein sources used in Experiment 1 on a dry weight basis.

Protein[1] source	% Dry matter	% Crude protein[2]	% Crude lipid[3]	% Ash
Experiment 1				
Kh+	83.80 ±0.07	59.89 ±0.14	7.72 ±0.28	25.81 ±0.02
Ksp	83.49 ±0.09	62.66 ±0.19	11.82 ±0.27	21.49 ±0.04
Ks	82.28 ±0.16	67.81 ±0.10	10.34 ±0.26	16.83 ±0.17

Mean ± SEM (standard error of the mean)

[1] Protein source codes:
Kh+ = hake offal press cake meal; Ks = hake offal press cake meal that was screened to reduce ash; and Ksp = hake offal press cake meal that was partially screened to reduce ash.

[2] Crude protein content determined by micro-Kjeldahl technique.

[3] Crude lipid content determined by Bligh and Dyer (1959) extraction method.

Table 2 - Composition of diets as fed in Experiment 1.

Ingredients	Diet[1]		
	Kh+	Ksp	Ks
	g/kg	g/kg	g/kg
Hake offal press cake meal	827.98		
Partially screened hake offal press cake meal		797.48	
Screened hake offal press cake meal			746.55
Dextrin	12.45	12.49	12.48
Cerelose	12.45	12.49	12.48
Alpha - cellulose	4.33	35.29	92.86
Vitamins[2]	17.32	17.37	17.36
Minerals[3]	17.32	17.37	17.36
Salmon oil[4]	80.45	79.72	73.80
Carboxymethyl cellulose	21.65	21.71	21.04
Ascorbic acid	1.73	1.74	1.74
Choline chloride (60%)	4.33	4.34	4.34

[1] Diet codes:
Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

[2] Vitamin supplement supplied the following levels of nutrient per kg dry diet: D-calcium pantothenate, 193.7 mg; pyridoxine HCl, 44.95 mg; riboflavin, 60 mg; niacin, 300 mg; folic acid, 20 mg; thiamin mononitrate, 40.65 mg; biotin, 3 mg; cyanocobalamine, 0.06 mg; menadione, 26.0 mg; dl-alpha-tocopheryl acetate, 600 IU; cholecalciferol, 2400 IU; retinol acetate, 10000 IU; inositol, 400 mg.

[3] Mineral supplement - see Table 4.

[4] Salmon oil stabilized with 500 mg ethoxyquin per kg oil

Table 3 - Proximate analysis of diets in Experiment 1 on a dry matter basis.

Diet[1]	% Dry matter	% Crude protein[2]	% Crude lipid[3]	% Ash
Kh+	84.58 ±0.03	46.13 ±1.22	13.89 ±0.36	22.11 ±0.06
Ksp	83.16 ±0.03	47.53 ±0.81	15.89 ±0.31	17.11 ±0.06
Ks	82.93 ±0.09	46.36 ±0.72	14.55 ±0.04	12.44 ±0.05

Mean ± SEM (standard error of the mean)

[1] Diet codes:

Kh+ = diet containing hake offal press cake meal; Ks = diet containing hake offal press cake meal that was screened to reduce ash; Ksp = diet containing hake offal press cake meal that was partially screened to reduce ash.

[2] Crude protein content determined by micro-Kjeldahl technique.

[3] Crude lipid content determined by Bligh and Dyer (1959) extraction method.

Table 4 - Supplemental mineral concentrations[1] used in Experiment 1.

Mineral supplement content[2]			
		Diet[3]	
Mineral	Kh+	Ksp	Ks
Ca	-	-	-
P	-	-	-
Mg	400.0	186.0	-
Mn	75.0	41.5	30.2
Zn	265.9	241.2	344.4
Co	2.0	1.8	2.4
Cu	5.0	7.0	10.5
Fe	-	-	-
F	5.0	5.0	5.0
I	5.0	5.0	5.0
Na	-	-	-
K	471.0	180.0	-
Se	0.1	0.1	0.1

[1] On a dry matter basis in mg/kg of diet

[2] Mineral supplement supplied minerals in the following forms:
manganese as $MnSO_4 \cdot H_2O$; zinc as $ZnSO_4 \cdot 7H_2O$; cobalt as $CoCl_2 \cdot 6H_2O$; copper as $CuSO_4 \cdot 5H_20$; iodine as KIO_3; fluorine as NaF; selenium as Na_2SeO_3; potassium as K_2SO_4; magnesium as $MgSO_4 \cdot 7H_2O$.

[3] Diet codes:
Kh+ = diet containing hake offal press cake meal, Ksp = diet containing hake offal press cake meal that was partially screened to reduce bone, Ks = diet containing hake offal press cake meal that was screened to reduce bone.

Table 5 - Dietary mineral concentrations[1] in Experiment 1.

Dietary content			
		Diet[2]	
Mineral	Kh+	Ksp	Ks
Ca	71159.0	42787.0	32354.0
P	32935.0	20122.8	15858.0
Mg	2548.0	1620.0	1288.0
Mn	120.7	72.8	55.0
Zn	350.0	317.0	423.0
Co	2.0	1.8	2.4
Cu	14.3	13.0	17.3
Fe	609.0	552.0	736.0
F	>5.0	>5.0	>5.0
I	>5.0	>5.0	>5.0
Na	7637.0	3520.0	3696.0
K	6000.0	2933.0	3058.0
Se	>0.1	>0.1	>0.1

[1] Protein source minerals and supplemental minerals on a dry matter basis in mg/kg of diet

[2] Diet codes:
Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

the chinook were dip-vaccinated and by observing the jumping behavior, degree of silvering, and fish weight. Transfer to salt water began on July 3, 1987, when the fish weight averaged 11 grams. The transfer to salt water took 16 days to complete. Every four days, the fresh water flow was reduced by 1/4 of the original flow rate and this was replaced by an equivalent flow of sea water. For the duration of the transfer, feeding was reduced to once a day.

To allow for future growth, half of the salmon were selected randomly and transferred to a second tank in late October. The second 3-metre diameter tank had the same combination of tarpaulin, nets, and water flow as the original tank.

On March 1, 1988, 240 fish were selected from the two tanks and distributed randomly five at a time into 6 indoor 200-litre tanks. The fish were captured with dip nets and held in large plastic pails containing aerated salt water. Approximately twenty fish at a time were netted from the pails and placed in a 10 litre bucket containing an anesthetic (0.35 ml 2-phenoxyethanol/ l of water). Each fish was individually weighed to the nearest 0.1 gram. The first 100 fish weighed were used to determine the average weight and weight distribution for the population, as well as the starting weight range for the experiment. Fish within the experimental weight range were isolated in a 10 litre bucket. Each time the number of fish in the bucket reached five, the fish were sequentially placed into each of the six 200-litre tanks until there were 80 fish per tank. The experimental fish weighed an average of 48 ± 8 grams.

Two days before the trial was to begin, it was announced that water used in the indoor tanks would be diverted to new facilities before the trial could be completed. Since the trial was delayed, on March 8, 1988, the fish in the 200-litre tanks were moved to an outdoor 3 meter diameter tank. The experiment was delayed until June 20 while the new indoor facilities and the water system were completed and evaluated for possible toxicants.

3.2.4 Experimental Design and Management Procedures

On June 1, 1988, the fish were anesthetized with 2-phenoxyethanol (0.35 ml/l) and weighed to the nearest 0.1 gram as described above. The weight distribution of the population was calculated and used to determine that fish weighing from 45 to 146 grams would be needed to ensure 30 fish per tank. Fish selected for the experiment were acclimated to the tanks for 21 days. In the first week, their diet was switched from Oregon Moist Pellet (OMP) to a dry commercial diet (EWOS Canada, Ltd.) by gradually substituting the dry diet for OMP. One week prior to the start of the experiment, the same technique was used to switch the fish from the dry diet to the experimental Ks diet. Except during sampling and weighing periods, all fish were feed to satiation twice daily from 8:30 to 9:30 am and 3:30 to 4:00 PM. Feed was withheld twenty-four hours before a sampling/weighing cycle.

The experiment was a completely randomized design in which three dietary treatments each had 2 replicates. The experiment ran 43 days from June 21 until August 2, 1988. Fish were sampled for plasma calcitonin concentration, plasma stanniocalcin concentration, gastrointestinal pH, and weight of gastrointestinal contents on days 0, 21, and 42. Individual weights(g) and fork lengths(cm) were also recorded for the fish killed on those days as per Experiment 1 section 3.2.5. Individual weights(g) and fork lengths (cm) for the rest of the fish were measured on days 1, 22, and 43. During the interval between days 0 and 21 some of the smaller fish appeared to lose weight, unlike the rest of the fish in the experiment, so were removed from the trial. Day 0 weights and lengths of the removed fish were identified and removed from the data. Daily feed intake and mortality were recorded for each tank.

Each replicate was a 200 litre tank that contained 30 fish. In the center of each tank, a system of double stand pipes ensured that waste feed and feces was flushed from the system. All tanks were covered by a nylon mesh with a second black nylon mesh placed over half of each tank to restrict light entry. The flow of sea water was set at 7 litres per minute and aeration was provided to each tank. The salinity ranged from 26-28

ppt while the dissolved oxygen and water temperature varied between 8-10 ppm and 8.5-12.5^{o}C, respectively. Lighting followed the natural photoperiod. Small shrimp-like organisms were found in the water on day 21. To remove the potential impact of these organisms as a food source, the water inflows were adjusted so that water was filtered by black nylon mesh before entering the tanks.

3.2.5 Sampling Protocol

On days 0, 21, and 42 the following sampling/weighing protocol was followed. Each tank had a three hour interval between feeding and sampling. There was a 15 minute delay between the feeding of each given tank to allow for the complete sampling of fish from one tank before sampling of the next tank would begin. Four fish were taken from each tank and placed into a 10 litre pail. The fish were then taken to the wet lab, netted, and stunned by a blow to the head. Fish weights and lengths were then taken and recorded to the first decimal place.

3.2.5.1 Blood Sampling

For two of the four fish, individual blood samples were taken from the caudal artery/vein just posterior to the anal fin. Blood was withdrawn using 3 cc ammonium heparinized syringes and 19.0 mm 20 gauge needles and then transferred to 5 ml test tubes. The blood, which was kept on ice for a maximum of 20 minutes, was centrifuged at 3,000 RPM for 10 minutes to separate the plasma. Plasma was transferred to 1 1/2 ml microfuge tubes in 450 µl (for calcitonin determination) and/or 250 µl (for stanniocalcin determination) aliquots, depending on the volume of plasma, and placed in dry ice. Samples were stored at -40^{o} C until analyzed for the hormones.

3.2.5.2 Removal of Gastrointestinal Contents

Before gastrointestinal contents were removed, all four fish were killed with a blow to the head. The gastrointestinal tract was dissected out and divided into 3 sections - the stomach, the intestine, and the rectum. Each section was clamped off and the pH taken with a Digesena model PHR-146 combination micro pH electrode. When not in

use, the probe was kept in a neutral buffer sitting in an ice bath. The probe entered the stomach from the anterior end and was inserted into the middle of the stomach contents. Intestinal pH was measured about 2 1/2 to 3 cm from the posterior end. The digesta from each section were weighed, chilled with dry ice, and stored in plastic vials at -20° C. To increase the number of pH readings taken, the sampling procedure was repeated on day 44 with the remaining fish. The time between tank group feedings was increased to half an hour because of the larger number of fish involved. No blood samples were taken because of the possibility that the stress from three days of handling may have affected hormonal levels.

3.2.5.3 Sampling of Remaining Fish

On the following day, the weights and lengths of the remaining fish were measured. One tank at a time, the fish were netted, placed in a 100 litre plastic pail, and transferred to the wet lab where aeration of the water was provided. Ten fish at a time were netted and anethesized as described as in section 3.2.5. Weights and lengths were recorded to the first decimal place. The fish were then placed in an aerated 10 litre pail where they could be monitored while they were allowed to recover from the 2-phenoxyethanol. Once recovered, the fish were returned to their respective tanks.

3.2.6 Analytical Protocols

The dry matter, crude protein, crude lipid, and ash percentages were determined for all diets and meals. All results were calculated on a dry matter basis and are provided in Tables 1 and 2. Dry matter was also determined for the digesta samples. Analyses of plasma titres of calcitonin and stanniocalcin were arranged through Dr. Fargher of the UBC Department of Physiology. Calcitonin and stanniocalcin titres were determined according to the procedures of Deftos et al. (1984) and Gellersen et al. (1988), respectively, as described below.

3.2.6.1 Dry Matter

Dry matter was determined by placing 2 gram samples into pre-weighed aluminum pans which were subsequently transferred to a drying oven set at 85° C. After 22 hours, the pans were removed, allowed to cool in a desiccator, and weighed.

3.2.6.2 Ash

One gram samples, taken from the material used for dry matter determination, were weighed into pre-weighed crucibles. The crucibles were placed in a muffle furnace and heated to 600° C for 4 hours. Ash was then determined by weighing the remaining residue.

3.2.6.3 Lipid

Lipid concentrations were determined using a modified version of the Bligh and Dyer (1959) extraction procedure. Approximately 2 grams of sample were combined with 10 ml of chloroform, 20 ml of methanol, 10 ml of water, and 1 gram of filter-aid and mixed in a blender for two minutes. An additional 10 ml of chloroform were added to the mixture and blended for another 30 seconds. The mixture was filtered under a vacuum though Whatman filter paper #1 into a vacuum flask. The blender was carefully rinsed with a 1:1 solution of methanol and chloroform, and the solution was also passed through the filter.

The flask contents were then poured into a 50 ml graduated cylinder and mixed with an additional 5 ml of water. The flask was also rinsed with the 1:1 solution which was then added to the graduated cylinder. The cylinders were then covered and allowed to sit overnight as the phases separated. The volume of chloroform/lipid was recorded and the methanol/water phase was removed by suction. Duplicate 5 ml aliquots were removed from the chloroform/lipid layer and placed into pre-weighed aluminum dishes for drying.

The dishes were heated on a hot plate under a fumehood to remove the chloroform. The samples were then placed for 1 hour in a drying oven at 100° C. They

were then allowed to cool in a desiccator before being weighed. All determinations were conducted in triplicate.

3.2.6.4 Protein

A 0.1 g sample was placed on nitrogen-free weighing paper and added to digestion tubes containing 10 ml of concentrated H_2SO_4, a mercury catalyst, boiling chips, and 2-5 ml of hydrogen peroxide. The mixture was digested for an hour at 430° C and then allowed to cool. Water was added to bring the total volume in the tubes to 75 ml. A 4 ml aliquot of each solution was analyzed using a Technicon Autoanalyzer (method number II)

3.2.6.6 Dry Matter of Gastrointestinal Contents

Digesta samples were freeze-dried for 72 hours and then weighed. After freeze drying, some samples were too small to provide an accurate dry matter determination. Moisture determinations (remaining moisture after freeze drying) were performed on the larger samples and the percent moisture found was assumed to be the same as for the smaller samples.

3.2.6.7 Calcitonin Assay Procedure

The radioimmunoassy procedure for calcitonin followed the protocol of Deftos et al. (1974). The first part of the procedure involved the radioiodination of calcitonin as described by Deftos (1971) and the production of calcitonin antiserum. Labeled hormone was separated from unlabeled or damaged hormone by adsorption to silica followed by elution with 20% acetone and 1% acetic acid.

Salmon calcitonin antiserum was produced by injecting guinea pigs with 1-2 mg of salmon calcitonin, suspended in complete Freund's adjuvant, at 2 to 4-week intervals. Antiserum was collected by periodic cardiac punctures.

Standard curves were produced using salmon calcitonin standards, ^{125}I-calcitonin, and salmon calcitonin antiserum under nonequilibrium conditions (3 days pre-incubation, 2-4 days incubation with tracer). The volume of tracer (diluted at 15,000:1)

and standards in the incubation tubes were adjusted to 0.5 ml with a mixture of 10% human plasma in a 0.05M phosphate buffer containing 0.01% disodium ethylene diamine tetraacetic acid. Incubation, at 4^{0} C for 2-4 days, was followed by phase separation with dioxane precipitation. Both the precipitate and supernate were then measured with an auto gamma counter and standard curves were produced.

To assay plasma samples, the assay was repeated with fish plasma samples of not more than 100 µl in place of standards. Calcitonin levels were then determined from the standard curves.

3.2.6.8 Stanniocalcin Assay Procedure

Stanniocalcin determination followed the procedure of Wagner et al. (1988). Iodination of the stanniocalcin was accomplished with lactoperoxidase. Unreactive iodide was removed by passage through a column of Sepharose. An immunoaffinity column was used to remove damaged [^{125}I]antiserum. A radioimmunoassy (RIA) curve was prepared by mixing 100 µl salmon stanniocalcin antiserum (1:16,000 initial dilution), 150 µl of 1% normal rabbit serum, 100 µl stanniocalcin standards (4-2,000 ng/ml and 100 µl of diluent buffer into borosilicate tubes. The nonspecific binding and zero binding tubes received buffer in place of the antiserum and stanniocalcin standards. The tubes were incubated for 24 hours at 4^{o}C. An additional 100 µl of [^{125}I]iodostanniocalcin was added and the tubes incubated for another 48 hours. Bound and free hormone were separated by combining 0.5 U goat antirabbit γ-globulin in 100 µl diluent buffer at 4^{0} C for 24 hours. Two ml of diluent sera were added to each tube and the mixture was centrifuged at 1,000 x g for half an hour. The solutions were then decanted and counted. Curves were then produced and used for the RIA determination.

3.2.7 Statistical Procedures

All statistical procedures were done using SYSTAT version 5.02. The data were analyzed by One Way Analysis of Variance (ANOVA)with significance set at $p < 0.05$. Testing for homogeneity of variance was done with Bartlett's Test of Homogeneity and

with the procedure described on pages 232-233 of the SYSTAT Statistics Manual. Values which were calculated as percentages, but fell outside the range of 20 - 70%, were transformed with an arcsin transformation before being analyzed by ANOVA.

3.3 RESULTS

3.3.1 Analysis of Diets and Protein Sources

The proximate compositions of the protein sources (hake, partially screened hake, and screened hake) and diets (Kh+, Ksp, and Ks) are given in Tables 1 and 2, respectively. The proximate values for the diets differed somewhat from the expected values (Table 3) for protein (48.0% of dry diet), lipid (17.0% of dry diet), and ash (Kh+ = 20.67%, Ksp = 16.41, and Ks = 11.89).

Supplemental and dietary mineral profiles are listed in Tables 4 and 5. The dietary mineral profiles show dietary content and may not accurately reflect the availability of the minerals. Partially and completely screening the hake press cake meal (Section 3.2.1 Fish Meals) reduced ash levels in the Ksp and Ks diets by 5% and 9.67%, respectively. Dietary calcium concentrations declined from 7.1% in the Kh+ diet to 4.3% in the Ksp diet and 3.2% in the Ks diet. Phosphorus levels declined proportionately with the calcium so a calcium to phosphorus ratio of 2:1 was maintained in all the diets without supplementation. The proportional amount of dietary magnesium loss due to screening was less than that for calcium so the level of supplementation in the diet needed to maintain the calcium to magnesium ratio declined as the amount of screening increased. Screening reduced the potassium concentrations between the hake press cake meal diet (Kh+) and the partially screened diet (Ksp). Increased potassium concentrations between the Ksp diet and the completely screened diet (Ks), however, indicate that the 100 openings/mm screen actually served to concentrate the potassium. Native zinc concentrations stayed consistent in all diets indicating that none of the zinc was removed by the screening process.

3.3.2 Mortality, Growth and Condition Factor

Percent mortality of fish over the duration of the experiment was 1.9% for each dietary treatment. Since individual fish weights and gastrointestinal contents were determined on days 21 and 42, it was possible to include their adjusted weights (without

gastrointestinal contents) with the weights of fish taken on days 22 and 43. Mean body weights within and between replicates are listed in Table 6. Growth, using between tank means, is also represented in Figure 1. Even though the mean weight of fish fed the Kh+ diet on day 43 was 5 grams higher than those of the fish fed the other diets (which only differed from each other by 0.2 grams), there was no significant difference in the mean body weight of fish due to dietary treatment. There was also no significant difference in the mean body weight of fish fed the different diets on days 1 and 22.

Average weight gains and percent body weight gains at the end of the experiment are shown in Table 7. Since the removal of fish on day 21 changed tank averages by as much as 3.5 grams, values given are based upon the sum of the gain or percent gain for the intervals day 1 to 21 and day 22 to 42. For both the average gain and percent gain, there were no significant differences between diets. Although the means for fish ingesting the Ksp and Ks diets were much closer to each other than to the Kh+ diet, the wide variation between replicate means confounded identification of any potential dietary effects.

The initial mean lengths as well as the change in mean lengths and percent change in mean lengths at the end of the experiment are provided in Table 8. Diet had no significant effect on change in length or percent change in length. Similar to the trend found for mean final body weights, the Kh+ diet supported the largest percent increase in length (6.1%) and the other two diets had almost identical values at 4.7% for the Ksp diet and 4.6% for the Ks diet.

Condition factors (Table 9) were not significantly influenced by dietary treatments. As with the other measurements of growth, fish fed the Kh+ diet had the largest change in condition factor with a 4.2% increase, but the similarity in the changes seen for condition factors of fish fed the Ksp and Ks diets was not as close as with the other measurements.

Fish fed the Kh+ diet had the largest specific growth rate for the duration of the experiment and for each of the day 0-21and day 22-42 intervals (See Table 10 and Figure 2). However, diet did not appear to have any significant effect.

3.3.3 Feed Consumption and Feed Efficiency

As shown in Table 11, fish fed the Kh+ diet had the largest average feed consumption. Even though the consumption of the Ksp and Ks diets only differed by 0.6 grams per fish over the duration of the experiment (compared to a difference of 7.0 to 7.6 grams with the Kh+ diet), feed consumption was not significantly different between fish fed the test diets ($p > 0.05$).Between days 0-21, fish fed the Ksp diet had the best feed efficiency at 0.614 (See Table 12 and Figure 2). This trend was reversed between days 22-42 where fish fed this diet had the lowest value at 0.408. Relative to the values between days 0-21, the feed efficiencies between days 22-42 declined for all groups regardless of diet, but fish fed with the Kh+ diet exhibited the least decline at 0.025. Over the 42 days of the experiment, fish fed the Kh+ diet had the best feed efficiency at 0.596, but this value was almost identical to that for fish fed the Ks diet (0.595). Differences in feed efficiency between fish fed the test diets during each interval and for the duration of the experiment were not significant ($p > 0.05$).

3.3.4 Gastrointestinal pH

As shown in Table 13 and Figure 3, pH values for the stomach, small intestine, and rectum did not differ significantly ($p>0.05$) due to dietary treatment. The greatest variation in pH values occurred in the stomach (4.86-5.2), but the differential declined as the readings were taken further along the gut (8.71 - 8.84 in the intestine and 8.86 - 8.90 in the rectum). In the stomach, pH values appeared to be unaffected by the amount of stomach digesta on both a wet and dry matter basis (data not shown).

Table 6 - Mean body weights of dietary groups at 21-day intervals in Experiment 1.

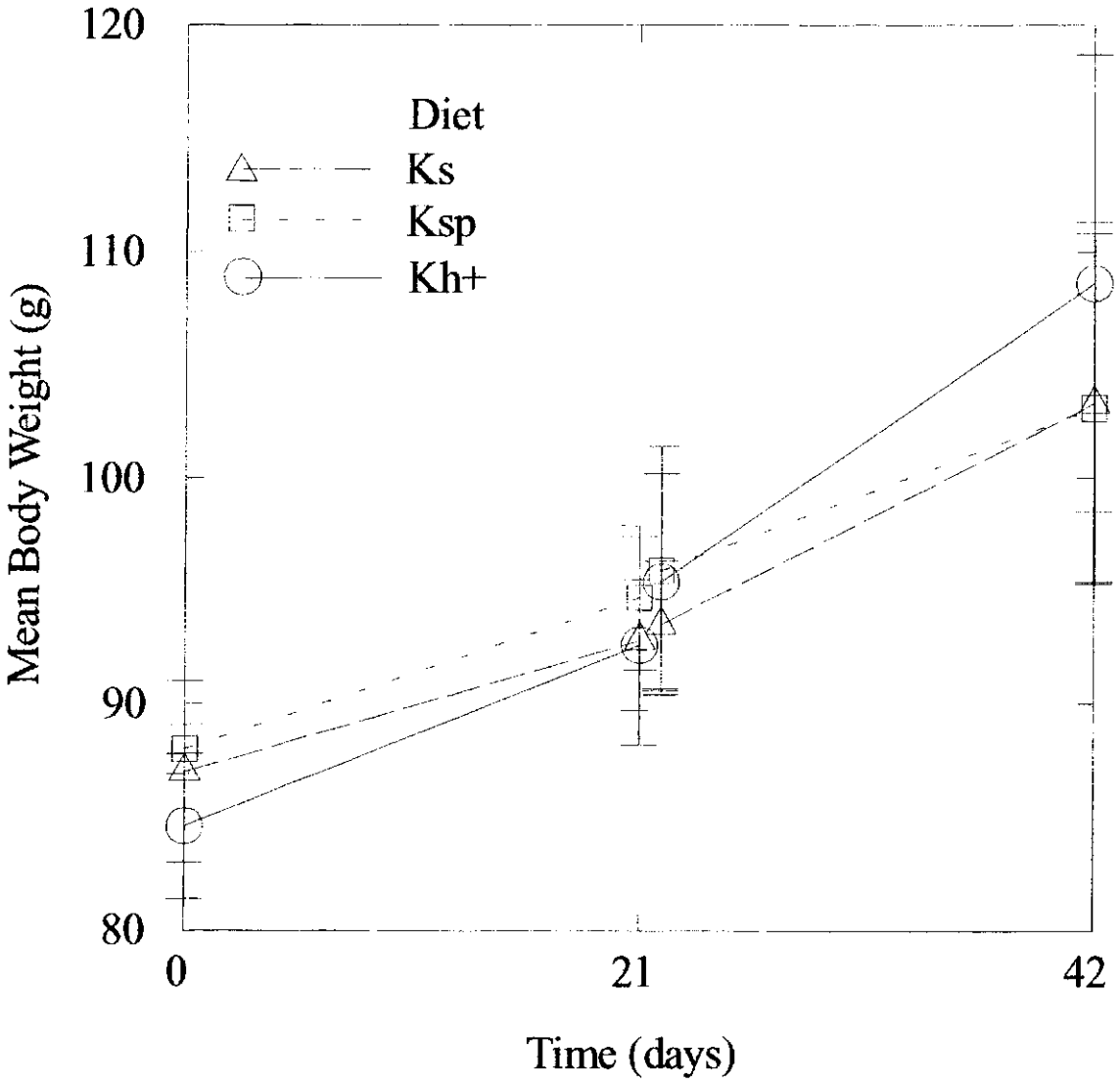
	Time interval in days			
Diet[1]	Day 0	Day 21[2]	Day 22[2]	Day 42
	(g)	(g)	(g)	(g)
Kh+				
Replicate 1	87.7 ±3.8	95.4 ±6.5	96.3 ±8.1	113.6 ± 9.7
Replicate 2	81.4 ±3.6	89.7 ±3.8	90.7 ±4.2	97.7 ± 4.6
Mean	84.6 ±3.2	92.6 ±2.9	95.4 ±4.8	108.5 ±10.1
Ksp				
Replicate 1	86.9 ±4.4	91.4 ±5.0	90.3 ±6.4	95.9 ±7.8
Replicate 2	89.1 ±2.9	97.9 ±3.0	101.4 ±3.4	110.2 ±4.4
Mean	88.0 ±1.1	94.7 ±3.2	95.9 ±5.5	103.1 ±7.1
Ks				
Replicate 1	91.0 ±4.8	97.4 ±5.2	100.1 ±6.2	108.5 ±5.9
Replicate 2	83.0 ±4.2	88.2 ±4.4	90.6 ±4.4	98.1 ±4.6
Mean	87.0 ±4.0	92.8 ±4.6	93.5 ±2.8	103.3 ±8.0

Replicate ± SEM (standard error of within replicate mean)
Mean ± SEM (standard error of the replicate mean)

[1] Diet codes:
Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

[2] Changes in average weight between days 21 and 22 reflect the effects of removal of fish.

Figure 1: Growth of Chinook Salmon in Experiment 1. Fish were Fed One of Three Diets Containing Different Concentrations of Ash for 42 Days.



Mean ± SEM (standard error of the mean), N=2

Diet codes: Kh+ = diet containing hake offal press cake meal, Ksp = diet containing hake offal press cake meal that was partially screened to reduce bone, Ks = diet containing hake offal press cake meal that was screened to reduce bone.

Table 7- Average body weight gain and percent body weight gain of dietary groups at the end of Experiment 1.

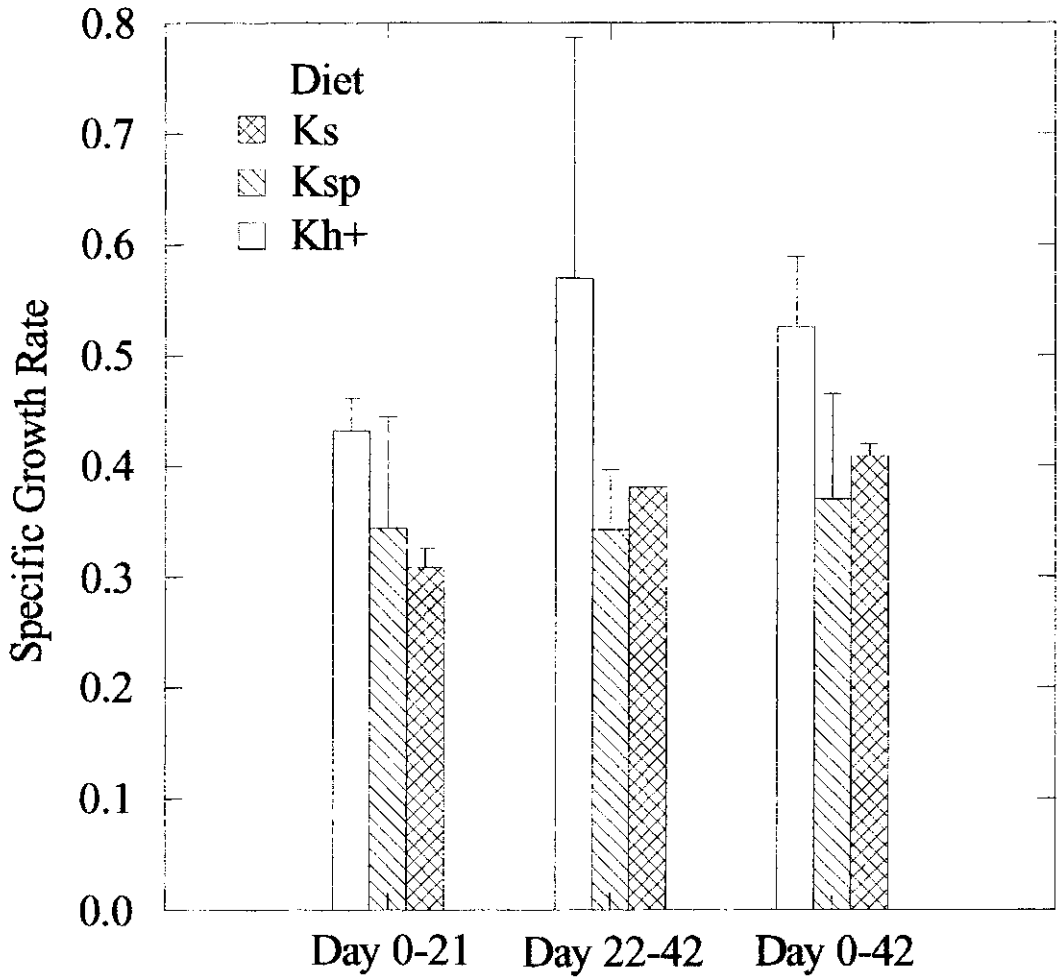
Diet[1]	Body weight gain[2] (g)	% Body weight gain[2] (%)
Kh+		
Replicate 1	25.0	26.8
Replicate 2	15.3	17.9
Mean	20.1 ±4.9	22.4
Ksp		
Replicate 1	10.2	11.4
Replicate 2	17.5	18.5
Mean	13.9 ±3.7	15.0
Ks		
Replicate 1	14.8	15.4
Replicate 2	12.8	14.6
Mean	13.8 ±1.0	15.0

Mean ± SEM (standard error of the replicate mean)

[1] Diet codes:
Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

[2] Sum of both 21-day interval gains. Percent body weight gain = (W2-W1)/W1*100 where W2 = final weight and W1 = initial weight. Note weights are on a wet weight basis.

Figure 2: Specific Growth Rates of Chinook Salmon in Experiment 1. Fish were Fed One of Three Diets with Different Concentrations of Ash for 42 Days.



Mean ± SEM (standard error of the mean), N=2

Diet codes: Kh+ = diet containing hake offal press cake meal, Ksp = diet containing hake offal press cake meal that was partially screened to reduce bone, Ks = diet containing hake offal press cake meal that was screened to reduce bone.

Table 8 - Initial length, change in length and percent change in length of dietary groups at the end of Experiment 1.

