Nutritional evaluation of rendered animal by-products and blends as suitable partial alternatives for fish meal in diets for rainbow trout (*Oncorhynchus mykiss*)

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The research investigations describe experiments with fish conducted with compliance with the Animal Scientific Procedures act 1986 under Project Licence number: 30/2135
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Fasakin, E.A., Serwata, R.D., Davies, S.J., 2005. Comparative utilization of rendered animal derived products with or without composite mixture of soybean meal in hybrid tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) diets. Aquaculture, 249: 329-338

2001 WAS conference (Aquaculture America 2001, Coronado Springs, Florida) **Poster presentation**: Relative performance of hydrolysed feathermeal in diets for African catfish (*Clarias Gariepinus*).

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Poster presentation: efficacy of untreated and enzymatically treated Phaffia rhodozyma as a dietary source of astaxanthin for rainbow trout.

2004 WAS Conference (Aquaculture Australasia 2004, Sydney, Australia)
Poster presentation: Evaluation of plant protein concentrates for fish: Digestibility trials in rainbow trout.
2004 WAS Conference (Aquaculture Australasia 2004, Sydney, Australia)

Poster presentation: Digestibility evaluation of selected animal by products for applications in diets for sea bass (*Dicentrachus labrax*).

2007 WAS Conference (Aquaculture America, 2007, San Antonio, