THE DIGESTIBILITY OF BLOOD MEAL PROTEIN BY CHINOOK SALMON (*Oncorhynchus tshawytscha*)

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ABSTRACT

The objective of the present study was to establish whether commercial sources of dried blood meals available in New Zealand offer useful sources of digestible protein for chinook salmon.

Three blood meals were evaluated to assess their suitability as ingredients in salmon feed. *In vitro* digestibility was determined using the pepsin and pronase digestibility assays. *In vivo* digestibility was determined by feeding diets to chinook salmon formulated to contain 20% dried blood meal (batch dried, ring dried or spray dried), and 80% of a basal diet and by correcting for the digestible protein supplied by the basal diet. Chromic oxide was included in the diets as an indigestible marker. The apparent digestibilities of crude protein and amino acids were determined based on samples of faeces collected after 7 days of feeding the respective test diet to chinook salmon. Feces were collected by a manual stripping of the hindgut

Crude protein contents were uniformly high, at 92.0%, 93.1% and 89.6% for the batch, ring and spray dried blood meals respectively. Amino acid contents were also generally high, with the exception of the essential amino acid methionine. All crude protein digestibility determinations ranked the blood meals as: spray dried > ring dried > batch dried. *In vitro* N digestibility values determined by pronase assay were 60.5%, 25.5% and 9.0% for spray, ring and batch dried bloods, respectively. The pronase assay yielded significantly ($P<0.001$) higher digestibility values than did the pepsin assay in the case of ring and spray dried blood meals, corresponding values for crude protein digestibility with the pepsin assay were 27.8%, 18.9% and 8.4% for spray, ring and batch dried bloods, respectively. The *in vivo* apparent faecal digestibility of protein in the dried blood meals determined in the chinook salmon were highest for the spray dried blood meal (68.4%), lower in ring dried blood meal (46.3%), and very low in the batch dried blood meal. The amino acid digestibilities were similarly ranked, with the difference between ring and spray dried blood meal being statistically significant for 9 of 17 assayed amino acids ($P<0.05$).

A secondary aim of the study was an evaluation of the laboratory rat as a model animal for determining protein digestibility in the chinook salmon. Batch dried blood meal was fed to rats in an experimental design closely following that set for the chinook salmon, and the apparent digestibility of nitrogen and amino acids were determined based on sampling of ileal digesta. Ileal crude protein digestibility for this batch dried blood meal in the rat was also very low (3.9%), suggesting that the rat may be a suitable model animal for prediction of protein digestibility in chinook salmon.

Differences in the protein digestibility of the blood meals appeared to be inversely related to the time spent in processing and drying. It appears that spray and ring dried blood meals may be suitable for inclusion in chinook salmon diets in partial replacement of fishmeal proteins.

Keywords: Salmon, rat, protein, amino acid, digestibility, blood meal

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World production

The last twenty years have seen a rapid increase in world aquaculture systems. Farmed salmon, catfish, tilapia, shrimp, shellfish and other species now make a significant contribution to our seafood consumption. The world production of cultivated fish in 1991 was estimated at 16 million tonnes and represented 16% of the total world fish production (NZ Fishing Industry Board, NZFIB, 1994).

Salmon make a key contribution to this total. The 1993 world catch of salmon was estimated at more than 828,000 metric tonnes (Globefish, 1993). Production volumes of farmed salmon are advancing rapidly on the wild catch. Farmed salmon contributed 495,000 tonnes to 1994 salmon production volumes, a sizeable increase on the few hundred tonnes produced in the industry's founding years in the 1970's (Forster, 1995). Leading farmed salmon producing countries are Norway, UK, Japan, Canada and Chile, with Norway contributing more than 50% of the harvested tonnage (Table 1.1). Species farmed are predominantly Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Other salmon species of economic importance include the Pacific salmon species of Chinook (*O. tshawytscha*), Sockeye (*O. nerka*), Chum (*O. keta)*, Pink (*O. gorbuscha*) and Coho (*O. kisutch*). Atlantic salmon dominate over Pacific salmon in world production due to the latter's lower survival in seawater, disease susceptibility and early maturity (Munro, 1990).

The present study is concerned with salmon culture in New Zealand. New Zealand is a minor player in world salmon aquaculture, farming predominantly chinook salmon. Estimates from the NZ Salmon Farmers Association (NZSFA) place 1994 production figures for chinook salmon at 4,550 tonnes, which represents less than 1% of the world's total production of farmed salmon. Many companies rely strongly on the world market for sales revenue and volume. New Zealand farmed salmon is a significant export (Table 1.2). In 1993, 2,591 tonnes of chinook salmon were exported at a FOB (free on board) value of $26 million dollars (NZFIB, 1996). In the past most of New Zealand's salmon production was exported to the United States, but in recent years the balance has shifted from 75% of exports sold to the United States in 1986 to less than 1% in 1991 (Wildman, 1992), with Japan now the predominant market.

Table 1.1: World Production of Farmed Salmon 1988-1993

(Source Globefish (1993); NZ data estimates from New Zealand Salmon Farmers Association, NZSFA; New Zealand Fishing Industry Board, NZFIB).

World Production Farmed Salmon (1000 tonnes)						
	1988	**1989**	**1990**	**1991**	**1992**	**1993**
Atlantic salmon						
Norway	80.0	117.0	150.0	160.0	141.0	180.0
UK	18.0	29.0	33.0	40.6	38.8	43.0
Ireland	4.7	6.2	10.0	8.0	10.5	12.0
Chile	0.6	1.7	4.0	17.7	24.0	16.0
Faroe Is	4.0	3.0	4.0	20.0	18.0	14.0
Ireland	1.3	1.5	3.0	5.0	6.0	10.0
Canada	1.6	1.6	2.0	2.5	10.8	17.0
Australia	1.0	2.0	4.0	2.5	2.6	2.6
Sub-total	112.5	162.5	210.5	264.6	257.9	297.6
Chinook Salmon						
Japan	14.0	18.0	21.0	24.0	24.0	25.0
Canada	6.0	12.4	13.0	16.1	12.0	8.0
Chile	4.0	7.0	9.0	19.1	23.0	25.0
USA	2.7	1.9	2.5	5.0	7.0	7.5
New Zealand	1.1	1.1	1.2	2.0	3.4	3.8
Total	**139.0**	**202.8**	**257.2**	**331.8**	**327.3**	**366.9**

Table 1.2: **New Zealand Chinook Salmon Exports, 1993.**

(Source New Zealand Fishing Industry Board, NZFIB)

Product	**Kilos**
Frozen whole	997,373
Chilled whole	695,876
Chilled headed and gutted	26,144
Frozen headed and gutted	632,248
Chilled other	59,429
Frozen other	18,154
Smoked, brined, salted, dried	154,056
Prepared, preserved (cans, jars)	8,530
Total	**2,591,810**

The two largest salmon operations in New Zealand currently produce approximately 4,000 tonnes of chinook salmon per annum (1995-1996 figures) using sea cages located in the Marlborough Sounds, Pelorus Sounds, and Stewart Island. A number of small to medium sized sea and fresh water salmon farming ventures also operate. Their production volumes range between 100 - 600 tonnes/annum.

The salmon stocks on which aquaculture operations are now based are the result of introductions around 1901 from a hatchery on the Sacremento River in North America (McDowall, 1994). After a number of attempts, populations of chinook and sockeye were successfully established in rivers along the east coast of the South Island (Wildman, 1992), with chinook stocks proving the most amenable to aquaculture. Historically in New Zealand salmon were cultured for wild stock enhancement, but by 1975 small scale fresh water pond operations rearing stock for harvest were well established. In 1982 the first salmon sea-pen was established on Stewart Island by The New Zealand Salmon Company. Small populations of sockeye salmon are today also cultured, and experimental populations of Atlantic salmon introduced in the early 1900's are held by the government agency, NIWA (National Institute of Water and Atmosphere), although Atlantic salmon never established successful river runs. Research has been undertaken on Atlantic salmon sea-pen culture, but this has achieved limited success, an outcome attributed to the use of source stock of a poor genetic base (Wildman, 1992). The commercial culture of trout for harvest in New Zealand is not permitted. Trout hatcheries exist and operate under the control of Fish and Game Councils for the express purposes of research, restocking programmes for catchments and to enhance sports fisheries.

Farm Production systems

Both Atlantic and Pacific salmon are anadromous, that is, they exhibit two distinct life stages, one spent in fresh water (egg and juvenile) and the other under sea conditions (adult). In the wild, salmon hatch and grow for around one year in the headwaters of rivers, before they migrate to sea. They remain feeding and growing in coastal waters or oceans until the onset of sexual maturity and the urge to spawn returns them to their parent rivers.

In cultured salmon, eggs and milt are collected from parent broodstock in autumn, and fertilised eggs are incubated in fresh water hatcheries. Hatched fry are cultured in hatchery raceways, ponds or tanks fed by gravity or by pumped water until they are ready to go to sea as smolts at 6 to 18 months. Salmon and other anadromous fish undergo a special metamorphosis known as smoltification which enables them to adapt without undue stress to the change from fresh to saltwater during their natural migration. Smoltification takes place in juvenile salmon in the

spring as water temperatures rise and day length increases, and comprises physiological, morphological and behavioural changes which influence farming practices. The most significant of these is the deposition of guanin in the skin which appears to act as a barrier to osmotic exchange and prevents the loss of water through the skin. Specialised basal cells in the gills proliferate and increase the fish's ability to excrete salt. The salmon becomes visually more like an adult, with adult parr markings on the skin. Feeding and schooling behaviour changes.

Freshwater production systems can carry fish through to harvest weights, or to large 2-year smolts for transfer to sea cages. However, in most NZ salmon farming operations, smolt are transferred to seawater after smoltification at 20 - 100g (Chinook salmon) or 80-200g (Atlantic salmon farmed overseas) and are grown in net cages. These cages have been increasing in size in recent years. A common size range today is 750 - 2000 m^3. The construction materials of the cage collars from which the net bag holding the fish is suspended is quite varied, ranging from wood and polystyrene, all-plastic, steel and plastic, to compliant all-rubber structures. Groups of six or more cages may be joined in flotillas, and aligned and constructed with walkways above the cages. Ancillary facilities include feed stores, long stay crew cabins, landing points and feed delivery and control systems. A variety of accessory equipment to assist in cage management has been introduced, including various forms of automatic feeders, fish pumps, underwater cameras to monitor fish behaviour, automatic graders and automatic net washers.

Seawater cage culture is exposed to many management difficulties and environmental hazards. Changing nets fouled with growth generally remains largely a manual process, and checking and repair of nets requires regular diving. The ability to assess the numbers of fish in cages other than by direct handling which causes stress is a serious management limitation. Depending on the site and geographical location, environmental hazards may include some of the following: algal blooms, storm damage, extremes of temperature, predation by birds and mammals, pollution, sulphide poisoning by accumulation of waste food and faeces below cages, collision with boats, stress associated with handling, and disease. Many diseases have and continue to be recorded with varying severity in a number of countries. The most significant of these are vibrio (significant in Norway), furunculosis (Scotland & Norway), sea lice, bacterial kidney disease (BKD) (widespread). Control of these diseases is by a combination of prophylactic management measures (reduced stocking densities, restricted transfer of stock etc), and therapeutic agents (antibiotics and vaccines). New Zealand has a unique advantage in salmon culture in that these diseases have either not been encountered or are infrequently recorded. New Zealand's largely disease free status has been maintained by the Animals Act (Section 13) which prohibits the importation of animals (and fish) without authorisation of the Minister of Agriculture and

Fisheries. In practice the stringent requirements required to obtain authorisation have prevented importation of salmonids or ova since the original importations.

The growth rate of the salmon depends on many factors, some of which are, stock origin, smolt size and vigour, stocking densities, water temperatures, feed quality and general farm management. Quinn & Unwin (1993) concluded that the NZ stocks of chinook grow more rapidly than their North American counterparts, up to an age of 3-4 years, but mature earlier, sometimes at 2 years; earlier maturation may be a result of this more rapid early growth. Most salmon production aims at achieving the fastest growth, as heavier fish (over 2-4kg) are more in demand and achieve higher prices/kg. Salmon can be ready for harvest at the 2-3kg range in less than 12 months at sea, often they are held longer for up to 21 months (Forster, 1995). To avoid early sexual maturation of salmon prior to achievement of market weight, hatcheries can produce all-female stock by testosterone treatment of female broodstock and/or subjecting eggs to an environmental shock (eg. heat, cold or chemical). Sex-reversed females produce X-only milt, which crossed with ova will produce female fry (Munro, 1990).

Harvesting is usually accomplished by pumping or brailling fish into a live holding tank on a boat and transporting them live to a processing plant (Foster, 1995). If this is not possible fish are killed at the farm and then transported in ice to the plant.

Aquaculture feeds

The salmon aquaculture industry is not simply a composite of farmers, processors and marketers. There is a vast network of organisations and companies that provide services and products to the industry. As of 1994, there were over 2,000 providers to the aquaculture industry (Anon, 1995), offering products including feeds, vaccines, fish diagnostics, medication and equipment (pumps, nets, feeders). Aquaculture feeds are a rapidly expanding part of these service industries. From a world production of 3.6 million tonnes in 1988, aquaculture feed manufacture is expected to increase to as much as 14 million tonnes by the year 2000 (Meyers, 1994). In 1993 11,919 tonnes of aquaculture feeds were manufactured in New Zealand (NZ Feed Manufacturers Association, NZFMA), primarily for the salmon industry.

Feed is the largest expense on a salmon farm, making up anywhere between 35 - 50 percent of total costs (Forster, 1995), depending on location and farm efficiency. Salmon feeds contain a variety of ingredients, usually fish meals and oils, other animal and vegetable meals, binders, vitamins and minerals. Pigments are fed to colour flesh to saleable levels. Feed processing generally involves extrusion that yields pellets which have an especially high resistance to

fragmentation during handling and the capacity to hold high levels of oil. Feed is supplied to the caged fish, either by hand or by using automatic feeders.

Feed prices seem to be similar internationally because many of the raw ingredients in salmon feed, principally fish meal and vitamins, are globally traded commodities. Chile has the lowest feed costs because fish meal is locally produced, with low transport and labour costs. New Zealand has much higher feed costs as extrusion processing is not yet available in New Zealand for salmon feeds, and extruded feeds are currently imported.

Since feed now represents such a significant proportion of farm costs, even small increases in efficiency can yield a significant cost reduction. These cost reductions are critical as salmon producers strive to reduce costs and maintain margins against a background of falling international prices. There are two basic ways in which gains can be made. Either the cost of feed itself can be reduced, or it can be used more efficiently (eg., to achieve lower feed conversion ratios (FCRs)). Options to reduce feed costs per kg of salmon produced are as follows:

(1) To lower the cost of feed by the use of lower cost ingredients, or by reducing manufacturing costs and margins

(2) To improve feed utilisation by improving formulations to give a better balance of nutrients and higher feed digestibility (ie use of high oil diets)

(3) To reduce feed wastage when feeding

(4) To operate with genetically improved strains of salmon with improved growth rate and/or food conversion efficiency.

Aims and objectives

Salmon culture in New Zealand, as in other countries, faces a number of challenges. International fresh salmon prices have fallen in recent years. This has been associated with over-production in both Norway and Scotland, and rising competition from Canada and Chile (Globefish, 1993).

The large contribution made by salmon feed to total production costs, suggests that reduced feed cost and improvements in feed utilisation by salmon can have a significant impact on the overall profitability of salmon farming. Judicious selection of feed ingredients is an important factor in dietary utilisation and diet cost. However, there is uncertainty as to what feedstuffs and characteristics of feedstuffs are desirable in fish feeds and little information is available on ingredient nutrient digestibility, energy availability and the presence or absence of growth and antinutritional factors. Still less is known about the economics or cost effectiveness of different diets on performance.

The success of aquaculture in the future will require reduced dependence on fishmeal as the primary ingredient, and increasing use of other plant and animal proteins. Fish meal is a well balanced and highly digestible protein, however its future use in fish feed is tenuous. Overfishing of important fishmeal species and increasing demand for fishmeal is likely to affect future aquaculture feed prices and production economics (Rumsey, 1994). In New Zealand our agricultural base provides a significant source of animal meals such as meat meal, meat and bone meal, and blood meal. The competitive price of these in relation to fishmeal offers a potential alternative in diet formulation, but little is known of their nutritional value for fish. In pigs and chickens, measurements of nutrient digestibility have played a major role in forecasting the feeding value of individual ingredients and compound feeds. These measurement techniques have only recently been modified for application to aquatic species, and nutritional information indicating the value of ingredients for fish species is sparse and where it exists it is seldom applicable to local ingredients. Such data are essential to achieving production and efficiency improvements required in the highly competitive climate of salmon culture and marketing today.

Blood meal is an important commercial source of animal protein in New Zealand and internationally. The aim of this work was to establish whether commercial sources of blood meals available in New Zealand offer useful, digestible sources of protein to chinook salmon. This involved taking three blood meals produced by three different manufacturing processes and evaluating them for quality using as the primary criteria *in vitro* and *in vivo* amino acid and protein digestibility assays. The *in vivo* studies involved both the rat as a model animal and chinook salmon as the target species.

CHAPTER 1

LITERATURE REVIEW:

PROTEIN DIGESTION IN SALMONIDS AND THE USE OF BLOOD MEAL AS A FEED INGREDIENT

1.1 Introduction

It is the aim of this review to summarise available information on protein digestion in farmed salmonids, and to describe and examine practical methods for the assessment of dietary protein digestibility. Blood meal, its significance to the New Zealand aquaculture industry, and its importance as a component in commercial salmonid formulations is then discussed. Salmon species covered by this review are given in Table 1.3.

1.2 Salmonid digestive system

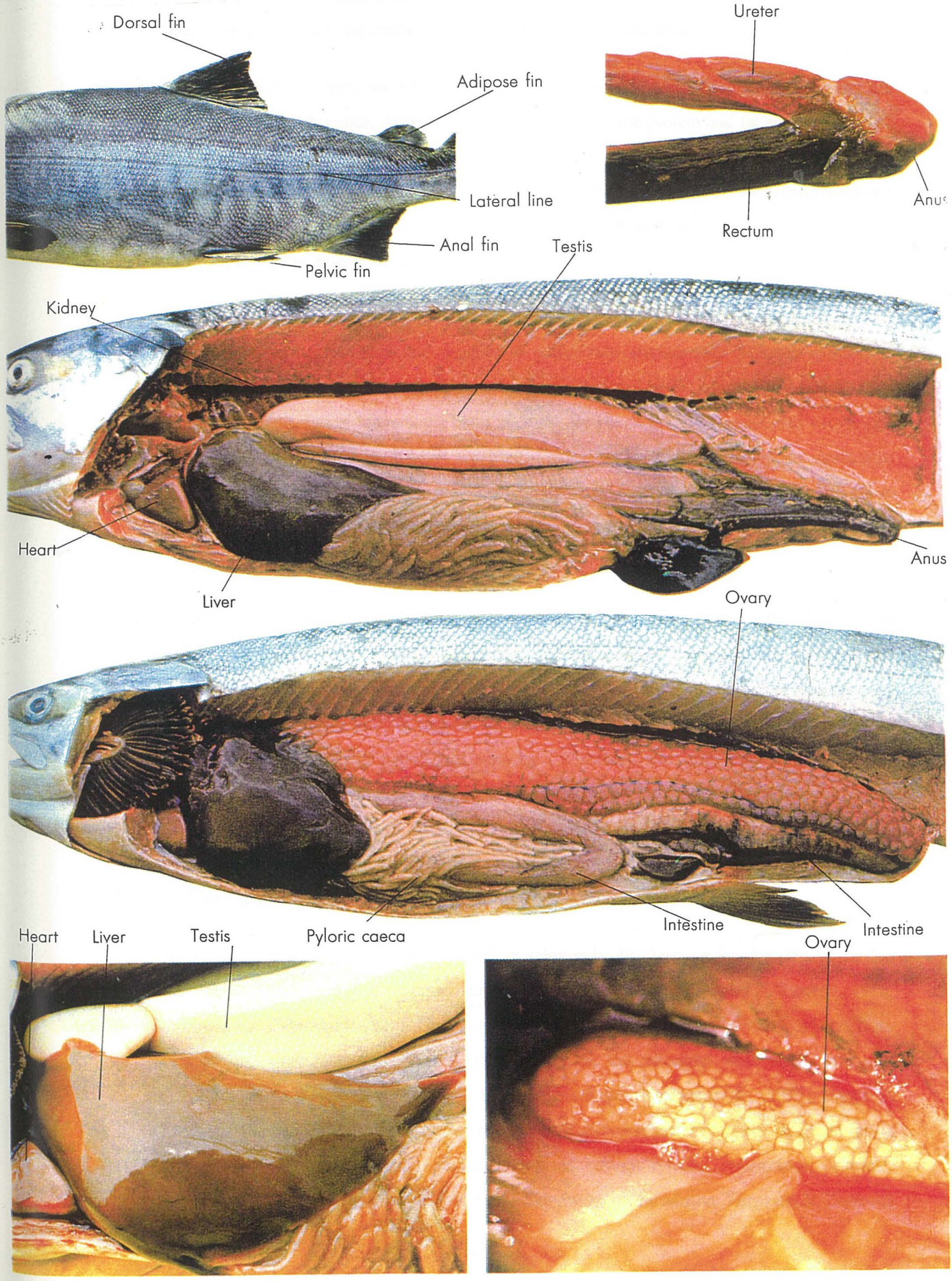
Digestion in teleost fishes, which encompasses salmonids, has been comprehensively reviewed by several authors (Barrington, 1957; Kapoor et al., 1975; Halver, 1989; Wilson, 1989). Halver (1989) states that "Fish maintain an intimate relationship between form and function of their gut and the food resource", and the general structure and physiology of the digestive system of the carnivorous salmonids is no exception. A short, wide oesophagus moves food to the stomach for digestion under acid conditions, followed by absorption in the mildly alkaline or neutral midgut region (including pyloric caeca, pancreas, gall bladder), and finally the hindgut prior to excretion of wastes (Figure 1).

In rainbow trout the pH of the stomach contents has been found to be 3.5-4.5 rising to pH 7.0 and above in the intestine starting from the pyloric caeca (Ash, 1980). The stomach is the most important site of digestion, and the presence of pepsin and HCL is well documented (Halver, 1989). The rate of proteolytic activity of specific trout pepsin is rapid, and at equivalent temperatures (between 0-37°C), greater than that of either its porcine or canine counterparts (Twining et al., 1983). However, comparison of proteolytic rates for rainbow trout pepsin at 15°C with dog and pig pepsins at 37°C, shows that at the respective temperatures at which these processes occur *in vivo*, all have similar activities. Additional gastric enzymes besides pepsin have been reported in salmonids. Lindsay and Harris (1980) looked at 42 species of fish and reported a high rate of incidence of cellulase, but no evidence of cellulase in rainbow trout. Prejs & Blaszczyk (1977) reported cellulase activity in the digestive tract of one species of salmonidae (*Coregonus albula*). Both authors conclude that the cellulotic activity is mainly exogenous rather

Table 1.3: Salmon Species Included in the Literature Review.

Common Name	Species
***Salmo* genus**	
Cutthroat trout	*S.clarkii*
Brown trout	*S.trutta*
Atlantic salmon	*S.salar*
***Oncorhynchus* genus**	
Rainbow trout	*O.mykiss* (prev *S.gairdneri*)
Chinook salmon	*O.tshawytscha*
Chum salmon	*O.keta*
Coho salmon	*O.kisutch*
Sockeye salmon	*O.nerka*
Pink salmon	*O.gorbuscha*
Masu salmon	*O.masu*
***Salvelinus* genus**	
Arctic char	*S.alpinus*

Figure 1: The Salmonid Digestive System



than endogenous in origin. Lindsay et al., (1984) demonstrated the presence of endogenous chitinase in the stomach and chitobiase in the intestine of rainbow trout.

Some absorption has been shown to occur in the stomach. In one of the first studies on the absorptive sites in salmonids, Austreng (1978), found that some protein and fat absorption occur in the stomach in rainbow trout, but that the intestine is the primary site, a finding confirmed by Dabrowski & Dabrowska (1981). As salmonids are carnivorous fish, microorganisms do not play an important role in the gastric digestion of food, although their presence in the gut is confirmed in the literature (Halver, 1989).

The pyloric caeca are evaginations of the anterior intestine, found in some, but not all, species of fish including salmonids. Size and number of caeca vary considerably between species. In rainbow trout their average length is 50.5mm (Ulla & Gjedrem, 1985) and they are longer in the pyloric region and shorter towards the large intestine. Bergot et al. (1981) examined two strains of rainbow trout with slightly different numbers of pyloric caeca and showed that the strain with the greater number of pyloric caeca grew faster on the same amount of feed than fish with fewer caeca and had increased food conversion ratios. Also, they found older fish had more caeca than younger fish.

It is unclear what physiological function the pyloric caeca play in fish, although their histological form, (being similar in cell structure to the small intestine), suggests a role in the digestion and absorption of nutrients. Austreng (1978) showed that a considerable part of protein and fat digestion occurred in the anterior part of the small intestine of rainbow trout, including caeca. Bauermeister et al., (1979) reported that caeca have a primary role in lipid absorption, and this was confirmed by Ezeasor & Stokoe (1981). Ulla & Gjedrem (1985) found no correlation between the number and length of caeca in rainbow trout, and fat and protein digestibility. A significant correlation was found between fat and protein digestibility and intestine length, suggesting minor involvement of the pyloric caeca.

The lining of the midgut is deeply folded in salmonids. Its function is as a site of further digestion, as well as being the primary site for amino acid and fat absorption (Austreng, 1978; Dabrowska & Dabrowski, 1981). The intestine and pyloric caeca source digestive enzymes from multiple sites, including the pancreas, the gallbladder, the intestinal wall, food- and gut-microflora. In their reviews on digestive function in teleosts, Kapoor et al., (1975), Fange & Grove, (1979) and Ash (1985) identified a number of enzymes that have been isolated from a wide variety of fishes. These included trypsin, chymotrypsin, collegenase, elastase, carboxypeptidase, carboxylesterase, lipases and carbohydrases. In salmon, Torrissen & Torrissen

(1984, 1985) identified tryptic enzymes in rainbow trout and Atlantic salmon. Other digestive enzymes are in all probability not dissimilar to other fishes of similar feeding habit. In general, workers reviewing the literature (Ash, 1985) have concluded that the properties of these proteolytic enzymes, and the transport systems involved in the absorption of the products of digestion, are qualitatively similar to those of higher vertebrates.

Austreng (1978) found that 60% of the digestible protein is absorbed in the first quarter of the gut of rainbow trout (in the area of the pyloric caeca); in the second quarter the resorption rate goes up to 85%. It has been well documented that there are pronounced specificities of *in vitro* and *in vivo* amino acid absorption depending on the posterior or anterior part of the intestine (Boge et al., 1979; Ingham & Arme, 1977; Hokazono et al., 1979; Dabrowski & Dabrowska, 1981), and it is generally accepted that the anterior portion of intestine absorbs L-forms of amino acids more effectively than the posterior one. In addition, reviews by Ash & McLean (1989) and McLean & Donaldson (1990) convincingly demonstrate that absorptive epithelial cells located in the gut of many, and possibly all fish, possess the ability to absorb protein in an intact form. In rainbow trout it is believed that rectal cells are involved in this process (Ezeasor & Stokoe, 1981). Carbohydrate absorption is low in the stomach and anterior part of the intestine, but increases thereafter (Austreng, 1978).