Diet[1]	Length day 0 (cm)	Change in length (cm)	% Change in length (%)
Kh+			
Replicate 1	19.1 ±0.2	1.3	6.8
Replicate 2	18.8 ±0.2	1.0	5.3
Mean	19.0 ±0.1	1.2 ±0.1	6.1
Ksp			
Replicate 1	19.2 ±0.3	0.5	2.6
Replicate 2	19.4 ±0.2	1.3	6.7
Mean	19.3 ±0.1	1.0 ±0.3	4.7
Ks			
Replicate 1	19.3 ±0.3	1.1	5.7
Replicate 2	19.0 ±0.3	0.7	3.4
Mean	19.2 ±0.1	0.9 ±0.1	4.6

Replicate ± SEM (standard error of the within replicate mean)
Mean ± SEM (standard error of the replicate mean)

[1] Diet codes:
Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

Table 9 - Initial condition factor[1], change in condition factor and percent change in condition factor of dietary groups at the end of Experiment 1.

Diet[2]	Condition factor[1] day 0	Condition factor[1] day 84	% Change in condition factor
			(%)
Kh+			
Replicate 1	1.21 ±0.02	1.27	4.9
Replicate 2	1.20 ±0.02	1.24	3.4
Mean	1.20 ±0.00	1.26 ±0.01	4.2
Ksp			
Replicate 1	1.21 ±0.01	1.20	-0.4
Replicate 2	1.22 ±0.01	1.23	0.9
Mean	1.21 ±0.01	1.22 ±0.01	0.3
Ks			
Replicate 1	1.23 ±0.01	1.24	0.9
Replicate 2	1.19 ±0.02	1.25	5.5
Mean	1.21 ±0.02	1.25 ±0.01	3.2

Replicate ± SEM (standard error of the within replicate mean)
Mean ± SEM (standard error of the replicate mean)

[1] Condition factor = $W / L^3 \times 200$, where W = weight (g) and L = length (cm).
[2] Diet codes:
Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

Table 10 - Specific growth rates for the dietary groups over 21-day intervals and the 42-day duration of Experiment 1.

Diet[2]	Time interval in days		
	Days 0 - 21[1]	Days 22 - 42[1]	Days 0 - 42[1]
	%/day	%/day	%/day
Kh+			
Replicate 1	0.401	0.787	0.615
Replicate 2	0.462	0.353	0.434
Mean	0.432 ±0.030	0.570 ±0.217	0.525 ±0.064
Ksp			
Replicate 1	0.243	0.287	0.236
Replicate 2	0.446	0.397	0.505
Mean	0.344 ±0.101	0.342 ±0.055	0.370 ±0.095
Ks			
Replicate 1	0.325	0.381	0.419
Replicate 2	0.292	0.381	0.398
Mean	0.309 ±0.017	0.381 ±0.000	0.409 ±0.011

Mean ± SEM (standard error of the replicate mean)

[1] Specific growth rate = 100 x (lnW2 - lnW1) / T where
W2 = final weight, W1 = initial weight, and T = time. Note: weights are on a wet weight basis.

[2] Diet codes:
Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

3.3.5 Hormones - Calcitonin and Stanniocalcin

Values are provided in Table 14. Due to the amount of blood obtained from the fish and the amount of plasma needed to run each procedure, only one hormonal determination could be done for most fish. Comparisons between fish which had both determinations were too few to have any meaningful interpretations.

Individual values for calcitonin were subject to large experimental errors. Two determinations were conducted on each sample, but the difference between their values was often considerable. For example, one value of 3121 pg/ml had a standard error of 1282.7. The values for samples taken on day 21 and day 42 were combined for each diet to increase the sample size. There were no significant differences between dietary treatments. Attempts were made to correlate fish size, intestinal contents (in each segment sampled and for the entire gastrointestinal tract), and fish size/intestinal contents with the hormonal levels (not shown). No patterns emerged and the correlations were all not significant.

The fish with the highest level of calcitonin (12100 pg/ml) was observed to be a mature (or maturing) male. Some other fish with high levels of calcitonin were also noted to have signs of gonadal development, but the relationship was not consistent for all fish with high calcitonin concentrations.

The values for stanniocalcin were much less variable within and between treatments than those for calcitonin (See Table 14). The largest within treatment variations were 18.3, 57.3, and 91 pg/ml for fish fed the Kh+, Ksp, and Ks diets, respectively. There were no significant differences between dietary treatments. As with the calcitonin data, attempts were made to correlate fish size, intestinal contents (in each segment sampled and for the entire gastrointestinal tract), and fish size/intestinal contents with the hormonal levels (not shown). No correlations were found to be significant.

Table 11- Average feed consumption[2] for dietary groups over 21-day intervals and the 42-day duration of Experiment 1.

Diet[1]	Time interval in days		
	Days 0 - 21 (g/fish)	Days 22 - 42 (g/fish)	Days 0 - 42 (g/fish)
Kh+			
Replicate 1	12.9	24.1	37.0
Replicate 2	14.9	18.1	33.0
Mean	13.9 ±1.0	21.1 ±3.0	35.0 ±2.0
Ksp			
Replicate 1	8.9	15.7	24.6
Replicate 2	13.3	19.2	31.5
Mean	10.6 ±1.7	17.4 ±1.7	28.0 ±3.4
Ks			
Replicate 1	10.7	17.6	28.3
Replicate 2	11.1	15.4	26.5
Mean	10.9 ±0.2	16.5 ±1.1	27.4 ±0.9

Mean ± SEM (standard error of the replicate mean)

[1] Diet codes:

Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

[2] On a dry matter basis

Table 12- Feed efficiency for dietary groups over 21-day intervals and the 42-day duration of Experiment 1.

Diet[2]	Time interval in days		
	Days 0 - 21[1]	Days 21 - 42[1]	Days 0 - 42[1]
Kh+			
Replicate 1	0.597	0.718	0.699
Replicate 2	0.556	0.385	0.493
Mean	0.577 ±0.021	0.552 ±0.166	0.596 ±0.103
Ksp			
Replicate 1	0.514	0.357	0.368
Replicate 2	0.712	0.460	0.669
Mean	0.614 ±0.099	0.408 ±0.052	0.519 ±0.150
Ks			
Replicate 1	0.603	0.473	0.619
Replicate 2	0.473	0.490	0.570
Mean	0.538 ±0.065	0.481 ±0.009	0.595 ±0.025

Mean ± SEM (standard error of the replicate mean)

[1] Feed efficiency = (W2 - W1) / Fc where W2 = final fish weight, W1 = initial fish weight, and Fc = feed consumption on a dry matter basis. Note: fish weights are on a wet weight basis.

[2] Diet codes:

Kh+ = hake offal press cake meal, Ksp = hake offal press cake meal that was partially screened to reduce bone, Ks = hake offal press cake meal that was screened to reduce bone.

Table 13 - pH readings for the stomach, small intestine and rectum of fish sampled in Experiment 1.

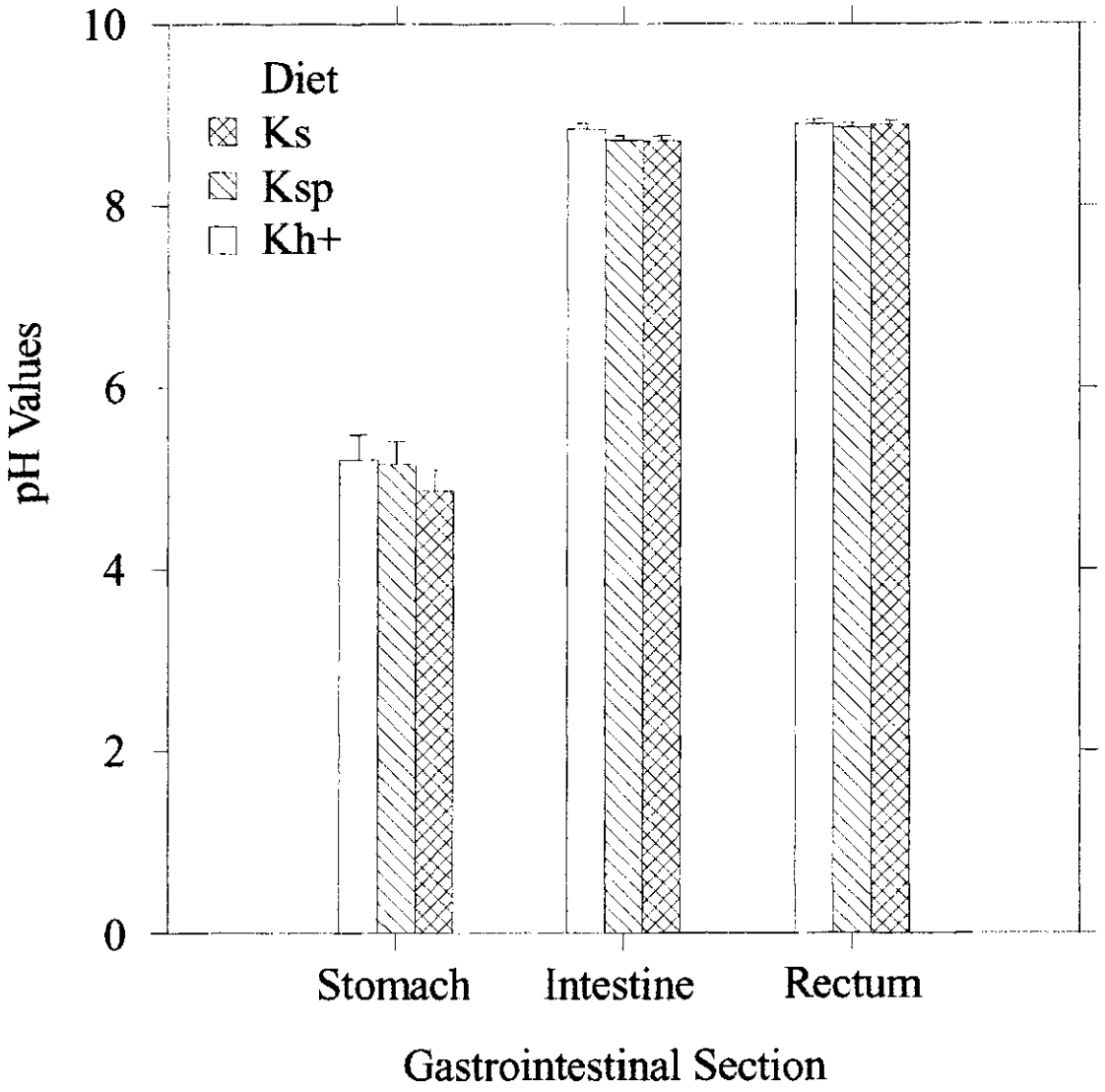
Diet[1]	Stomach pH	Intestinal pH	Rectal pH
Kh+			
Replicate 1 (12)	5.62 ±0.33	8.94 ±0.09	8.89 ±0.07
Replicate 2 (16)	4.88 ±0.44	8.76 ±0.08	8.90 ±0.08
Mean (28)	5.20 ±0.28	8.84 ±0.06	8.90 ±0.05
Ksp			
Replicate 1 (15)	5.31 ±0.35	8.74 ±0.07	8.93 ±0.07
Replicate 2 (16)	5.00 ±0.39	8.69 ±0.08	8.79 ±0.06
Mean (31)	5.15 ±0.25	8.72 ±0.05	8.86 ±0.05
Ks			
Replicate 1 (16)	5.24 ±0.33	8.67 ±0.07	8.87 ±0.06
Replicate 2 (13)	4.34 ±0.26	8.76 ±0.08	8.91 ±0.06
Mean (29)	4.86 ±0.23	8.71 ±0.05	8.89 ±0.05

Replicate ± SEM (standard error of the within replicate mean)
Mean ± SEM (standard error of the mean for all samples)
( ) = Number of samples taken

[1]Diet codes:

Kh+ = Hake offal press cake meal; Ks = Hake offal press cake meal that was screened to reduce ash; Ksp = Hake offal press cake meal that was partially screened to reduce ash.

Figure 3: Gastrointestinal pH Values of Chinook Salmon in Experiment 1. Fish were Fed One of Three Diets Containing Different Concentrations of Ash for 42 Days.



Mean ± SEM (standard error of the mean), N=2

Diet codes: Kh+ = diet containing hake offal press cake meal, Ksp = diet containing hake offal press cake meal that was partially screened to reduce bone, Ks = diet containing hake offal press cake meal that was screened to reduce bone.

Table 14 - Plasma calcitonin and stanniocalcin concentrations in relation to diet treatment in Experiment 1.

Diet[1]	Calcitonin (pg/ml)	Stanniocalcin (pg/ml)
Day 0	(4)	(5)
Mean	3682 ±775	17.5 ±2.1
Kh+	(14)	(13)
Mean	4429 ±726	25.4 ±6.6
Ksp	(12)	(14)
Mean	3932 ±906	24.9 ±3.7
Ks	(14)	(13)
Mean	3368 ±456	18.9 ±1.4

Mean ± SEM (standard error of the mean)
( ) = Number of samples taken

[1]Diet codes:
Kh+ = Hake offal press cake meal; Ks = Hake offal press cake meal that was screened to reduce ash; Ksp = Hake offal press cake meal that was partially screened to reduce ash.

3.3.6 Gross Morphological Changes

All fish (mortalities, fish used for blood and stomach sampling, and fish remaining at the end of the experiment) were dissected and examined for gross morphological changes. Seven of the fish had definite signs of gonadal development while an additional six may have had the start of gonadal development. One fish had an undiagnosed condition which was characterized by white pin-head sized areas of necrosis on the liver, heart, and spleen. Six of the fish selected for sampling had hearts which had a definite golden tinge. The hearts might have been slightly enlarged, but otherwise the fish were normal in appearance.

3.4 DISCUSSION

3.4.1 General

Under the conditions of this study, reduction of the concentration of dietary ash by partial or extreme removal of bone from the hake offal press cake meal had no impact on the nutritional quality of hake offal press cake meal for sea water-adapted chinook salmon. The dietary ash concentrations also had no impact on the plasma concentrations of calcemic hormones (calcitonin and teleocalcin), gastrointestinal pH, or gross morphological appearance of the fish.

An estimate of the average weight of the fish on day 0, which ranged from 45 to 146 grams, was used to determine pellet size. As a result, some of the smaller fish may have had difficulty in eating the pellets. This was indicated by two factors. First, the fish that lost weight (1.9% of total fish) between days 0 and 21 were amongst the smallest on day 0. Secondly, when these fish were dissected their gastrointestinal tracts were either empty or contained small shrimp-like organisms which had entered the tanks through the water supply. Hence, consideration of the small fish were removed from the experiment.

The determination of the availabilities and interactions of minerals in the diets of fish is complicated by the ability of fish to obtain minerals directly from their environment (Hilton, 1989). In sea water-adapted fish, significant amounts of waterborne minerals may be obtained from both gill and intestinal routes (Perry and Wood, 1985; Sundell and Bjornsson, 1988). Most of the minerals taken up across the intestine, with the exception of calcium, are monovalent. Bjornsson and Nilsson (1985) and Sundell and Bjornsson (1988) have found in Atlantic cod that 30-70% of the ingested calcium is absorbed while magnesium and sulfates tend to remain in the intestinal fluids.

In the present study it was assumed that most of the minerals in the hake offal press cake meal would be highly available to the fish and, hence, emphasis was placed on

maintaining similar mineral ratios between diets while satisfying the known mineral needs of salmonids (Lall, 1989). Care was also taken to fortify the diets with trace minerals such as zinc and manganese. The latter was thought to be especially important since Watanabe et al. (1980) noted that fresh water-adapted trout and chum salmon fed diets containing white fish meal as the sole source of dietary minerals developed cataracts and exophthalmus, and had depressed growth after thirteen weeks. This was thought to be a consequence of low trace mineral availability in white fish meal. Satoh et al. (1983) reached the same conclusion since they found that freshwater rainbow trout had suboptimal growth and development unless the diets were supplemented with zinc, manganese, copper, and magnesium.

3.4.2 Growth and Condition Factor

Although there were no significant differences in fish growth and condition factor due to dietary treatment, fish fed the Kh+ diet consistently had the highest values for specific growth rate and linear growth. The results demonstrate that the concentrations of supplementary minerals used in the test diets were sufficient to support normal growth and development. Reduced zinc availability in the presence of high levels of tricalcium carbonate, as noted by Satoh et al. (1987b) with freshwater trout, was not evident in this experiment regardless of the dietary calcium level. If an interaction were occurring between dietary zinc and calcium, the sea water-adapted chinook may have still been able to absorb sufficient zinc to meet their requirements or to enhance zinc uptake across the gill to compensate. Although freshwater trout are able to absorb zinc across the gill, the amount of uptake is insufficient to compensate for severe dietary zinc deficiencies (Spry et al., 1988).

3.4.3 Feed Consumption and Feed Efficiency

Similar to the growth measurements, feed consumption (g/fish) was greater in fish fed the Kh+ diet than in those consuming the other diets. The difference, however, was not significant. The increased feed consumption did not translate into greater feed

efficiency and there was considerable variation in feed efficiency between replicates within the dietary treatments.

3.4.4 Hormones

If calcium were taken up across the gastrointestinal tract, it would be anticipated that calcitonin and/or stanniocalcin concentrations would increase in response. However, the titres of each of the hormones were found to be unrelated to dietary treatment. Both hormones showed a reduction in titre as the dietary ash levels declined, but the trends were not significant and the values were subject to wide variations within dietary treatments. It is possible that the levels of calcium in the test diets elicited the maximal hormonal production and lower concentrations of the hormones would only be seen in fish fed diets of lower calcium content. Maximum plasma titres of each of the hormones may have also been missed because sequential measurements were not taken. The timing for blood sampling was based upon the advice of Dr. Copp from the UBC Department of Physiology.

Fish with the highest levels of calcitonin tended to be undergoing maturation. The fish with the highest calcitonin titres had the greatest degree of gonadal development of the fish sampled. These results agree with the findings of Watts et al. (1975) who found that calcitonin levels increased as sockeye salmon (*Oncorhynchus nerka*) sexually matured. It is therefore possible that the fish maturation was confounding dietary effects on plasma calcitonin titres.

Stanniocalcin is known to act upon the gill and this hormone may also act at the level of the intestine (Takagi et al., 1985). The results from this study would seem to indicate that the intestine is not the target tissue of the hormone.

Hormones other than stanniocalcin and calcitonin may be directly involved with calcium uptake across the gut. In the Atlantic cod, 25-hydroxycholecalciferol was found to stimulate calcium uptake across the intestine while 24, 25-dihydroxycholecalciferol was found to decrease calcium uptake.(Sundell and Bjornsson, 1990) More research

directed specifically at the interaction between calcemic hormones and intestinal calcium uptake in sea water-adapted chinook would help to clarify the situation.

3.4.5 Gastrointestinal pH

Stomach digestion (acidic) in salmonids, in comparison to stomachless fish, permits greater intestinal proteolysis, enhances the level of small peptides in the digesta, and allows a shorter intestinal length to accomplish digestion (Grabner and Hofer, 1989). Gastrointestinal pH's were measured to determine if the buffering capacity of high dietary ash levels would alter pH.

Usher et al. (1990) found a positive relationship between increased stomach acidity and feed intake in Atlantic salmon smolts adapted to sea water. Further, they concluded that ingesting sea water (pH 8) decreased stomach pH in non-feeding smolts and HCl secretion stimulated by feed ingestion was sufficient to acidify the salt water and ingesta. Although average pH values reported in this experiment were more alkaline than those reported by Usher et al. (1990) (foregut = 4.65, midgut = 7.80 and hindgut = 7.66), they appeared to be unaffected by dietary ash concentrations, or by the absence or presence of digesta in the stomach. Except for the stomach pH values of non-feeding fish, the findings in this study confirm and extend those of Usher et al. (1990).

3.4.6 Renal Calcinosis

At the outset of this study, it was uncertain whether high dietary ash and calcium levels would cause renal calcinosis in sea water-adapted chinook salmon. Romestand et al. (1986), for instance, found that trout fed diets supplemented with cadmium, calcium and cholecalciferol had significant changes in the condition factor, number of erythrocytes, and levels of some blood components (cholesterol, protein, sugar, calcium) as well as increased incidence of uremia and nephrocalcinosis. The latter occurred especially when high gradients of cadmium and calcium were present in the trout diets. Hicks et al. (1984), also working with trout, found that elevated levels of selenium caused nephrocalcinosis and, in extreme cases, increased kidney calcium and magnesium,

and liver magnesium. Enhanced incidence of nephrocalcinosis in salmonids has also been reported to be due to low dietary concentrations of magnesium in relation to calcium and phosphorus (Cowey et al. 1977), elevated levels of carbon dioxide in the water (Smart et al., 1979), and the presence of proliferative kidney disease (Ferguson and Richards, 1979). These studies differ from those by Berge and Austreng (1989) and Tacon et al. (1983) which found that high levels of dietary ash (35%) or dietary calcium (6-7%) did not induce renal calcinosis adversely affect growth performance of rainbow trout.

It is noteworthy that of all of the fish dissected, only one appeared to have overt signs of renal calcinosis. The fish was amongst those removed on day 21 (due to weight loss) which had shrimp-like organisms in their gastrointestinal tracts. It appears, therefore, that the dietary conditions employed in this study did not induce nephrocalcinosis in chinook salmon held in sea water and this is contrary to what would be expected for salmonids held in freshwater. It should be emphasized, however, that the present experiment was of short duration and further research on the influence of dietary ash levels on renal calcinosis is warranted.

3.5 Conclusions - Experiment 1

The reduction of dietary ash levels from 23.1% to 13.4% had no significant effect upon chinook salmon growth, appetite or feed efficiency under the conditions of this study.

The concentrations of calcitonin and teleocalcin in the plasma, gastrointestinal pH values and the gross morphology of the fish were unaffected by changes in the concentration of dietary ash. If the diets had any effects on hormonal secretion, they were masked by the large fluctuations of hormonal levels between fish given the same dietary treatments. Increases in the plasma titres of calcitonin appeared to be linked to the degree of fish maturation. Mean stomach acidity was identical across all dietary treatments, and acid secretion was sufficient to acidify stomach contents.

4.0 EXPERIMENT 2

Evaluation of Deboned and Non-Deboned Hake Offal Press Cake Meals, Relative to Deboned and Non-Deboned Roe Herring Press Cake Meals, as Protein Sources for Sea Water-Adapted Chinook Salmon.

4.1 OBJECTIVES

This experiment was conducted to compare the nutritive value of hake offal press cake meal to roe herring press cake meal at each of two dietary protein concentrations (48 and 35%) and to assess the effects of mechanical deboning of each of the raw materials on the nutritive values of the meals using chinook salmon in sea water.

4.2 MATERIALS AND METHODS

4.2.1 Fish Meals

The four press cake meals studied were produced from hake offal, deboned hake offal, roe herring offal, and deboned roe herring offal, respectively. An additional protein source, namely minced freeze dried hake fillets, was also included in the study. All of the offals were obtained from B.C. Packers and handled as described in Experiment 1, section 3.2.1. The herring offal was composed of female carcasses from which the roe had been stripped. The hake offal composition was identical to that described in Experiment 1, section 3.2.1. Deboned meals were produced from the hake and herring offals by chopping them, processing them with a mechanical deboner, and then pressing the deboned flesh into frozen blocks. Meal production, storage, addition of antioxidant, and grinding followed the protocols as described in Experiment 1. The meal produced from the deboned herring offal had to be ground with a small laboratory hammer mill because its high oil content did not permit grinding by the Fitz mill.

Minced freeze-dried hake fillets were prepared as follows. After the hake fillets were freeze-dried, they were kept frozen at -20^{o} C. Subsequently, they were minced with a meat grinder on Aug. 10, 1988, refrozen, and then finely ground on Aug. 15 in a Fitz hammer mill. A component of the minced fillet (possibly collagen) was refractory to grinding and eventually occluded the holes of the screen in the grinding chamber.

Whenever the flow of ground product was severely restricted by the occlusion of the screen holes, refractory component was removed from the chamber. The material was not added back to the ground material because of potential complications which may have arisen during diet preparation. Ethoxyquin (antioxidant) was added at the same level as employed for the stabilization of the meals in experiment 1.

4.2.2 Diets

All diets were formulated to be isocaloric (36.8 kcal of estimated metabolizable energy/ kg dry diet) and they were produced as described in Experiment 1 section 3.2.2 except that the pellet size was 1/8 inch. The proximate compositions of the meals, test diet formulations, and diet proximate compositions are given in Tables 15, 16, and 17, respectively. Table 18 provides information on the supplemental levels of minerals employed while Table 19 gives the total dietary mineral contents. Coding for the diets was as follows: H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

4.2.3 Pre-experimental Treatment of Fish

Four thousand Robertson Creek chinook, averaging 5-6 g in weight, arrived on March 29, 1988, at the DFP, West Vancouver Laboratory. The fish were kept outdoors in two 2.5 meter diameter (6000 litre) tanks in which the water was aerated and the tanks were covered by a dark nylon mesh net. Fresh well water (11^{o} C) was supplied to the tanks at a rate of 20 litres per minute. In mid May the flow rate was increased to 35 litres per minute. Fish were dip-vaccinated against *Vibriosis* (*ordalli* and *anguillarum*) on April 5 and one month later on May 5. Fish were fed OMP.

The timing for transferring the chinook to salt water was determined by jumping behavior, degree of silvering, weight, and the time interval from when the fish were last dip-vaccinated. The transfer to salt water, which began on July 3, 1988, followed the

Table 15 - Proximate analysis of dietary protein sources used in Experiment 2, on a dry weight basis.

Protein[1] source	% Dry matter	% Crude protein[2]	% Crude lipid[3]	% Ash
H+	89.90 ±0.10	72.14 ±0.18	18.51 ±0.99	11.62 ±0.14
H-	86.68 ±0.06	79.51 ±0.35	17.63 ±0.89	5.84 ±0.06
K+	81.46 ±0.18	64.27 ±0.21	7.72 ±0.28	25.85 ±0.11
K-	83.60 ±0.29	78.63 ±0.66	9.34 ±0.41	9.15 ±0.07
F+	98.42 ±0.09	79.90 ±1.42	5.62 ±0.09	11.17 ±0.06

Mean ± SEM (standard error of the mean)

[1] Protein source codes:
H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; "+" = non-deboned protein source; and "-" = deboned protein source.

[2] Crude protein content determined by micro-Kjeldahl technique.

[3] Crude lipid content determined by Bligh and Dyer (1959) extraction method.

Table 16 - Composition of diets as fed in Experiment 2.

Ingredient (g/kg)	Diet[1]								
	Hh+	Hh-	Hl+	Hl-	Kh+	Kh-	Kl+	Kl-	Fh+
B.C. herring press cake meal	678.5		504.1						
Deboned B.C. herring press cake meal		628.7		469.0					
Hake offal press cake meal					773.0		586.4		
Deboned hake offal press cake meal						644.2		483.6	
Minced freeze-dried hake fillets									572.7
Dextrin	57.1	56.2	58.2	57.5	52.5	54.8	54.6	56.4	61.5
Cerelose	62.8	61.8	64.0	63.2	57.8	60.3	60.1	62.1	67.7
Alpha - cellulose	122.7	160.8	197.8	225.4	-	116.7	100.2	191.1	127.6
Vitamins[2]	18.35	18.1	18.70	18.47	16.9	17.6	17.6	18.1	19.8
Minerals[3]	27.5	27.4	28.1	27.7	25.3	26.4	26.3	27.2	29.7
Salmon oil[4]	-	14.9	95.5	105.5	44.2	48.2	123.2	128.8	85.4
Carboxymethyl cellulose	26.6	26.2	27.1	26.8	24.5	25.6	25.5	26.3	28.7
Ascorbic acid	1.8	1.8	1.9	1.9	1.7	1.8	1.8	1.8	2.0
Choline chloride (60%)	4.6	4.5	4.7	4.6	4.2	4.4	4.4	4.5	4.9

[1] Diet codes:
H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[2] Vitamin supplement supplied the following levels of nutrient per kg dry diet: D-calcium pantothenate, 193.7 mg; pyridoxine HCl, 44.95 mg; riboflavin, 60 mg; niacin, 300 mg; folic acid, 20 mg; thiamin mononitrate, 40.65 mg; biotin, 3 mg; cyanocobalamine, 0.06 mg; menadione, 26.0 mg; dl-alpha-tocopheryl acetate, 600 IU; cholecalciferol, 2400 IU; retinol acetate, 10000 IU; inositol, 400 mg.

[3] Mineral supplement - see Table 18.

[4] Salmon oil stabilized with 500 mg ethoxyquin per kg oil.

Table 17 - Proximate analysis of Experiment 2 diets on a dry matter basis.

Diet[1]	% Dry matter	% Crude protein[2]	% Crude lipid[3]	% Ash
Hh+	89.05	49.42	11.86	10.05
	±0.29	±1.36	±0.21	±0.02
Hh-	86.59	47.23	11.77	7.68
	±0.15	±1.30	±0.21	±0.07
Hl+	90.10	31.71	16.62	8.58
	±0.17	±0.55	±0.06	±0.04
Hl-	89.08	30.89	16.27	8.02
	±0.15	±0.84	±0.02	±0.01
Kh+	85.06	47.48	11.26	22.21
	±0.04	±0.76	±0.08	±0.13
Kh-	86.81	48.65	12.11	9.12
	±0.08	±0.70	±0.01	±0.05
Kl+	86.82	39.48	16.19	20.26
	±0.05	±0.32	±0.32	±0.03
Kl-	88.24	32.55	16.26	8.62
	±0.11	±1.24	±0.25	±0.05
Fh+	90.28	47.05	10.69	9.55
	±0.19	±0.60	±0.06	±0.10

Mean ± SEM (standard error of the mean)

[1] Diet codes:

H = B.C. herring press cake meal; K = Hake offal press cake meal; F = Minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[2] Crude protein content determined by micro-Kjeldahl technique.

[3] Crude lipid content determined by Bligh and Dyer (1959) extraction method.

Table 18 - Supplemental mineral concentrations[1] used in Experiment 2.

Mineral supplement content [2]									
	Diet[3]								
	Hh+	Hh-	Hl+	Hl-	Kh+	Kh-	Kl+	Kl-	Fh+
Ca	-	9351	-	10627	-	-	-	3502	3639
P	1414	3148	801	5116	11824	1775	9740	4000	-
Mg	997	545	709	739	3071	72	2517	374	392
Mn	128	75	92	75	301	75	247	74	79
Zn	207	212	212	221	185	183	187	197	229
Co	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Cu	3.8	5.1	5.8	6.6	-	4.8	2.3	3.7	8.9
Fe	-	107	93	199	6.8	177	49	221	410
F	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
I	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Na	-	371	485	1333	1296	-	1334	-	4034
K	4526	4042	5686	6453	14926	9494	12663	8204	-
Se	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[1] On a dry matter basis in mg/kg of diet.

[2] Mineral supplement supplied minerals in the following forms:
manganese as $MnSO_4 \cdot H_2O$; zinc as $ZnSO_4 \cdot 7H_2O$; cobalt as $CoCl_2 \cdot 6H_2O$; copper as $CuSO_4 \cdot 5H_20$; iodine as KIO_3; fluorine as NaF; iron as $FeSO_4 \cdot 7H_2O$; selenium as Na_2SeO_3; potassium as K_2SO_4; magnesium as $MgSO_4 \cdot 7H_2O$; calcium as $CaCO_3$; phosphorus as KH_2PO_4; sodium as NaCl.

[3] Diet codes:
H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h= high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

Table 19 - Experiment 2 dietary mineral concentrations[1].

Dietary content (mg/kg)									
	Diet[2]								
	Hh+	Hh-	Hl+	Hl-	Kh+	Kh-	Kl+	Kl-	Fh+
Ca	25100	15200	17200	14500	70700	13100	53000	15200	19900
P	18600	10800	12500	10200	45400	10300	36300	11400	13900
Mg	2670	1410	1790	1440	5810	1380	4700	1580	1920
Mn	138	88.1	111.6	87.8	339	84.1	292	97	110
Zn	247	215	242	212	255	205	280	293	226
Co	1.1	1.2	1.4	1.7	0.2	1.9	0.2	0.76	0.56
Cu	9.7	9.77	8.5	6.99	13.6	16.9	9.10	6.52	11.5
Fe	590	510	660	490	730	600	550	500	520
F	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0
I	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0	>5.0
Na	5030	6900	4480	5680	10700	4840	9900	5700	10300
K	11200	16100	9780	12200	23300	11200	22000	15000	15800
Se	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1

[1] Protein source minerals and supplemental minerals on a dry matter basis in mg/kg of diet.

[2] Diet codes:

H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

protocol used in Experiment 1 section 3.2.3. Later in the month, the fish were switched from OMP to a commercial dry diet (EWOS) as described in Experiment 1, section 3.2.3.

4.2.4 Experimental Design and Management Procedures

On September 2, 1988, 100 fish were sampled from the outdoor tanks to determine the weight distribution of the population. Based upon the results, a weight range of 18.5 to 42.1 g was used to select fish for the experiment. Approximately 100 fish were netted at a time and transferred into large plastic buckets. The buckets were then transported into the wet lab where the fish were anesthetized and sequentially distributed to the experimental tanks as described in Experiment 1. The selection process continued until there were 105 fish in each of 18 1100-litre tanks.