The hindgut is an extension of the midgut with gradually diminishing digestive/absorptive functions, near neutral pH, and with increased levels of mucus production. In rainbow trout, the lining of the hindgut is deeply folded (Halver, 1989) which probably increases food retention and therefore the period of absorption (Ezeasor & Stokoe, 1981). The sides of the folds have vacuolated cells suggestive of protein or other macromolecule uptake by pinocytosis (Ezeasor & Stokoe, 1981). The hindgut has been implicated in amino acid absorption; the extent of which has not yet been quantified. Dabrowski & Dabrowska (1981) found elevated levels of free amino acids in rainbow trout rectum, and Austreng (1978) confirms that absorption of protein occurs far back in the rectum. This conclusion has not received universal agreement. Van Den Ingh et al., (1991) induced pronounced morphological changes in the distal intestine by feeding soya-bean meal to Atlantic salmon, yet found no effect on growth performance. This observation led to his conclusion that the distal intestine normally has almost no quantitative function in protein absorption.

Bacterial activity has been detected in the digestive tract of salmonids, in densities higher than in the surrounding waters, although this has not been confirmed as of endemic origin (Achene et al., 1989). Typical numbers of bacteria in fish intestines are 10^8 aerobic heterotrophic bacteria g^{-1} and approximately 10^5 anaerobic bacteria g^{-1} (Ringo et al., 1995). Lesel et al., (1989) report that in

rainbow trout at normal rearing temperature, the number of bacteria is limited to 10^5 to 10^7 germs.g^{-1} wet weight of digestive compartment. These levels are substantially lower than those reported for endothermic animals such as humans. While the digestive tract of endotherms is colonised mainly by obligate anaerobes, the predominant bacterial genera/species isolated from most fish guts have been anaerobes or facultative anaerobes. Trust & Sparrow (1974) and Trust et al. (1979) isolated the aerobic bacteria *Clostridium, Bacteroides*, from the gut of rainbow trout. Other species identified in salmonids include *Lactobacillus, Fusobacterium, Aeromonas, Enterobacter* and *Pseudomonas*. Some investigations have suggested that microorganisms have a beneficial effect on the digestive processes of fish (eg. microbial breakdown of cellulose; Stickney & Shumway (1974), Trust et al., (1979) and lipids (Ringo et al., 1995)), and act as protection against pathogens (Ringo et al., 1995). Direct evidence for a nutritional role for these bacteria is not found in most reviews on this topic, and their role in digestion is generally assumed to be negligible.

The period of gastric digestion has been examined, with reported times varying from 36 hours at 15°C for full gastric evacuation in rainbow trout (Windell & Norris, 1969), to a food passage time of 15-16 hr at 18°C and 24-26 hr at 9-10°C in 80g rainbow trout (Fauconneau et al., 1983). Variability in results can be partly attributed to the effect of temperature on the rate of gastric emptying, with an increased rate of digestion with increasing temperature (Brett & Higgs, 1970). For gastric emptying time in sockeye salmon, not including time to pass through the intestine, a satiation meal of pelleted food decreased from 147 hr at 3°C to 18 hr at 23°C. The effect of temperature is believed to be related directly to enzyme activity at various temperatures (Halver, 1989). This is reflected in seasonal changes in proteolytic activity in the gut content of some fish. In the roach, amylase activity varied with environmental temperature while protease activity increased after the spring thaw and then decreased again immediately afterwards (Hofer, 1979).

Studies on the rate of digestion and gastric evacuation time not only reveal some of the underlying relationships controlling growth but also provide rationale for fish feeding practices. For example, Brett & Higgs (1970) found that for sockeye at 5°C, the stomach is only quarter empty 1 day after satiation, and concluded that below this temperature, feeding once a week should be entirely adequate. Multiple daily feedings were recommended once the temperature exceeds 15°C.

Digestive capacity changes during early development. The digestive tract of salmonids develops as a simple tube, first adjacent to the yolk material and then enveloping it. By the time of first feeding, the salmonid digestive tract is already well differentiated into a stomach, pyloric caeca and a short intestine (Lauff & Hofer, 1984). Rainbow trout develop proteolytic enzyme activities

soon after hatching, both pepsin- and trypsin-like, in stomach and intestines respectively (Pfeffer, 1982). There is evidence that proteolytic activity (trypsin and chymotrypsin) increases with age (Lauff & Hofer, 1984). In juvenile fish, weighing less than 0.5g, the rate of this development depends on the protein content of the diet, the increase being considerably greater in tryptic than in peptic activities (Kawai & Ikeda, 1973). Nutrient digestibility continues to increase with body weight. The capacity of young 18g rainbow trout to digest both protein and carbohydrate is significantly lower than that of larger trout (Windell et al., 1978b).

Maturation of anadromous salmonids also involves a significant change in their metabolism. The gonads increase in size over a relatively short period of months from less than 1% of body weight to about 20-25%, mainly during a period in which the fish do not feed. Torrissen & Torrissen (1985) observed the total activities of digestive proteases of maturing Atlantic salmon, and found higher enzyme activities in early maturation and lower activities in the later stages, until maturity. They attributed these variations to changed feed consumption. Torrissen & Torrissen (1984) noted that differences in fish size did not affect protease activity in either mature or immature fish.

1.3 Salmonid digestion and nutrition

The science of fish nutrition is considered to have originated in the 1940's & 1950's when several groups in the USA began to publish reports on the nutrition of salmonid fishes (Jauncey, 1993). The experiments, and diets derived from these, were often adapted from those used in terrestrial animals. Since then the mass of data on fish and specifically salmonid nutrition has increased massively. Numerous textbooks are available on the subject (Halver 1989; Steffens 1989, Lovell 1989) and a number of international symposia have been published (Cowey et al. 1985; Lied 1989; Takeda & Watanabe 1990; Kaushik & Luquet 1993). Review papers are abundant (Lall, 1991; Jauncey, 1993) and hundreds of research papers are published in journals such as "Aquaculture" and "Fish Nutrition".

The nutritional requirements of fish for growth, reproduction and normal physiological functions are similar to those of land animals. There are exceptions; for example many land animals can synthesise vitamin C whereas fish cannot, many fish have a requirement for n-3 fatty acids whereas warm blooded animals require n-6, and fish can absorb minerals from the environment. One of the striking differences in nutrition between fish and farm animals is that fish have lower dietary energy requirements for growth. As a consequence, fish are among the most efficient of animals in converting feed to body tissue. Rainbow trout, when fed high-quality diets, require not more than 1.5kg feed (air-dry weight) per kilogram of gain (wet weight) compared to a broiler

chicken (2.5:1) or swine (4.0:1), (Halver, 1989). The reason for this disparity is that fish have lower energy expenditures for maintenance. Fasting heat production in sub-adult rainbow trout (8.85 $BW^{.82}$ kcal per fish per day) is around 10% of Brody's values of 70 $BW^{.75}$ for mammals and 83 $BW^{.75}$ for birds (Lovell, 1994). Several factors contribute to this:

(1) A physically supportive environmental medium that requires less energy for movement and that obviates land animals' requirement for antigravitational musculature.

(2) Poikilothermy. The body temperature is the same as the surrounding environment. No energy is expended on body temperature regulation and metabolic rate is dependent on water temperature.

(3) Reduced energy cost of conversion of ammonia to urea or uric acid for excretion. Fish excrete 85% of their waste nitrogen as ammonia through the gills.

Energy sources in the diet include fats, proteins and carbohydrates, with both fats and proteins generally being highly available energy sources for fish. Fats contain more energy per unit weight than any other biological compound, and are used efficiently to supply energy. The protein allowances in fish diets are appreciably higher than those in the diets of terrestrial warm-blooded animals (NRC, 1993), and protein is also a highly available energy source for fish. No specific dietary requirement for carbohydrates has been demonstrated in fish, and its value as an energy source is variable among species (Steffens, 1989). If carbohydrates are not provided in the diet other more expensive compounds, such as protein and lipids, are catabolized for energy (NRC, 1993). In practical diet formulations, non-protein sources of energy generally supply most of the energy needs, with protein being used primarily for tissue protein growth.

In terms of nutrient requirements, it is generally assumed that there is little variation among fish species (NRC, 1993). In a practical sense, nutritional requirements determined for salmonid species including Atlantic salmon, chinook salmon and rainbow trout have all been applied in formulating species specific diets, and it is believed (NRC, 1993) that this has served adequately as a basis for efficient, productive and cost-effective diets. As more information becomes available, the requirements of various species and for specific productive functions will be refined.

Protein

Most animals which have been domesticated as farm animals are either omnivorous or herbivorous. By contrast most fish are carnivorous and many which are farmed, including

salmonids, have a high requirement for dietary protein and amino acids (Pike et al., 1990). Mammals and birds typically achieve maximum growth rate on diets containing 12-25% protein, whereas fish require diets with 35-55% protein to reach maximum growth rate, with the requirement for chinook salmon reported to be 40% (DeLong et al., 1958).

Proteins are composed of some 20 amino acids linked into chains by peptide bonds. The requirement for dietary protein has two components

1. A need for indispensable amino acids that the fish cannot synthesise.

2. A sufficient supply of either dispensable amino acids or of sufficient amino nitrogen to enable the fish to synthesise them

Thus the requirement is not for protein *per se*, but for a sufficient and balanced supply of amino acids. As with protein, the amino acid requirement of fish is therefore higher than for other farmed animals (Table 1.4). The amino acid content of feed proteins may vary markedly, and it is important to select a mixture of feed proteins that will primarily meet the amino acid requirements of the fish. Table 1.4 gives the estimated requirements for the 10 amino acids known to be essential in fish.

The optimal dietary protein level for fish, as for other animals, is influenced in part by factors associated with the diet (digestibility, amino acid composition, optimal dietary protein to energy balance). In addition animal factors (eg age, sex, size) and the environment (eg water temperature) have significant influences. Protein requirements decrease as the fish approaches maturity. The optimal dietary protein level for very young salmonids (fry) is 45-50% of the diet, while smolts (20-200g) require 40% and adults require about 35% dietary protein (NRC, 1981). Changes in water temperature have also been shown to affect the protein requirements of fish. Chinook salmon were found to require 40% protein at 8°C and 55% protein at 15°C (DeLong et al., 1958).

Lipid

Lipids have two fundamental roles in salmonid diets; the provision of energy and the supply of essential fatty acids (EFA's). They also assist in the absorption of fat soluble vitamins. Lipids are highly digestible to salmonids with values of 85 - 95% when fed alone or in mixed diets (Austreng, 1978; Cho & Slinger, 1979). A number of reviews have been published on lipid nutrition in fish (Watanabe 1982, Greene & Selivonchick 1987).

Table 1.4: **The Essential Amino Acid Requirements (% air dry diet) of the Chinook Salmon and those of Other Omnivorous Animals.**
(Source Mertz, 1969)

Amino acid	Chinook	Chick	Piglet	Rat
Arginine	2.4	1.1	0.2	0.2
Histidine	0.7	0.3	0.2	0.4
Isoleucine	0.9	0.8	0.6	0.5
Leucine	1.6	1.2	0.6	0.9
Lysine	2.0	1.1	0.6	1.0
Methionine	1.6	0.8	0.6	0.6
Phenylalanine	2.1	1.3	0.5	0.9
Threonine	0.9	0.6	0.4	0.5
Tryptophan	0.2	0.2	0.2	0.2
Valine	1.3	0.8	0.4	0.4

Lipids serve as an important source of dietary energy for salmonids, with a role in sparing the use of more expensive proteins as an energy source (Reinitz et al., 1978; Takeuchi et al., 1978). The upper and lower limits of lipids that can be included in the diet have not yet been fully defined, and recommendations cannot be made without considering the type of lipid as well as the protein and overall energy content of the diet. Other factors may modify inclusion, including digestible energy/crude protein (DE/CP) ratio, processing technology, influence on nutrient intake, carcass fat accumulation and rancidity of oils. Takeuchi et al. (1978) found that protein could be reduced from 48 to 35% in rainbow trout diets without any reduction in weight gain if lipid concentration was increased from 15 - 20%. He estimated the optimum ratio of lipid to protein as 18% : 35%. Fish oils are now used in most salmonid diets as the primary energy source, and maximum inclusion levels are constantly being increased in practical salmon diets. Carnivores, such as salmon, have a natural diet rich in triglycerides and adapt to high fat diets (Steffens, 1989). In Europe feed manufacturers are currently producing Atlantic salmon diets containing up to 35% lipid.

The second role of lipids is in the provision of essential fatty acids. Fatty acids are often designated by numbering, and this convention is used here. The notation involves three numbers given in sequence, the first denoting the number of carbon atoms; the second, following a colon, the number of double bonds; and the third, designated as n- indicates the number of carbon atoms between the methyl terminal and the first double bond.

Only plants can synthesise the linoleic acid 18:2n-6 and linolenic acid 18:3n-3, fish and terrestrial animals are incapable of *de-novo* synthesis of these n-6 and n-3 fatty acids, making dietary sources essential for normal growth and survival. For land animals tissues are rich in n-6 and, as might be expected, these are the EFA's. Fish tissues, in contrast, are rich in n-3. In salmonids, the fatty acid requirements have been defined (Table 1.5).

Fish nutrition is concerned principally with the polyunsaturated fatty acids (PUFA's), particularly linolenic acid, 18:3n-3, and the highly unsaturated fatty acids (HUFA's), which include eicosapentaenoic (EPA), 20:5n-3, and docosahexaenoic acid (DHA), 22:6n-3. Fish vary in their ability to convert 18:3n-3 to longer chain HUFA's, a major difference appears to exist between freshwater and marine fish. In general, freshwater fish require either linoleic acid, 18:2n-6, or linolenic acid, 18:3n-3, or both, whereas marine fish require EPA, 20:5n-3, and/or DHA, 22:6n-3 (Owen et al., 1975). The evidence suggests that while rainbow trout find 18:3n-3 an effective EFA source, it is less effective for marine fish (implicating chinook salmon) due to their reduced ability for bio-conversion of 18:3n-3 to HUFA's 20:3n-5 and 22:3n-6 (Kanazawa, 1985). The estimated requirement for 18:3n-3 as shown in Table 1.5 may be in part due to the fact this fatty

Table 1.5: The Essential Fatty Acid Requirements of Salmonids

Species	Fatty Acid	Level	Reference
Rainbow	18:3n-3	0.8-1.6%	Castell et al 1972, Watanabe et al. 1974
Rainbow	18:3n-3	20% of lipid	Takeuchi & Watanabe 1977
Rainbow	20:5n-3 or 22:6n3	10% of lipid	Takeuchi & Watanabe 1977
Rainbow	18:2n-6	required	Cowey 1992
Chinook	18:3n-3	required	Nicolaides & Woodall 1962
Coho	n-3	1.0-2.5%	Yu & Sinnhuber 1979

acid is conventionally used as the n-3 source in purified research diets. In practical salmon diets n-3 HUFA's, such as 20:5n-3 and 22:6n-3 are an important source of EFA's, and Takeuchi & Watanabe (1977) found provision of this in the diet of rainbow trout reduces the EFA requirement from 20% 18:3n-3 (Table 1.5).

There is convincing evidence that the degree of unsaturation does not appreciably affect digestibility or utilisation of fats and oils as energy sources - almost any fat/oil is adequate (Steffens 1989). However, the requirement of salmon for PUFA's and HUFA's restricts salmonid diets to the use of marine oils. This is because n-3 fatty acids are found in terrestrial animal fats only in trace amounts, they are present at low levels in plant oils (except linseed oil) and are common only in marine fish oils, with HUFA's virtually restricted to this source (Steffens 1989).

Carbohydrate

There is no dietary requirement for carbohydrates recorded in fish (NRC, 1993). Poor utilisation of this nutrient group is reported in salmonids (Steffens 1989). Growth rate in rainbow trout has been reported to increase with decreasing dietary carbohydrate level (Refstie & Austreng, 1981), although the reported maximum digestible carbohydrate level for practical salmonid diets varies. Buhler & Halver (1961) fed 48% carbohydrate (dextrin) to chinook salmon with no deleterious effects. Brauge et al. (1994) conclude that 230g rainbow trout grown in seawater were able to utilise up to 25% of digestible carbohydrate in a diet efficiently, while Hemre et al. (1995) recommended that feeds for Atlantic salmon should contain 5 - 22% dietary carbohydrate, with an optimum level of 9%.

Much of the variation in recommended maximum carbohydrate levels can be attributed to the observation that fish growth and the digestibility of carbohydrates in the diet of salmonids varies inversely with the molecular complexity of carbohydrate (Buhler & Halver, 1961; Smith, 1971). Apparent digestibility ranged from 2.8% for alpha cellulose to 86% for glucose in rainbow trout (Smith, 1971). Reported starch digestibility in rainbow trout for native, pre-cooked and gelatinized starch varied respectively through 38%, 50% and 87% (Bergot & Breque, 1983; Lindsay et al., 1984). The high digestibility of glucose makes it a more suitable carbohydrate source in salmonid diets, Bergot (1979) demonstrated that trout can tolerate a high level (30%) of glucose in the diet without adverse effects on growth and feed conversion.

Other factors also affect the digestibility of dietary carbohydrate. Inclusion level is a significant factor. Bergot & Breque (1983) reported decreased starch digestibility with increased levels of dietary inclusion. Other reports indicate some adaptability to elevated dietary carbohydrates, with

an increased pancreatic amylase activity accompanying an increased amount of dietary starch (Achene et al., 1989). The digestibility of the carbohydrate component of diets can be increased by extrusion processing (Hilton et al., 1981). Arnesen & Krogdahl (1993) reported improved starch digestibility in pre-extruded wheat products fed to Atlantic salmon.

Fibre (cellulose, lignin and other indigestible plant matter) is poorly utilised by fish. Davies (1985) extensively reviews this subject. Buhler & Halver (1961) observed depressed growth rates in chinook salmon reared on diets with increasing levels of cellulose, while Hilton et al. (1983) found that rainbow trout showed a significant growth depression associated with increased intake and reduced gastric retention times at levels of 10 and 20% cellulose. Their studies showed that the digestibility of cellulose is zero, and concluded that formulated diets should be no more than 10% cellulose.

The low capacity of the salmonid digestive system, the relatively short food retention time and the relatively low body temperature allow little opportunity for microbial action on high fibre diets. It has been concluded that the contribution made by bacterial flora, and in particular of the amylase producing bacteria, to the digestion of dietary carbohydrates in trout (Bergot & Breque, 1983; Achene et al., 1989), is not significant. This has been partly attributed to the lack of endemic gut cellulase. Stickney & Shumway (1974) reported widely varying cellulase activity in the digestive tracts of 62 fish species. However they did not consider *Salmonidae*. They found no cellulase in the gut of any species of offshore fish. Prejs & Blaszczyk (1977) reported cellulase activity in the digestive tract of salmonidae (*Coregonus albula*) however enzyme activity correlated with the amount of dead plant material in fish tracts and not to the actual amount of cellulose in the gut. This suggests that cellulase activity in the intestinal tracts of fish in these studies is due to the microflora of ingested plant material, not endemic gut flora.

Chitin is a polymer which is a component of many invertebrate exoskeletons. Regardless of its presence as a food component in many wild salmonid diets, and the demonstrated presence of endogenous chitinase in the stomach and chitobiase in the intestine of rainbow trout (Lindsay et al., 1984), its reported digestibility is close to zero, and it causes growth depression when fed to rainbow trout (Lindsay et al., 1984).

Despite the reported disadvantages and poor utilisation, some level of digestible carbohydrate is used in aquaculture diets to improve pellet binding (NRC, 1981), and as a cheap source of dietary energy. This needs to be balanced against an upper limit to carbohydrate inclusion, above which poor FCR's (food conversion ratios) are found due to increased dietary intake and reduced growth (Hemre et al., 1995). It is often economically desirable to try to incorporate into diets for

the commercial production of salmonids the maximum carbohydrate level which is efficiently utilised by the fish, provided it is consistent with good fish production and health.

Other

In addition to the main nutrients given above, fish have dietary requirements for other nutrients, particularly vitamins and minerals.

Eleven water-soluble vitamins (B group, L-ascorbic acid, choline, myo-inositol) and four fat-soluble vitamins (A, D, E, K) are known to be required by coldwater fishes (NRC, 1981). Compared with most birds and mammals the gastrointestinal tract of fish does not contain a typical pattern of established microorganisms (Margolis, 1953). Accordingly it cannot be assumed that fish obtain any appreciable quantities of vitamins from microbial synthesis in the intestine. Therefore these vitamins are generally fortified in aquaculture feeds.

In addition to vitamins, at least 7 major minerals and 15 trace elements are essential for animal life. Although most of these are probably also required by fish, only 9 dietary minerals (calcium, phosphorus, iron, iodine, selenium, magnesium, zinc, copper, manganese) have been shown to be required (NRC, 1993). Trace minerals are often added to salmonid diets, although at least part of the salmonid requirement for the minerals calcium, cobalt, iron, magnesium, potassium, sodium, zinc and others can be obtained directly from water by exchange across the gill membrane. Certain minerals such as chlorides, phosphates and sulphates are effectively obtained from feed sources (NRC, 1981).

There is also a wide range of non-nutrient factors added to diets, including water, binders, antioxidants, anti fungal agents, chemotherapeutants, pigments, hormones, growth enhancers and feeding stimulants.

1.4 Protein digestibility and its experimental determination

Very few nutrients are utilised by an animal in the form in which they are ingested. The nutritive value of a feed is dependent not only upon the nutrient content, but also upon the ability of an animal to digest, absorb and utilise the nutrients in the feed. This ability is affected by a number of factors, including the type of animal and the feed material.

Availability refers to the amount of a nutrient in a diet that is absorbed in a form suitable for utilisation. Quantitative data estimating the proportion of amino acids in the ingested protein that can be utilised for metabolic functions are limited. The most common method for determining availability is by formulating a series of diets limiting to varying degrees in one amino acid.

Growth response to the limiting amino acid is compared to that using standard reference diets, and a slope ratio assay provides an estimate of the relative bioavailability (Batterham et al., 1979). This method is slow, expensive, and not universally accepted (Jackson, 1990).

As an alternative to quantitatively assessing availability, it is more practical to establish the extent to which ingested nutrients or amino acids can be absorbed from the gastrointestinal tract, namely the digestibility coefficient. Digestibility is one of the most common estimates of nutrient availability made in nutritional studies. Although circumstances do exist whereby digested nutrients may not be available to the animal, such as the well documented Maillard or browning reaction which occurs between lysine and reducing sugars rendering it unavailable under conditions of extreme processing, diet formulation on the basis of digestibility is close to reflecting the amount of a nutrient available for metabolic and productive functions. Strictly defined, digestibility describes the fraction of nutrient or energy in the ingested feedstuff that is not excreted in the faeces and which is, therefore, assumed to be absorbed by the animal. Simplistically, in a digestibility trial, the food under investigation is given to the animal in known amounts and the output of faeces measured. The apparent digestibility (AD) is calculated from this. However, faeces contain not only undigested food residues, but also include metabolic products derived from body tissues (digestive enzymes, microorganisms, mucus cells, epithelium etc.). This component is known as the endogenous faecal nitrogen (EFN). The excretion in faeces of substances not arising directly from the feed may lead to underestimation of the proportion of food actually absorbed by the animal. The values obtained in digestibility trials that include correction for EFN losses are therefore called true digestibility (TD) to distinguish them from calculations of apparent digestibility. Apparent digestibility values are simpler to calculate and provide satisfactory estimates of nutrient absorption in most cases, therefore most references in this literature review are to apparent digestibility.

Feedstuff digestibility assessment in fish is desirable for (1) least-cost diet formulation (2) nutrient requirement research (3) screening of feed ingredient quality. Digestibility studies have been used extensively to determine nutrient requirements of domestic animals, and the standard methods employed have been adapted to fish. Literature covering protein digestibility in salmonids has been reviewed (Cowey, 1975). Estimates of feedstuff digestibility by fish are not extensive but are available (Cho & Slinger, 1979; Watanabe & Pongmaneerat, 1991; Hajen et al., 1993b). Only a fraction of these, however, refer to chinook salmon. Atlantic salmon and rainbow trout are the primary farmed species and therefore the principal fish used in experimental studies. Lack of data can also be attributed to the special difficulties encountered with aquatic *in vivo* trials. Fish are a more difficult subject, being harder to maintain in a laboratory and have a body temperature dependent on the surrounding water temperature, which may in turn affect

digestibility. They are generally of small size when used in feeding experiments, and there are special problems associated with collecting waste products from their aquatic environment. Practical feeding trials to date have provided fish nutritionists with a wealth of data, allowing formulation of productive and cost-effective feeds for commercially important species. However, much of the existing data need to be fine-tuned with regard to effects of species, fish size, management, environment, ingredients processing and diet composition on digestibility in practical diets.

There are almost as many different methods for calculating feed digestibility as there are workers in this field, and these methods may be classified under two main headings. The first is *in vitro*, where the feed is digested in an enzyme solution in the laboratory. The second is *in vivo*, carried out on live fish and measured as the difference between the amount of nutrient in the diet, and in the excreta, divided by the amount in the diet. Chemical methods, while giving some indication of comparative feed quality, do not always indicate the absolute availability of nutrients in a feed to a particular species. A long term feeding trial, although time-consuming and expensive, is the most dependable method for measuring the value of a feed.

1.4.1 *In vitro* determination

In vitro determination of protein digestibility is rapid, inexpensive, and circumvents the difficulties associated with live fish experiments. There are a number of *in vitro* test systems which have shown potential. The most reliable of these methods use individual proteolytic enzymes or combinations of them. These are reviewed by Satterlee et al., (1977, 1979) and Boisen & Eggum (1991). The basis of enzymatic techniques is to simulate *in vivo* digestion, and they are designed either to give maximal digestibility values or to measure the initial rate of hydrolysis. Despite the obvious fact that duplication of *in vivo* results can never be completely possible given the many factors involved in digestibility, some *in vitro* techniques have shown reasonable accuracy.

Individual enzymes which have been used include pepsin (Sheffner et al., 1956), pronase (Ford & Salter, 1966; Dawson, 1988), trypsin (Maga et al., 1973) and pancreatin (Menden & Cremer, 1966).

Pepsin is an important specific enzyme which is present in the stomach of most animals, including salmonids (Halver, 1989; Twining et al., 1983), and was the first enzyme to be used to determine protein digestibility *in vitro*. It is the most widely used single enzyme method and is recognised officially (Association of Official Analytical Chemists, 1984). The standard AOAC method simulates the peptic digestion in the gastrointestinal tract by digesting 2g of sample at

37°C with 0.2% pepsin in a suitable acid medium at pH 1.9. Measurement of soluble protein is by the Kjeldahl method. Pepsin digestibility has been used extensively throughout the feed manufacturing industry as a quick reference value for ranking the digestibility of feed proteins. The fishing research groups at the Torry Research Station, Aberdeen and Fishing Industry Research Institute (FIRI), Capetown have developed this procedure for fishmeals in aquaculture diets. Unreliability of the AOAC method for fishmeals was mainly attributed to the relative strengths of pepsin:sample ratio used and also the fact that no correction was made for acid hydrolysis. Revised procedures for pepsin digestion were developed at the Torry Research Station and FIRI and involve the use of low pepsin concentrations of 0.0002% and 0.002% respectively (Lovern et al., 1964; Droesti et al., 1964). Anderson et al. (1993) found the Torry method when applied to fishmeals gave a wider range of pepsin digestibility values and small differences in meal quality were more readily detected. The acid-corrected AOAC method generally failed to differentiate protein quality. The Torry and FIRI methods have been extensively tested and found to show good sensitivity when applied to heat treated fish meals, and correlate well with net protein utilisation for rats, growth coefficient in chicks, and available lysine (Lovern et al., 1964; Droesti et al., 1964). Dawson (1988) found limitations to the accuracy of pepsin digestibility. The pepsin method was found to have unusually high standard deviations, particularly for blood meals. Hegedus et al. (1989) found estimations of the crude protein content and *in vitro* pepsin digestibility of blood meal inadequate in predicting the available protein content of blood meal, and other protein meals. Similar limitations of pepsin digestibility as a method for predicting biological availability have been reported by other authors. Anderson et al. (1993) reported that in fishmeals pepsin digestibility values were a useful index of protein quality in so far as they ranked fishmeals in the same order as biological tests.