The tanks were grouped into two parallel rows of nine tanks each. A system of double stand pipes in the center of each tank was used to ensure adequate flushing of waste feed and feces from the system. Every tank was supplied with aerated water (dissolved oxygen = 8.5-10 ppm, temperature = 10.5- 12.5^0 C), a nylon mesh cover, and sea water (salinity = 26-28 ppt) flowing at a rate of 20 litres per minute. Lighting followed the natural photoperiod.

On September 5, a surge of toxic material from decaying biological matter in the water system killed a significant number of fish in some tanks. Surviving fish were allowed to recover before being redistributed on September 13. The redistribution resulted in 90 fish per tank.

The fish were acclimated to the tanks and feeding regime until the start of the experiment on October 18, 1988. Fish were fed to satiation twice daily between 8:30 and 10:30 am and 3:15 and 4:00 PM. The shorter feeding time in the afternoon reflected reduced hunger of the fish relative to the situation for the morning feedings. One week before the experiment, all fish were switched, as per protocol in Experiment 1, to a basal diet which contained deboned herring as the sole protein source..

The experiment was conducted as a completely randomized design with a 2x2x2 factorial layout containing two replicate groups per dietary treatment. The test factors were protein level (high and low), deboning (non-deboned or deboned), and protein source (hake offal and herring offal) for a total of 8 dietary treatments. An additional diet containing freeze dried hake fillets was included as a positive control. The experimental duration was 84 days (October 18, 1988, to January 10, 1989). Individual weights (g) and fork lengths (cm) were measured and recorded to the first decimal place on days 0, 21, 42, 63, and 84. Fish were sampled for whole body proximate analysis on days 0, 42, and 84. Daily feed intake and mortality were recorded for each group.

4.2.5 Sampling Protocol

Fish collection and handling followed the procedures described in Experiment 1 section 3.2.5. On October 17, after the morning feeding, a total of 6 fish were removed randomly from six of the tanks (1 fish per tank). The gastrointestinal contents were removed as described in Experiment 1 and the carcasses were frozen for later proximate analyses. On day 42 six fish per diet were selected randomly, killed, and frozen on dry ice for later proximate analysis as described in Experiment 1. The same procedure of sampling fish for proximate analysis was repeated on day 84.

Before weighing, feed was withheld for twenty four hours. On October 18 and 19, respectively, fish from the first and second row of tanks were weighed. During the weighing, damaged or unhealthy fish were removed and excluded from the experiment. At the start of the experiment, each tank had a total of 80 fish. All fish were weighed and measured on days 21, 42, 63, and 84 using the protocols described in Experiment 1.

Part way through the experiment, it became evident that disease was affecting some groups. On day 21, six unhealthy looking fish were selected and sent to the Department of Fisheries and Oceans, Pacific Biological Station in Naniamo, B.C. for examination. The fish were diagnosed as having *vibriosis* and a 10-day dietary treatment of 0.12 g of oxytetracycline/kg fish biomass/day was recommended for all groups.

Salmon oil was used as a carrier for the drug to improve dispersal and adherence to the feed. The carrier increased the level of oil in the diets by approximately 1%. The drug and feeds were blended in small plastic bags which were shaken by hand for 10 minutes. To ensure that sufficient amounts of drug were consumed by the fish, calculations of the required amount of drug per kilogram diet were based upon 1/2 the normal daily feed intake for each group. Any additional feeding needed to reach the point of satiation was done with the respective non-medicated feed.

4.2.6 Analytical Protocols

Determinations of dry matter, crude protein, crude lipid, and ash concentrations in the diets, meals, and whole carcasses were conducted as described in Experiment 1 section 3.2.6.

4.2.6.1 Preparation of Sample for Whole Body Proximate Analysis

Each fish which was selected for whole body proximate analysis on days 0 and 84 was analyzed individually. Homogeneous samples for analysis were obtained by processing the fish with a Polytron homogenizer.

Optimal processing was achieved with the following steps. First, each fish was weighed and sliced into 6 to 13 mm thick cross sections. The fish and an equivalent weight of water were then added to a square bottomed bottle. The bottle selected had to have a neck wide enough to accept the Polytron mixer. Also, it had to be sufficiently narrow so that the stirring vortex could move all material and it had to have enough depth to prevent material from escaping out of the top during mixing.

After the fish were partially ground at low settings, a Polytron setting of 8 was used. Mixing was continued until all material appeared to be uniform. Each homogenate was poured into a pre-weighed plastic weighing dish and the total weight was recorded. The homogenate was then frozen and freeze-dried for 72 hours. Finally, the material was weighed and ground with a mortar and pestle.

Since the ratio of added water to fish weight as well as the weight of homogenate before and after freeze drying was known, it was possible to calculate how much of the fish's moisture had been removed by freeze-drying.

4.2.6.2 Lipid

Lipid determination varied slightly from the protocol which was described in Experiment 1 section 3.2.6.3. Six ml of water were added to the graduated cylinder (instead of 5 ml) and 7 ml of water were added to the mixture of chloroform, methanol, and sample (instead of 10).

4.2.6.3 Amino Acids

A standard amino acid hydrolysis procedure was used for breaking down the dietary proteins for subsequent amino acid determination. The equivalent of approximately 25 mg of protein was mixed with 20 ml of 3 N HCl and placed in an autoclave at 121° C and 15 psi for 17 hours. HCl was removed using a rotary evaporator placed in a 60° C water bath. Fifteen ml of 0.2 M sodium citrate buffer were added to the sample and the solution was filtered twice through a medium porosity Gooch apparatus. The filtrate was added to 3 ml of 1 N NaOH and then the volume was made up to 50 ml with the sodium citrate buffer. The solution was refrigerated overnight, refiltered through the Gooch apparatus, and then filtered a final time through 0.22 μ filter.

Fifty μl of the solution was injected into a Beckman 6300 Automatic Amino Acid Analyzer. A sequence of 2 temperatures (50 and 65° C), three buffers (Na-A, Na-B, and Na-C in order of increasing pH) and a cation exchange column were used. Sodium hydroxide was used to regenerate the column after each sample. A buffer flow rate of 15 ml/hr and a solvent flow rate of 8 ml/hr were used.

The samples were compared against a Sigma Chemical Company (St Louis, MO) A-2908 amino acid standard. The standard was diluted to 5 nmole per 50 μl. By comparing the standard to the samples and knowing the mg of diet per 50 ml of solution,

the level of amino acids in the sample were calculated. The levels of the sulfur containing amino acids (methionine, and cystine) and tryptophan were not determined.

4.2.7 Statistical Procedures

All statistical procedures were conducted using SYSTAT version 5.02. Due to single degrees of freedom, ANOVA was conducted using the means model as described by Searle (1987), Malcolm Greig (1992), and the SYSTAT Statistics Manual (pages 303- 313) (See Table 33). For the analysis, significance was set at $p < 0.05$. Values which were calculated as percentages, but fell outside the range of 20 - 70%, were transformed with an arcsin transformation before being analyzed by ANOVA

To control type I errors (falsely accepting a value as significant), the Bonferroni procedure for pairwise comparisons, as described in the SYSTAT Statistics Manual (pages 223, 239, and 275), was used to compare values found significant by ANOVA. Significance for the procedure was set at $p < 0.05$ divided by the number of values being compared.

4.3 RESULTS

Unless otherwise stated: a) the Bonferroni pairwise procedure, used when analysis of variance found significant effects, found no significant differences in the pairwise comparisons and b) statistical analyses mentioned below are for the effects of protein level, protein source, and deboning of the offals on the data.

4.3.1 Analysis of Diets and Protein Sources

The proximate compositions of the protein sources (hake offal meal, deboned hake offal meal, herring offal meal, deboned herring offal meal, and minced hake fillets) and the diets (Kh+, Kh-, Kl+, Kl-, Hh+, Hh-, Hl+, Hl-, and Fh+) are provided in Tables 15 and 17. All diets, except for the Kl- and Fh+, were reasonably close to the expected value for protein (48% and 35% for high and low protein, diets respectively) and lipid (12.3% for high protein diets and 19.2% for the low protein diets) values. A mixing error with the Kl+ diet resulted in a 7% elevation of the protein concentration above the other low protein diets. The Fh+ pellets were harder than those of the other diets and they did not easily absorb the lipid which was applied externally (See Experiment 1, section 3.2.2).

Supplemental and dietary mineral profiles are listed in Table 18 and 19. Deboning reduced the ash concentrations in the herring meal by 49.7% and in the hake meal by 64.6%. Ash concentrations in the high protein herring diets differed by 23.6% while the those in the high protein hake diets differed by 58.9%. The corresponding differences in ash for the low protein diets were 6.5% and 57.5%, respectively.

All diets, except for Kh+, Kl+, and Hh+, had dietary calcium concentrations ranging from 1.31 - 1.99%. The Kh+, Kl+, and Hh+ diets had roughly 4 times, 3 times and 1.5 times, respectively, the calcium concentrations of the other diets. An attempt was made to maintain a calcium to phosphorus ratio of approximately 1.4:1.0 and a phosphorus to magnesium ratio of roughly 7.3:1.0 in all diets. Supplemental calcium and phosphorus were added to the Hh-, Hl-, and Kl- diets to maintain constant ratios between

these minerals. The Fh+ diet needed only supplemental calcium since there was sufficient native phosphorus provided by the minced hake fillets.

Dietayr amino acid concentrations are provided in Tables 20 and 21. When amino acids were expressed as a percentage of protein it was found that deboning the hake offal increased the concentration of phenylalanine, tyrosine, and valine and decreased the concentrations of glycine and proline in the hake meal. Although the differences were slight, deboning the hake offal may have also increased the concentrations of isoleucine, leucine, and lysine. This contrasts with the herring meals where deboning decreased the concentrations of glycine and proline, and slightly increased the leucine and lysine concentrations.

When the amino acid contents of diets containing deboned meals were compared on a percent of protein basis, the hake diets were found to have lower concentrations of lysine and taurine and higher concentrations of histidine. In contrast, the diets with non-deboned hake meal were higher in glycine and histidine, and lower in lysine and taurine than the diets containing non-deboned herring meal. Compared to the rest of the diets, the hake fillets diet had higher concentrations of glutamic acid and lysine. The percentage of phenylalanine in the hake fillet diet was the same as in the non-deboned hake diets, while the percentage of glycine, isoleucine, leucine, proline, tyrosine were the same as in the deboned hake diets.

4.3.2 Measures of Growth - Weight, Percent Body Weight Gain, Body Weight Gain, Length, Percent Length Gain, Condition Factor, Percent Change in Condition Factor and Specific Growth Rate

Average weights of fish on days 0, 21, 42, 63, and 84 are provided in Table 22 and depicted in Figure 4. By day 21, dietary protein concentration was significantly affecting the body weights ($p<0.05$). The average fish weights on days 42, 63, and 84 were found to be significantly affected by the interaction between protein source,

deboning, and protein concentration. Fish weights on days 42, 63, and 84 were significantly influenced by the effect of diet ($p < 0.05$).

On days 21, 42, 63, and 84, the average weights of fish fed the high protein hake and herring diets had the following relative performances: a) with one exception on day 84, fish fed the high protein herring diets were consistently heavier than the fish on the high protein hake diets, b) the fish consuming the diet containing deboned herring meal were consistently heavier than those on the diet containing non-deboned herring meal, and c) fish feeding on the diet produced with deboned hake meal were consistently smaller than the fish fed the diet made with non-deboned hake meal. Similar characteristics were not observed with fish fed the low protein diets.

Body weight gain (Table 23), change in length (Table 24), and specific growth rate (Table 25 and Figure 5), over the duration of the experiment were significantly affected by the interaction between protein concentration, protein source, and deboning. For these measures, the relative performances of fish fed the high protein diets were the same as described above for fish weights except for the relative performances between fish fed the diets with the deboned hake and herring meals. The performance of fish fed the deboned herring meal diets were consistently equal or superior to the performance of fish fed the deboned hake meal diets. For fish fed the low protein diets the following relative performances were observed: a) fish fed hake diets outperformed those fed herring diets b) fish fed the deboned hake meal diet tended to slightly outperform fish fed the non-deboned hake meal diet for most measures, and c) feeding fish the deboned herring meal diet tended to slightly reduce performance relative to fish fed the non-deboned herring meal diet.

Protein concentration was found to be the only significant factor ($p < 0.05$) affecting percent body weight gain (Table 23) and percent change in length (Table 24) over the 84 days of the experiment . The largest percent gains and percent changes in length were supported by the high protein diets. For the same time period, the change in

Table 20 - Dietary amino acid content expressed as % of total dietary protein in Experiment 2.

Amino Acid	Diet				
	Hh+	Hh-	Hl+	Hl-	Fh+
Ala	5.11	5.04	5.92	5.50	5.62
Arg	5.67	6.32	7.24	6.89	5.45
Asp	7.84	7.78	8.53	8.53	8.98
Glu	11.22	11.36	12.45	12.30	13.83
Gly	5.07	4.31	5.58	4.64	5.14
His	1.87	1.96	2.11	2.14	2.13
Iso	3.55	3.70	3.94	4.00	3.91
Leu	6.46	6.73	7.19	7.31	7.14
Lys	6.70	6.96	7.45	7.59	7.98
Phe	3.44	3.48	3.76	3.74	3.53
Pro	3.72	3.37	4.07	3.65	3.46
Ser	3.45	3.39	3.88	3.66	3.77
Tau	0.40	0.52	0.62	0.54	0.26
Thr	3.62	3.66	4.14	3.96	3.76
Tyr	2.86	2.94	3.15	3.16	3.02
Val	4.12	4.14	4.49	4.45	4.19

Amino Acid	Diet			
	Kh+	Kh-	Kl+	Kl-
Ala	5.77	5.12	4.96	5.29
Arg	5.82	5.22	4.34	5.20
Asp	8.37	8.33	7.25	8.73
Glu	11.92	12.23	10.25	12.63
Gly	7.13	4.91	6.29	5.07
His	2.34	2.31	2.02	2.40
Iso	3.41	3.80	2.98	3.91
Leu	6.30	6.88	5.40	7.09
Lys	6.03	6.50	5.17	6.71
Phe	3.58	4.84	3.11	3.75
Pro	4.62	3.56	3.95	3.65
Ser	4.01	3.75	3.43	3.88
Tau	0.35	0.28	0.32	0.25
Thr	3.77	3.79	3.17	3.88
Tyr	2.57	3.44	2.21	3.02
Val	3.94	4.25	3.40	4.34

[1] Diet codes:

P = Pre-experiment diet; H = B.C. herring press cake meal;
K = Hake offal press cake meal; F = Minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non deboned protein source; and "-" = deboned protein source.

Table 21 - Amino acid content in g/kg diet in Experiment 2.

Amino Acid	Diet					
	Hh+	Hh-	Hl+	Hl-	Fh+	
Ala	25.3	23.8	18.8	17.0	26.4	
Arg	28.0	29.8	22.9	21.3	25.7	
Asp	38.7	36.8	27.1	26.3	42.2	
Glu	55.4	53.6	39.5	38.0	65.0	
Gly	25.1	20.4	17.7	14.3	24.2	
His	9.3	9.2	6.9	6.6	10.0	
Iso	17.5	17.5	12.5	12.4	18.4	
Leu	31.9	31.8	22.8	22.6	33.6	
Lys	33.1	32.9	23.6	23.4	37.5	
Phe	17.0	16.5	11.9	11.6	16.6	
Pro	18.4	15.9	12.9	11.3	16.3	
Ser	17.0	16.0	12.3	11.3	17.7	
Tau	2.0	2.5	2.0	1.7	1.2	
Thr	17.9	17.3	13.1	12.2	17.7	
Tyr	14.2	13.9	10.0	9.8	14.2	
Val	19.9	19.5	14.2	13.7	19.7	

Amino Acid	Diet					
	Kh+	Kh-	Kl+	Kl-	Req[2]	Req[3]
Ala	27.4	24.9	19.6	17.2	--	--
Arg	27.6	25.4	17.2	16.9	21	28.8
Asp	39.8	40.5	28.6	28.4	--	--
Glu	56.6	59.5	40.5	41.1	--	--
Gly	33.8	23.9	24.8	16.5	--	--
His	11.1	11.3	8.0	7.8	6.1	8.4
Iso	16.2	18.5	11.8	12.7	7.9	10.8
Leu	29.9	33.5	21.3	23.1	14	19.2
Lys	28.6	31.6	20.4	21.9	17.5	24
Phe	17.0	23.5	12.3	12.2	17.8[4]	24.5[4]
Pro	21.9	17.3	15.6	11.9	--	--
Ser	19.0	18.2	13.5	12.6	--	--
Tau	1.7	1.4	1.3	0.8	--	--
Thr	17.9	18.5	12.5	12.6	7.9	10.8
Tyr	12.2	16.7	8.7	9.8	--	--
Val	18.7	20.7	13.4	14.1	11.4	15.6

[1] Diet codes:

P = Pre-experiment diet; H = B.C. herring press cake meal; K = Hake offal press cake meal; F = Minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non deboned protein source; and "-" = deboned protein source.

[2] Values taken from Cowey (1988) and modified for dietary protein levels of 35%.

[3] Values taken from Cowey (1988) and modified for dietary protein levels of 48%.

[4] Modified combined tyrosine/phenylalanine requirement taken from Nose and Murai (1990)

condition factor and percent change in condition factor (Table 26) were unaffected by protein concentration, protein source, or deboning.

When all the measures of growth were analyzed for the effect of diet, change in condition factor and percent change in condition factor were the only measures not significantly affected.

Fish fed the hake fillet diet had the largest percent body weight gain, specific growth rate, and percent change in condition factor over the duration of the experiment.

4.3.3 Measures of Feed Utilization - Feed Intake, Protein Efficiency Ratio, Feed Efficiency, Productive Protein Value, Instantaneous Protein Gain, Instantaneous Lipid Gain

For most measures of feed utilization, fish fed the high protein diets had the same relative performances as described for specific growth rates in section 4.3.2. The only exception was the productive protein value of fish fed the high protein hake diets. In this case, fish fed the non-deboned hake meal outperformed the fish fed the deboned hake meal. Similarly, all measures of feed utilization for fish fed the low protein diets had the same relative performance as described for the low protein diets in section 4.3.2.

The feed efficiency (Table 28 and Figure 6), protein efficiency ratio (Table 29 and Figure 7), productive protein value (Table 30), and instantaneous protein gain (Table 27) over the 84 days of the experiment were affected by the interaction between protein source, protein concentration, and deboning.

Feed intake (Table 27) and instantaneous lipid gain (Table 30) were affected by protein concentration. The effect of protein concentration on fish feed intake agrees with visual observations of fish feeding behavior during the experiment. Fish on the low protein diets initially fed as vigorously as those on the high protein diets, but they stopped feeding sooner. Following cessation of feeding, the chinook on the low protein diets still actively pursued and captured pellets. The fish, however, mouthed the pellet(s) and eventually expelled them. Shortly after displaying the mouthing behavior, the

chinook lost all interest in the feed. This behavior contrasted with the fish on the high protein diets which gradually decreased feeding behavior as they reached the point of satiation. Fish on the hake fillet diet initially behaved like the fish on the low protein diets, but after a week their food consumption and behavior matched that of fish fed the other high protein diets.

In comparison to fish fed the other diets, fish fed the hake fillet diet had the best performances for feed efficiency and protein efficiency ratio.

Of all the measures of feed utilization, protein efficiency ratio was the only value not significantly affected by diet. In the case of productive protein value, Bonferroni Pairwise Comparisons found that fish fed the Kh+ diet were significantly different from those fed the Kl- and Hl+ diets ($p < 0.05$).

4.3.4 Body Composition - Whole Body Proximate Composition and Body Protein Level

Terminal percentages of dry matter, ash, and nitrogen-free extract in the carcass (Table 31) were unaffected by protein source, dietary protein concentration, or deboning. Lipid content, however, was affected by dietary protein level ($p<0.05$) and protein content was affected by protein concentration, protein source, and deboning. Fish fed the lower protein diets had consistently higher lipid levels in their bodies.

Body protein level (Table 30) was significantly influenced by the interaction between protein concentration, protein source, and deboning. The relative performances of the fish fed the high and low protein diets were the same as described for specific growth rate in section 4.3.2. Analyses for dietary effect revealed that body protein level was significantly affected by diet ($p<0.05$).

4.3.5 Mortality and Disease

An outbreak of *Vibriosis* in the experimental fish necessitated a 10 day dietary treatment with oxytetracycline for all fish groups. Percent mortality values are shown in Table 32. The interaction between protein source, deboning and protein density was

Table 22 -Mean body weights of dietary groups at 21-day intervals in Experiment 2.

Diet[1]	Day Number				
	0	21	42	63	84
Hh+	33.4 ±0.6	36.5 ±0.3	39.7 ±1.1	44.2 ±0.1	47.3 ±0.3
Hh-[2]	34.5	40.8	44.8	49.7	53.8
Hl+	33.5 ±0.3	35.3±0.0	36.7 ±0.2	39.2 ±0.4	41.3 ±1.0
Hl-	30.4 ±0.3	31.9±0.1	32.6 ±0.2	34.8 ±0.2	36.7 ±0.6
Kh+	31.3 ±1.2	35.9 ±0.5	38.9 ±0.7	42.7 ±0.3	47.4 ±1.1
Kh-[2]	31.4	34.3	36.5	40.0	42.8
Kl+	32.0 ±1.6	33.9 ±2.0	35.0 ±2.3	38.3 ±2.2	41.4 ±2.3
Kl-	31.2 ±1.8	33.6±2.3	35.6 ±1.9	38.4 ±1.9	40.5 ±1.9
Fh+	30.6 ±1.0	35.1 ±3.5	38.1 ±4.1	43.7 ±5.5	47.9±5.6

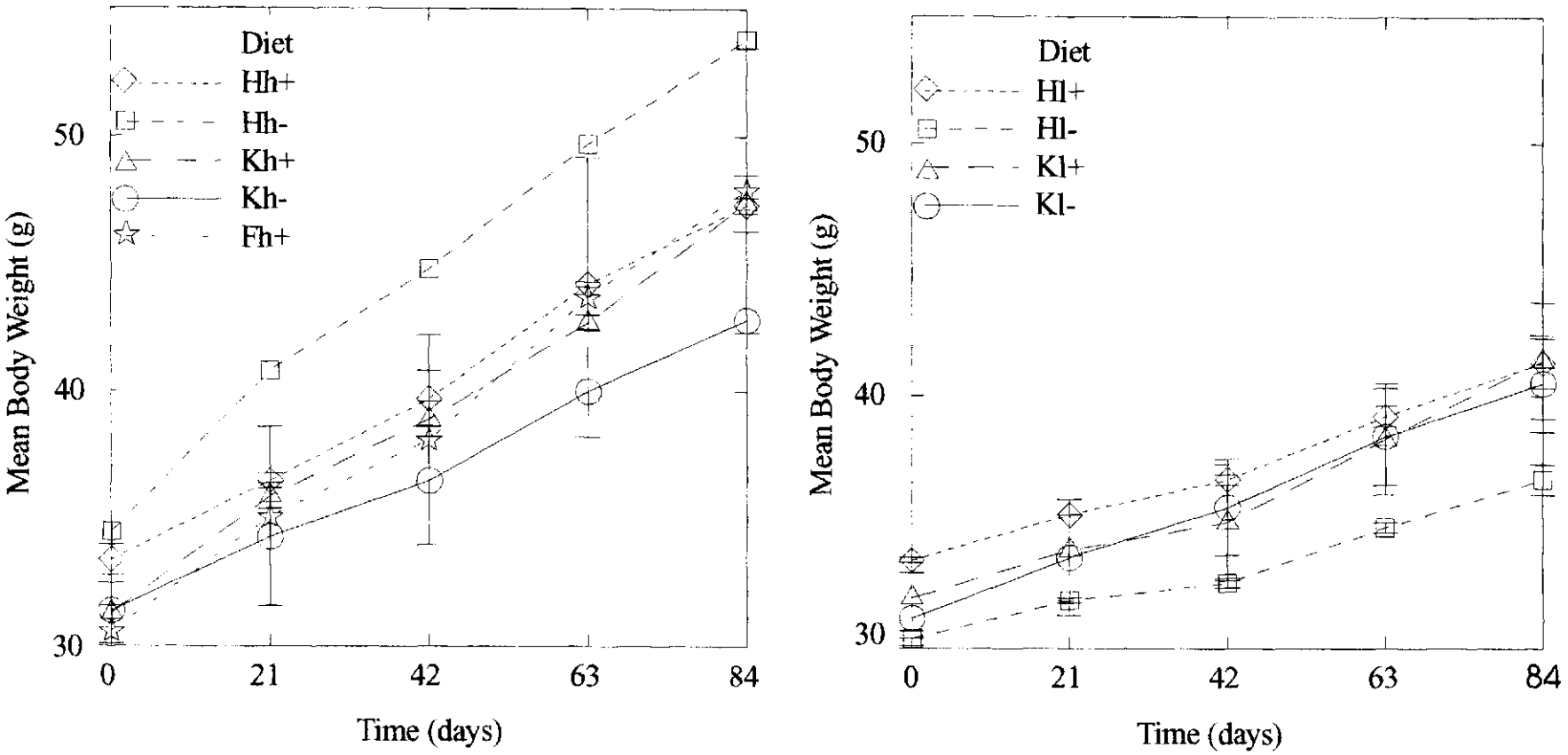
Mean ± SEM (standard error of the mean)

[1] Diet codes:

H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[2] Replicate lost to disease.

Figure 4: Mean Body Weights of Chinook Salmon in Experiment 2. Fish were Fed One of Nine Diets with Either Hake Offal, Herring Offal, or Hake Fillets as the Sole Protein Source for 84 Days.



Mean ± SEM (standard error of the mean), N=2, N=1 for Hh- and Kh-

Diet codes: H = B.C. herring press cake meal; K = Hake offal press cake meal; F = Minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non- deboned protein source; and "-" = deboned protein source.

Table 23 - Average body weight gain and percent body weight gain of dietary groups at the end of the 84-day Experiment 2.

Diet[1]	Body weight gain	% Body weight gain
	g	
Hh+	13.7 ±0.8	41.1
Hh-[2]	19.4	56.2
Hl+	7.6 ±1.0	22.8
Hl-	6.2 ±0.2	20.4
Kh+	15.6 ±2.5	49.9
Kh-[2]	11.2	35.5
Kl+	9.3 ±0.9	29.2
Kl-	9.2 ±0.0	29.4
Fh+	17.3 ±4.6	56.5

Mean ± SEM (standard error of the mean)

[1] Diet codes:
H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[2] Replicate lost to disease

Table 24 - Starting length, change in length and percent change in length of dietary groups at the end of the 84-day Experiment 2.

Diet[1]	Length day 0	Change in length	% Change in length
Hh+	14.0 ±0.0	1.8 ±0.1	12.5
Hh-[2]	14.2	2.2	15.5
Hl+	14.0 ±0.1	1.2 ±0.2	8.2
Hl-	13.7 ±0.0	1.1 ±0.1	7.7
Kh+	13.8 ±0.2	1.8 ±0.2	13.1
Kh-[2]	13.9	1.4	10.1
Kl+	13.8 ±0.1	1.3 ±0.1	9.1
Kl-	13.8 ±0.1	1.2 ±0.1	8.4
Fh+	13.7 ±0.1	2.0 ±0.5	14.2

Mean ± SEM (standard error of the mean)

[1] Diet codes:

H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[2] Replicate lost to disease.

Table 25 - Specific growth rates[1] for dietary groups over 21-day intervals and the 84-day duration of Experiment 2.

	Time intervals in days				
Diet[2]	0 - 21[1]	22 - 42[1]	43- 63[1]	64 - 84[1]	0 - 84[1]
Hh+	0.428 ±0.118	0.393 ±0.091	0.495 ±0.120	0.320 ±0.013	0.415 ±0.027
Hh-[3]	0.791	0.440	0.512	0.383	0.528
Hl+	0.249 ±0.019	0.182 ±0.015	0.296 ±0.022	0.246 ±0.061	0.250 ±0.033
Hl-	0.236 ±0.056	0.093 ±0.013	0.300 ±0.044	0.253 ±0.041	0.226 ±0.007
Kh+	0.653 ±0.114	0.378 ±0.021	0.407 ±0.138	0.483 ±0.083	0.493 ±0.073
Kh-[3]	0.415	0.278	0.424	0.325	0.367
Kl+	0.270 ±0.044	0.141 ±0.038	0.427 ±0.021	0.377 ±0.010	0.307 ±0.008
Kl-	0.352 ±0.049	0.259 ±0.053	0.343 ±0.042	0.272 ±0.023	0.313 ±0.013
Fh+	0.635 ±0.327	0.377 ±0.040	0.642 ±0.073	0.445 ±0.041	0.528 ±0.103

Mean ± SEM (standard error of the mean)

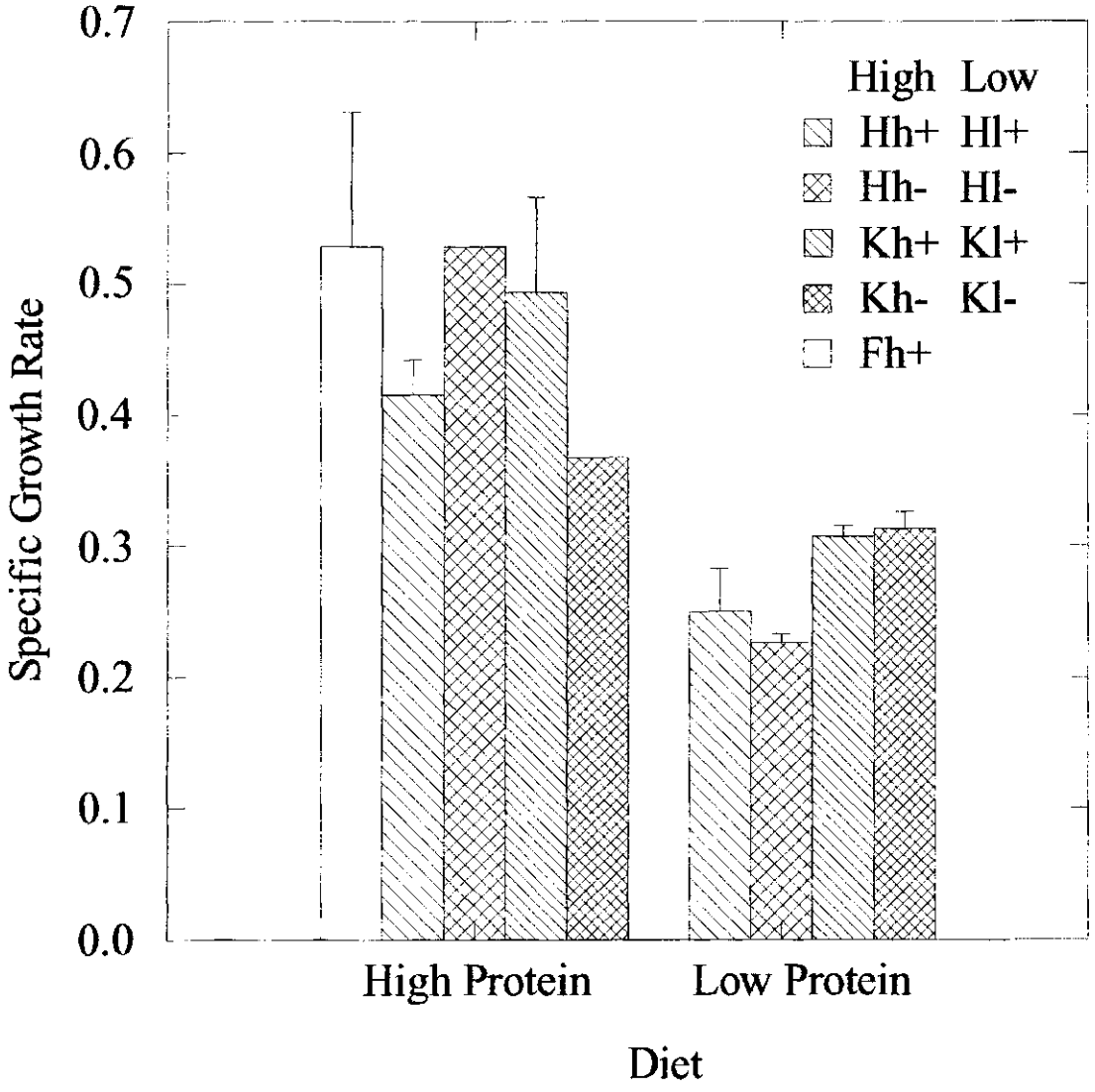
[1] Specific growth rate = [(lnW2 - lnW1) / T] * 100 where W2 = final weight (g), W1 = initial weight (g), and T = time in days.

[2] Diet codes:

H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[3] Replicate lost to disease.

Figure 5: Specific Growth Rate of Chinook Salmon in Experiment 2. Fish were Fed One of Nine Diets with Either Hake Offal, Herring Offal, or Hake Fillets as the Sole Protein Source for 84 Days



Mean ± SEM (standard error of the mean), N=2, N=1 for Hh- and Kh-

Diet codes: H = B.C. herring press cake meal; K = Hake offal press cake meal; F = Minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non- deboned protein source; and "-" = deboned protein source.

Table 26 - Initial condition factor[1], change in condition factor and percent change in condition factor of dietary groups at the end of 84-day Experiment 2.