Pronase is a protease extracted from the bacterium *Streptomyces griseus* and has been used as an alternative to animal proteolytic enzymes in investigations of the digestibility of food proteins (Ford & Slater, 1966; Taverner & Farrell, 1981; Dawson ,1988). Pronase contains a large number of enzymes capable of hydrolysing most of the specific substrates of pepsin, trypsin, chymotrypsin, papain, cathepsin C, carboxypeptidase and several others. In earlier work evaluating this method, Taverner & Farrell (1981) reported good agreement between pronase and *in vivo* estimates of digestibility in pigs. Pronase digestibility is routinely used in New Zealand to evaluate the protein quality of fishmeals, meat meals and other animal protein sources for animal feeds. Dawson (1988) concluded that the pronase method was simple to use and gave much better replication than pepsin digestibility in the evaluation of blood meal (Table 1.6). In addition, the pronase method gave a wider spread of values between the different samples than did the pepsin digestibility method. However, correlation with rat ileal digestibility values was

Table 1.6: The Pepsin and Pronase Digestibility (% of dry matter) of Blood Meal Samples (± SD).

(Source Dawson, 1988).

Blood meal	Pepsin dig.	Pronase dig.
BM 1	52.66 (13.49)	29.21 (1.67)
BM 2	64.50 (5.11)	26.01 (0.70)
BM 3	23.40 (0.33)	12.01 (0.70)
BM 4	35.35 (3.49)	25.59 (0.60)
BM 5	28.85 (0.88)	9.62 (1.45)
BM 6	64.67 (-)	42.60 (1.80)
BM 7	103.26 (0.50)	67.89 (1.43)
BM 8	76.49 (13.75)	35.12 (1.85)

Results are the means of the three values except where a dash appears for standard deviation

low, although he attributed this to inaccurate *in vivo* values. Moughan et al. (1989) found that although the pronase assay showed high precision, it also showed low correlation with rat *in vivo* values.

A criticism of the single enzyme method has been that the enzyme has specific cleavage sites and is not characteristic of the hydrolysis that occurs in the gastrointestinal tract. Thus various combinations of enzymes or protease preparations containing a spectrum of enzymes have also been evaluated. Some of these have included pepsin-pancreatin (Akeson & Stahmann, 1964), pepsin-papain (Ford & Salter, 1966), successively pepsin-pancreatin-erepsin (Ford & Salter, 1966), or protease extracts from the gut of fish (Grabner, 1985; Grabner & Hofer, 1985; Eid & Matty, 1989; Dimes et al., 1994a, 1994b; Dong et al., 1993).

These multi-enzyme systems have been used with varying success (Moughan et al., 1989; Boisen & Eggum, 1991). The pH stat and pH drop methods are multi-enzyme systems that use a solution of trypsin, chymotrypsin and peptidase (Hsu et al., 1977). They follow the principle that during proteolysis, protons are released from the cleaved peptide bonds, resulting in a decrease in pH in a suspension. Assuming a correlation between the initial rate of peptide release and protein digestibility, the latter can be predicted by (1) recording the decrease in pH (pH-drop method), or (2) keeping the pH constant with continuous addition of NaOH and measuring NaOH consumption (pH-stat method).

Digestibility values obtained with the pH-drop method were found by Moughan et al. (1989) to show a high degree of precision, and a significant relationship was shown between pH-drop and *in vivo* ileal digestibility of meat and bone meal in rats. Boisen & Eggum (1991) state that compared with the pH-drop method, the pH-stat method improves the prediction of digestibility. Dawson (1988) found in meat meal the pH-stat method correlated well with *in vivo* values in rats. Anderson et al. (1993) reported it to be a useful rapid evaluation tool for evaluating protein quality in fishmeals, but agreement with *in vivo* digestibility in Atlantic salmon was incomplete.

Horn et al. (1976) asserted that "*in vitro* protein decomposition should be, in fact, a copy of natural conditions in order to obtain the truest possible picture of the first phase of protein metabolism". *In vitro* digestion tests are normally carried out with arbitrary substrate concentrations at temperatures around 37°C and for digestion periods of 24 to 48 h. The Torry pepsin digestibility method uses 45°C for 16 h. In contrast, the physiological temperature optimum for salmonids was specified by Brett (1971) to be 15°C. The passage time of food through the alimentary tract (evacuation time) in trout of medium weight is reported as 30 to 40 h (Grove et al., 1978; Windell et al., 1969.) *In vitro* tests designed for use in mammalian systems

may have significant limitations when used as a basis for accurate predictions about the value of proteins for fish utilisation.

Some more recent studies (Grabner, 1985; Grabner & Hofer, 1985) have recognised the demand for an *in vitro* digestibility test with a fish-specific physiological orientation, that more closely replicates the conditions in the digestive system of fish. Grabner (1985) used a method which involved incubation of the test protein with trout digestive fluids in a titration, with the subsequent separation and analysis of soluble digestion products. He found the solubilisation of peptides using this method agreed with *in vivo* results from Austreng (1978). Eid & Matty (1989) incubated protein sources with carp intestinal extract *in vitro* to determine digestibility and also found strong correlation with *in vivo* results. Dimes et al. (1994a,b) estimated *in vitro* digestibility of salmonid feeds by an adapted pH-stat method using enzyme fractions from trout pyloric caeca. They found good agreement with growth of fish, and concluded that this method was an improvement in the prediction of *in vitro* digestibility of salmonid feeds over the 3-enzyme pH-stat method. Dong et al. (1993) compared the method using trout pyloric caeca enzymes with other *in vitro* methods and found the enzyme method most sensitive and more closely correlated with *in vivo* results.

The validity of *in vitro* techniques depends on a high correlation with *in vivo* values under standardised conditions using identical methods. Where *in vitro* methods are shown to be capable of predicting *in vivo* values determined using animal assays, then they can have an application in the routine and rapid analysis of feed ingredients.

1.4.2 *In vivo* determination

Despite the time and expense involved, long-term *in vivo* feeding trials are still recognised as the most dependable method for measuring the value and digestibility of fish feeds. *In vivo* determination of nutrient digestibility is made by comparing the amount of nutrient ingested with that excreted. The percentage apparent digestibility (AD) of a nutrient is expressed by the equation:

$$AD = \frac{\text{Nutrient consumed - Nutrient in faeces}}{\text{Nutrient consumed}} \times 100$$

Apparent digestibilities do not always reflect nutrient availability, as faeces contain not only undigested food residues, but also include metabolic products (EFN) derived from body tissues. Taking this fraction into account, true protein digestibility (TD) as a percent is calculated from the equation:

$$TD = \frac{\text{Nutrient consumed - Nutrient in faeces - Endogenous nutrient in faeces}}{\text{Nutrient consumed}} \times 100$$

Reported values for EFN and TD values in fish are infrequent, Nose (1967) found that 3.1% of faecal nitrogen collected from rainbow trout came from endogenous sources. Where these values have been estimated, traditional evaluation methods for EFN have been used, that is, by feeding a protein-free diet or by aid of regression analysis. Dabrowski & Dabrowska (1981) fed fish a nitrogen free diet and measured the faecal nitrogen excreted. Rychly & Spannhof (1979) determined TD in rainbow trout by taking into account the regression coefficient of a curve drawn from faecal nitrogen losses in fish fed graded amounts of dietary protein, and estimating endogenous protein by extrapolation to zero. Other methods are available, but have not yet been applied in fish nutrition studies. In pigs De Lange et al. (1990) used a ^{15}N-isotope dilution technique to determine the endogenous protein and amino acid losses, and suggested re-evaluation of previous traditional methods. The amount of endogenous faecal nitrogen estimated was found to vary slightly depending on the evaluation methods used (Ogino et al., 1973). In the determination of true availability of amino acids, both Rychly & Spannhof (1979) and Dabrowska & Dabrowski (1981) found that TD values were higher in comparison to the respective AD. In practice, most fish digestibility studies calculate AD rather than TD values. Kaushik (1989) in respect to endogenous faecal nitrogen concludes "While the assessment of such metabolic faecal nitrogen can throw some light on the potential contamination of faeces, and hence on the estimation of apparent digestibility, it is quantitatively negligible and will bear little significance in nitrogen balance studies under normal feeding conditions". In agreement, Ogino et al. (1973) note that the difference between TD and AD of protein will be marked only when protein intake is low.

Nutrient digestibility in fish can be determined in two ways, either directly, when the total amount of the particular nutrient ingested and the amount excreted is measured, or indirectly, in which the ratio of an indicator and the nutrient in both diet and faeces is measured.

The total recovery of unconsumed food and faeces on a routine basis, either manually or using specially designed apparatus, is difficult and time consuming, and has limited the use of direct measurement methods. In one of the first digestibility studies Phillips et al. (1948) force fed trout with known amounts of a test diet, then at various intervals killed fish and analysed contents of their digestive tract. Later, the development of metabolism chambers for fish (Post et al., 1965) made it easier to extend nutrient balance and digestion trials to fish. Smith (1971), in a modification of the original metabolism chamber of Post et al. (1965), held fish in a fixed position for collection of faeces, gill excretions and urine. The fish were confined in a tube,

force-fed daily, and the water surrounding the fish in the front of the chamber was separated by a rubber dam from that at the rear of the chamber. Criticism of this method has focused on the stress suffered by test fish due to the restriction of swimming activity and force feeding, which may in turn compromise feed utilisation and digestion. Use of this method has been restricted to rainbow trout and attempts to adapt it to other species have been so far unsuccessful. Smith (1971) himself noted during his original research: "Most fish adapted to the close confinement of the chambers. Occasionally a fish would struggle and fight the chamber until exhausted. Some fish regurgitated all or part of the feed ... Some abrasion and loss of slime occurred in the area of the diaphragm". In the study, all fish that failed to adapt to the chamber were discarded.

Most practical fish digestibility studies circumvent problems with total recovery of food and faeces by utilising inert markers incorporated into the feed specifically for the purpose of digestibility calculation. This indicator method involves measurement of the ratios of nutrient to some indigestible component (the indicator) in the feed and in the faeces. For reliability of results, the indicator must be carefully selected and have the following characteristics. It must:

- be indigestible,
- be unaltered chemically during passage through the gut,
- be nontoxic to fish,
- be conveniently analysed,
- be capable of passing through the gut uniformly with other digesta.

Digestibility in fish has been determined using the inert marker chromic oxide (Austreng, 1978; Bird, 1992), or indigenous dietary components including cellulose (Buddington, 1979), hydrolysis resistant ash 'HRA' (De Silva & Perera, 1983), ash (Leavitt, 1985; De Silva & Perera, 1984), crude fibre 'CF' and hydrolysis resistant matter 'HROM' (De Silva & Perera, 1983; Bird, 1992).

The chromic oxide indicator method is the method of choice for most fish digestibility trials, although it is not without its critics. Inaccuracies can occur when quantitative recovery of the indicator does not occur. Bowen (1978) encountered a number of difficulties and reported a high degree of variation in digestive efficiencies when this indicator was used for diets containing living algae and bacteria. Leavitt (1985) found differential passage rate of chromium pigment in the American lobster. Dabrowski & Dabrowska (1981) found chromium oxide used in digestibility studies was removed selectively faster from the stomach of rainbow trout than other food components, with the potential for an increase in digestibility coefficients in subsequent parts of the digestive tract.

If diets are prepared correctly and readily consumed by fish, methods employing chromic oxide have a number of advantages. Quantitative feeding and collection of faeces are unnecessary, experimental fish can live undisturbed in normal conditions until the end of the experiment, and a large number of fish can be used eliminating individual differences and allowing sufficient faeces for evaluation. Austreng (1978) and Windell et al. (1978a) have both confirmed the suitability of this procedure for measuring digestibilities in fish.

Other indicators are used to a lesser degree, with some uncertainty as to their total reliability, and doubts as to their indigestibility. HRA is composed of mineral ash resistant to acid digestion. Ash was evaluated by Leavitt (1985) as a marker in the American Lobster. He found it an unsatisfactory tracer as it was absorbed through the gut wall. Crude fibre and HROM basically refer to the same group of materials. Cellulose and chitin are the two main components of HROM and cellulose and lignin are major components of the crude fibre fraction. The use of chitin and cellulose (HROM) as a dietary reference for digestibility calculations in fish is based on the reported absence of endogenous cellulase in the gut of fish (Stickney & Shumway, 1974). The assimilation of HROM by different species remains controversial and contradictory. Bowen (1981) reported assimilation of a small fraction of HROM in the detrital aggregate by Tilapia. Smith (1971) reports small but significant digestion of cellulose in rainbow trout. Buddington (1980) reported that HROM may be digested to a very minor extent (digestibility of between 1.05% and -0.29%) in rainbow trout and tilapia.

De La Noue & Choubert (1986) compared direct and indirect digestibility measurement, and concluded that provided the faecal collection method is suitable both can be used to obtain reliable digestibility figures. A number of authors have compared the various alternative marker methods for accuracy. De Silva & Perera (1983) compared the digestibility of the aquatic macrophyte by *Etroplus suratensis* using HRA, CF, and HROM, and found the recovery of HROM in the faeces was nearest to 100%, suggesting it to be the better marker.

Whatever the choice of method in a fish digestibility trial, any determination encounters technical difficulty in the separation of fish faeces from the water and the avoidance of its contamination by uneaten feed and nutrient losses by leaching. Leaching of nutrients from faecal material is a source of error in digestibility estimation (Smith et al., 1980). Windell et al. (1978a) found significant nutrient leaching from faeces during the first hour after defaecation, with limited additional leaching up to 4 hours. The accurate collection of faeces for analysis is an important part of digestibility studies, and to overcome some of the practical difficulties several techniques have been devised, including, dissection, suction, siphoning, metabolism chambers, decantation and continuous filtration.

In one of the first digestibility studies in salmonids, Nose (1960) applied the chromic oxide indicator method to rainbow trout and collected faecal samples by manually stripping the fish and gently squeezing out the faecal material from the rectum. This simple technique avoids nutrient leaching in the water, and is used after the fish has been anaesthetised. Windell et al. (1978a) obtained samples by applying suction to the anus or by dissecting fish. The disadvantages of forced evacuation techniques include the following

- Significant handling and consequent stress to the fish.
- Undetermined error arising from contamination of faeces with milt, ova, blood, urine and body slime.
- A reduction of the transit time of intestinal contents with possible concomitant reduction in nutrient absorption (Austreng, 1978; Windell et al., 1978a).
- The assumption that digestion and absorption are complete when material has reached the distal end of the intestine. Austreng (1978) and Windell et al. (1978a) have demonstrated absorption occurs in the rectum of rainbow trout, and other authors (Ash & McLean, 1989) have described the ability of enterocytes from the posterior intestine to ingest intact proteins by pinocytosis.
- The consequences of possible daily fluctuations in digestibility (De Silva & Perera, 1983, 1984) are that stripping and intestinal dissection may not provide a representative sample.

With the aim of avoiding any manipulation of fishes and the dissolution and leaching of faeces, other researchers have proposed devices for the separation and recovery of faeces based on the assumption that insoluble matter that can be settled or filtered from the aquarium is of faecal origin and soluble excretions are of metabolic origin. Beamish (1972) collected faeces with forceps when they were available in pellet form. When pellets broke up in the water they were siphoned from the tank and collected in a millipore filter. Smith (1971) used a fully enclosed metabolic chamber, while Ogino et al. (1973) collected faeces by passing the effluent water from the fish tanks through a filtration column. Cho & Slinger (1979) used a "Guelph system" consisting of a collection tube for faeces. Cho et al. (1982) used a settling column and Choubert et al. (1979, 1982) a mechanically rotating screen to filter out faecal material.

These methods offer the advantage of allowing the fish to feed normally, avoid excessive handling, allow repeated collections and determinations over a long period of time, and permit evaluation of different diets. At the same time observations of growth rate and carcass analysis

can be made. A primary consideration in the design of many of these systems is the brief contact of faeces with the water and therefore avoidance of nutrient leaching. Some have achieved notable success in this regard. The system of Choubert et al. (1982) removed faeces from the water within 6-15 s of ejection. However others still expose faeces to potential leaching. The Guelph system gave consistently higher nutrient digestibility values in comparison with the stripping technique, (Cho & Slinger, 1979; Hajen et al., 1993a). Hajen et al. (1993a) found that apparent digestibility values increased markedly with increased exposure time to the water, and that most losses occurred after exposure for greater than 6 hours. Smith et al., (1980) found an analysis of solids alone did not give a true account of nitrogen in the faeces of rainbow trout held in a metabolism chamber. Apparent digestibility of nitrogen based on solids was about 10 percentage points higher than the digestibility based on the nitrogen in the total homogenised faecal suspension, presumably due to nutrient leaching. Windell et al. (1978a) compared the faecal collection methods of dissection, anal suction, stripping and netting sediment, and found the most reliable digestibility estimates are obtained when faeces are collected by intestinal dissection or anal suction, provided only well formed pellets are removed.

For routine and accurate estimation of apparent digestibility of feedstuffs in fish using *in vivo* trials, methodology needs to be standardised, and the experimental design must be practical and able to be replicated in a variety of experimental facilities. The wide range of experimental design and methodologies employed in fish nutrition studies to date has meant that comparison of results is often difficult. Consideration needs to be made of pre-trial conditions, including acclimation periods, feeding programs and experimental fish stocks. Diets must be prepared correctly and readily consumed by fish. It is important that food availability, in itself, does not act as a factor limiting the growth of the fish and this implies that *ad libitum* feeding programs are required. A frequent problem to be avoided in conducting experimental feeding trials is insufficient numbers - too few treatments, replications, fish or tissue samples. This makes statistical analysis difficult, especially if mortality occurs, (which should be anticipated).

Generally, most researchers agree on the measurement and reporting of apparent digestibilities for feedstuffs. The use of the inert digestibility marker, chromic oxide is widespread, and results in accurate digestibility values (Austreng, 1978; Windell et al., 1978a). The marker circumvents problems associated with quantitative feeding and collection of faeces. Methods of faecal collection available are varied, but slaughter and subsequent dissection of the lower rectum to obtain faecal samples has shown reasonable accuracy. Where fish need to be maintained for subsequent experimentation stripping faecal contents from live fish is often used. These methods avoid nutrient leaching, and do not require complicated faecal collection and settling equipment in fish tanks.

Digestibility trials with other animals

Because of significant differences in habitat and physiology between terrestrial and aquatic species, it is preferable to base nutritional conclusions on chinook salmon from data obtained with that same species, eliminating a potentially significant source of error. In practice, however, digestibility trials on salmonids are relatively complicated, costly and time-consuming compared with those on many terrestrial animals, and demand facilities that are in practice unavailable to many companies involved in salmon feeding and production.

To avoid some of these difficulties, in the past digestibility values obtained from mink or rats have been used in the practical assessment of commercial fish diets. The rat is considered a suitable mammalian model for nutritional research, and has been shown to be a useful model for the pig in the nutritional evaluation of feed ingredients (Moughan et al., 1984, 1987). Protein digestibility in the rat, as with other animals, can be determined according to the ileal or faecal analysis methods. In rats ileal protein digestibility is a common measure, and is considered to provide a more accurate estimate of amino acid and protein digestibility than the faecal approach because this method measures digestibility prior to microbial degradation and synthesis of amino acids in the large intestine. The comparison of rat ileal digestibility values with faecal digestibility has shown faecal sampling to overestimate the digestibility of protein due to protein degradation by the hindgut microflora, and subsequent poor utilisation of these breakdown products (Moughan et al. 1984). In salmon bacterial activity has been recorded, although this has not been confirmed as of endemic origin (Achene et al., 1989), and numbers of bacteria are typically low. Some studies have implicated microorganisms in the microbial breakdown of cellulose (Stickney & Shumway, 1974; Trust et al., 1979) and lipids (Ringo et al., 1995). Direct evidence for a nutritional role for these bacteria is not found in most reviews on this topic, and their role in digestion is generally assumed to be negligible. For this reason, faecal digestibility measures are considered an accurate assessment of digestibility in salmon.

Overseas, mink are used extensively as a model for fish for determining digestibility in feedstuffs (Skrede, 1977; 1979; Skrede et al., 1980; Skrede & Krogdahl, 1985; Borsting, 1992). In Norway, for instance, mink protein digestibility values are used to confer the LT 94 seal to their premium fishmeals (Romero et al., 1994). Deacon (1992) notes that salmonids are similar to mink in that they have a short digestive tract, and that digestibility values for proteins from trout, mink and Atlantic salmon are comparable. Skrede et al. (1980) confirm this, but only under certain experimental conditions. In a comparison of the digestibility of cod fillet protein between chickens, mink, foxes and rainbow trout, true faecal digestibility values showed fairly small differences between species, although there was an overall tendency towards higher values in the

fur animals and rainbow trout than in chickens. In contrast, the digestibility of protein and individual amino acids in meat and bone meal were considerably lower in rainbow trout and mink - their digestive systems were the most sensitive to protein sources of low digestibility. Romero et al. (1994) found good agreement between protein digestibility for fishmeal estimated in rainbow trout and mink. However, Springate & Gallimore (1992b) comment that mink and rat are not good indicators of the digestibility of fishmeal in salmon and trout, as they tend to digest them more efficiently than salmonids.

1.5 Factors affecting protein digestibility

Protein digestibility and its experimental determination may be affected by a number of factors including, previous nutritional history, feeding rates, diet, species, fish size, disease status, management, environment and water temperature.

The previous nutritional and environmental history of the fish is important, as tissue stores of some nutrients may influence the digestibility of others. Trial designs often include a period of starvation or acclimation prior to feeding trials. Acclimation times will depend on the fish used, but are often around 1 month. Longer periods of acclimation may be necessary for wild fish, which are unaccustomed to confinement and the feeding of pelleted diets, possibly resulting in an altered response in feeding trials. Previous starvation to clear the alimentary tract may influence digestibility. Increasing periods of starvation prior to feeding have been shown to decrease the rate of digestion. Hastings (1969) believes that a slow passage of food from the stomach, under conditions of previous starvation, may be an efficiency mechanism to allow enzymes and digestive juices to attain a level of excretion essential for the amount of substrate present.

Feeding rate may also influence digestibility, although this effect is not confirmed for protein. Windell et al. (1978b) found rainbow trout fed at 1.6% of their weight showed significantly lower digestibility for total dry matter, carbohydrate and energy, although no similar effect was apparent for protein and lipid. Storebakken & Austreng (1987) found that the level of food intake had no significant effect on the apparent protein digestibility for rainbow trout of 0.5-1.0kg body weight. These data agree with those reported for brown trout. Elliott (1976) fed meals ranging from 10% to 100% of the maximum ration and absorption efficiency decreased with increasing level of energy intake. At very low or high levels of intake, digestion efficiency may change. Hastings (1969) notes that gastric evacuation increases with the level of food intake, and that as food intake increases, apparent digestibility increases, then decreases for abnormally high intakes. Windell et al. (1978b) confirm that at large meal sizes, digestive and absorptive efficiencies decrease.

Digestibility of food is closely related to its chemical composition, and the composition of other feedstuffs ingested with it. The apparent protein digestibility varies with protein intake. In carp, digestibility declined steeply when N intake fell below 100mg/100g liveweight/day, while increasing dietary protein content in Tilapia (*Sarotherodon niloticus*) decreased protein digestibility (Steffens, 1989). In rainbow trout apparent protein digestibility increased with increasing protein level (Austreng & Refstie, 1979). With a known and uniform food intake, various techniques of forced feeding are sometimes used in trials. Hastings (1969) cites a personal communication from Windell noting that forced feeding increases the rate of digestion, and also increases variation between fish. While high dietary ash up to 17.5% (Shearer et al., 1992) and fat to 30% (Johnsen et al., 1993) failed to affect protein digestibility in Atlantic salmon, in rainbow trout increased levels of starch reduced protein digestibility (Kitamikado et al., 1964). Austreng et al. (1977) found that the utilisation of protein was poorer in rainbow trout fed high levels of dietary carbohydrate, while Smith (1971) found cellulose interfered with the digestion and absorption of protein, reducing digestibilities. Where glucose was the primary carbohydrate utilised, Refstie & Austreng (1981) found the opposite result. Apparent protein digestibility increased with increasing dietary carbohydrate.

Antinutritional factors and toxins in the diet have also been reported to affect digestibility in salmon. For example, trypsin inhibitor found in some insufficiently heat-treated soyabean meals has been shown to interfere with protein digestion (Dabrowski & Kozak, 1979). Phytic acid in oil seed meals may bind to calcium, magnesium and zinc reducing their availability to the animal (Smith, 1977) and amylase inhibitor in wheat can inhibit amylase activity and carbohydrate digestibility in trout (Hofer & Sturmbauer, 1985). Other examples involving other compounds are available in the literature (Halver, 1989).

The quality of the protein source is also highly significant. In general, most protein containing feedstuffs have high digestibility (75 - 95%, NRC 1993). Differences exist, however, between protein types and sources. Cho & Slinger (1979) report protein digestibility values of 40-96% for protein sources fed to rainbow trout. Another important factor affecting the digestibility of nutrients is the method of food processing. Whole soya beans fed to channel catfish were completely indigestible. When coarsely ground they had a digestibility of 31%, while fully ground soya-bean meal was 72% digested (Hastings, 1969). Extrusion processing generally increases carbohydrate digestibility (Arnesen & Krogdahl, 1993) while in contrast overheating fishmeal greatly reduces its nutritive value (Finley, 1989).

Different species of fish can be expected to digest the same nutrients with different efficiency. Differences in protein digestibility between individuals can also occur within a given species.

Austreng & Refstie (1979) reported significant differences in protein digestibility between different families of rainbow trout. Such information presents good opportunities for selective breeding, but may also represent a source of error in digestibility trials.

Fish size is also an important factor. Small fish appear to be poorer at digesting protein than larger ones. Windell et al. (1978b) found significantly lower protein digestibilities in small (18g) rainbow trout, while Kitamikado et al. (1964) reported that fish up to 6g liveweight exhibited significantly poorer protein digestibility than those in the weight range 10-100g. This was attributed to decreased proteolytic enzyme activity in young trout. Most digestibility experiments use small fish, it has the advantage of using less space and obtaining faster responses, but the results are applied indiscriminately to all sizes of fish. Since most of the feed consumed, and the majority of the commercial cost of fish farming, is in larger fish, nutrient availabilities on large fish need to be available.

Apparent protein digestibility estimated from *in vivo* salmon trials may show daily variability (Negas et al., 1995). Daily rhythms in a number of metabolic processes have been demonstrated in animals. De Silva & Perera (1983) reported for the first time the possible existence of rhythmicity in digestion. De Silva & Perera (1984) confirmed the existence of rhythms by demonstrating that a day or two of high digestibility in Tilapia fry (*S. niloticus*) was generally followed by a day of low digestibility

Water temperature and salinity may also affect protein digestibility. Windell et al (1978b) did not observe any difference in digestibility for larger rainbow trout (207g and 586g liveweight) at 11^0C and 15^0C, but smaller fish (18g) exhibited clearly diminished values at 7^0C. Cho & Slinger (1979) also found a higher protein digestibility at 18^0C than at 9^0C-15^0C.

In salinity studies Dabrowski et al. (1986) concluded that in rainbow trout held in fresh or sea water, ions did not seem to interfere with protein digestion and nutrient absorption in the alimentary tract. In contrast, Usher et al. (1990) found apparent nitrogen digestibility to be 9-10% lower in seawater adapted Atlantic salmon smolts compared with freshwater adapted smolts. Although no significant difference in enzyme activity was found, mid and hindgut pH values were significantly higher in seawater than freshwater adapted smolts.

There are a wide variety of factors which can affect digestibility values. Absolute comparisons of digestibility values obtained in different studies are only valid if experimental conditions are standardised, otherwise variations in experimental method could be responsible for digestibility variation.