Diet[2]	Condition factor[1] day 0	Change in condition factor	% Change in condition factor
Hh+	1.21 ±0.02	0.00 ±0.01	0.0
Hh-[3]	1.20	0.01	0.8
Hl+	1.20 ±0.02	-0.02 ±0.01	-1.7
Hl-	1.17 ±0.01	-0.03 ±0.01	-2.6
Kh+	1.19 ±0.00	0.04 ±0.03	3.4
Kh-[3]	1.17	0.01	0.9
Kl+	1.21 ±0.03	-0.01 ±0.01	-0.8
Kl-	1.19 ±0.04	0.01 ±0.03	0.8
Fh+	1.18 ±0.00	0.04 ±0.01	3.4

Mean ± SEM (standard error of the mean)

[1] Condition factor = $W / L^3 \times 100$, where W = weight (g) and L = length (cm).

[2] Diet codes:

H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[3] Replicate lost to disease.

shown ($p < 0.05$) to significantly impact fish survival. The results, however, may be biased because two replicates eliminated from the experiment were being fed high protein diets. One group, fed deboned hake meal, was dropped from the trial because mortality was over 80%. A second group, fed deboned herring meal, was also dropped from the experiment, but this was due to poor feed efficiencies (resulting from disease) rather then high percent moralities. The relative performances of fish consuming the high protein and low protein diets were the same as described in section 4.3.2. for specific growth rate. Percent mortality ranged from 17.3 to 31.9%.

Table 27 - Average feed consumption[1] for dietary groups over 21-day intervals and for the 84-day duration of Experiment 2.

	Feed consumption per fish				
Diet[2]	Days 0 - 21	Days 21 - 42	Days 42 - 63	Days 63 - 84	Days 0 - 84
	g	g	g	g	g
Hh+	6.53 ±0.79	5.35 ±0.63	7.08 ±0.03	6.46 ±0.21	25.43 ±1.24
Hh-[3]	8.33	5.69	7.71	6.44	28.17
Hl+	5.96 ±0.16	4.16 ±0.13	6.18 ±0.11	5.99 ±0.03	22.29 ±0.12
Hl-	5.33 ±0.03	3.56 ±0.04	5.34 ±0.18	4.65 ±0.04	18.88 ±0.29
Kh+	8.25 ±0.71	5.54 ±0.03	7.54 ±0.40	7.69 ±0.92	29.02 ±2.00
Kh-[3]	6.85	5.53	6.67	6.00	25.04
Kl+	6.28 ±0.49	4.00 ±0.47	6.41 ±0.16	5.91 ±0.85	22.60 ±1.98
Kl-	6.29 ±0.70	4.59 ±0.28	6.09 ±0.13	5.07 ±0.10	22.04 ±0.66
Fh+	5.82 ±1.90	4.58 ±0.63	6.64 ±0.65	5.92 ±0.15	22.96 ±3.33

Mean ± SEM (standard error of the means)

[1] On a dry matter basis.

[2] Diet codes:

H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[3] Replicate lost to disease.

Table 28 - Feed efficiency for dietary groups over 21-day intervals and the 84-day duration of experiment 2.

Diet[2]	Feed efficiency[1]				
	Days 0 - 21	Days 21 - 42	Days 42- 63	Days 63 - 84	Days 0 - 84
Hh+	0.472 ±0.074	0.581 ±0.079	0.613 ±0.143	0.447 ±0.037	0.539 ±0.005
Hh-[3]	0.749	0.693	0.657	0.645	0.689
Hl+	0.302 ±0.030	0.331 ±0.017	0.381 ±0.025	0.349 ±0.090	0.342 ±0.043
Hl-	0.290 ±0.069	0.178 ±0.028	0.396 ±0.045	0.409 ±0.067	0.328 ±0.005
Kh+	0.552 ±0.035	0.537 ±0.036	0.456 ±0.130	0.591 ±0.040	0.535 ±0.049
Kh-[3]	0.418	0.374	0.510	0.470	0.445
Kl+	0.297 ±0.041	0.253 ±0.055	0.511 ±0.008	0.542 ±0.062	0.413 ±0.002
Kl-	0.380 ±0.035	0.403 ±0.035	0.436 ±0.040	0.443 ±0.023	0.416 ±0.014
Fh+	0.711 ±0.205	0.633 ±0.047	0.830 ±0.112	0.714 ±0.003	0.739 ±0.092

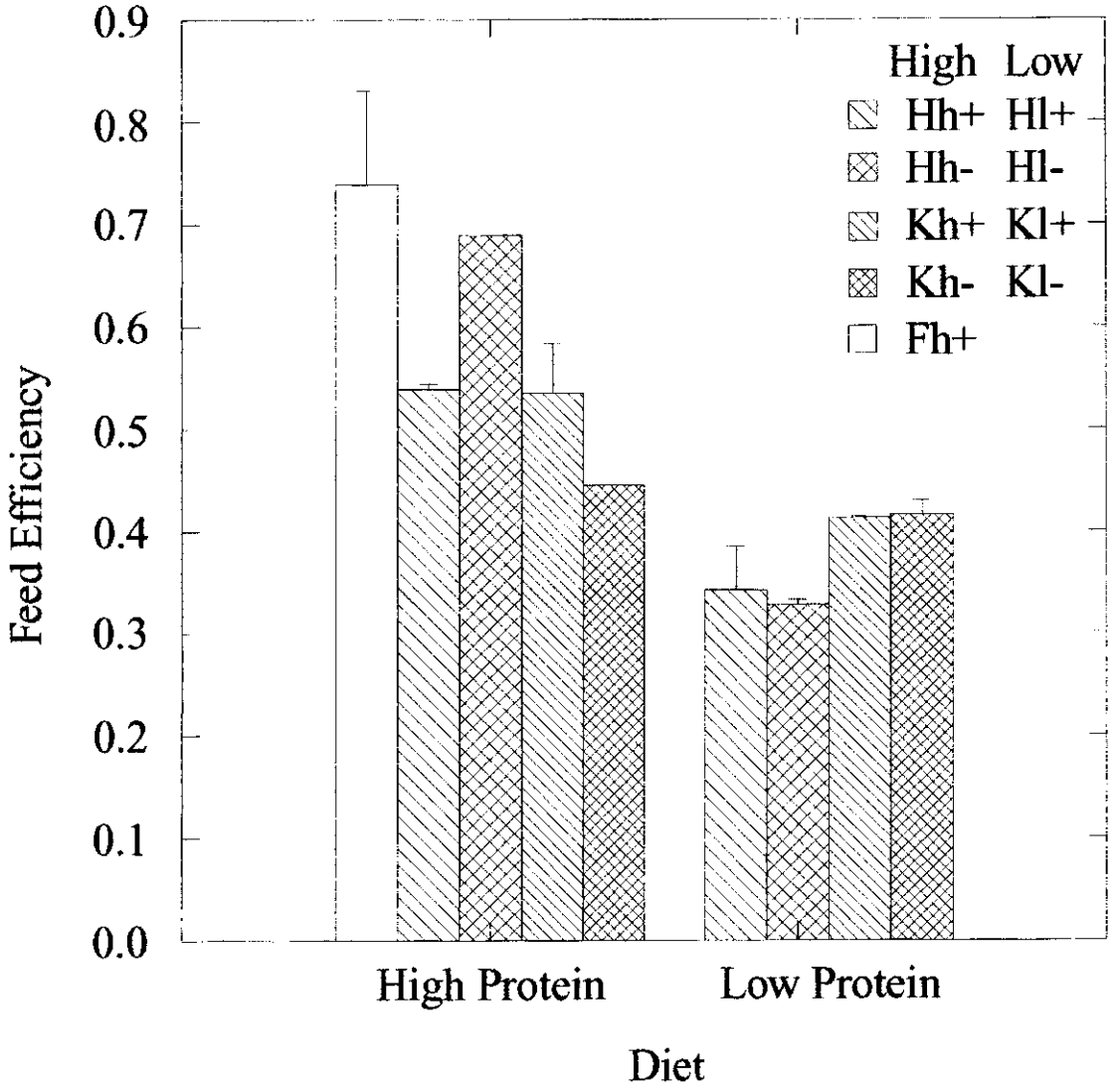
Mean ± SEM (standard error of the mean)

[1] Feed efficiency = (W2 - W1)/Fc where
W2 = final fish weight, W1 = initial fish weight, and Fc = dry feed consumption. Note: fish weights are on a wet weight basis.

[2] Diet codes:
H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; "-" = deboned protein source.

[3] Replicate lost to disease

Figure 6: Feed Efficiencies of Chinook Salmon in Experiment 2. Fish were Fed One of Nine Diets with Either Hake Offal, Herring Offal, or Hake Fillets as the Sole Protein Source for 84 Days



Mean ± SEM (standard error of the mean), N=2, N=1 for Hh- and Kh-
Diet codes: H = B.C. herring press cake meal; K = Hake offal press cake meal; F = Minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non- deboned protein source; and "-" = deboned protein source.

Table 29 - Protein efficiency ratio for dietary groups over 21-day intervals and the 84-day duration of Experiment 2.

Diet[2]	Protein efficiency ratio[1]				
	Days 0 - 21	Days 22 - 42	Days 43- 63	Days 64 - 84	Days 0 - 84
Hh+	0.95 ±0.15	1.18 ±0.16	1.24 ±0.29	0.97 ±0.08	1.11 ±0.01
Hh-[3]	1.59	1.47	1.39	1.37	1.45
Hl+	0.95 ±0.10	1.04 ±0.05	1.20 ±0.08	1.10 ±0.28	1.11 ±0.15
Hl-	0.94 ±0.22	0.58 ±0.09	1.28 ±0.15	1.32 ±0.22	1.09 ±0.03
Kh+	1.16 ±0.07	1.13 ±0.08	0.96 ±0.27	1.24 ±0.09	1.16 ±0.09
Kh-[3]	0.86	0.77	1.05	0.97	0.93
Kl+	0.75 ±0.10	0.64 ±0.14	1.29 ±0.02	1.37 ±0.16	1.06 ±0.01
Kl-	1.17 ±0.11	1.24 ±0.11	1.34 ±0.12	1.36 ±0.07	1.30 ±0.02
F++	1.51 ±0.43	1.35 ±0.10	1.76 ±0.24	1.52 ±0.01	1.58 ±0.20

Mean ± SEM (standard error of the mean)

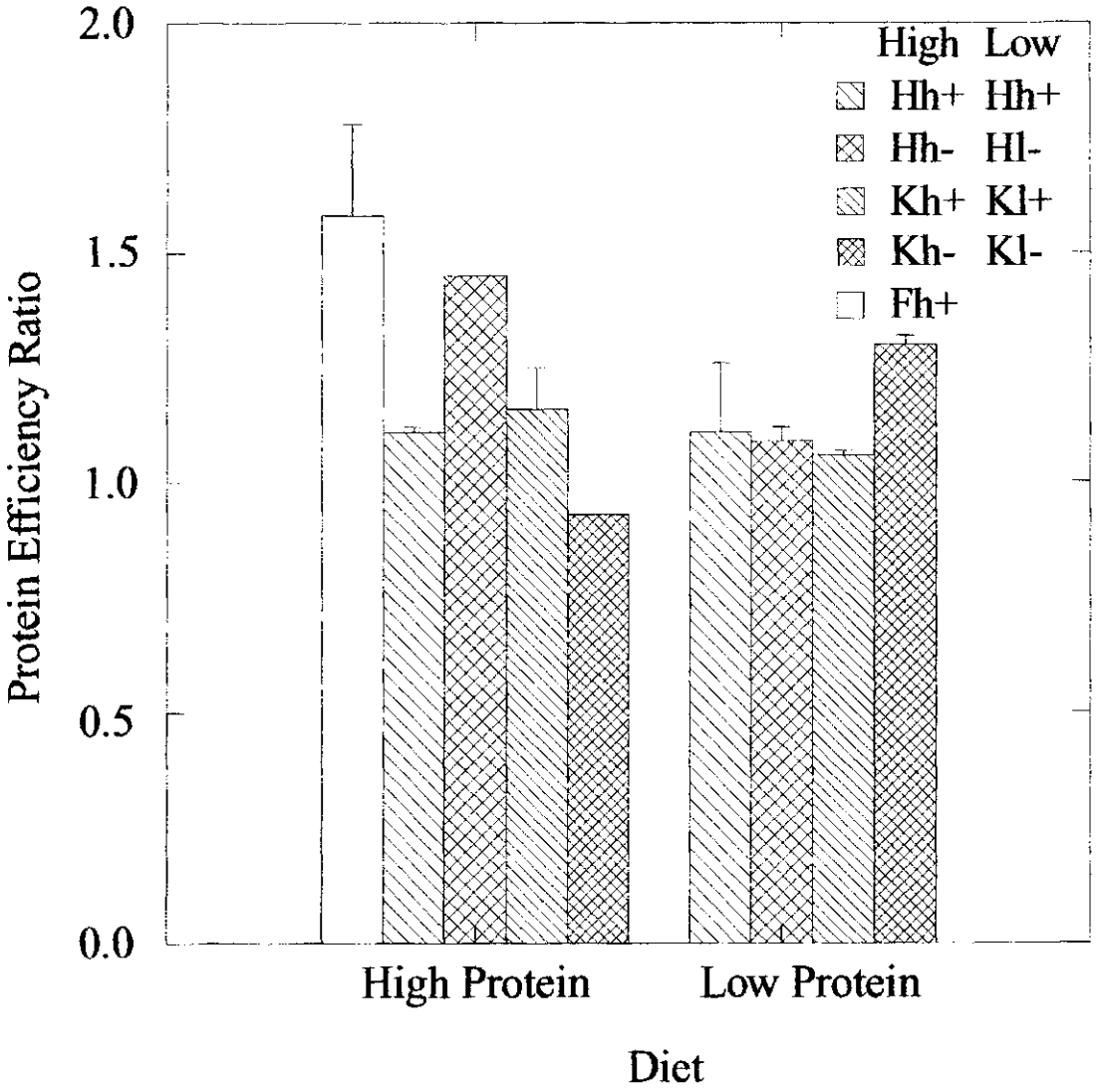
[1] Protein efficiency ratio = body weight gain (g) / protein intake (g).

[2] Diet codes:

H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[3] Replicate lost to disease.

Figure 7: Protein Efficiency Ratios of Chinook Salmon in Experiment 2. Fish were Fed One of Nine Diets with Either Hake Offal, Herring Offal, or Hake Fillets as the Sole Protein Source for 84 Days.



Mean ± SEM (standard error of the mean), N=2, N=1 for Hh- and Kh-

Diet codes: H = B.C. herring press cake meal; K = Hake offal press cake meal; F = Minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non- deboned protein source; and "-" = deboned protein source.

Table 30 - Mean body protein weights, productive protein value, instantaneous protein gain, and instantaneous lipid gain for dietary groups over the 84-day duration of Experiment 2.

Diet[1]	Body protein level day 0	Body protein level day 84	Productive protein value[2]	Instant. protein gain[3]	Instant lipid gain[4]
	g	g			
Hh+	3.80 ±0.06	6.91 ±0.04	24.7 ±0.4	0.711 ±0.027	0.462 ±0.027
Hh-[5]	3.94	7.71	28.3	0.800	0.588
Hl+	3.81 ±0.02	6.14 ±0.15	32.9 ±2.1	0.567 ±0.033	0.450 ±0.033
Hl-	3.46 ±0.03	5.15 ±0.08	29.0 ±0.3	0.474 ±0.007	0.453 ±0.007
Kh+	3.57 ±0.14	6.20 ±0.14	19.0 ±1.1	0.730 ±0.073	0.522 ±0.073
Kh-[5]	3.58	6.10	20.7	0.635	0.374
Kl+	3.65 ±0.18	5.94 ±0.33	25.7 ±0.5	0.579 ±0.008	0.572 ±0.008
Kl-	3.55 ±0.21	5.81 ±0.28	31.4 ±0.0	0.586 ±0.013	0.709 ±0.013
Fh+	3.48 ±0.11	6.84 ±0.70	30.8 ±2.0	0.796 ±0.103	0.654 ±0.103

Mean ± SEM (standard error of the mean)

[1] Diet codes:
H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h= high protein diet; l = low protein diet; "+" = non-deboned protein source; "-" = deboned protein source.

[2] Productive protein value = protein gain (g) / protein intake x 100.

[3] Instantaneous protein gain = [(lnP2 - lnP1) / T] * 100 where P2 = final weight of body protein (g), P1 = initial weight of body protein (g), and T = time in days.

[4] Instantaneous lipid gain = [(lnL2 - lnL1) / T] * 100 where L2 = final weight of body lipid (g), L1 = initial weight of body lipid (g), and T = time in days.

[5] Replicate lost to disease.

Table 31 - Proximate compositions of whole fish[1] in Experiment 2.