1.6 Commercial diet formulation

In spite of the inherent difficulties, a body of data on nutrient contents and digestibilities has amassed.

> "A primary objective in dietary formulation for fish is to provide a nutritionally balanced mixture of ingredients to support the maintenance, growth, reproduction and health of the animal at an acceptable cost" Source: NRC 1993

Feeding fish in their aqueous environment involves considerations beyond those for feeding land animals. These aspects include the nutrient contribution from natural aquatic organisms, effects of feeding and diet composition on dissolved oxygen and other water quality factors, and the loss of nutrients if the feed is not consumed immediately. Fish feeds require special processing methods to achieve physical properties for feeding in water, and variation in feeding behaviour requires special feeding regimens for various species.

Wild salmonids are carnivorous, feeding on zooplankton, insects and fish. Early trout and salmon diets were attempts to duplicate the composition of natural food, and the earliest formulations were wet feeds consisting of trash fish and slaughter house wastes (Fowler & Burrows, 1971). Later, use of raw fish products was de-emphasised as there were indications that these were vectors for transmitting fish diseases. Wood & Wallis (1955) demonstrated that kidney disease could be transmitted orally by feeding infected raw salmon products. As dietary requirements for finfish became more generally known, various animal and vegetable protein meals, binders, vitamins, minerals and fish oils were added to diets either to increase the dietary value or as binders. Common formulations used were the Oregon Moist Pellet developed in 1959 (Hublo, 1963), followed by the Abernathy Moist Pellet (Fowler & Burrows, 1971). Subsequently, these and other rations used for early trout culture were extended to meet the needs of Atlantic salmon culture in Norway in the early 1980's, and later in other parts of Europe, Asia and America.

"Moist" feeds, as these formulations have become known, contain 60-65% dry matter and have not been heated or dried. They are prepared by adding moisture and a hydrocolloidal binding agent, such as guar gum, carboxymethyl-cellulose or gelatinized starch with ground wet animal and/or fish tissue and dry ingredients (vitamins, pigments etc), and using a grinder to form the mixture into soft, moist pellets. Moist feeds are softer, more palatable and better accepted by several cultivated salmonids than alternative formulations. There is also a metabolic basis for the addition of fresh water to feeds to correct osmotic imbalance in marine salmonids, and a number of European fish farms add freshwater to dry pellets prior to feeding for this reason (Ghittino, 1979). However, moist feeds have a number of significant disadvantages (Anon, 1992).

- Environmental pollution leading to feed wastage, poor water quality and disease. This caused severe problems for the Danish trout industry, where moist feeds are now outlawed.

- Diet storage and handling. The feed must either be made fresh each day or stored frozen because the high moisture content allows rapid spoilage.

- Increased labour costs when home mixing of diets is involved.

- Nutritional problems encountered with home mixed moist diets due to inexperienced diet formulation and quality control.

- Disease such as kidney disease, nocardiosis and ichthyophoniasis.

Today moist salmonid diets still play an important role in areas disposing of large amounts of suitable and cheap fish. However the above problems combined with the rapid growth of the salmon industry mean that moist pellets are often inadequate and uneconomical, and are replaced by high density "dry" diets containing 90% dry matter. The two most common processing methods for dry diets involve the use of varying degrees of heat, moisture and pressure.

In the 1970's this technology was applied primarily to produce standard pressure-steam pellets. Steam pelleting involves the use of moisture, heat and pressure to compress ingredients into compact pellets. Hot steam is added to the feed mash during pelleting, to elevate temperatures to between 70 - 90°C for compression, after which pellets are cooled and dried.

In the early 1980's extruded pellets became an increasingly significant feed for salmonids. Extrusion is a process by which the feed mix, in the form of a dough, is forced through a small orifice at very high pressure and temperature (100 - 150°C). As the material is squeezed through the die holes the external pressure decreases, water vaporises and causes expansion of the pellet. The pellet then passes through a hot drying process to reduce moisture. Research into the productivity of fish fed extruded feeds has shown a number of advantages.

- Physical characteristics of the feed are improved. These improvements include the following. (i) Superior durability and water stability (Hilton et al., 1981). (ii) Reduced fines, and hence reduced water pollution and vitamin leeching losses (Slinger et al., 1979). (iii) Improved pellet floatability (Stickney, 1979).

- Production of high energy diets has become possible. Extruded pellets can absorb and hold higher levels of fat exceeding 30%, more than was previously possible with

conventional steam pellets. High fat levels can improve feed conversion efficiencies for some species of finfish by increasing efficiency of protein utilisation (Reinitz et al., 1978; Takeuchi et al., 1978).

Other performance advantages have been attributed to:

- elimination of some anti-nutritional factors (eg anti-trypsin in soybean meal) and bacteria (salmonella) in diets due to the high extrusion temperatures (Wee, 1991)

- and to improved bio-availability of carbohydrates when fed to rainbow trout (Hilton et al., 1981), due to improved starch gelatinisation during processing. This also reduces pollution due to increased digestibility and lower faecal output.

Generally, feeding of extruded diets has been linked with improved productivity in salmonids. Hilton et al. (1981) found an increased gastric emptying time and lower weight gains but higher feed efficiency when extruded feed was given to rainbow trout. The depressed feed intake was attributed to the low density of extruded feed, or to excessively high levels of digestible carbohydrate. Wee (1991) reports that the growth patterns of fish fed extruded feed are equivalent or slightly better than with a steam pelleted diet. Food conversion ratio (FCR) was significantly reduced, and a lower intake of extruded feed was required than for a steam pelleted feed, for a similar performance. Certainly, under controlled laboratory conditions, FCR's of lower than 1:1 can regularly be achieved with commercial extruded feeds, and some farms in Norway claim they achieve 1:1 now with Atlantic salmon (Forster, 1995). Based on these and other reported results, most farming systems in Norway, Chile, Scotland, Canada and Australasia now predominantly use extruded feed. In New Zealand, the much smaller aquaculture industry makes the economics of extrusion technology less available to the industry as a whole. Until 1996 over 95% of industry feed was steam pelleted, with protein levels of 42-50%, and oil content ranging from 9-18%, supplied by a single manufacturer, Northern Roller Mills (NRM) NZ Ltd. In 1996 significant importations of extruded diets containing 22-25% oil were made by individual fish farming companies. NRM (NZ) Ltd are moving towards installation of an extruded aquaculture feed plant in New Zealand.

The main reported disadvantage of extruded feeds has been the higher cost of production. This is due to the higher capital and operating costs, and also the need for vitamin and amino acid supplementation due to increased processing losses (Wee, 1991). Reduced vitamin stability occurs during extrusion processing, particularly for vitamin C (Hilton et al., 1977). Pigment losses of 10-15% also occur during extrusion processing (Roche Products (NZ) Ltd, pers comm.). The outcome of this, as recorded in Eurofish Report (12 May 1994), is that with the

higher relative costs of extruded feeds, (in terms of final $ costs/kg fish produced), extruded feeds may not have the efficiencies at first reported.

Feed costs and efficiencies are a driving force in commercial diet formulations when 35-50% of on-farm costs are feed costs. Least cost diet formulation using linear programming methods is commonly used to derive diets for fish, as for other farmed animals. For dietary formulation using least cost methods, the following information is required.

- The nutrient requirements of the animal.
- Nutrient and energy contents of ingredients.
- Minimum and maximum restrictions on concentrations of ingredients.
- The prices of ingredients.

To satisfy the high dietary requirements of salmon for protein, modern fish diets rely heavily on fish meal. A typical grower salmonid feed contains 40-70% fish meal, and even higher concentrations, of up to 80% fish meal, are used in starter feeds. Fish meal is used intensively because it has many attributes which make it an almost ideal ingredient for inclusion in carnivorous fish feed formulations. It has a highly digestible protein content, an amino acid profile that matches that of fish food, and therefore satisfies the nutritional requirements of many fish species, is a good source of energy, essential fatty acids, certain minerals and vitamins, and has been attributed with containing other nutritional "growth" factors.

In 1991, six million tonnes of fish meal were produced, of which only around 840,000 tonnes or 14% were used for aquaculture (Springate & Gallimore, 1992a). The International Fish Meal Manufacturers Association in London projects that worldwide fishmeal production will decline by about 5% over the next decade due to depletion of stocks caused by overfishing. When this is combined with projected increases in aquaculture, predicted at 2 to 3 times by the year 2000 (Barlow 1989), and other intensive farming systems requiring formulated feeds, the predicted result is that upwards pressure on fishmeal supplies will force prices up, significantly increasing the cost of raising fish in the future. It appears mandatory that other ingredients be found to individually or in combination replace part or all of the fishmeal component in salmonid diets. Protein sources that will probably become more important in the future are plant meals (soya bean, cottonseed, canola (rapeseed), sunflower, peanut, copra, linseed, palm kernel meals), single cell proteins and animal by-product protein meals (meat and bone meal, blood meal, feather meal).

Substitution of fish meal protein sources with other animal and vegetable proteins has to date met with mixed success (Fowler & Banks, 1976; Tacon & Jackson, 1985; Gomes et al., 1995). Generally, a proportion of the fish meal in salmonid diets can be successfully substituted with careful formulation, and most commercial salmon diets contain some animal and vegetable meals. This will most often mean a combination of several protein sources to achieve a balance of nutrients suited to the needs of the species, and taking due account of any inclusion limitations which may be imposed by antinutritional or palatability factors. However, it is clear from the literature that it is far easier to substitute the first 10% of the fishmeal in a salmon diet than the next and that it gets progressively more difficult as the level diminishes.

The choice of ingredients is pivotal to the success of the formulation. It is generally accepted that aquatic animals are more sensitive to feed quality than terrestrial animals (Akiyama, 1993). It follows that the production of consistent, good quality aquaculture feeds depends not only on feed processing technology but also on formulation and feed ingredient quality. While a body of research has developed around the traditional ingredients used in fish feeding, particularly fishmeals, many alternative products now being considered for aquaculture diets have not been similarly examined, and little or nothing is known about the value of these products for fish.

For a feed manufacturer, literature searches and chemical analysis form the starting point in the determination of the nutritive value of an ingredient and final formulated feed. However, to determine the actual availability of nutrients in that ingredient, measures of digestibility are required. These should ideally relate to the ingredient, feed processing conditions and production conditions (environmental conditions, feeding system, fish age and species etc) under which the finished feed will be fed. *In vitro* digestibility trials are an option, but ideally *in vivo* trials on the relevant species should be considered. In reality, feed manufacturers rarely have the available resources for *in-vivo* trials on individual ingredients, and rely on chemical analyses of protein quality and available published digestibility values for common feedstuffs (Cho & Slinger, 1979; NRC, 1981; Jauncey, 1993; Hajen et al., 1993a,b)

1.7 Blood meal

In any country, least cost formulation will favour the use of suitable locally available ingredients over comparatively expensive imported ingredients. For example, in Canada soya bean meals are grown and included in salmon diets. In New Zealand, meat production forms a significant part of the economy. In the 1993-1994 season (ending September 30, 1994), 25,192,752 lambs were processed for export, 4,762,750 sheep, 1,712,871 beef cattle and 150,961 goats, returning NZ$4.2 billion dollars (NZ Meat Producer, 23(2)).

Of the product volume entering a meat plant, only 30 - 40% of the animal is used for human food. Rendering of inedible wastes currently accounts for 45% of the product volume, and returns about 10% of the product value (NZ Meat Producer, 23(2)). Three - four percent of the animal's live weight is blood (Swan 1992), so large quantities of rendered blood meal are recoverable daily at slaughter plants. In New Zealand, blood meals are rendered at a number of slaughter plants. In 1981 estimated production was 8,000 tonnes (Reid, 1981).

In commercial blood meal processing, proteins in the blood are coagulated, separated to remove water and dried to produce blood meal. Animal type and age may slightly influence blood composition, but it is generally high in protein (85-92%) with a moisture content of 8-10%. There are a number of categories used to define blood processing methods. Swan (1992) identifies the 3 most common methods of processing inedible blood as follows:

1. Apply indirect heat to the whole blood to boil off most of the water. This method is very energy inefficient and produces a denatured product with very low solubility. It is often used at smaller, older plants, or at plants that have not updated their equipment.

2. Inject steam to bring the temperature to about 90°C to coagulate the blood proteins. Then remove most of the water in a decanter and dry the solids, eg in a ring dryer, a rotating drum cooker, or a batch dryer. This method uses less energy than method (1) because about half of the water is removed mechanically. A denatured protein powder is produced, the solubility of which depends on the type of dryer used. The powders produced in ring dryers are usually more soluble. In the case of batch dried blood, the process is as for ring dried blood, with the exception that blood is not fed into the drier continuously, but dried in batches of fixed volume. High temperatures and/or long times during processing lower the nutritional value of the blood meal.

3. Concentrate whole blood by ultrafiltration, then dry the concentrate in a spouted-bed dryer. A very soluble, "spray dried" powder or blood flour is produced.

The feed potential of blood meal has been recognised for many years, and its use in stock feed has been supported by feeding experiments where good results have been achieved with the use of small amounts in diets for ruminants (Stock & Klopfenstein, 1979; Loerch et al., 1983; Bani et al., 1993), pigs (Fitzpatric & Bayley, 1977; Pearson 1992, 1993, 1994), poultry (Squibb & Braham, 1955) and salmon (Fowler & Banks, 1976; Asgard & Austreng, 1986).

The crude protein contents of blood meal is uniformly high at 82 - 92%, and amino acid levels are also high, particularly for leucine and lysine (Table 1.7). The amino acid profile of blood

meal is generally favourable, although some imbalances associated with animal feeding have been reported which restrict inclusion levels in practical diets (Asgard & Austreng, 1986).

Table 1.7: Crude Protein and Essential Amino Acid Contents (% air-dry weight) for Seven New Zealand Blood Meals (BM1 - BM7) and a UK Blood Meal (UK8).

(Compliled from data from Pearson, 1992; Crawshaw, 1993).

Amino acid	BM1	BM2	BM3	BM4	BM5	BM6	BM7	UK8	Average
Protein	89.9	86.7	89.4	87.3	92.2	81.9	86.9	87.0	87.7
Threonine	4.7	4.8	4.9	4.6	5.1	4.0	4.6	5.0	4.7
Valine	7.9	8.0	8.3	7.9	8.5	7.2	7.9	7.7	7.9
Isoleucine	0.6	0.7	0.8	0.6	1.0	0.8	0.8	1.5	0.8
Leucine	10.7	10.1	12.1	10.5	13.0	9.8	11.6	12.9	11.3
Tyrosine	3.1	3.0	2.9	2.9	3.2	2.9	2.8	3.0	3.0
Phenylalanin	6.8	6.7	6.2	6.3	6.7	5.9	6.1	6.7	6.4
Histidine	5.2	5.3	5.9	5.5	6.2	4.8	5.3	6.3	5.6
Lysine	8.5	8.1	8.3	7.8	8.7	7.6	8.1	9.1	8.3
Methionine	1.2	1.2	1.2	1.2	1.4	1.1	1.3	1.1	1.2

New Zealand blood meals from Richmond, Aoteoroa, Belfast, Mataora, Takapau, Horotiu, Rangiuru, UK blood meal from Prosper De Mulder

Fitzpatrick & Bayley (1977) found lower growth in young pigs fed blood meal as opposed to soya protein flour diets, despite similar digestibilities in the two protein sources. They attributed this to imbalance in the levels of essential amino acids in blood diets, particularly high levels of leucine and valine compared to isoleucine. In salmonids a sub-optimal amino acid profile results from the low content of the essential amino acids methionine, cystine, isoleucine and tryptophan (Lambden et al., 1976a; Asgard & Austreng, 1986), and high leucine content (Asgard & Austreng, 1986). This precludes blood meal from being a primary protein source in salmon diets. It has been demonstrated that in chinook salmon, as in other species, an excess of dietary leucine can act as an antimetabolite of isoleucine and can thereby increase the requirement for isoleucine (Chance et al., 1964). However if the blood protein does not make up a major part of the dietary protein, the low content of the deficient amino acids can be balanced by a high content of these amino acids in other feed components, and it is possible to formulate diets with an acceptable balance of amino acids.

Total protein and amino acid values for blood meal overstate the amounts of these nutrients which are available to animals. In contrast with the consistently high crude protein and amino acid values reported in blood meals, digestibilities are extremely variable and often very low, for example 54 - 92% for protein (Table 1.8), with some reported digestibilities even lower at 15.43% (Pearson, 1993). Historically, this has restricted inclusion in many stock rations to less than 3-6%, as higher levels were considered to depress performance (Crawshaw, 1993).

Low digestibilities in blood meal are frequently attributable to processing conditions, in particular overheating during cooking and/or drying. Overheated blood, characterised by black heat hardened or burnt blood, is consistent with low pepsin and pronase digestibility (Dawson, 1988). Lambden et al (1976a,b) found that drying at temperatures up to 140°C had little effect on amino acid content of blood, but heating at 250°C for 1 hr had a marked effect, reducing total recovery of amino acids by 70%. Protein damage was identified as occurring above 175°C. Methionine and isoleucine, already low in blood, were reduced, arginine and threonine were destroyed, and lysine content was halved. However, at these high temperatures Kjeldahl protein content remained at 93%, showing that the protein content *per se* of severely heat damaged blood gives little indication of its nutritional value.

Pearson (1993) related digestibility values to commercial processing methods, and concluded that processing methods such as the batch system, which requires long drying times, results in meals of lower quality. Ring driers frequently produced blood meals of a high digestibility, the best products exceeding 90% for ileal protein digestibility. This association has been suggested previously. Waibel et al. (1974) found that lysine in commercial "batch dried" blood meal was

Table 1.8: Apparent Protein and Essential Amino Acid Digestibilities (%) for Seven New Zealand Blood Meals (BM1 - BM7) and a UK Blood Meal (UK8).
(Compiled from data from Pearson, 1992; Crawshaw, 1993)

Amino acid	BM	BM	BM	BM	BM	BM	BM7	UK8	Average
Protein	54.0	67.6	82.9	72.8	87.0	79.8	91.8	97.0	79.1
Threonine	65.1	44.0	77.3	75.4	80.9	79.5	85.7	87.0	74.4
Valine	73.7	64.2	80.9	76.4	81.3	82.3	91.6	92.0	80.3
Isoleucine	87.3	77.4	95.8	91.6	86.0	86.1	92.0	70.0	85.8
Leucine	70.1	56.5	82.4	80.1	85.1	84.0	92.7	93.0	80.5
Tyrosine	75.2	58.0	81.2	81.0	82.9	81.9	81.7	-	77.4
Phenylalanin	70.8	57.8	78.6	79.5	82.2	82.6	90.1	92.0	79.2
Histidine	72.7	56.1	86.0	85.1	87.0	84.8	92.3	94.0	82.2
Lysine	71.1	54.9	85.7	84.7	87.6	84.4	92.9	93.0	81.8
Methionine	72.9	66.1	85.6	88.3	94.5	88.1	89.1	-	83.5

New Zealand blood meals from Richmond, Aoteoroa, Belfast, Mataora, Takapau, Horotiu, Rangiuru, UK blood meal from Prosper De Mulder

less than 20% available to rats, chicks and turkeys whereas in "ring-dried" blood meal, between 80% and 90% of the lysine was available. Parsons et al. (1975) found that 90% of the lysine in ring-dried blood meal was available to young pigs. Spray dried blood meal also compares favourably to other commercial preparations, Bersting (1992) found that spray dried blood meal fed to minks showed 79.7% protein digestibility compared with 52.2% for batch dried blood. A direct relationship between drying time and protein digestibility in blood meal was shown by Meads et al. (1995). He found drying time alone to be sufficient to predict apparent ileal digestibilities of crude protein (ADCP) using the following regression equation:

ADCP % = 89.9 - 0.266 TD (where TD = drying time in minutes).

That temperature and/or drying times are the only factors responsible for low digestibilities is debatable. Although Pearson (1993) endorsed the association between time in the drier and digestibility, in a later report, Pearson (1994) found that drying temperature alone was not the causal factor for poor quality in blood meals.

Despite the identified problems, the use of blood meal in salmonid diets is common practice in New Zealand. The suitability of this substitution is supported by overseas studies. Fowler & Banks (1976) replaced fish meal with spray-dried blood meal in starter diets for chinook salmon. They concluded that the effect was positive when up to 5% of the fish meal was replaced by blood meal, but there were pathological effects when as much as 17.5% of the fish meal was replaced by blood meal. In rainbow trout, Luzier et al. (1995) found diets for rainbow trout can be formulated with 22.7% blood powder substituted for herring meal without significant loss of growth or reduction in food conversion. Asgard & Austreng (1986) found that blood, frozen or preserved by formic acid (ensiled), could be used to replace fish offal in moist diets without negative effects on growth, health, chemical composition and organoleptic properties in rainbow trout and Atlantic salmon. Successful substitution was at up to 24.5% of the diet. Generally the utilisation of protein by rainbow trout and carp is as good as that by other farm animals, and blood meal digestibilities have been reported for several species of finfish (Table 1.9). In New Zealand, blood meal is currently included in formulated commercial salmon diets at levels varying from 0 to 5%.

1.8 Conclusion

Protein is an important nutrient in the diet of farmed salmonids, requiring 35-55% dietary protein to achieve maximum growth rates. Blood meal has been used as a source of protein and amino acids in animal and fish feeds, in partial replacement or to supplement the use of fishmeal

Table 1.9: Reported Apparent Digestibility Values (%) for Blood Meal Crude Protein in Finfish

Species	Blood type	Digestibility	Reference
Chinook	Continuous	29.4	Hajen et al., 1993b
Rainbow	Canadian	39.8	Cho & Slinger, 1979
Catfish	American	74.0	Brown et al., 1985
Rainbow	Spray dried	84.8	Luzier et al., 1995
Rainbow	Spray dried	86.0	Smith et al., 1980
Rainbow	Ring dried	89.4	Smith et al., 1980
Rainbow	Ensild/frozen	97.0	Asgard & Austreng, 1986

proteins. In New Zealand, significant quantities of blood meal, 8,000 t/annum in 1981 (Reid, 1981) are produced by our meat industry.

The value of blood meal may be diminished where excessive heat treatment during processing have reduced protein and amino acid digestibility. Crude protein and amino acid analysis is a poor predictor of the availability of nutrients in formulated feeds, and measurement of digestibility is the most practical and accurate predictor of nutrient availability. *In vitro* determination of protein and amino acid digestibility is rapid and inexpensive, and a number of techniques have shown accuracy in the prediction of *in vivo* digestibilities. *In vivo* determination of digestibility is the most accurate method for measuring the value of a feedstuff, but in fish is expensive and associated with a number of problems, in particular the collection and recovery of faeces.

Identification of digestible sources of blood meals offers an opportunity for the formulation of economic salmon diets where this ingredient could replace other traditional protein sources such as fishmeal. Ultimately *in vivo* determination of digestibility is required to verify the feed value of blood meals, but for feed manufacturers a simpler, quicker and less expensive technique would be of value for routine and regular testing of blood meals. *In vitro* digestibility assays may offer such an opportunity, as may the use of model species such as the rat in *in vivo* digestibility trials. If such alternative methods are shown to accurately predict nutrient digestibility, then there will be a concomitant gain in feed quality and salmon productivity.

CHAPTER 2

CHEMICAL COMPOSITION AND *IN VITRO* PROTEIN DIGESTIBILITY FOR THREE NEW ZEALAND DRIED BLOOD MEALS.

2.1 Introduction

Blood meal is not a major source of protein in salmon diet formulation overseas, due to its low supply. In 1986, Norway, which is a major salmon producer, produced only a few hundred tonnes of blood (Asgard & Austreng, 1986) and its use in compound salmon diets was uncommon. UK production is estimated at around 8,000 tonnes per annum, which is about 20% of the potential yield (Crawshaw, 1993). New Zealand is also a major producer of blood meal. In 1981 estimated production was 8,000 tonnes, with a value of NZ$4 million (Reid, 1981).

The major local use of blood meal in New Zealand is as a raw material for animal feeds. Estimates provided by the NZ Feed Manufacturers Association show that 3,189 tonnes of blood meal were used in compound feed production in 1993, all of which was of domestic origin. This was 3.3% of the protein sources used, and 0.75% of the total raw materials. Salmon feeds are identified as part of this usage.

Not all blood meals will perform equally in the diets of salmon, because of differences in nutrient availability, which can have a profound effect on salmon productivity. Differences in digestibility of commercial blood meals are largely caused by the processing methods employed in their preparation (Hajen et al., 1983b; Pearson 1993, 1994; Meads et al., 1995). Moreover, identification of the quality of commercial blood meals is difficult, and methods employed vary from simple visual or chemical analysis, to more accurate and expensive *in vivo* feeding trials.

Methods of assessing quality fall into three categories:

1. Physical inspection

   Colour, texture/particle size, odour, solubility, processing information.

2. Chemical analysis

   Proximate analysis, amino acid analysis, *in vitro* digestibility.

3. Biological measurement

   Animal growth trials, *in vivo* digestibility.

Blood meal should always be free from contamination, of uniform particle size and colour, and without pungent or rancid odours. Processing conditions should minimise heat and heat induced protein damage and meal should not be burnt. Chemical assays, such as for amino acids or pepsin and pronase digestibility will give some indication of nutritional value (Dawson, 1988; Pearson, 1993, 1994), while growth trials and animal digestibility studies which provide *in vivo* measures of the utilisation of blood meal by animals are more accurate indicators of quality.

A problem faced by the feed manufacturing industry is to find routine, accurate, quantitative and cost effective methods for measuring feed quality. Using *in vivo* fish bioassays for assessing the protein quality of feeds has proven to be too time consuming and expensive for routine use by the feed and aquaculture industries given the various problems routinely encountered with fish digestibility trials. To date the simplest and cheapest methods which are commonly used to assess blood meal quality are crude protein analysis, amino acid analysis, pepsin digestibility and pronase digestibility (Dawson, 1988; Pearson, 1992, 1994). Dawson (1988), however, found that the correlation of pepsin and pronase *in vitro* digestibilities with *in vivo* values was generally low for blood meals, despite a high degree of correlation being shown for other feed ingredients such as meat meal.

The investigation reported here firstly involved a survey of blood meal production volumes and methods in New Zealand, upon which the selection of sources of blood meal for experimental purposes was based. Further, samples of blood meal typical of the three main commercial methods of production (spray, batch and ring drying) were submitted to proximate chemical analysis, and for pepsin and pronase digestibility assay.

2.2 Materials and Methods

2.2.1 Blood Meal Sources

A questionnaire was mailed to all meat processing plants in New Zealand (addresses sourced from lists of approved meat processing facilities available from MAF Regulatory Authority) to compile information on the production of blood meal in New Zealand, including the location of blood meal processing plants, volume of production and processing methods. Forty-seven questionnaires (refer Appendix 1) were posted. Three commercially processed blood meals were selected for comparison, based on the type of processing method employed. The source plants and the production methods for the blood meals selected for experimental purposes, together with their identification codes, were as follows:

Blood meal A	Richmond Takapau; ring dried

Blood meal B	Affco Whangarei; spray dried
Blood meal C	Alliance Smithfield; batch dried

Fresh blood meal samples were collected from two of the above rendering plants, and were stored for two weeks prior to trial. In the case of blood meal B, the spray drying plant in Whangarei closed in late 1995 and so blood meal B was sourced from freezer stocks and thus was 10 months old at the time of trial.

Meal A was derived from sheep and cattle. The raw blood was coagulated to around 60% moisture by direct steam injection, before passing through a decanter to separate the solid component from the liquids. Coagulator steam pressure was approximately 2 Bar, with a temperature range from 100 to 110°C. The coagulated blood was screw fed into a direct gas fired ring drier, in which the end point temperature range lay between 120 and 130°C. The time spent in the drier was dependent on the moisture level at point of drying, but the process is carried out at above 90°C for not less than 10 minutes. The drier flow rate was 1.2 tonnes per hour. From there blood was screw fed into the bagging bin for packing.

Meal B was also sourced from sheep and cattle. The blood was concentrated by ultrafiltration, then coagulated and homogenised. Blood was then fed into the specialised spouted bed dryer. In this equipment atomised blood was sprayed onto beads suspended in air. The beads were coated in a thin film of dried blood, which were carried out in the hot air stream. Residence time in the spouted bed drier was around 30 seconds. The dried blood was then separated from the air into fine spray dried blood powder.

Meal C was sheep based only. The raw blood was coagulated using 6 Bar steam pressure, then the solids separated in a decanter. The coagulated blood was then batch fed from the bin to a Niven batch dryer. Drying times varied from 2.5 to 3.5 hours, at temperatures between 105 and 110°C. Blood was dried to 5% moisture. After drying, the blood was hammer-milled to reduce particle size, and tallow was added at a rate of 40 litre per tonne to reduce dustiness.

2.2.2 Chemical Analysis

Duplicate samples of each blood meal were subjected to a proximate chemical analysis and to analysis for amino acids. This was carried out at the Department of Animal Science Nutrition Laboratory, Massey University.

Protein

The total nitrogen content of samples was determined in duplicate using the Kjeldahl method (Association of Official Analytical Chemists, 1980). Nitrogen (N) content was measured on a Tecator Kjeltec 1030 Analyser and protein content calculated as N x 6.25.

Amino Acids

Amino acids were determined in duplicate on 10 mg samples using the method as described in detail by Hendriks et al. (1986). Samples were hydrolysed in 1 mL of 6 mol/l glass distilled HCL (containing 0.1% phenol) for 24 h at 110 °C ± 2°C in glass tubes, sealed under vacuum. Amino acids were detected on an HPLC system using absorbance at 570 nm (440 nm for proline). Amino acids were identified by retention time against a standard amino acid mixture.

Cysteine and methionine were analysed as cysteic acid and methionine sulphone by oxidation with 2 mL of performic acid (1 part 30% H_2O_2 to 9 parts 88% formic acid) for 16 h at 0°C and neutralisation with 0.3 ml of 50% (wt/wt) HBr prior to hydrolysis.

Tryptophan, which is destroyed during acid hydrolysis, was not determined. No corrections were made for loss of amino acids during hydrolysis, and free amino acid molecular weights were used in calculating the amino acid yields.

Moisture

Approximately 1-2g of sample was oven dried at 105°C overnight (15 hours). The percentage weight loss was equated with water loss.

2.2.3 Pepsin and Pronase N Digestibility

Pepsin and pronase N digestibilities were determined on 6 random samples of each of blood meals A, B and C at the Animal Sciences Department, Lincoln University, according to methods as described by Dawson (1988) and as given in full in Appendix 2.

The pepsin digestibility method was developed at the Torry Research Station, Aberdeen. Samples of 1g are incubated in 0.0002% pepsin (of 1:10,000 activity) for 16h at 45°C. Post incubation, the sample is filtered and the nitrogen content of the filtrate determined. True pepsin digestibility (TPD %) is calculated using the following expression:

$$TPD\% = \frac{\text{(Nitrogen in sample - Nitrogen in residue) x 100}}{\text{Nitrogen in sample}}$$

and where: Nitrogen in residue = $N_a - N_{a \cdot p} = N_p$

where: N_a = N in acid only treated residues

where: N_{a-p} = N in acid plus pepsin treated residues

where: N_p = N in residues resulting from pepsin digestion

The pronase method used for pronase digestibility determination was that of Dawson (1988), adapted from the original method of Taverner & Farrell (1981). With this method the sample (1g) is incubated with a 0.02% pronase solution for 16 h at 27°C. The undigested proteinaceous material was then precipitated, centrifuged and the soluble nitrogen of the supernatant determined. Digestibility is determined as:

$$\text{Pronase Dig \%} = \frac{\text{N in supernatant}}{\text{Total N}} \times 100$$

Data analysis

The pepsin and pronase digestibilities of the blood meals were subjected to analysis of variance (ANOVA) using the SAS general linear model, with blood, enzyme and blood x enzyme as variables. Student's *t* test was used to determine levels of significance between blood meals and digestibility assays.

2.3 Results

2.3.1 Blood Meal Questionnaire

Of the 47 questionnaires sent, replies were received from 32 companies. The balance of information from the remaining companies was obtained by follow-up phone calls. As of 1 January 1995 it was determined that 23 plants process blood meal in New Zealand; 12 in the North Island and 11 in the South Island (Figures 2 and 3).

Several of the plants lacked facilities for blood meal processing, and either sold or contracted blood meal production to nearby plants and companies with processing capability. A number of plants process blood all year round, but in general processing is seasonal, occurring between November and June when slaughter volumes are highest.

FIGURE 2: Blood Rendering Plants in the North Island of New Zealand as on January 1, 1995

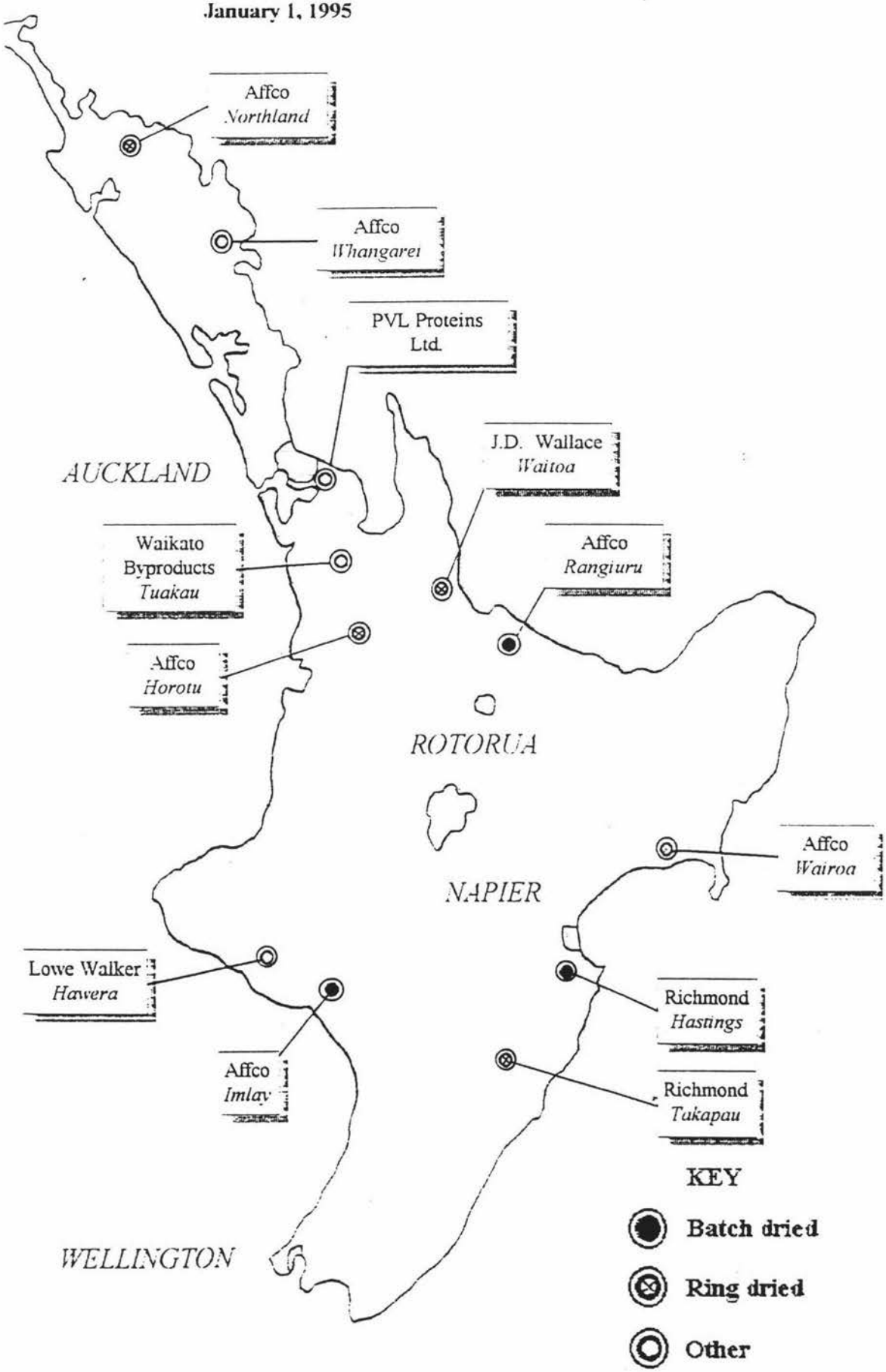
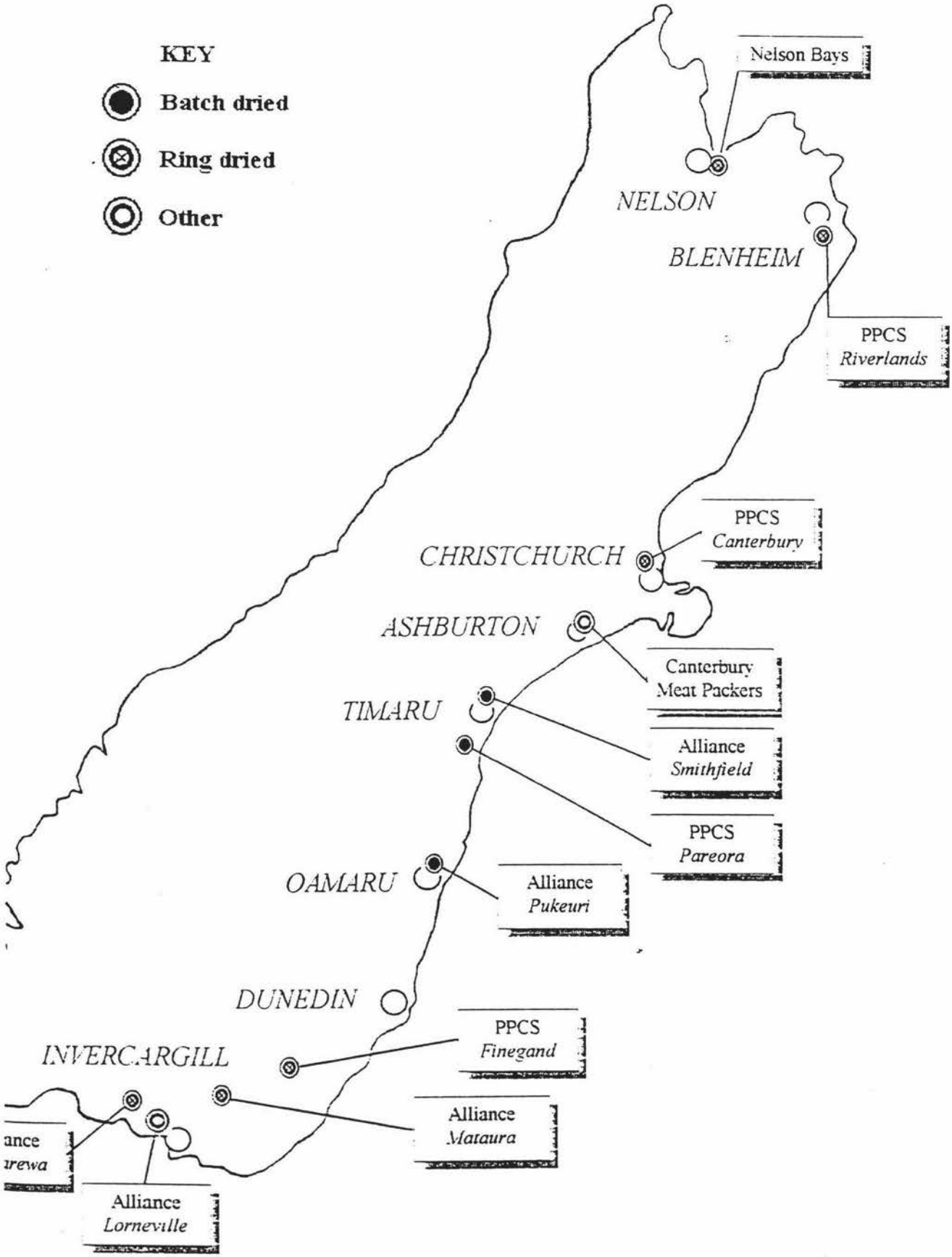


FIGURE 3: Blood Rendering Plants in the South Island of New Zealand as on January 1, 1995



Blood processing methods varied, with ring driers being most common (used at 11 of the 23 plants). The reported quality of blood meal produced at the various plants was generally similar in chemical profile. Protein was usually recorded at the rendering plant at 90-96%, with moisture from 4-10%.

The volume of blood meal produced annually in New Zealand is difficult to accurately determine. The information was not available from meat industry data, and over 50% of respondents to the questionnaire considered this information commercially sensitive and would not release the information. However, based on extrapolation from the volumes provided in the survey, the 1981 published estimate of 8,000 tonnes (Reid, 1981) would also appear to be a realistic estimate of the volumes in 1995. A full summary of the survey results is presented in Table 5.1, Appendix 3.

2.3.2 Chemical Analysis

Results from the chemical analysis of the blood meals are given in Table 2.1.

The crude protein contents of the blood meals were uniformly high, ranging from 89.6% to 93.1%. Moisture ranged from 6.8% to 9.3%. Amino acid levels were also generally high. No one blood meal recorded consistently high amino acid levels in comparison with the others, although of the 9 essential amino acids tested, 6 of the highest values were recorded for the batch dried blood meal (Blood meal C). Proline was low in ring dried blood meal A at 1.47%, in comparison with blood meals B and C at 3.34% and 3.65% respectively.

2.3.3 Pepsin and Pronase N Digestibility

The mean pepsin and pronase N digestibilities for the three blood meals are given in Table 2.2. Values for individual replicates for the three blood meals are given in Table 5.2, Appendix 4.

Both the pepsin and pronase methods ranked N digestibility of the blood meals as B (spray dried) > A (ring dried) > C (batch dried). The digestibility of batch dried blood meal was very low. Differences between the digestibilities of blood meals A, B and C were statistically significant ($P<0.001$).

Statistical analysis of the data revealed a significant ($P<0.001$) interaction between blood meals and digestibility assay. Pepsin digestibility values were significantly lower than pronase values for blood meals A and B ($P<0.001$), but not for blood meal C. The standard errors were generally low, indicating that the *in vitro* methods have a high degree of precision.

Table 2.1 **Determined Chemical Composition (% air dry weight) of Blood Meals A, B and C.**

Chemical	Blood Meal A	Blood Meal B	Blood Meal C
Crude Protein	93.06	89.62	92.00
Moisture	6.98	9.29	6.76
Amino Acid			
Arginine	3.63	3.49	3.77
Histidine	5.13	5.47	5.67
Isoleucine	0.80	0.66	0.85
Leucine	10.96	11.40	11.70
Lysine	7.37	8.22	7.89
Methionine	1.11	1.31	1.22
Phenylalanine	5.88	6.53	6.21
Threonine	3.79	4.23	4.32
Valine	7.69	7.90	8.30
Alanine	6.57	7.08	6.67
Aspartic acid	8.60	9.05	9.54
Cysteine	0.88	0.75	0.79
Glutamic acid	7.87	7.97	7.93
Glycine	3.57	3.67	3.84
Proline	1.47	3.34	3.65
Serine	3.52	3.94	3.46
Tyrosine	2.58	2.63	2.85

Table 2.2 **Mean (±SE) Pepsin and Pronase N Digestibility (%) for the Blood Meal Samples.**

Blood	Pepsin	Pronase	Level of Significance[1]
A	18.9 (0.81)	25.5 (0.25)	* P<0.001
B	27.8 (0.73)	60.5 (0.94)	* P<0.001
C	8.4 (0.16)	9.0 (0.23)	NS

[1] NS, non-significant (P>0.05); *, significant (P<0.001)

2.4 Discussion

New Zealand has a large primary industry based on the slaughter and processing of lambs, cattle and deer. By-products of this industry are meat and blood meals, which are valuable sources of protein for inclusion in animal feeds. There are 23 plants which process blood meal in New Zealand. They do so by a variety of methods; principally ring drying, but also batch drying, spray drying and other processing methods (eg low temperature rendering). Particular attributes of the processing used in rendering, (particularly drying times), have been shown to be negatively correlated with the quality of blood meal as determined by *in vivo* N digestibility in the rat (Pearson, 1992; 1993; Meads et al., 1995).

The blood meals used in the presently described study were chosen for their very different processing methods. Blood meal C was batch dried, spending 2.5-3.5 hours residence time at 105-110°C. Blood meal A was ring dried, experiencing only 10 minutes drying at lesser temperatures of 90-130°C. The spray dried blood meal (Blood meal B) received the least damaging heat treatment, with only 30 seconds in the cooking and drying processes. Spray dried blood is regarded as highly digestible and desirable for animal feeds. Its exposure to short term drying maintains high protein and amino acid quality. Ring dried blood meal is the most common processing method currently used in New Zealand.

The gross chemical compositions of the three blood meals were similar, and typical of published values (NRC, 1993; Crawshaw, 1993; Dawson, 1988; Pearson, 1992; 1993). Crude protein values at 89.6 to 93.1% were consistently high, falling within the ranges of 82.5% - 93.4% reported by Pearson (1993) in his analysis of numerous NZ blood meals. Lambden et al. (1976a) found that Kjeldahl N values of blood meal are not greatly affected by exposure of the blood meal to heat, and that N levels alone do not provide a true indication of the nutritional value of dried blood. Hegedus et al. (1989) confirmed the inadequacy of crude protein as an indicator of available protein in blood and other protein meals. In the present work availability was assessed by commercially available *in vitro* digestibility procedures and involving digestion of the test blood meals with pepsin and pronase. A concern with the use of pepsin, particularly in the evaluation of fishmeals and other high protein meals, has been the strength of pepsin solution used (Lovern et al., 1964). In the past the poor correlation of pepsin digestibilities with animal assays has been attributed to the very high levels of pepsin used in these experiments, resulting in virtually all the protein in the sample being digested, giving no real indication of protein quality. Two pepsin digestibility methods are currently in common use. These are the standard AOAC method and a modification (as used in this work) developed by the Torry Research Station in Aberdeen for measuring fishmeal protein quality. The two are very similar, with the main

difference being that the AOAC method recommends a 0.2% pepsin solution and the Torry method a 0.0002% solution. Anderson et al. (1993) found that the Torry method when applied to fishmeals gave a wider range of pepsin digestibility values with the result that small differences in meal quality were more readily detected. Dawson (1988) used this method to evaluate *in vitro* digestibility of New Zealand blood meals. Results on 8 blood meals ranged from 0.75 to 26.77%. In the current work pepsin digestibilities for experimental blood meals A, B and C were all low, ranging from 8.4% for the batch dried blood meal C, to 27.8% for the spray dried blood meal B.

The pronase method uses a protease extracted from the bacterium *Streptomyces griseus* and has been used by Ford & Slater (1966) as an alternative to animal proteolytic enzymes in investigations of the digestibility of food proteins. It has given good correlations with *in vivo* protein digestibility in rats (Saunders & Kohler, 1972), and Dawson (1988) found it more accurate than pepsin digestibility in measuring the *in vitro* digestibility of NZ blood meals. In the present study, pronase digestibility ranked the blood meals in the same order of digestibility as did the pepsin method, (spray blood B > ring blood A > and batch blood C).

Despite resulting in similar ranking of blood meal quality, pronase digestibilities were consistently higher than pepsin digestibilities. For blood meals A and B, these differences were statistically significant. Blood meal C had a very low digestibility (pepsin 8.4%, pronase 9.0%). Overall, the pronase method gave a wider spread of values between the different blood meals than did the pepsin digestibility method. Dawson (1988) also found that the pronase method gave a higher spread of values, which he attributed to an increased sensitivity to digestion time with the pronase method. Experimental results showed no consistent trend for pronase digestibility to be more precise than pepsin digestibility, with similar standard errors reported for the pepsin and pronase methods. This was contrary to the findings of Dawson (1988), who found that for a range of blood meals (pronase digestibilities from 9.6 to 67.9%), the pronase method gave much better replication than did the pepsin method.

The variation in the *in vitro* digestibility values for the three blood meal types is attributed to differences in the processing conditions, particularly the length of time and the temperatures used during drying. This result is consistent with other research. Both Pearson (1993) and Meads et al., (1995) have noted a reduced digestibility of blood meals which have been dried at high temperatures for long time periods.

The present results suggest that spray dried blood meal is likely to be the most suitable blood material for fishmeal replacement in chinook salmon diets. On the other hand, the high crude

protein and amino acid levels in all blood meals tested appeared to favour their use in controlled quantities in salmon diets without differentiation. Crude protein levels of 89.62% - 93.06% in the blood meals were higher than the reported average for high quality whole herring meal (72.0%, NRC 1993). The levels of most amino acids were higher in blood meals than reported averages for high quality fishmeals (NRC, 1993), making blood meals generally a richer source of amino acids than fishmeal. The exceptions were the essential amino acids arginine and methionine, where values for blood meals (3.49% - 3.77% and 1.11% - 1.31% respectively) were lower than those reported by NRC (1993) for fishmeal (arginine 4.54%, methionine 2.08%). Lambden et al. (1976a) and Asgard & Austreng (1986) reported a low content of the essential amino acids methionine, cysteine, isoleucine and tryptophan in blood meal which precluded blood meal as a primary protein source in salmon diets. Chance et al. (1964) reported that in chinook salmon, as in other species, an excess of dietary leucine can act as an antimetabolite of isoleucine and can thereby increase the requirement for isoleucine. With the high levels of leucine found in experimental blood meals, this imbalance could result in isoleucine deficiencies when blood meal is included in salmon diets, despite adequate assayed levels of isoleucine in bloods. These deficiencies and surpluses could be corrected for in the dietary least cost formulation through balancing using other protein compounds. *In vitro* digestibility measurements, however, favoured some bloods over others. The high digestibility of spray dried blood (blood meal B) makes it the most suitable replacement for fishmeal. Very low pepsin and pronase digestibility values obtained for batch dried blood (blood meal C) make it least suitable as an ingredient in high quality salmon feeds, and, compared with fishmeal, a relatively expensive source of digestible protein. Literature recommendations are that spray dried blood meal may be included at levels varying from 5% (Fowler & Banks, 1976) to 22.7% (Luzier et al., 1995). Based on the experimental results, ring dried blood meal would be suitable for inclusion at lower dietary levels than spray dried bloods but at higher levels than batch dried blood. The presently discussed results determined using *in vitro* methodology need to be confirmed using *in vivo* digestibility methods.

CHAPTER 3

IN VIVO DIGESTIBILITY OF PROTEIN FOR THREE NEW ZEALAND DRIED BLOOD MEALS

3.1 Introduction

If alternative protein feedstuffs, such as blood meal, are to be used in the formulation of "least cost" diets for fish, it is desirable to know more about these feedstuffs than the information provided by standard chemical analyses alone. *In vitro* digestibility assays have some value for the prediction of *in vivo* nutrient digestibility, although statistical correlations with *in vivo* trials may be low (Moughan et al., 1989; Boisen & Eggum, 1991). In contrast, *in vivo* digestibility trials, although more time-consuming and expensive, are still recognised as the most dependable method for measuring the digestibility of nutrients in feeds.

For domestic animals quick and simple digestibility trials are carried out routinely, and the standard methods employed have been adapted to fish. For fish the aquatic environment makes the measurement of feed intake and faeces output difficult and therefore estimates of feedstuff digestibility by fish are not extensive (Cho & Slinger, 1979; Smith et al., 1980; Watanabe & Pongmaneerat, 1991; Anderson et al., 1993; Luzier et al., 1995; Gomes et al., 1995). Atlantic salmon and rainbow trout are the primary farmed species and therefore the principal fish used in experimental studies, and only a fraction of the reported digestibility studies have been carried out on chinook salmon (Fowler & Banks, 1976; Hajen et al., 1993b). *In vivo* digestibility data which assist efficient diet formulation using New Zealand feed ingredients, though available for species such as chickens and pigs, are not available for chinook salmon. Such data are required for the formulation of high performance diets and to achieve high efficiency in the utilisation of ingredients.

To circumvent the need for determining digestibility in fish, digestibility values obtained from mink or rats have been used to assist in the formulation of commercial fish diets. The rat is considered a suitable mammalian model for nutritional research, and has been shown to be a useful model for the pig in the nutritional evaluation of feed ingredients (Moughan & Smith, 1984). In a number of overseas studies, mink are used in determining the digestibility of feedstuffs (Skrede, 1977; 1979; Skrede & Krogdahl, 1985; Borsting, 1992) and in Norway, mink protein digestibility values are used commercially to confer a quality seal to premium fishmeal (Romero et al., 1994). The validity of using comparative species is not confirmed. Deacon (1992) notes that protein digestibility values obtained for trout, mink and Atlantic salmon are

comparable. Skrede et al. (1980) confirm this under some experimental conditions, but Springate & Gallimore (1992b) comment that digestibility values for fishmeal determined using the mink and rat do not reflect the digestibility of fishmeal assessed in salmon and trout. According to the latter workers, the former species tend to digest protein more efficiently.

The digestibility of feed proteins may be affected by a number of factors, including the conditions used during their processing. The digestibility of protein in commercially produced blood meals has been found to vary considerably with the manufacturing process (Dawson, 1988; Borsting, 1992), particularly with respect to drying times and temperatures (Pearson, 1993; Meads et al., 1995). In salmon, reported protein digestibility of various commercial blood meals varies from 39.8% (Cho & Slinger, 1979) to 89.4% (Smith et al., 1980). Again, to date there are no known publications of protein digestibility values for New Zealand blood meals for chinook salmon, despite the routine use of blood meal in New Zealand chinook salmon diets (NRM (NZ) Ltd).

In the present work the digestibility of protein and amino acids for three dried blood meals (ring dried, spray dried and batch dried) was assessed in chinook salmon, using salmon held in small tanks and with the manual collection of faeces. Comparison was also made between fish apparent faecal amino acid digestibility and rat apparent ileal amino acid digestibility for one blood meal. The aim of the study was to quantify protein digestibility in chinook salmon for three commercial blood meals. A secondary objective was to assess the suitability of the rat as an animal model for the prediction of protein digestibility in salmon.

3.2 Materials and Method

3.2.1 Blood Meals

Three commercially produced blood meals (as described in Chapter 2) were used:

Blood meal A	Richmond Takapau; ring dried
Blood meal B	Affco Whangarei; spray dried
Blood meal C	Alliance Smithfield; batch dried

3.2.2 Chemical Analysis

All analyses were carried out at the Department of Animal Science Nutrition Laboratory Massey University, following the procedures described in Chapter 2 section 2.2.2.

Nitrogen content of rat digesta and amino acid content of both rat and chinook salmon digesta were determined as described in Chapter 2 section 2.2.2. Fat was determined by Soxhlet ether

extraction according to the method outlined by the Association of Official Analytical Chemists (1980).

Chromic oxide (Cr_2O_3) was used as a digestibility marker. Concentrations in diets and digesta were determined on duplicate 100 mg samples using atomic absorption spectrophotometry (Costigan & Ellis, 1987).

3.2.3 Digestibility in Chinook Salmon

Diets

A basal diet was formulated by NRM (NZ) Ltd to meet the nutritional requirements of chinook salmon (NRC, 1993) and contained 49% protein and 18% lipid. All the diets were mixed and steam pelleted using a 6mm die at the Massey University Feed Processing Unit. All diets contained chromic oxide, as an indigestible marker at an inclusion level of 0.6g per 100g of diet.

Four diets were prepared, the basal diet (Diet D), and three test diets (Diets A, B and C) each containing 20% of blood meal A, B or C respectively substituted for the basal diet. The ingredient and chemical compositions of the test and basal salmon diets are given in Table 3.1. The basal diet was fed along with the test blood meal to simulate practical feeding conditions and to encourage full feeding and normal growth by the salmon (Cho & Slinger, 1979).