Diet of Fish[2]	% Dry Matter	% Protein[3]	% Lipid[3]	% Ash[3]	% N-free extract[4]
(Day 0)	17.43 ±1.31	65.38 ±1.19	23.73 ±0.86	11.98 ±1.32	-1.07 ±1.09
Hh+	21.07 ±0.53	69.33 ±0.92	20.43 ±0.26	10.35 ±0.07	-0.11 ±1.12
Hh-	20.82 ±0.40	68.78 ±2.50	20.89 ±0.78	10.66 ±0.75	-0.32 ±4.04
Hl+	22.11 ±0.36	67.29 ±1.82	22.13 ±0.59	9.99 ±0.09	0.79 ±2.30
Hl-	21.63 ±1.18	64.85 ±1.57	23.14 ±0.24	10.74 ±0.43	1.28 ±1.39
Kh+	19.43 ±1.42	67.29 ±2.86	21.81 ±3.33	10.59 ±0.08	0.31 ±4.76
Kh-	21.36 ±1.02	67.93 ±1.23	19.81 ±2.33	11.01 ±0.65	1.24 ±4.75
Kl+	21.82 ±1.03	65.67 ±3.43	23.69 ±1.54	10.77 ±0.11	-0.13 ±5.08
Kl-	22.46 ±0.89	63.82 ±4.36	25.68 ±3.10	9.24 ±0.02	1.25 ±7.04
Fh+	21.28 ±0.46	67.07 ±3.49	21.62 ±2.16	10.02 ±0.64	1.28 ±1.71

Mean ± SEM (standard error of the mean)

[1] Individually analyzed 6 whole fish sampled on day 0 and 6 whole fish per diet sampled on day 84. The averages for the day 0 fish were combined since all fish were fed the same diets prior to day 0.

[2] Diet codes:
H = B.C. herring press cake meal; K = Hake offal press cake meal; F = Minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[3] On a dry matter basis

[4] Nitrogen-free extract on a dry matter basis. Calculated by subtracting the percent protein, lipid, and ash for each fish from 100%.

Table 32 - Percent mortality over the 84-day duration of Experiment 2.

Diet[1]	% Mortality (%)
Hh+	22.6 ±4.8
Hh-[3]	17.3
Hl+	30.0 ±4.8
Hl-	31.9 ±4.8
Kh+	17.9 ±4.8
Kh-[3]	26.5
Kl+	31.3 ±4.8
Kl-	19.6 ±4.8
Fh+	26.3 ±4.8

Mean ± SEM (standard error of the mean)

[1] Diet codes:

H = B.C. herring press cake meal; K = hake offal press cake meal; F = minced freeze-dried hake fillets; h = high protein diet; l = low protein diet; "+" = non-deboned protein source; and "-" = deboned protein source.

[3] Replicate lost to disease.

Table 33 - Summary of F-test probabilities from the analysis for the effects of protein concentration, protein source, deboning and diet on the parameters measured in Experiment 2.

Measurement	Dietary Factors[1] A	B	C	A x B	A x C	B x C	A x B x C	E
Weight day 0	.485	.367	.155	.195	.756	.245	.393	.998
Weight day 21	.800	.018	.140	.155	.500	.109	.065	.102
Weight day 42	.857	.003	.113	.195	.521	.052	.029	.030
Weight day 63	.690	000	.070	.113	.370	.011	.015	.006
Weight day 84	.462	.000	.172	.148	.143	.016	.016	.005
Body weight gain	.821	.000	.781	.555	.028	.051	.019	.004
Percent body weight gain	.804	.001	.593	.957	.108	.100	.054	.012
Change in length	.719	.000	.355	.472	.036	.054	.036	.006
Percent change in length	.701	.001	.502	.609	.065	.053	.059	.010
Specific Growth Rate Day 0-84	.816	.001	.579	.952	.103	.091	.049	.011
Condition factor day 0	.450	.856	.736	.818	.923	.304	.781	.997
Change in condition factor	.961	.106	.113	.525	.936	.910	.237	.288
Percent change in condition factor	.942	.108	.108	.894	.961	.532	.239	.209
Feed consumption day 0-84	.249	.002	.371	.529	.488	.381	.057	.037
Feed efficiency day 0-84	.592	.000	.344	.042	.450	.003	.025	.003
Protein efficiency ratio day 0-84	.191	.718	.254	.632	.240	.038	.012	.177
Body protein level day 0	.482	.347	.153	.194	.751	.245	.381	.998
Body protein level day 84	.532	.001	.025	.027	.945	.004	.030	.005
Productive protein value	.059	.000	.001	.296	.032	.044	.009	.001
Instantaneous protein gain	.329	.077	.092	.193	.258	.003	.010	.021
Instantaneous lipid gain	.866	.002	.432	.214	.921	.021	.106	.013
Carcass proximate - dry matter	.534	.150	.623	.805	.418	.646	.761	.672
Carcass proximate - protein	.532	.001	.025	.027	.945	.004	.003	.050
Carcass proximate - lipid	.776	.050	.401	.390	.773	.468	.507	.363
Carcass proximate - ash	.966	.211	.927	.305	.158	.366	.126	.298
Carcass proximate - nitrogen free extract	.680	.711	.908	.914	.713	.926	.826	998
Mortality	.585	.043	.573	.289	.890	.206	.045	.127

[1]Dietary Factor Codes:

A = bone; B = protein concentration; C = protein source; A x B = bone/protein concentration interaction; A x C = bone/protein source interaction; B x C = protein concentration/protein source interaction; A x B x C = bone/protein concentration/protein source interaction; E = diet.

4.4 DISCUSSION

4.4.1 General

Sea water-adapted chinook salmon were noted in this study to utilize hake offal press cake meal as effectively as herring press cake meal. With the high protein diets, deboning the offals appeared to reduce the utilization of hake meal and improve the utilization of the herring meal. Differences in growth between fish fed the diets were likely related to changes in the dietary concentration of arginine caused by the deboning. Irrespective of the source of marine protein, reducing the dietary protein concentration reduced growth, decreased feed intake and increased the percent of carcass lipid of the chinook salmon. Similar results were found by Silver et al. (1992).

With one exception, when the F test in the ANOVA indicated significant interactions, Bonferroni Pairwise Procedures were unable to distinguish which dietary factors(s) caused the effect(s).

4.4.2 Amino Acid Profiles

The amino acids which differed in concentration between the hake fillets and the non-deboned hake offal press cake meal (with the exception of histidine) mimicked the patterns reported by Shahidi et al. (1991) for cod discards and fillets. The actual concentrations of amino acids, however, differed between the species. The deboning of hake offal increased the similarity between the hake meal and hake fillet amino acid profiles with the exception of phenylalanine, glutamic acid, and lysine. Protein lost to the deboning process was likely high in collagen as indicated by the declines in the percent proline and glycine in the hake meals (Morrison and Boyd, 1979). Grinding may have altered the amino acid profile of the hake fillets. Whenever the fillets were ground, a portion of the product composed of long stringy filaments (likely collagen) was left behind.

All hake offal based diets, the hake fillet based diet, and the high protein diet derived from non-deboned herring offal were deficient in arginine on a g/kg diet basis.

Dietary concentrations of methionine, cysteine and tryptophan were not determined. It is unknown whether differences in the amounts of these amino acids between diets influenced fish performance. It should be noted that the concentrations of dietary amino acids provided in Table 19 may not reflect their availability to the fish.

4.4.3 Measures of Growth - Weight, Percent Body Weight Gain, Body Weight Gain, Length, Percent Length Gain, Condition Factor, Percent Change in Condition Factor and Specific Growth Rate

Although significant effects of experimental factors could not be statistically analyzed on an individual basis for all measures of growth (because of interactions), fish fed the high protein diets consistently outperformed those fed the low protein diets. However, the effects of protein source and deboning on weight gains and specific growth rates of the fish were not consistent.

With the high protein diets, deboning potentially improved the performance of fish fed the herring meals and reduced the performance of those fed the hake meals. The diets containing the non-deboned hake and herring meals supported similar growth performances in the fish. This contrasts with the low protein diets where deboning had little if any impact on fish performance. Instead, fish fed the hake meal diets tended to outperform the fish on the herring meal diets.

The observed interaction between deboning and protein source for with fish fed the high protein diets may have been caused by deboning-induced changes in the dietary arginine concentrations. This possibility is supported by two observations. First, changes in the dietary arginine concentrations were mirrored by changes in fish performance. Secondly, arginine was the only amino acid in the diets in which concentrations were less than the estimated requirement for chinook salmon.

The high protein diets containing non-deboned herring and hake meal were both equally deficient in arginine (28.0 and 27.6 mg/kg diet, respectively) and supported the same growth performance in the fish. After deboning, however, the herring meal was no longer deficient in arginine (29.8 g/kg diet) and supported increased levels of growth in

the fish. Alternately, the deboned hake meal was even more deficient in arginine (25.4 g/kg diet) and this was reflected in the growth performance of the fish fed this diet.

Assuming that both protein sources were readily and equally digestible, the concentration of protein in the high protein diets (48%) would have been in excess of the amount needed to meet the needs for essential amino acids (Kim et al, 1991). Under these conditions, arginine would appear to be the limiting factor for growth and the cause of the interaction between deboning, protein source, and changes in the growth of the fish.

With the low protein diets, the fish fed the hake diets tended to outperform those fed the herring diets despite the apparent arginine deficiency in the hake meal diets. This would suggest that one or more factors other than arginine were limiting the growth of the herring diet fed fish. It is conceivable that the herring meals were less digestible than the hake meals in this circumstance. When protein concentrations in diets are equal but sub-optimal for growth, both the digestibility of the protein (total protein available to the fish) as well as the concentrations of essential amino acids can be the limiting factors for growth (Kim et al, 1991; Steffens, 1981). It could be speculated that except for arginine concentrations, the protein quality of the hake offal press cake meals were superior to the herring offal press cake meals (See section 4.4.7).

In the experiment, it was unlikely that minerals were the cause of the interaction between protein source, protein concentration, deboning and growth. Although the availabilities of potassium, magnesium, manganese, and zinc (especially in the presence of tricalcium phosphate) in white fish meal are relatively low for fresh water-adapted salmonids, fortification of the diets with these minerals should have avoided any deficiencies (assuming other dietary requirements are supplied by the diet) (Shearer, 1988; Satoh et al, 1987b, 1991). Lall and Bishop (1977) reported that Atlantic salmon raised in sea water required supplemental dietary zinc, copper, cobalt, iron, manganese,

iodine, and fluorine for optimal performance, but sodium, magnesium, and potassium supplementation was not required.

The relative importance of the calcium to phosphorus ratio in the diets of fish is unclear. Some studies have found that dietary calcium has no effect on phosphorus requirements of fish (Andrews et al., 1973; Lovell, 1978; Reinitz et al., 1978) while others report that optimal calcium to phosphorus ratios exist (Nose and Arai, 1976). If the calcium to phosphorus ratio is critical in determining dietary phosphorus utilization, the calcium to phosphorus ratios used in this study (1.4:1 and 2:1) likely did not result in any phosphorus deficiency.

4.4.4 Measures of Feed Utilization - Feed Intake, Protein Efficiency Ratio, Feed Efficiency, Productive Protein Value, Instantaneous Protein Gain, Instantaneous Lipid Gain

All measures of feed utilization by the fish, except instantaneous lipid gains, were significantly affected by the interaction between protein concentration, protein source, and deboning. Relative performances between the fish fed the different high protein diets were the same as described in section 4.4.3. In fish fed the high protein diets, the differences in feed consumption, protein utilization, and feed utilization were probably caused by the differences in dietary arginine concentrations.

Apparent differences between the performance of fish fed the low protein hake and herring diets were reflected in the relative feed efficiencies. However, the protein efficiency ratios and the productive protein values indicated that there were no differences in fish performance. If fish were utilizing the hake diets more effectively that the herring diets it would be expected that measures of feed and protein utilization would reflect this. Since the effects of the low protein diets on the growth of the fish could not be statistically analyzed (because of dietary factor interactions), it can only be concluded that the hake offal was at least as good a protein source as the herring offal.

4.4.5 Body Composition - Whole Body Proximate Composition and Body Protein Level

Body protein levels in fish fed the high protein diets reflected the trends seen in section 4.4.2. Body protein levels in fish fed the low protein diets, however, did not appear to change in response to deboning or protein source. Silver et al. (1992) observed that chinook salmon in sea water deposited more lipid relative to protein as the ratio of lipid to protein in the diet increased. The same response occurred with the chinook salmon in this trial.

4.4.6 Mortality and Disease

The effect of the *vibriosis* outbreak did not appear to favour any specific dietary treatment or interaction. It was impossible to separate out the effects of disease and mortality due to other causes. Overall, a higher degree of mortality seemed to occur in fish fed the low protein diets. However, the results were probably biased because of the removal of one replicate from each of the high protein deboned hake and herring meal treatments.

4.4.7 Hake vs. Herring

Freshness of marine protein sources can affect the nutritional quality of the final product (Pike et al., 1990). Much of the ground fish caught on the West coast is allowed to deteriorate before it is processed into fish meal. Often, there is a five day interval between capture and landing, but intervals of up to ten days are known to occur. Once the fish are landed and processed, the waste product is viewed as having little value and treated accordingly. In Vancouver, some processors only receive 2 cents per pound for the offal while others on Vancouver Island must pay the fish meal plant to take the offal. Offal can often sit for several days in outdoor bins before going to the fish meal plant (Field, 1990; Sitt, 1991).

The roe herring are often caught and landed within a day. After landing, most of the fish are sexed, and the females are quickly frozen and stored. Males are sent to the

fish meal plants and processed. The females are thawed under different conditions as processors try to improve roe firmness while minimizing roe damage. The different thawing conditions may impact the freshness of the herring. Herring offal is viewed by the processors as having some value and is treated with more care than the ground fish offal (Field, 1990; Sitt, 1991).

Due to the degradation of the hake flesh by the parasite *Kudoa paniformis*, the hake must be landed and processed within two days to have acceptable quality for human consumption (Silver, 1989). The rapid capture and processing means that the hake offal has the potential to match and possibly exceed the freshness of the roe herring going into the fish meal plant. This may be difficult to achieve, however, because of the distance of the plants processing the hake from the fish meal plants and the effects of *K. paniformis* (Field, 1990).

In the present study, the freshness of the hake and the herring offal was probably slightly less than that of offal coming directly off the processing line. Producing the hake and herring meals under the same conditions and with the same fish meal machine probably negated any differences in the nutritive value of the meals caused by processing.

Since the performance of chinook fed the hake and herring diets at a given protein concentration could not be isolated and evaluated statistically (due to the interactions between protein concentration, protein source and deboning), the following comments are based upon the trends seen across the different measures used to evaluate the hake and herring diets.

For fish fed the low protein diets, hake offal and herring offal were equal in their nutritive value. Depending upon how the herring was thawed before being deroed (proprietary information), its relative freshness going into the fish meal machine might have been below that of the hake. Deboning and the resulting dietary ash concentrations had no impact on the utilization of the offals by the fish.

For fish fed the high protein diets, arginine appeared to be the limiting factor for growth. Deboning of the hake offal might have reduced the nutritional quality of the hake meal by reducing the concentration of arginine. Conversely, deboning of the herring offal potentially improved the nutritional value by increasing the concentration of arginine.

So long as the freshness of the hake offal matches that of the herring offal, experimental results indicate that the hake meal is at least as good a protein source as the herring meal. Deboning the hake offal appears to be unnecessary, nutritionally speaking, since it did not improve the nutritional value of the hake meal. The only apparent limitation with the hake offal press cake meals was the suboptimal arginine concentrations relative to the requirements of the chinook salmon.

4.5 CONCLUSIONS - EXPERIMENT 2

Although overall dietary effects, such as the interaction between protein source and deboning, were found to be significant, differences between specific diets could not be determined.

There were consistent differences between the weight, feed consumption, and carcass lipid content of fish fed the high and low protein diets. The latter fish had depressed weights, less feed consumption, and higher concentrations of carcass lipid. The differences were likely caused by the protein-energy ratios and essential amino acid concentrations of the low protein diets as well as the feeding behavior of fish on the low protein diets. (Kim et al, 1991; Silver et al., 1992).

For fish fed the high protein diets, deboning seemed to reduce the weight gain, specific growth rate, protein efficiency ratio, instantaneous protein gain, instantaneous lipid gain, feed efficiency, total body protein, and productive protein value of fish fed the hake diet realtive to fish fed with the deboned herring meal diet. The results probably reflected the changes in dietary arginine concentrations caused by the deboning process.

5.0 OVERALL CONCLUSIONS

Nutritionally speaking, deboning the hake offal appeared to be an unnecessary procedure. The chinook salmon seemed to be able to utilize hake offal press cake meal at least as effectively as the herring offal press cake meal. So long as the freshness of the hake offal is maintained, hake offal press cake meal can be readily utilized in the diets of sea-water adapted chinook salmon.

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