Animals, housing and feeding

Eleven month old female chinook salmon were sourced from The New Zealand King Salmon Co. Ltd. Hatchery at Takaka, and held for experimentation at Crop and Food Crown Research Institute NZ Ltd (CFCRI), Nelson.

Prior to the study the fish were kept on a commercial steam-pelleted salmon diet (NRM NZ Ltd.; 45% protein, 17% fat). Salmon were acclimated at the CFCRI for 14 days before the commencement of experimentation.

Salmon were stocked at random in four circular $1m^3$ polythene lined fibreglass tanks. Twelve salmon averaging 450g body weight (weight range 300g - 700g) were held in each tank. Of these ten (drawn randomly) were used at each sampling, while the additional two fish allowed for any potential mortalities. Sea water with aeration was supplied to each tank at a flow rate of 7-10 litres/min. Water temperatures were uncontrolled, but were measured daily. Dissolved oxygen was measured weekly using an automatic Dissolved Oxygen probe and meter. Fish were subjected to natural photoperiod.

Table 3.1 Ingredient and Chemical Compositions of the Experimental Diets (air dry weight basis) for Chinook Salmon.

		DIET		
	A	B	C	D
Ingredient (g)				
Enerva fishmeal	51.200	51.200	51.200	64.00
Blood meal[a]	19.880	19.880	19.880	0.00
Pollard	9.200	9.200	9.200	11.50
Fish oil	9.032	9.032	9.032	11.29
Wheat meal	5.520	5.520	5.520	6.90
Skimmilk powder	4.000	4.000	4.000	5.00
Rovimix 25 Vit C	0.048	0.048	0.048	0.06
Chromic oxide	0.600	0.600	0.600	0.60
Betaine anhydrous	0.200	0.200	0.200	0.25
Mineral premix[b]	0.200	0.200	0.200	0.25
Vitamin premix[b]	0.120	0.120	0.120	0.15
TOTAL	100.000	100.000	100.000	100.00
Nutrient (%)				
Crude protein	58.90	57.80	59.15	49.53
Fat	15.73	15.46	15.82	18.14
Moisture	7.60	7.39	7.39	8.22
Amino acid (g/100g)				
Arginine	3.18	3.17	3.05	2.82
Histidine	2.52	2.56	2.48	2.13
Isoleucine	1.97	2.01	1.84	2.02
Leucine	5.59	5.49	5.02	3.59
Lysine	4.74	4.79	4.36	3.59
Methionine	1.40	1.64	1.25	0.92
Phenylalanine	3.00	3.05	2.71	1.90
Threonine	2.42	2.55	2.28	1.87
Valine	3.97	3.89	3.60	2.49
Alanine	3.92	3.75	3.40	2.85
Aspartic acid	5.68	5.62	5.17	4.18
Cysteine	0.49	0.49	0.50	0.38
Glutamic acid	7.32	7.16	6.46	6.06
Glycine	3.13	2.99	2.81	2.87
Proline	1.03	2.38	2.27	2.06
Serine	2.08	2.20	1.92	1.58
Tyrosine	1.96	1.96	1.83	1.50

[a]One of three experimental blood meals, A, B or C collected from plants in New Zealand (section 3.2.1)

[b]Special salmon mineral and vitamin premix, Pantech, Auckland.

Diets A, B, C and D were allocated at random to the four treatment tanks. Fish were hand-fed the trial diets for 7 days. Feeding was to satiation, to a maximum of 200g per tank per day (3.7% of the body weight per day). Three feedings were carried out daily from 08:00 - 10:00, 12:00 - 13:00 and 16:00 - 17:00 hours. On the 7th day faecal samples were collected and the salmon were removed from the tanks. Tanks were cleaned by siphoning solids, brushing algal growth, and completely replacing the water. At this stage the new salmon from the acclimation tanks were introduced to the experimental tanks. This procedure was repeated 3 more times over the next 21 days, to give a total of 4 replicates (each of 10 fish per diet). The design is represented as follows:

	Tank 1	**Tank 2**	**Tank 3**	**Tank 4**
Days 1-7	Diet B	Diet A	Diet C	Diet D
Days 7-14	Diet A	Diet C	Diet D	Diet B
Days 14-21	Diet C	Diet D	Diet B	Diet A
Days 21-28	Diet D	Diet B	Diet A	Diet C

Slaughter, collection of faeces and sample handling

On the 7th day of each experimental period, faecal sampling commenced at 14:00 hours. Prior to faecal collection the fish were anaesthetised using 20ml Aqui-S (active ingredient 50% isoeugenol; manufacturer Fish Transport Systems Ltd., Petone) in each $1m^3$ tank. Faeces were collected from the hind gut region by gently stripping the ventral abdominal region from the pelvic fin to the anus as described by Austreng (1978). At each sampling time faeces from 10 fish in each treatment were taken. Faeces from randomly drawn salmon (n=5) were pooled to give 2 samples of faeces for each diet in each replicate. Faeces were pooled at stripping directly into sealable plastic bags and immediately frozen at -20°C. Salmon weights were taken at the final sampling (Table 5.3, Appendix 5). Following stripping, salmon were held in large tanks.

Prior to chemical analysis, all samples (faeces and diet) were freeze-dried to a constant weight and then finely ground (less than 1mm mesh) for the duplicate determination of total N, amino acids and chromium.

Data Analysis

The estimates of amino acid digestibility in the test diets were calculated from the dietary ratio of amino acid to chromium relative to the corresponding ratio in the faeces, using the formulation:

$$\text{Apparent Digestibility (AD) \%} = \frac{\frac{\text{AA in diet}}{\text{Cr in diet}} - \frac{\text{AA in faeces}}{\text{Cr in faeces}}}{\frac{\text{AA in diet}}{\text{Cr in diet}}} \times 100$$

The apparent digestibility of the respective nutrient in blood meal was calculated from AD values for the test and basal diets using the following relationship:

Digestibility of test diet = Digestibility of basal + Digestibility of blood + Interaction

The use of a basal diet assumed that there were no interactions between the components of diet during digestion. The apparent nutrient digestibility in blood meal was derived using the following calculation:

$$\text{AD Blood \%} = \frac{((0.2 \times a) \div (0.8 \times b) \times Dig_t) - ((0.8 \times b) \times Dig_d)}{(0.2 \times a)}$$

Where a = amount of nutrient in blood

b = amount of nutrient in basal diet

Dig_t = digestibility of nutrient in test diet

Dig_d = digestibility of nutrient in basal diet

Protein digestibility was calculated arithmetically as the sum of digestible amino acids.

The statistical model for data analysis was:

$$Digestibility_{ijkl} = u + Tank_i + Date_j + Diet_k + e_{ijkl}$$

The amino acid digestibilities for the blood meals and diets were subjected to analysis of variance (ANOVA) using the SAS general linear models computer programme. Duncan's Multiple Range Test was used to determine levels of significance between blood meals and experimental diets.

3.2.4 Ileal digestibility in the rat

Diets

The experimental diets were mixed and steam pelleted using a 6mm die at the Massey University Feed Processing Unit. To each diet chromic oxide was added as an indigestible marker at the rate of 0.6g per 100g of diet.

Two diets were prepared, (Diet C and Diet D, Table 3.2). Both the rat and salmon test diets and the experimental methods were standardised as much as practical. The basal rat diet (Diet D) was formulated to have an ingredient composition similar in all respects to the salmon basal diet

Table 3.2 Ingredient and Chemical Compositions of the Experimental Diets (air dry weight basis) for the Rat.

	Diet C	Diet D
Ingredient (g)		
Cornstarch	40.000	50.00
Enerva fishmeal	25.448	31.81
Blood meal[a]	19.880	0.00
Pollard	4.576	5.72
Fish oil	4.488	5.61
Wheat	2.744	3.43
Skimmilk	1.992	2.49
Rovimix 25 Vit C	0.024	0.03
Chromic oxide	0.600	0.60
Betaine anhydrous	0.196	0.12
Mineral premix[b]	0.196	0.12
Vitamin premix[b]	0.056	0.07
TOTAL	100.000	100.00
Nutrient (%)		
Crude protein	37.78	26.02
Fat	8.59	9.60
Moisture	7.40	6.29
Amino acid (g/100g)		
Arginine	1.95	1.30
Histidine	1.86	0.91
Isoleucine	1.11	1.15
Leucine	4.20	1.99
Lysine	2.98	1.64
Methionine	0.88	0.79
Phenylalanine	2.45	1.22
Threonine	1.63	0.85
Valine	2.82	1.28
Alanine	2.61	1.44
Aspartic acid	1.83	2.12
Cysteine	0.34	0.22
Glutamic acid	4.19	2.98
Glycine	1.91	1.34
Proline	1.61	1.06
Serine	1.28	0.60
Tyrosine	1.19	0.73

[a]Experimental blood meal C collected from a plant in New Zealand (section 3.2.1).

[b]Special rat mineral and vitamin premix, Pantech, Auckland.

except that a rat vitamin/mineral premix was used and at a marginally different inclusion level and cornstarch was used to dilute the diets by 50% (50g starch added to 50g mixture). The experimental diet (Diet C) was formulated as 80% basal and 20% blood meal C.

Animals, housing and feeding

The experiment was carried out at the Small Animal Production Unit, Massey University, Palmerston North. Eighty male and female Sprague-Dawley rats, between 80 and 157g in body weight and with a mean body weight of 124g, were kept individually in raised stainless steel cages with wire mesh floors, held in racks with ten cages per row. The room was temperature controlled (21°C ± 1°C) with a reversed 12 h light/dark cycle.

The rats were randomly allocated to either the experimental (Diet C) or basal diet (Diet D) such that there were 40 rats per diet. Rats were then randomly allocated to cages.

Each rat had free access for a 1 hour period to its respective diet three times a day (09:00 - 10:00, 12:00 - 13:00, 16:00 - 17:00) each day for a 7 or 8 day period. The experiment was designed to run for a 7 day period, as with the salmon, but it was logistically not possible to slaughter and sample eighty rats on one day. For this reason half the rats in each diet treatment group were slaughtered on the 8th day. Rat live weights were recorded at the start of the trial and at slaughter (Appendix 6, Table 5.4). Water was available *ad libitum*.

Slaughter and collection of digesta

On the 7th and 8th day, rats were killed 1 hour after the final feeding (asphyxiation in carbon dioxide gas followed by decapitation). Digesta were removed from the terminal 20 cm of ileum by flushing with distilled water. Ileal digesta from groups of 5 rats (3 males and 2 females) were pooled to give 8 experimental observations for each diet. Digesta samples were frozen (-20°C) in sealed plastic bags immediately after collection.

Prior to analysis, all samples (digesta and diet) were freeze-dried to a constant weight and then finely ground (less than 1mm mesh) for duplicate determination of nitrogen, amino acids and chromium.

Data Analysis

Apparent digestibilities of the nutrients in the diets and blood meal C were calculated as described in the previous section (refer section 3.2.3 Data Analysis).

$$\text{Digestibility}_{ijkl} = u + \text{Species}_i + \text{Diet}_j + e_{ijk}$$

The apparent amino acid digestibilities for Diet C were subjected to analysis of variance (ANOVA) using the SAS general linear models computer programme.

3.3 Results

3.3.1 Digestibility in chinook salmon

Salmon behaviour in the experimental tanks was generally settled, and care was taken throughout the trial to minimise disturbance around the tanks. Previously, when fish were held in small experimental tanks cleaning was found to disturb the fish and reduce feeding activity for 12-24 hours. Therefore tanks were cleaned only after sampling, and not at all during each 7 day experimental period. There were no mortalities on any dietary treatment during the trial.

Daily water temperatures for the trial period ranged from 14.7°C to 16.8°C, with an average of 15.3°C. Dissolved oxygen (DO) averaged 7.25 mg/l, ranging from 6.9 mg/l to 7.45 mg/l. Both temperature and DO values were well within the accepted tolerance range for chinook salmon.

Partly because of the infrequency of cleaning, water visibility was low and it was difficult to monitor and respond to feeding behaviour while hand-feeding. Therefore, on all days fish were fed the complete 200g feed allocated per tank. Feeding behaviour on any particular day was variable. On some days salmon fed more vigorously and aggressively than on others. No link could be discerned between feeding and the experimental variables (dissolved oxygen, temperature, time etc) which were measured. Variation in feed intake was supported by the faecal sampling results. For the test period 14-21 days, feeding on the day of sampling was poor, and it was found on sampling that only 4-6 fish in each treatment had faecal contents. A minimum of 2 fish were bulked to give a sample. Sample volumes were insufficient for N analysis, therefore protein digestibility was calculated as the sum of digestible amino acids.

There was no statistically significant effect of tank on AA digestibility. A significant effect of treatment date was found only for the amino acids isoleucine, cysteine and proline ($P<0.05$). As a result the digestibilities for the basal diets were combined over all treatment days and the average was used in the calculation of the blood meal digestibilities.

Blood meal amino acid digestibilities calculated from initial chemical analysis were highly variable across replicates and in the case of blood meal C resulted in predominantly negative digestibility values. A review of the data indicated that a source of error could be the apparently low determined chromium values (Appendix 7, Table 5.5). Chromium was added to the experimental diets at 0.6% air dry weight, while analytical dietary analysis found mean dietary

chromium (on a dry matter basis) of 0.442%, (0.408% air dry weight). Given expected losses of chromium on diet mixing, this value appears reasonable. The coefficient of variation (CV) for dietary chromium (4 mean diet values) was 2.9%. In contrast, the CV's for faecal chromium values for diets A, B, C and D were much higher at 29.38%, 28.07%, 32.57% and 17.43% respectively. Because of the variability in the faecal chromium contents, chromium and dry matter analysis were repeated for all samples. Repeated chromium values were not consistently different to the originals, and therefore original and repeated values were pooled for the final data analysis (Appendix 7, Table 5.5).

The mean apparent amino acid digestibilities and calculated protein digestibility for blood meals A and B are shown in Table 3.3. Full digestibility results, including blood meal nutrient digestibilities of every sample, are given in Appendix 8, Table 5.6. Amino acid digestibilities for spray dried blood meal B were higher than that for ring dried blood meal A. For the amino acids histidine, leucine, lysine, phenylalanine, threonine, valine, alanine, proline and serine these differences were statistically significant ($P<0.05$). Isoleucine showed consistently low digestibility for both blood meals. Low digestibility of isoleucine is probably an artefact of the low isoleucine levels inherent in blood, and error attributable to the difference method employed here.

Results from blood meal C are not shown, as amino acid digestibilities calculated from the experimental data were negative. It appears that the digestibility of protein and amino acids in batch dried blood meal C was very low in chinook salmon. The results obtained may be attributable to problems in faecal sampling, though this would appear unlikely given the reasonable results obtained for diets A and B. The low faecal recoveries throughout the experimental period, particularly on days 14-21, may have compromised collection of representative faecal samples for analysis. Experimental error may also have been compounded by the error inherent in the method used whereby digestibilities were calculated by difference. The digestibility of protein and amino acids in diet C was very low, and when estimating a small difference between two large numbers, mathematically this results in a greater error.

The data were re-examined using the digestibility values for test diets A, B, C and basal diet D, without correcting for the digestibility of the basal diet (Table 3.4). This avoided any error contributed by the difference method, and provided useable data for diet C. For all amino acids mean diet digestibilities were ranked: basal diet D > Diet B > Diet A > Diet C, with the differences between diet A and B being statistically non significant. There was a statistically significant difference between diet C and the other diets for digestibility of all amino acids.

Table 3.3 Mean (±SE) Apparent Faecal Digestibility of Protein and Amino Acids in Two Blood Meals for Chinook Salmon .

Component	Apparent Digestibility (%) [1]	
	A	B
Protein [2]	46.3	68.4
Amino acid		
Arginine	31.4±10.62[a]	40.9±10.92[a]
Histidine	53.7±6.49[a]	73.7±6.54[b]
Isoleucine	2.6±19.89[a]	-5.1±18.03[a]
Leucine	62.5±6.14[a]	80.7±6.35[b]
Lysine	60.0±6.10[a]	75.8±7.49[b]
Methionine	45.4±10.14[a]	56.5±8.50[a]
Phenylalanine	60.0±6.33[a]	77.9±5.24[b]
Threonine	54.8±8.43[a]	76.3±7.22[b]
Valine	61.5±6.30[a]	79.6±5.87[b]
Alanine	60.7±7.01[a]	78.7±7.35[b]
Aspartic acid	54.8±7.18[a]	68.3±6.55[a]
Cysteine	14.3±13.52[a]	15.9±14.74[a]
Glutamic acid	45.5±10.22[a]	59.4±10.52[a]
Glycine	37.6±12.88[a]	56.2±9.58[a]
Proline	11.1±20.28[a]	64.4±8.93[b]
Serine	54.7±8.05[a]	72.1±6.19[b]
Tyrosine	47.3±9.54[a]	54.4±7.64[a]

[1] Digestibility of nutrient in the blood meal after correction for the basal diet.

[2] Calculated from the sum of amino acid digestibilities.

[a-c] Means within the same row, not sharing the same superscript are significantly different ($P<0.05$).

Table 3.4 Mean (±SE) Apparent Faecal Digestibility of Amino Acids in the Experimental Diets for Chinook Salmon.

	Apparent Digestibility (%) [1]			
Amino Acid	**A**	**B**	**C**	**D**
Arginine	76.6±2.53^{a}	78.5±2.58^{a}	53.7±1.87^{b}	90.1±0.67^{c}
Histidine	75.2±2.44^{a}	82.5±2.56^{ab}	25.7±3.07^{c}	88.2±0.79^{b}
Isoleucine	78.7±2.19^{a}	78.7±2.49^{a}	72.5±0.71^{b}	87.8±0.72^{c}
Leucine	76.7±2.66^{a}	84.6±2.81^{a}	27.9±3.94^{b}	87.6±1.05^{a}
Lysine	79.5±2.07^{a}	84.5±2.73^{a}	41.5±3.12^{b}	89.5±0.74^{a}
Methionine	74.8±2.18^{a}	76.3±2.24^{a}	60.0±1.89^{b}	83.3±1.26^{c}
Phenylalanine	74.1±2.76^{a}	81.8±2.42^{a}	25.8±3.73^{b}	85.1±1.12^{a}
Threonine	74.2±2.84^{a}	81.2±2.61^{a}	32.3±3.82^{b}	84.0±1.21^{a}
Valine	75.6±2.74^{a}	83.5±2.60^{a}	27.3±3.90^{b}	86.5±1.16^{a}
Alanine	78.4±2.56^{a}	84.8±2.81^{a}	36.6±3.33^{b}	88.6±0.87^{a}
Aspartic acid	69.3±2.44^{a}	73.7±2.30^{a}	28.3±2.96^{b}	76.7±1.43^{a}
Cysteine	45.77±4.99^{a}	48.0±4.90^{a}	20.7±2.61^{b}	64.0±2.24^{c}
Glutamic acid	77.9±2.50^{a}	81.3±2.61^{ab}	53.5±1.95^{c}	88.5±0.77^{b}
Glycine	73.2±3.05^{a}	77.4±2.32^{ab}	46.3±1.94^{c}	84.2±0.89^{b}
Proline	75.4±3.07^{a}	80.4±2.57^{a}	44.8±2.67^{b}	86.8±0.94^{c}
Serine	72.9±2.87^{a}	78.9±2.37^{a}	29.1±5.40^{b}	83.0±1.30^{a}
Tyrosine	72.8±2.76^{a}	75.0±2.33^{ab}	43.5±2.36^{c}	84.0±1.03^{b}

[1] Digestibility of nutrient in the experimental diet as fed (basal plus blood meal).

$^{a-c}$ Means within the same row, not sharing the same superscript are significantly different ($P<0.05$).

3.3.2 Ileal digestibility in rats

The rats remained healthy, consumed the diet readily and gained in weight over the 7 day trial period (Appendix 6, Table 5.4) There was a single mortality on day 6 of one male rat fed Diet D.

The rat digestibility data were treated in the same way as described in section 3.3.1 for the salmon. The digestibilities for the basal diets were combined over all treatment days and the mean used in the calculation of the blood meal nutrient digestibilities. Chromium and dry matter analyses were repeated for all samples, and original and repeated values were pooled for the final data analysis (Appendix 7, Table 5.5).

The mean apparent digestibility of protein and amino acids in blood meal C was low in the rat (Table 3.5). Digestibility values for protein and amino acids in diet C obtained from chinook salmon were not available to be statistically compared with values obtained in the rat, but chinook salmon digestibility values were also generally low.

The data were re-examined using the digestibility values for diet C (basal + blood meal C) in the chinook salmon (Table 3.4) and diet C (basal + blood meal C) without correcting for the digestibility of the basal diet (Table 3.6). A significant species difference between the rat and chinook salmon for digestibility of diet C was found for only 5 of the 17 assayed amino acids, these were leucine, tyrosine, phenylalanine, histidine, and cysteine. In all 5 amino acids, the magnitude of the digestibility difference between species was small, with the lower dietary digestibility values being recorded in chinook salmon.

3.4 Discussion

The lack of customised facilities for the sea water digestibility trials in chinook posed some difficulties in the study. Tanks passing effluent through a series of mechanically rotating screens (Choubert et al., 1982) or the Guelph system utilising a faecal collection column (Cho & Slinger, 1979) have been found to be highly reliable methods for estimation of digestibility in salmonids. However, facilities allowing the complete collection of faeces were not available and practical alternatives that provided accurate digestibility data were needed. The chromic oxide indicator method in which the indicator is measured using faecal samples obtained by intestinal dissection or stripping is an accepted methodology in salmon digestibility trials (Nose, 1960; Austreng, 1978; Hajen et al., 1983a). This was the method employed in the present experiment, although some problems were encountered. Variable feeding behaviour led to a low recovery of faeces on some sampling days, which in turn restricted material available for nitrogen analysis and calculation of crude protein digestibility. Daily variation of up to 50% in feed intake is well

Table 3.5 Mean (±SE) Apparent Ileal Digestibility of Protein and Amino Acids in Blood Meal C for the Rat.

Amino Acid	Apparent Digestibility (%)
Protein	3.9±1.72
Amino acid	
Arginine	13.8±3.32
Histidine	8.3±3.27
Isoleucine	11.1±4.31
Leucine	13.5±2.99
Lysine	11.8±3.43
Methionine	11.3±4.21
Phenylalanine	17.0±3.16
Threonine	9.9±3.66
Valine	10.3±3.07
Alanine	13.2±3.25
Aspartic acid	1.8±3.86
Cysteine	3.9±2.12
Glutamic acid	6.0±3.55
Glycine	8.4±3.75
Proline	6.3±3.65
Serine	5.1±6.49
Tyrosine	17.6±4.57

Table 3.6 Mean (±SE) Apparent Ileal Digestibility of Amino Acids in Experimental Diet C for the Rat and Chinook Salmon.

	Apparent Digestibility (%) [1]	
Amino acid	**Diet C Rat**	**Diet C Chinook**
Arginine	57.9±1.33[a]	53.7±1.87[a]
Histidine	41.3±1.84[a]	25.7±3.07[b]
Isoleucine	71.0±0.79[a]	72.5±0.71[a]
Leucine	41.9±1.83[a]	27.9±3.94[b]
Lysine	48.1±1.61[a]	41.5±3.12[a]
Methionine	66.6±0.55[a]	60.0±1.89[a]
Phenylalanine	44.2±1.93[a]	25.8±3.73[b]
Threonine	40.9±1.99[a]	32.3±3.82[a]
Valine	38.6±1.88[a]	27.3±3.90[a]
Alanine	46.6±1.73[a]	36.6±3.33[a]
Aspartic acid	32.2±2.06[a]	28.3±2.96[a]
Cysteine	33.9±1.01[a]	20.7±2.61[b]
Glutamic acid	53.4±1.35[a]	53.5±1.95[a]
Glycine	46.4±1.75[a]	46.3±1.94[a]
Proline	45.0±1.71[a]	44.8±2.67[a]
Serine	40.2±2.44[a]	29.1±5.40[a]
Tyrosine	51.9±2.23[a]	43.5±2.36[b]

[1] Digestibility of nutrient in the experimental diet as fed (basal plus blood meal).

[a-c] Means within the same row, not sharing the same superscript are significantly different ($P<0.05$).

known commercially (Andrew Campbell, NZKSC; pers. comm.) and has also been reported in the literature (Negas et al., 1995). Larger tanks with capacity for holding a greater number of salmon for sampling may have reduced the impact of this problem by ensuring adequate faeces were collected for all chemical analyses.

Insufficient faecal material may have impacted upon the accuracy of the protein and amino acid digestibility values in salmon. The standard errors of these values were high (Table 3.3), and in the case of blood meal C values were also highly negative. Calculated protein digestibilities were 46.3% for ring dried blood meal A and 68.4% for spray dried blood meal B. Blood meal C showed low digestibilities, but no values are reported. Published results on the digestibility coefficients of crude protein of blood meal by salmonids are extremely variable (Table 1.9). Reported digestibilities of spray dried blood meal vary from 69.1% when included at 25% in the test diet, to 86.0% when included at 50% in the test diet (Smith et al., 1980). In ring dried blood meal reported crude protein digestibilities vary from 29.4% (Hajen et al., 1993b) to 89.4% (Smith et al., 1980). Hajen et al. (1993b) attributed the low digestibility reported in blood meal tested to excessive heat during processing. Digestibility values in salmon for batch dried blood meal have not been previously reported. In mink, Borsting (1992) compared digestibility coefficients in batch dried and spray dried blood, and found them to be 0.64 and 0.89 respectively, he concluded the former to be unsuitable for fur animal feed.

Low digestibilities were found for all amino acids in the present study. Apparent digestibilities were highest in spray dried blood meal (Blood B), followed by ring dried blood (Blood A), and were lowest in batch dried blood (Blood C). Differences between blood meals A and B were statistically significant for 9 of the 17 assayed amino acids. Comparison of digestibility values for experimental diets, including data from diet C, showed a similar digestibility ranking for all amino acids, with the differences between diets A and B being statistically non significant. Digestibility of all amino acids in diet C containing batch dried blood meal was significantly lower than that for diets A or B. While protein digestibility values are available for blood meal in salmonids (Cho & Slinger, 1979; Smith et al., 1980; Brown et al., 1985; Asgard & Austreng, 1986; Luzier et al., 1995), amino acid digestibility was not reported in these studies, and therefore these values are not available for comparison with the present experimental findings. In other species data are available on the digestibility of amino acids in blood meal. In the rat, ileal digestibilities of essential amino acids in blood meals from 7 different New Zealand plants ranged from 44.0% to 92.9% (Pearson, 1992).

Experimentally determined amino acid digestibilities in blood meals were very variable within dietary replicates. This can be seen by the high standard errors of mean digestibility values (Table

3.3). In addition, the accuracy of results is questionable as blood meal C recorded consistently negative amino acid digestibility values for salmon. This initially appeared to be the result of low chromium levels in faecal samples. Repeated chromium analysis failed to identify significant analytical variation, with results in general agreement with original assayed chromium values. Mean amino acid digestibilities recorded in the rat for blood meal C were positive, with lower standard errors. The apparent digestibility data were more variable for the salmon, (SE ranging from ±5.24 to ±19.89), than for the rat, (SE range from ±1.72 to ±6.49). This variance difference between species indicates that the outcome may be attributable to a factor associated with the salmon experimental design rather than to laboratory analytical techniques.

The implication is that some sampling or other experimental factor in the salmon digestibility trial is involved in the problems experienced with these results. The use of chromic oxide as an indicator in salmonid digestibility trials has been repeatedly validated (Austreng, 1978; Hajen et al., 1993a). In the literature, doubts have been raised about the accuracy of the technique, Bowen (1978) reported the differential passage of chromic oxide in the gut, while in the lobster Leavitt (1985) found chromic oxide was absorbed through the intestinal wall. The method of faecal collection may also be a source of error. Collection of faeces by stripping has been shown to result in lower digestibility values than those determined using faeces voided from fish and collected in the water column (Cho & Slinger, 1979; Hajen et al., 1993). Although faecal collection by stripping avoids the problem of leaching, there may, according to Austreng (1978), be a reduction of the transit time of intestinal contents with possible concomitant reduction in nutrient absorption. The use of faeces removed from the fish by stripping assumes that digestion and absorption are complete when the material has reached the distal end of the intestine. This is generally held to be true, with bacterial activity in the large intestine in salmonids reportedly negligible. However the hindgut has been implicated in amino acid absorption (Austreng, 1978; Dabrowski & Dabrowska, 1981), the extent of which has not yet been quantified. Austreng (1978) concluded the difference between the methods of collection of faeces in the water column and collection by stripping is not significant if stripping occurs in the hindmost part of the rectum, ie. from the ventral fin to the anus. Another minor possible source of error may be due to daily fluctuations in digestibility which has been reported by some authors (De Silva & Perera, 1983; Negas et al., 1995). This suggests that stripping fish once will not provide a representative sample. The achievement of representative faecal samples may also have been compromised by the sampling day with poor faecal recovery, and low faecal recoveries over the entire experimental period. That sampling error was a significant error source in the presently reported results does, however, appear unlikely given the reasonable digestibility values obtained for diets A and B.

The greater error associated with digestibility values for blood meal C may have been compounded by the method used for determining digestibility values by calculating differences between a basal and test diet. Where digestibilities are very low, this error may have a significant impact on results. When blood meal digestibility was not corrected using basal diet digestibility, amino acid digestibility values for the diets clearly showed a significantly lower digestibility of the experimental diet C containing batch dried blood meal. The use of basal diets in fish digestibility studies is a common methodology (Cho & Slinger, 1979; Asgard & Austreng, 1985; Hajen et al., 1993b). An alternative method used to determine digestibility of feedstuffs is to feed semi-purified basal diets (Brown et al., 1985).

The blood meals used in the presently reported experiments were chosen for their very different processing methods. Blood meal C was batch dried, spending 2.5-3.5 hours residence time at 105-110°C. Blood meal A was ring dried, experiencing only 10 minutes drying at lesser temperatures of 90°C. The spray dried blood meal (Blood meal B) received the least damaging heat treatment, with only 30 seconds in cooking and drying processes. In other species and on other feed ingredients there is evidence that heat treatment during processing will reduce the digestibility of protein and amino acids (eg March & Hickling, 1982; Pike et al., 1990). The processing method used in rendering, particularly drying times, has been shown to be negatively correlated with the quality of blood meal as determined by rat ileal N digestibility (Meads et al., 1995). In the rat, crude protein digestibility of amino acids in spray dried blood of 94.6%, in ring dried blood meal of 70.2%-92.0% and in batch dried blood meal of 17.0%-65.1% have been recorded (Pearson, 1993). Pike et al. (1990) report heat treatment of fishmeals had a severe effect on protein digestibility, amino acid digestibility, feed intake, growth and feed conversion in chinook salmon. In low temperature fishmeal these authors reported that the apparent digestibility of essential amino acids ranged from 76.4% to 93.7%. Based on the present experimental findings of decreasing amino acid digestibility in spray, ring and batch dried blood meals respectively, evidence of similar ranking in crude protein digestibility in other species, and findings of poor amino acid digestibility in salmonids in heat treated fishmeal, it is concluded that heat treatment applied during processing will probably reduce amino acid digestibility in salmonids.

The high digestibility of spray dried blood (blood meal B) makes it the most suitable protein ingredient in salmon diets, but even here some low amino acid digestibility values were recorded. Very low digestibility values obtained for diets containing batch dried blood (diet C) make it unsuitable as an ingredient in high quality salmon feeds, and, compared with fishmeal, a relatively expensive source of digestible protein. Literature recommendations are that spray dried blood meal may be included at levels varying from 5% (Fowler & Banks, 1976) to 22.7%

(Luzier et al., 1995). Based on the experimental results, ring dried blood meal would also be suitable for inclusion at low dietary levels.

In the second part of this study, the laboratory rat was evaluated for suitability as a model animal to allow the determination of apparent amino acid digestibility in chinook salmon. For an inter-species comparison of digestibility to be valid, the species must be examined under comparable conditions. Every effort was taken during the design of this study to maintain such comparability, where possible. The body weights of both salmon and rats were selected to correspond to that of an immature life stage in both animals. Feeding in both species was *ad libitum*, three times daily. The experimental period was set at 7 days for both animals. In practice, the different species used and environment in which the animals needed to be maintained meant that comparability was only possible in a limited way. One difference in method involved faecal sampling in salmon and ileal sampling in rats for digestibility analysis. In rats ileal protein digestibility is considered to provide a more accurate estimate of amino acid and protein digestibility, a comparison of rat ileal digestibility values with faecal digestibility showed the latter to overestimate the digestibility of protein due to protein degradation by the hindgut microflora, and subsequent poor utilisation of these breakdown products (Moughan et al. 1984). In salmon bacterial activity has been recorded, although this has not been confirmed as of endemic origin (Achene et al., 1989), numbers are typically low, and their role in digestion is generally assumed to be negligible. For this reason, faecal digestibility measures are considered an accurate assessment of digestibility in salmon. Differences in experimental techniques and designs may have had an effect on digestibility values.

Ileal digestibility of protein and amino acids in blood meal C for the rat was very low, ranging from 3.9% for protein to a high of 17.6% for tyrosine. The variability of digestibility of crude protein assessed by rat ileal digestibility in New Zealand blood meals is well recorded, as is the low protein and amino acid digestibility in batch dried blood meals (Pearson, 1993; Meads et al., 1995). Chinook salmon also showed low digestibility of blood meal C. A comparison of digestibility of amino acids in diets C in the rat and salmon found agreement between species for all amino acids tested with the exceptions of leucine, tyrosine, phenylalanine, histidine and cysteine. Digestibility of these amino acids was slightly but significantly lower in chinook salmon. Other studies examining the agreement between rat and salmonid digestibility values in feedstuffs have not been found, despite anecdotal evidence that rats may provide comparable digestibility values (Romero et al., 1994). The rat has been shown to have value as a model for other species. There is general agreement between the rat and pig for the ileal digestibility of protein and amino acids in several feed ingredients (Moughan et al., 1984; Donkoh et al.; 1994 Moughan et al., 1984; 1987). This is not the case for all feedstuffs, Moughan et al. (1984) found

significant differences between rats and pigs for the apparent ileal digestibility of protein in peas. Without further comparative digestibility trials no definitive conclusion can be reached on the suitability of the rat as a model for digestibility of feedstuffs in salmon. However, the laboratory rat assay offers a rapid and inexpensive technique for determining digestibility of feedstuffs. If its validity can be confirmed more generally it will provide an opportunity to improve prediction of digestibility of locally manufactured feedstuffs for use in the diets of salmonids. Both the rat and chinook salmon showed very low protein and amino acid digestibility in batch dried blood meal in the presently reported study. These initial results indicate that the rat may be a suitable model animal to evaluate digestibility of blood meal in chinook salmon.

CHAPTER 4

GENERAL CONCLUSION

To satisfy the high dietary requirements of salmon for protein, formulated diets need to contain 40-70% fishmeal. With predictions of increasing fishmeal prices and volume shortages (Barlow, 1989), substitution of part of the fishmeal component of salmon diets with blood meal has been investigated (Asgard & Austreng, 1986; Hajen et al., 1993b; Luzier et al., 1995). Blood meals are an important source of protein in the New Zealand stock food and aquaculture industry. The digestibility of blood protein has been found to vary considerably with the manufacturing process used (Dawson, 1988; Pearson, 1993; Borsting, 1992; Meads et al., 1995), making it necessary to evaluate the protein and amino acid digestibility of blood meal prior to its inclusion in formulated salmon diets.

The present findings show that, at least for the blood meals examined, crude protein and amino acid gross compositional values are a poor indicator of protein quality. All blood meals contained uniformly high crude protein and amino acid levels. The pronase *in vitro* protein digestibility method provided a useful method of ranking the blood meals for feed quality quickly and cheaply. *In vitro* protein digestibilities measured using the pepsin and pronase methods were highest for spray dried blood (pepsin digestibility 27.8%, pronase digestibility 60.2%), lower for ring dried blood (pepsin digestibility 18.9%, pronase digestibility 25.5%), and very low for batch dried blood (pepsin digestibility 8.4%, pronase digestibility 9.0%).

For determining the amino acid digestibility of blood meal in chinook salmon, *in vivo* digestibility trials, although more difficult, should provide the greatest accuracy of results. However, experimental results from digestibility trials in chinook salmon showed a high degree of variation about the mean digestibility values, and in the case of blood meal C digestibility values were consistently negative. The reason for this was not clearly established in the present work, but may reflect a need to improve the sampling protocol to ensure that representative samples and adequate volumes of faeces are collected for analysis. In addition, estimating amino acid digestibility in a feedstuff by comparison with the basal diet may increase the error when the feedstuff tested has a very low digestibility. *In vivo* salmon trials ranked blood meal digestibility of protein and amino acid in the experimental diets and blood meals in the same order as did the *in vitro* assays, (spray dried blood meal/diets > ring dried blood meal/diets > batch dried blood meal/diets). In the case of the diet containing batch dried blood meal, amino acid digestibilities were significantly lower than in diets containing ring or spray dried blood meal.

The *in vitro* digestibility assays used in the present study, pepsin and pronase, ranked protein digestibility of blood meals in the same order as did the *in vivo* salmon digestibility trial, but more rapidly and at lower cost. However, actual protein digestibility values were very different between these methods. Rat digestibility trial protocols and facilities are commercially available for the evaluation of feedstuffs in New Zealand, and are also a less costly alternative to *in vivo* salmon trials. Amino acid digestibility values of diets containing blood meal C were statistically comparable between the rat and chinook salmon for all but 5 assayed amino acids. This offers the possibility that the rat may be a suitable animal model for the salmon in the evaluation of protein digestibility for feedstuffs.

For the replacement of fishmeal in salmon diets, the chemical profile of blood meal compares favourably with that of quality whole fishmeals (Table 4.1). Digestibility values, however, are consistently highest for spray dried blood meal and lowest for batch dried blood meal, clearly differentiating these as suitable protein feed ingredients in salmon diets.

For a full evaluation of blood meal quality for use in salmon diet formulations it is useful to determine both the availability of nutrients and the cost per unit of available nutrient. This calculation is shown in Table 4.2 for blood meals and fishmeal. When the cost/unit digestible protein is calculated, and at current prices, best value as a protein source is represented by fishmeal. Spray dried blood meal has the most value of the blood meals tested as a replacement for fishmeal, due to its high crude protein level, and high protein digestibility relative to the other blood meal sources. For batch dried blood meal a minimum 57% protein digestibility would be required to achieve a cost/unit digestible protein equivalent to fishmeal.

An important factor determining the digestibility of proteins in blood meals appeared to be processing conditions, with batch dried blood meal showing the lowest digestibility measures for both *in vitro* and *in vivo* methods. Further analysis of the effect of blood meal processing on digestibility in chinook salmon is required, but data reported here and from other species would seem to support this assertion. Spray dried blood meal and lower levels of ring dried blood meal appear to be suitable for inclusion in salmon diets, however the poor digestibility of batch dried blood meal confirms it as a poor protein source in salmon diets.

Further work needs to be conducted to replicate the *in vivo* salmon digestibility assay to reduce the variation of observations about the mean.

Table 4.1 Chemical Composition and Digestibility of Experimental Blood Meals Compared with Known Values for Whole Fishmeal.

	Blood A	Blood B	Blood C	Fishmeal	Reference
N Digestibility (%)					
Pepsin (Torry)	18.9	27.8	8.4	93.6	Anderson et al. 1993
Pronase	25.5	60.5	9.0	55.0	Johnston & Savage 1987
Salmon in vivo	46.3[a]	68.4[a]	-	90.5	Hajen et al. 1993b
Rat in vivo	-	-	3.9	82.0	Moughan et al. 1984
Chemical (% air dry)					
Crude protein	93.06	89.62	92.00	72.0	NRC 1993
Moisture	6.98	9.29	6.76	8.0	NRC 1993
Essential AA's					
Arginine	3.63	3.49	3.77	4.54	NRC 1993
Histidine	5.13	5.47	5.67	1.65	NRC 1993
Isoleucine	0.80	0.66	0.85	0.74	NRC 1993
Leucine	10.96	11.40	11.70	5.19	NRC 1993
Lysine	7.37	8.22	7.89	5.57	NRC 1993
Methionine	1.11	1.31	1.22	2.08	NRC 1993
Phenylalanine	5.88	6.53	6.21	2.71	NRC 1993
Threonine	3.79	4.23	4.32	2.90	NRC 1993
Valine	7.69	7.90	8.30	4.30	NRC 1993

[a] Calculated from digestible amino acid values

Table 4.2 Comparison of Blood Meals and Fishmeal on a Cost/Unit Digestible Protein Basis.

Blood A		**Blood B**		**Blood C**		**Fishmeal**[1]	
Cost/t[2]	$1050	Cost/t[2]	$1300	Cost/t[2]	$1000	Cost/t[2]	$1250
Protein (%)	93.1	Protein (%)	89.6	Protein (%)	92.0	Protein (%)	72.0
Cost/unit protein	$11.28	Cost/unit protein	$14.50	Cost/unit protein	$10.87	Cost/unit protein	$17.36
Digestibility[3]	46.3	Digestibility[3]	68.4	Digestibility[3]	0.00	Digestibility[3]	90.5
Cost/unit DP[4]	$24.36	Cost/unit DP[4]	$21.20	Cost/unit DP[4]	***	Cost/unit DP[4]	$19.18

[1] Fishmeal values taken from quality whole herring meal (NRC, 1993)

[2] Cost taken as estimate of 1995 values (pers comm. Salmon Services, 1994)

[3] *In vivo* protein digestibility calculated in salmon from digestible amino acid values

[4] Cost/unit DP = Cost/unit digestible protein

APPENDIX 1

BLOOD MEAL PRODUCTION QUESTIONNAIRE

Company name: _____ **Plant location:** _____

Main species used in blood production (cattle/sheep/deer etc): _____

_____

Tonnage blood produced 1993: _____ (Export: _____ Local: _____)

Months of the year rendering operates: _____

Production method (Spray-dried/ring dried/batch dried/other (please specify):

_____

_____

_____

Rendering/drying temperatures and times: _____

_____

Specifications: (Protein, moisture, lysine, digestibility, other (please specify):

_____

_____

_____

Other blood rendering plants in your geographical area:

_____

Comments: _____

_____

Questionnaire completed by: _____ **Title:** _____

APPENDIX 2

METHODS TO DETERMINE PEPSIN AND PRONASE N DIGESTIBILITY

A) Method for Pepsin Digestibility

Procedure carried out by Lincoln University using the method described by Dawson (1988), adapted from the Torry Research Station, Aberdeen:

Samples were not pre-ground to avoid unrealistically high values for the material which, if ground, would be made physically more accessible to digestion.

1. Weigh 1 g of sample into a 200 ml dispensing bottle

2. Make up 0.75 M HCL and place 2 litre batches in a water bath which was set at 45°C

3. Add sufficient Sigma pepsin (1:10,000 activity) to the pre-warmed acid to make a 0.0002% pepsin solution and carefully mix.

   (Do not make up more pepsin solution than can be dispensed in 2 h)

4. Dispense 150 ml of the pepsin solution into the dispensing bottle with pre-weighed sample. cap and mix. Place the bottle on its side in a 45°C shaking water bath and shake for exactly 16 h.

5. After 16 h filter the warm digestion mixture through a fibreglass filter paper (Whatman GFA 12.5 cm) using a Harvey filtering funnel and 1 g celite filtering aid. Wash the digest from the bottle and rinse the residue with 400 ml of hot distilled water.

6. Place the residue plus fibreglass filter paper in a suitable Kjeldahl digestion flask and digest to determine the nitrogen which remains after the digestion.

7. Repeat the above procedure using the acid without any added pepsin.

8. Determine the residual moisture and nitrogen content of the original sample.

9. Calculate the true pepsin digestibility (TPD).

$$TPD = 100 - \frac{(\text{N acid residue} - \text{N pepsin residue})}{\text{Total N in original dry weight of sample}} \times 100$$

Appendix 2 contd...

B) Method for Pronase Digestibility

Procedure carried out by Lincoln University using the method of Dawson (1988); adapted from Taverner & Farrell (1981).

1. 1 g samples were placed in a 50 ml dispensing bottle.

2. 4 mg pronase (B grade, Calbiochem), 20 ml 0.04 M borate buffer (Ph 8.0) and three drops of toluene were added to each sample.

3. The bottle was then placed in an oscillating constant temperature water-bath at 27°C for 16 hours.

4. At the end of the incubation period, 10 ml of sulphosalicylic (150 g/l) was added to the bottle to precipitate the unhydrolysed protein.

5. The bottle with its contents were shaken for another 20 minutes.

6. The contents of the bottle were then transferred to a 50 ml volumetric flask and made up to volume with distilled water.

7. The entire digesta sample was then centrifuged at 1000 g for 30 minutes.

8. The supernatant was sucked into a clean 50 ml dispensing bottle. Two 10 ml portions of the supernatant fraction were taken to determine soluble N using the Kjeldahl technique. Protein digestibility was determined as the ratio, soluble N: total sample N.

Table 5.1 Summary of New Zealand Blood Meal Production Survey Results

Number of companies surveyed: 47

Number of companies responding: 32

Number of NZ companies producing blood meal: 23

Company	1993 Production (t)	Production Dates	Species	Production method	% Protein	% Moisture
Affco Northland	not provided	Oct - Oct	Cattle, goats, sheep	Ring	not provided	not provided
Affco Whangarei	not provided	Oct - Oct	Cattle	Spray	not provided	not provided
PVL Proteins Ltd	250	Oct - Oct	Cattle, pig, sheep	Other	90%	10%
Waikato Byproducts	not provided	Oct - Oct	Cattle	Other (flow dry)	80-85%	10%
J.D.Wallace Waitoa	504	Oct - Oct	Cattle,deer, pigs, sheep	Ring	85%	10% max
Affco Horotiu	not provided	Oct - Oct	Cattle	Ring	90%	5-8%
Affco Rangiuru	>500	Oct - Oct	Cattle, sheep	Batch	not provided	not provided
Affco Wairoa	200	Oct - Jul	Cattle, goats, sheep	Ring	not provided	not provided
Lowe Walker Hawera	not provided	Oct - Oct	Cattle	Other	not provided	not provided
Affco Imlay	517	Oct - Oct	Cattle, sheep	Batch	90-95%	5-10%
Richmond Hastings	280	Oct - Aug	Cattle	Batch	94%	8.5%
Richmond Takapau	627	Oct - Oct	Cattle, sheep	Ring	92-97%	8-10%

Company	1993 tonnes	Production	Species	Production method	% Protein	% Moisture
Nelson Bays	not provided	Oct - Oct	Cattle, sheep	Ring	90 - 92%	6%
PPCS Riverlands	not provided	not provided	Cattle, sheep	Ring	85% min	not provided
PPCS Canterbury	not provided	not provided	Cattle, sheep	Ring	85% min	not provided
Canterbury Meat Packers	not provided	Sep - Jul	Sheep	Low temp	92-96%	6%
Alliance Smithfield	404	Oct - Sep	Cattle, pigs, sheep	Batch	89-92%	4-6%
PPCS Pareora	not provided	Nov - Jul	Deer, sheep	Batch	85%	8-10%
Alliance Pukeuri	432	Nov - Jun	Cattle, sheep	Batch	90-95%	5%
PPCS Finegand	not provided	Nov - Jul	Cattle, sheep	Ring	90% min	10% max
Alliance Mataura	not provided	Dec - Jun	Cattle, sheep	Ring	not provided	7%
Alliance Lorneville	641	Nov - Jun	Cattle, sheep	Other	91-96%	4-7%
Alliance Makarewa	131	Nov - Jun	Sheep	Ring	92-96%	6-8%

APPENDIX 4

Table 5.2 **Pepsin and Pronase N Digestibility (%) for Replicate Blood Meal Samples (R1-R6).**

Blood Meal	Pepsin N Digestibility (%)								
	R1	R2	R3	R4	R5	R6	Mean	SD	SE
A	17.	17.	18.	16.	21.	21.	18.85	1.99	0.81
B	28.	28.	26.	27.	30.	25.	27.78	1.79	0.73
C	8.4	8.5	8.2	8.3	7.7	8.9	8.37	0.40	0.16
Blood Meal	**Pronase N Digestibility (%)**								
	R1	R2	R3	R4	R5	R6	Mean	SD	SE
A	26.	26.	25.	25.	24.	25.	25.52	0.61	0.25
B	64.	59.	60.	57.	60.	61.	60.52	2.31	0.94
C	9.7	9.4	8.9	8.0	8.3	9.3	8.98	0.66	0.23

APPENDIX 5

Table 5.3 Final Experimental Salmon Weights (g).

Fish	1	2	3	4	5	Av	6	7	8	9	10	Av
Diet A												
Day 1-7	547	426	526	557	286	**468**	448	517	407	476	598	**489**
Day 7-14	404	551	495	523	580	**511**	433	537	394	435	325	**425**
Day 14-21	526	439	449	589	524	**505**	446	480	530	486	310	**450**
Day 21-28	710	535	610	487	679	**604**	518	533	553	595	322	**504**
Diet B												
Day 1-7	495	330	536	609	490	**492**	404	315	556	358	480	**423**
Day 7-14	485	467	475	517	469	**483**	359	516	626	601	335	**487**
Day 14-21	620	377	612	329	519	**491**	484	668	544	537	607	**568**
Day 21-28	625	485	600	535	590	**567**	368	400	415	615	585	**477**
Diet C												
Day 1-7	487	432	707	290	430	**469**	658	420	561	568	493	**540**
Day 7-14	484	470	594	301	527	**475**	335	325	508	637	315	**424**
Day 14-21	495	562	462	433	447	**480**	372	577	305	553	515	**464**
Day 21-28	544	585	355	601	355	**488**	456	430	658	585	503	**526**
Diet D												
Day 1-7	428	538	640	387	587	**516**	772	565	316	530	470	**531**
Day 7-14	541	525	421	463	348	**460**	523	520	334	304	394	**415**
Day 14-21	333	496	476	318	283	**381**	224	448	375	469	227	**349**
Day 21-28	478	630	575	544	508	**547**	790	639	604	560	580	**635**

APPENDIX 6

Table 5.4 Experimental Rat Weights at Start and at Time of Slaughter (g).

Cage	Diet	Sex	Initial wt (g)	Slaughter wt (g)	Slaughter day	Total feed eaten (g)
1[a]	C	M	143.1	220.6	7	146.1
2[a]	C	M	141.7	222.5	7	137.9
4[a]	C	M	116.1	204.3	7	153.5
49[a]	C	F	112.2	163.1	7	110.5
50[a]	C	F	117.6	148.5	7	92.8
5[b]	C	M	152.6	234.8	7	162.0
8[b]	C	M	134.5	230.8	7	152.2
9[b]	C	M	134.1	214.5	7	140.7
53[b]	C	F	127.6	180.1	7	131.3
54[b]	C	F	119.8	153.2	7	113.6
10[c]	C	M	130.5	214.3	7	144.5
13[c]	C	M	140.1	247.8	7	174.3
16[c]	C	M	140.5	220.4	7	148.1
56[c]	C	F	131.7	188.6	7	136.6
58[c]	C	F	126.4	188.4	7	133.8
18[d]	C	M	142.3	228.0	7	151.2
19[d]	C	M	139.0	231.1	7	157.7
22[d]	C	M	135.5	214.5	7	138.8
59[d]	C	F	109.2	168.0	7	134.6
62[d]	C	F	113.6	178.0	7	135.6
23[e]	C	M	109.8	201.7	8	164.9
25[e]	C	M	126.7	212.0	8	155.0
26[e]	C	M	125.9	212.6	8	148.5
63[e]	C	F	85.6	144.5	8	124.8
64[e]	C	F	111.1	165.6	8	130.0
28[f]	C	M	118.1	204.0	8	141.0
31[f]	C	M	143.2	243.7	8	185.5
33[f]	C	M	122.3	216.1	8	148.1
66[f]	C	F	118.2	184.6	8	150.0
67[f]	C	F	102.9	159.6	8	127.5
37[g]	C	M	150.8	246.1	8	168.0
41[g]	C	M	149.5	244.7	8	166.4
42[g]	C	M	149.5	226.4	8	156.0
69[g]	C	F	122.1	138.0	8	116.0
74[g]	C	F	86.2	157.9	8	130.2
45[h]	C	M	116.5	227.2	8	173.0

Appendix 6 Table 5.4 contd....

Cage	Diet	Sex	Initial wt (g)	Slaughter wt (g)	Slaughter day	Feed eaten (g)
47[h]	C	M	118.0	214.7	8	157.1
48[h]	C	M	101.8	199.3	8	148.2
76[h]	C	F	100.0	136.6	8	118.3
79[h]	C	F	112.5	171.0	8	132.8
3[i]	D	M	149.0	225.6	7	138.0
6[i]	D	M	143.6	225.2	7	144.0
7[i]	D	M	157.3	241.2	7	139.2
51[i]	D	F	112.6	173.5	7	118.8
52[i]	D	F	131.3	167.5	7	108.6
11[j]	D	M	147.5	257.9	7	166.4
12[j]	D	M	135.6	211.8	7	135.5
14[j]	D	M	138.4	192.7	7	121.5
55[j]	D	F	113.6	156.0	7	113.4
57[j]	D	F	136.3	191.6	7	121.2
15[k]	D	M	134.8	220.0	7	131.5
17[k]	D	M	143.5	241.8	7	153.9
20[k]	D	M	149.6	230.1	7	134.5
60[k]	D	F	125.5	183.9	7	125.1
61[k]	D	F	123.9	160.3	7	106.6
21[l]	D	M	124.3	184.9	7	117.9
24[l]	D	M	114.9	198.6	7	134.1
27[l]	D	M	115.4	189.7	7	125.9
65[l]	D	F	88.8	151.3	7	110.6
68[l]	D	F	120.9	178.9	7	144.7
29[m]	D	M	144.2	253.3	8	179.1
30[m]	D	M	146.3	250.2	8	165.3
32[m]	D	M	113.3	196.5	8	138.5
70[m]	D	F	104.1	164.3	8	130.3
71[m]	D	F	117.1	163.9	8	130.8
34[n]	D	M	101.3	176.7	8	122.2
35[n]	D	M	118.6	211.4	8	137.4
36[n]	D	M	144.2	DIED		
72[n]	D	F	99.1	166.5	8	130.4
73[n]	D	F	80.2	160.2	8	116.6
38[o]	D	M	86.6	190.9	8	137.3
39[o]	D	M	148.9	245.9	8	171.5
40[o]	D	M	134.2	231.4	8	146.4
75[o]	D	F	93.4	159.6	8	125.3

Appendix 6 Table 5.4 contd....

Cage	Diet	Sex	Initial wt (g)	Slaughter wt (g)	Slaughter day	Feed eaten (g)
77[a]	D	F	89.5	146.1	8	116.3
43[p]	D	M	124.7	222.8	8	160.4
44[p]	D	M	144.7	244.9	8	174.8
46[p]	D	M	108.5	194.7	8	145.7
78[p]	D	F	127.5	187.3	8	135.3
80[p]	D	F	143.2	203.3	8	138.3

[a] Rats sharing the same superscript were pooled (n=5) as a single analytical sample

APPENDIX 7

Table 5.5 Summary of Chromic Oxide. Original, Repeated and Pooled Data for Chinook Salmon and the Rat (% Dry Matter (DM), % Chromium (%Cr/DM), Mean ± SD).

	Original Data		Repeated Data		Pooled Data	
Sample Description	% DM	%Cr/DM	%DM	%Cr/DM	%DM	%Cr/DM
Salmon						
Diet A	92.45	0.4227	94.04	0.3880	93.24	0.4053
Diet B	92.48	0.4512	93.99	0.3992	93.23	0.4252
Diet C	92.76	0.4454	93.85	0.4226	93.30	0.4340
Diet D	91.59	0.4485	93.27	0.4061	92.43	0.4273
Mean of Diets ± SD	92.32±0.51	0.442±0.0131	93.79±0.35	0.404± 0.014	93.05 ±0.14	0.423± 0.012
DietA Faeces D1[a]	101.00	0.4661	93.89	0.5118	93.89	0.4889
DietA Faeces, D1 R2	93.04	0.7683	92.31	0.7516	92.67	0.7599
DietA Faeces, D2 R1	93.35	0.4794	92.38	0.4918	92.86	0.4856
DietA Faeces, D2 R2	91.12	0.2911	88.41	0.2915	89.76	0.2913
DietA Faeces, D3 R1	93.34	0.4800	93.24	0.5152	93.75	0.4976
DietA Faeces, D3 R2	96.16	0.7767	92.91	0.8445	94.52	0.8106
DietA Faeces, D4 R1	93.12	0.6053	90.82	0.5826	91.97	0.5939
DietA Faeces, D4 R2	94.35	0.5771	92.43	0.5985	93.39	0.5878
Mean Diet A ± SD	94.43±3.00	0.555±0.163	92.05±1.72	0.574 ±0.169	92.85±1.48	0.564± 0.165
DietB Faeces, D1 R1	97.48	0.7277	95.44	0.6622	96.46	0.6949
DietB Faeces, D1 R2	94.31	0.4776	90.34	0.5227	92.32	0.5001
DietB Faeces, D2 R1	92.20	0.7876	93.30	0.7252	92.75	0.7564
DietB Faeces, D2 R2	90.97	0.5104	89.67	0.5473	90.32	0.5288
DietB Faeces, D3 R1	90.86	0.5122	88.75	0.5068	89.80	0.5095
DietB Faeces, D3 R2	89.17	0.3732	88.52	0.4068	88.84	0.3900
DietB Faeces, D4 R1	93.69	0.7681	92.71	0.7947	93.20	0.7814
DietB Faeces, D4 R2	91.82	0.8415	90.60	0.9273	91.21	0.8844
Mean Diet B ± SD	92.56±2.57	0.625±0.175	91.17±2.42	0.637±0.173	91.86±2.39	0.631±0.172
DietC Faeces, D1 R1	92.62	0.5427	95.08	0.5489	93.85	0.5458
DietC Faeces, D1 R2	92.97	0.4499	92.19	0.4879	95.58	0.4689
DietC Faeces, D2 R1	92.60	0.4508	91.25	0.4761	91.92	0.4634
DietC Faeces, D2 R2	92.36	0.3377	92.65	0.3545	92.50	0.3461
DietC Faeces, D3 R1	89.38	0.3676	89.96	0.3904	89.67	0.3790
DietC Faeces, D3 R2	87.68	0.2290	89.43	0.1515	88.55	0.1902

APPENDIX 7 Table 5.5 contd...

	Original Data		Repeated Data		Pooled Data	
Sample Description	**% DM**	**%Cr/DM**	**%DM**	**%Cr/DM**	**%DM**	**%Cr/DM**
DietC Faeces, D4 R1	93.86	0.6124	93.63	0.6612	93.74	0.6368
DietC Faeces, D4 R2	94.58	0.6821	94.24	0.6899	94.41	0.6860
Mean Diet C ± SD	92.01±2.31	0.459±0.149	92.30±2.01	0.470±0.174	92.53±2.40	0.464±0.161
DietD Faeces, D1 R1	92.63	0.6933	93.00	0.6836	92.81	0.6884
DietD Faeces, D1 R2	91.83	0.8137	94.73	0.7528	93.28	0.7832
DietD Faeces, D2 R1	93.48	0.7476	95.06	0.7266	94.27	0.7371
DietD Faeces, D2 R2	89.43	0.7499	93.19	0.7205	91.31	0.7352
DietD Faeces, D3 R1	90.62	0.6484	92.96	0.6166	91.79	0.6325
DietD Faeces, D3 R2	89.29	0.4864	91.84	0.4894	90.56	0.4879
DietD Faeces, D4 R1	92.31	0.8949	94.80	0.8814	93.55	0.8881
DietD Faeces, D4 R2	90.69	0.8434	93.51	0.7765	92.10	0.8099
Mean Diet D ± SD	91.28±1.52	0.735±0.128	93.64±1.12	0.706±0.116	92.46±1.24	0.720±0.121
Rat						
Diet C	95.19	0.4261	94.28	0.4471	94.73	0.4366
Diet D	97.58	0.4274	95.25	0.4735	96.41	0.4504
Mean of Diets ± SD	96.38±1.69	0.427±0.001	94.76±0.68	0.460±0.019	95.57±1.19	0.443±0.010
Diet C Ileal, R1	94.19	1.2277	92.70	1.4160	93.44	1.3218
Diet C Ileal, R2	93.79	1.2443	93.68	1.3321	93.73	1.2882
Diet C Ileal, R3	94.36	1.3417	92.10	1.4602	94.73	1.4009
Diet C Ileal, R4	94.37	1.3353	94.09	1.2630	94.23	1.2991
Diet C Ileal, R5	94.39	1.3952	93.66	1.3988	94.02	1.3970
Diet C Ileal, R6	94.37	1.5346	94.03	1.2694	94.20	1.4020
Diet C Ileal, R7	93.82	1.3428	92.62	1.3031	93.22	1.3229
Diet C Ileal, R8	94.63	1.2565	93.65	1.4087	94.14	1.3326
Mean Diet C ± SD	94.24±0.29	1.335±0.100	93.32±0.74	1.356±0.074	93.96±0.48	1.346±0.047
Diet D Ileal, R1	94.00	2.4760	94.53	2.4523	94.26	2.4641
Diet D Ileal, R2	95.05	2.3871	91.46	2.6744	93.25	2.5307
Diet D Ileal, R3	94.64	3.1085	93.40	2.6908	94.02	2.8996
Diet D Ileal, R4	93.48	3.2370	95.45	2.7476	94.46	2.9923
Diet D Ileal, R5	93.19	3.1628	93.17	2.5699	93.18	2.8663
Diet D Ileal, R6	93.17	3.1927	92.46	2.6738	92.81	2.9332
Diet D Ileal, R7	93.01	2.9381	95.95	2.7771	94.48	2.8576
Diet D Ileal, R8	93.37	2.8408	95.99	2.5517	94.68	2.6962
Mean Diet D ± SD	93.74±0.75	2.918±0.329	94.05±0.69	2.642±0.109	93.89±0.71	2.780±0.195

[a] D1 = Day1-7, D2=Day 7-14, D3=Day 14-21, D4=day 21-28

[b] R1-R8 = Replicate samples, pooled from 5 animals

APPENDIX 8

Table 5.6: Apparent Digestibility (%) of Crude Protein and Amino Acids in Diets and Blood Meal for Replicate Samples Determined in Chinook Salmon

Sampling Day	1-7	1-7	7-14	7-14	14-21	14-21	21-28	21-28	
Component			**Digestibility (%) of Diet A**						**Mean ± SD**
Arginine	64.74	84.92	78.26	80.99	69.12	73.70	84.43	76.88	76.63±7.15
Histidine	67.86	86.22	75.16	80.07	66.37	72.96	81.49	71.68	75.22±6.91
Isoleucine	68.66	85.61	79.51	83.22	72.63	74.79	85.48	79.76	78.71±6.20
Leucine	66.08	86.73	76.69	84.13	68.78	74.49	83.64	73.39	76.74±7.51
Lysine	71.26	87.76	79.72	84.43	72.96	77.93	84.82	77.12	79.50±5.85
Methionine	68.10	82.29	77.22	79.89	66.39	69.94	80.92	73.84	74.82±6.16
Phenylalanine	64.13	84.90	74.17	81.20	65.10	70.55	81.76	71.35	74.14±7.80
Threonine	65.49	84.73	74.95	81.74	64.60	66.88	81.91	73.06	74.17±8.02
Valine	65.67	86.24	75.54	83.03	66.45	72.81	82.85	72.41	75.63±7.76
Alanine	66.07	87.19	78.82	85.09	71.93	76.71	85.04	76.22	78.38±7.25
Aspartic acid	59.94	80.16	70.10	73.53	61.38	66.14	75.16	67.75	69.27±6.90
Cysteine	39.98	64.72	46.79	58.19	26.53	26.55	56.00	46.57	45.67±14.10
Glutamic acid	66.50	86.12	79.23	83.12	69.68	75.44	85.04	78.31	77.93±7.08
Glycine	55.70	82.54	75.49	78.92	67.66	70.60	80.50	73.84	73.16±8.64
Proline	59.19	84.43	77.65	82.53	68.96	71.02	83.45	75.75	75.37±8.67
Serine	62.58	84.10	73.93	79.53	62.96	67.84	80.64	71.59	72.90±8.13
Tyrosine	65.42	83.31	73.89	77.94	61.69	66.13	80.96	72.64	72.75±7.81
Component			**Digestibility (%) of Blood Meal A**						**Mean ± SD**
Arginine	-14.04	68.84	14.51	52.72	3.94	22.78	66.85	35.83	31.43±30.03
Histidine	34.10	82.90	53.51	66.54	30.11	47.64	70.32	44.24	53.67±18.36
Isoleucine	-125.1	63.47	-4.39	36.80	-80.95	-56.90	61.98	-1.60	2.63±56.25
Leucine	37.85	85.54	62.36	79.54	44.09	57.26	78.42	54.72	62.47±17.36
Lysine	35.67	84.33	60.63	74.50	40.69	55.33	75.67	52.97	59.97±17.25
Methionine	17.62	78.83	56.94	68.46	10.22	25.54	72.92	42.38	45.36±28.68
Phenylalanine	37.01	84.66	60.05	76.18	39.24	51.75	77.46	53.57	59.99±17.90
Threonine	28.99	86.15	57.09	77.27	26.34	33.12	77.78	51.48	54.78±23.84
Valine	38.60	85.86	61.26	78.48	40.38	55.01	78.06	54.09	61.47±17.82
Alanine	26.99	84.79	61.88	79.05	43.02	56.10	78.91	54.76	60.69±19.84
Aspartic acid	27.39	86.92	57.30	67.39	31.63	45.64	72.19	50.37	54.85±20.32
Cysteine	-1.13	65.93	17.32	48.24	-37.59	-37.53	42.29	16.74	14.28±38.23
Glutamic acid	-1.19	78.89	50.75	66.62	11.77	35.30	74.46	47.01	45.45±28.91
Glycine	-36.06	77.14	47.43	61.89	14.39	26.80	68.53	40.45	37.57±36.44
Proline	-96.01	71.07	26.18	58.46	-31.38	-17.70	64.56	13.61	11.10±57.37
Serine	25.79	86.04	57.55	73.22	26.85	40.52	76.35	51.03	54.67±22.76
Tyrosine	22.18	81.77	50.40	63.88	9.77	24.55	79.93	46.24	47.34±26.98

Appendix 8, Table 5.6 contd...

Sampling Day	1-7	1-7	7-14	7-14	14-21	14-21	21-28	21-28	
Component			**Digestibility (%) of Diet B**						**Mean ± SD**
Arginine	65.06	77.33	75.88	74.46	77.89	85.53	83.28	88.28	78.46±7.30
Histidine	66.39	81.70	83.70	80.38	84.20	86.69	87.06	90.18	82.54±7.23
Isoleucine	63.65	74.33	79.81	78.41	81.17	83.61	81.92	86.35	78.66±7.03
Leucine	66.74	80.57	87.02	84.87	87.02	89.62	88.94	91.72	84.56±7.94
Lysine	66.98	81.65	87.19	84.02	87.59	88.18	88.85	91.68	84.52±7.71
Methionine	66.49	77.99	70.61	79.10	70.29	82.58	79.05	83.96	76.26±6.34
Phenylalanine	67.09	80.80	81.78	79.39	82.39	87.25	85.90	89.38	81.75±6.84
Threonine	65.05	77.21	84.37	80.94	82.43	85.33	85.49	88.83	81.21±7.39
Valine	67.11	80.58	85.74	83.69	86.12	88.61	84.62	91.29	83.47±7.34
Alanine	66.89	80.41	87.70	85.61	87.94	89.64	88.51	91.71	84.80±7.96
Aspartic acid	61.84	75.92	74.82	66.88	74.86	74.97	77.57	82.95	73.73±6.51
Cysteine	52.65	55.16	45.10	46.82	39.67	20.44	57.74	66.43	48.00±13.87
Glutamic acid	65.37	77.93	82.07	80.20	83.51	86.37	85.24	89.47	81.27±7.37
Glycine	63.70	75.76	79.23	74.59	79.08	80.70	80.13	86.28	77.44±6.56
Proline	64.92	76.34	83.57	79.70	81.05	83.86	84.18	89.18	80.35±7.27
Serine	64.77	77.09	79.64	76.43	79.31	82.99	83.39	87.17	78.85±6.71
Tyrosine	64.71	75.95	71.49	69.55	73.38	80.33	78.94	85.38	74.96±6.58
Component			**Digestibility (%) of Blood Meal B**						**Mean ± SD**
Arginine	-15.79	36.10	29.98	23.97	38.46	70.79	61.27	82.42	40.90±30.89
Histidine	32.40	71.56	76.66	68.17	77.95	84.30	85.26	93.23	73.69±18.50
Isoleucine	-232.7	-90.91	-18.17	-36.74	-0.16	32.17	9.83	68.61	-5.05±51.00
Leucine	40.42	71.68	86.26	81.39	86.25	92.13	90.58	96.87	80.70±17.95
Lysine	27.59	67.91	83.13	74.42	84.23	85.85	87.68	95.46	75.78±21.18
Methionine	19.41	63.04	35.06	67.26	33.85	80.47	67.07	85.70	56.48±24.04
Phenylalanine	46.12	75.82	77.93	72.76	79.25	89.79	86.86	94.40	77.87±14.81
Threonine	31.61	65.21	85.02	75.54	79.65	87.65	88.11	97.34	76.27±20.42
Valine	42.57	73.05	84.73	80.09	85.60	91.23	82.21	97.29	79.60±16.61
Alanine	31.94	67.26	86.30	80.84	86.93	91.36	88.42	96.78	78.73±20.80
Aspartic acid	34.41	74.48	71.35	48.76	71.49	71.79	79.20	94.51	68.25±18.54
Cysteine	29.86	37.39	7.14	12.33	-9.19	-66.98	45.17	71.27	15.87±41.69
Glutamic acid	-4.88	45.89	62.59	55.04	68.43	79.98	75.39	92.49	59.37±29.76
Glycine	-0.49	49.32	63.64	44.47	63.02	69.70	67.37	92.73	56.22±27.10
Proline	10.84	50.48	75.57	62.12	66.81	76.57	77.69	95.04	64.39±25.25
Serine	35.41	67.55	74.19	65.82	73.32	82.92	83.96	93.84	72.13±17.50
Tyrosine	20.66	57.60	42.94	36.58	49.16	71.98	67.44	88.58	54.37±21.62

Appendix 8, Table 5.6 contd...

Sampling Day	1-7	1-7	7-14	7-14	14-21	14-21	21-28	21-28	
Component			**Digestibility (%) of Diet C**						**Mean ± SD**
Arginine	51.75	48.21	56.82	51.81	52.70	*	51.15	63.33	53.67±4.96
Histidine	23.71	15.60	33.39	19.33	24.17	*	24.14	39.27	25.66±8.11
Isoleucine	70.51	71.04	73.72	73.20	72.47	*	70.74	75.68	72.48±1.88
Leucine	25.22	16.65	34.13	20.30	24.47	*	26.15	48.07	27.86±10.42
Lysine	38.59	32.68	45.66	35.30	39.96	*	40.83	57.74	41.54±8.25
Methionine	52.66	56.74	63.69	65.24	64.26	*	55.20	61.99	59.97±5.01
Phenylalanine	23.99	16.83	33.34	18.22	21.25	*	22.54	44.63	25.83±9.86
Threonine	30.79	24.21	37.82	24.78	28.29	*	30.29	49.78	32.28±8.95
Valine	23.41	16.13	33.72	20.25	24.60	*	25.47	47.24	27.26±10.32
Alanine	33.51	27.62	42.30	29.94	34.16	*	35.23	53.64	36.63±8.81
Aspartic acid	28.15	19.46	29.19	23.89	26.80	*	25.83	44.42	28.25±7.82
Cysteine	17.05	15.66	23.03	14.28	21.29	*	18.57	34.71	20.66±6.91
Glutamic acid	51.67	48.24	56.41	50.5	53.14	*	50.99	63.71	53.52±5.15
Glycine	44.61	40.81	48.49	44.08	45.20	*	44.29	56.77	46.32±5.12
Proline	42.19	37.33	48.60	40.04	43.33	*	43.43	58.78	44.81±7.06
Serine	33.30	26.48	38.53	25.85	29.27	*	30.12	50.37	29.13±14.29
Tyrosine	41.60	37.16	46.44	40.64	41.91	*	41.01	56.36	43.59±6.25
Component			**Digestibility (%) of Blood Meal C**						**Mean ± SD**
Arginine	-62.98	-77.11	-42.73	-62.74	-59.22	*	-65.38	-16.76	-55.27±19.79
Histidine	-73.21	-93.51	-49.00	-84.17	-72.06	*	-72.13	-34.27	-68.33±20.29
Isoleucine	-92.86	-87.34	-59.32	-64.80	-72.45	*	-90.47	-38.84	-72.30±19.68
Leucine	-51.31	-70.40	-31.49	-62.26	-52.99	*	-49.25	-0.44	-45.45±23.21
Lysine	-54.12	-70.79	-34.18	-63.39	-50.26	*	-47.81	-0.12	-45.81±23.27
Methionine	-39.76	-23.40	-4.47	-10.72	-6.76	*	-29.58	-2.35	-16.72±14.33
Phenylalanine	-50.85	-66.80	-30.04	-63.70	-56.95	*	-54.09	-4.92	-46.76±21.96
Threonine	-61.14	-79.07	-41.96	-77.54	-67.97	*	-62.51	-9.35	-57.08±24.40
Valine	-52.43	-68.45	-29.72	-59.39	-49.81	*	-47.88	-0.03	-43.96±22.70
Alanine	-60.71	-76.69	-36.88	-70.40	-58.95	*	-56.05	-6.13	-52.26±23.88
Aspartic acid	-56.88	-80.78	-54.01	-68.61	-60.61	*	-65.28	-12.12	-56.61±21.50
Cysteine	-72.20	-76.24	-54.85	-80.24	-59.90	*	-67.78	-20.98	-61.74±20.04
Glutamic acid	-60.83	-74.73	-41.58	-66.18	-54.83	*	-63.59	-11.98	-53.39±20.95
Glycine	-73.94	-89.09	-58.42	-76.06	-71.59	*	-75.23	-25.38	-67.10±20.46
Proline	-58.41	-74.23	-37.54	-65.42	-54.70	*	-54.39	-4.43	-49.87±22.98
Serine	-57.88	-77.22	-43.06	-78.99	-69.29	*	-66.91	-9.51	-57.55±24.46
Tyrosine	-47.93	-61.76	-32.85	-50.92	-46.96	*	-49.75	-1.97	-41.73±19.47

Appendix 8, Table 5.6 contd...

Sampling Day	1-7	1-7	7-14	7-14	14-21	14-21	21-28	21-28	
Component			**Digestibility (%) of Diet D**						**Mean ± SD**
Arginine	87.86	89.26	*	90.63	91.50	87.83	91.72	91.82	90.09±1.76
Histidine	85.94	87.72	*	89.66	90.30	84.93	89.92	89.05	88.22±2.09
Isoleucine	84.66	87.30	*	87.93	89.37	86.16	89.71	89.48	87.80±1.90
Leucine	84.17	88.65	*	88.17	90.08	83.25	89.61	89.52	87.63±2.77
Lysine	87.04	89.87	*	90.35	91.43	86.44	90.89	90.58	89.51±1.96
Methionine	79.09	80.83	*	82.54	86.34	80.69	87.34	86.51	83.33±3.34
Phenylalanine	81.96	86.39	*	85.67	86.90	79.87	87.63	87.10	85.07±2.97
Threonine	80.25	84.68	*	85.70	85.75	78.69	86.76	86.22	84.01±3.19
Valine	82.11	87.06	*	87.18	89.53	82.38	88.89	88.61	86.54±3.06
Alanine	85.38	88.70	*	89.20	91.00	85.39	90.31	90.02	88.57±2.30
Aspartic acid	72.45	76.12	*	80.32	79.81	70.78	79.58	77.76	76.69±3.78
Cysteine	57.86	64.94	*	64.19	67.52	54.24	70.30	69.03	64.01±5.93
Glutamic acid	85.57	88.41	*	88.77	90.50	85.80	90.24	90.02	88.47±2.05
Glycine	81.01	83.22	*	86.24	86.54	81.31	86.12	85.04	84.21±2.36
Proline	84.36	87.21	*	88.01	88.70	82.38	88.94	88.08	86.81±2.48
Serine	79.69	83.08	*	85.08	85.08	76.91	86.08	85.27	83.03±3.45
Tyrosine	81.16	85.09	*	84.10	84.10	79.69	87.26	86.44	83.98±2.72

APPENDIX 9

Table 5.7: Apparent Digestibility (%) of Crude Protein and Amino Acids in Diets and Blood Meal for Replicate Samples Determined in the Rat

Sampling Day	1-7	1-7	7-14	7-14	14-21	14-21	21-28	21-28	
Component			**Digestibility (%) of Diet C**						**Mean ± SD**
Arginine	59.74	53.90	58.55	55.45	52.48	63.79	60.96	58.30	57.90±3.77
Histidine	43.21	35.80	42.75	40.38	33.37	50.25	44.17	40.04	41.25±5.21
Isoleucine	72.13	68.77	71.37	70.23	67.11	74.25	71.75	72.22	70.98±2.23
Leucine	45.12	37.94	40.12	37.90	35.80	51.66	45.11	41.55	41.90±5.18
Lysine	49.52	43.49	48.95	47.06	41.28	56.29	50.59	47.61	48.10±4.55
Methionine	64.92	66.04	69.17	66.33	66.73	68.65	66.42	64.89	66.64±1.56
Phenylalanine	47.91	40.49	41.24	38.46	38.72	53.93	47.96	44.71	44.18±5.45
Threonine	41.98	33.68	40.85	40.39	33.11	50.36	45.00	41.77	40.89±5.62
Valine	41.37	33.59	38.33	36.01	31.34	48.49	41.36	38.07	38.57±5.32
Alanine	48.72	41.55	46.42	44.57	39.82	55.38	49.68	46.80	46.62±4.88
Aspartic acid	33.39	24.94	31.32	31.13	24.39	42.26	36.06	34.22	32.21±5.82
Cysteine	30.15	32.46	36.75	33.13	37.40	37.37	32.92	31.28	33.93±2.85
Glutamic acid	55.21	49.69	53.84	52.39	47.40	60.04	55.15	53.76	53.43±3.81
Glycine	47.54	39.45	47.03	46.66	39.10	53.95	49.44	47.99	46.40±4.96
Proline	47.48	39.02	45.20	40.69	40.49	53.68	47.45	46.22	45.03±4.83
Serine	39.99	30.93	39.17	40.42	31.18	51.33	45.94	43.00	40.24±6.90
Tyrosine	53.04	45.98	50.35	62.74	43.52	58.19	52.50	48.53	51.86±6.30
Component			**Digestibility (%) of Blood Meal C**						**Mean ± SD**
Arginine	18.40	3.85	15.44	7.70	0.30	28.51	21.45	14.81	13.81±9.40
Histidine	11.78	-1.41	10.97	6.75	-5.73	24.31	13.50	6.15	8.29±9.26
Isoleucine	17.42	-1.22	13.16	6.84	-10.43	29.12	15.29	17.91	11.14±12.20
Leucine	18.73	7.03	10.58	6.97	3.53	29.40	18.73	12.91	13.48±8.45
Lysine	15.91	4.17	14.80	11.12	-0.14	29.11	18.00	12.19	11.89±9.69
Methionine	-10.23	14.17	25.27	15.21	16.64	23.43	15.55	10.11	11.27±11.92
Phenylalanine	23.14	10.97	12.20	7.63	8.07	33.02	23.22	17.90	17.02±8.95
Threonine	11.92	-3.37	9.83	8.98	-4.43	27.36	17.48	11.53	9.91±10.36
Valine	14.82	2.12	9.87	6.08	-1.55	26.44	14.81	9.43	10.25±8.68
Alanine	26.99	84.79	61.88	79.05	43.02	56.10	78.91	54.76	60.69±19.84
Aspartic acid	27.39	86.92	57.30	67.39	31.63	45.64	72.19	50.37	54.85±20.32
Cysteine	-1.13	65.93	17.32	48.24	-37.59	-37.53	42.29	16.74	14.28±38.23
Glutamic acid	-1.19	78.89	50.75	66.62	11.77	35.30	74.46	47.01	45.45±28.91
Glycine	-36.06	77.14	47.43	61.89	14.39	26.80	68.53	40.45	37.57±36.44
Proline	-96.01	71.07	26.18	58.46	-31.38	-17.70	64.56	13.61	11.10±57.37
Serine	25.79	86.04	57.55	73.22	26.85	40.52	76.35	51.03	54.67±22.76
Tyrosine	22.18	81.77	50.40	63.88	9.77	24.55	79.93	46.24	47.34±26.98

Appendix 9 Table 5.7 contd...

Sampling Day	1-7	1-7	7-14	7-14	14-21	14-21	21-28	21-28	
Component			**Digestibility (%) of Diet D**						**Mean ± SD**
Arginine	86.79	87.13	88.98	88.22	86.66	88.55	86.68	86.40	87.43±0.99
Histidine	82.35	83.14	85.23	84.85	82.81	84.78	82.21	82.87	83.53±1.22
Isoleucine	83.80	84.07	85.90	84.47	82.92	85.42	83.29	83.59	84.18±1.03
Leucine	86.39	86.82	88.36	87.24	86.17	87.98	86.25	86.23	86.93±0.85
Lysine	84.23	84.96	86.63	85.74	83.91	86.24	83.78	84.19	84.96±1.11
Methionine	85.45	86.46	87.84	85.73	85.71	87.77	86.80	85.71	86.44±0.95
Phenylalanine	85.98	86.45	88.19	87.01	85.43	87.92	85.97	85.64	86.57±1.04
Threonine	76.78	77.67	79.99	78.89	76.16	79.65	75.70	76.42	77.66±1.66
Valine	82.54	82.88	85.29	84.00	82.17	85.05	82.62	82.29	83.36±1.25
Alanine	84.02	84.53	86.49	85.13	83.44	85.75	73.10	83.35	84.48±1.23
Aspartic acid	62.79	64.51	68.88	67.96	63.86	68.34	62.92	64.14	65.43±2.54
Cysteine	55.11	62.82	66.86	62.44	62.88	66.41	57.90	61.82	62.03±3.94
Glutamic acid	81.48	81.71	84.44	83.69	81.23	84.02	81.70	81.32	82.45±1.35
Glycine	71.36	72.18	76.07	74.52	71.20	75.06	70.33	70.61	72.67±2.22
Proline	78.40	77.87	81.27	80.57	76.57	82.12	7.17	78.90	79.11±2.00
Serine	71.61	71.76	75.87	74.80	71.51	75.22	71.00	71.58	72.92±2.00
Tyrosine	83.42	84.24	85.94	84.90	83.64	86.13	84.10	83.42	84.47±1.08

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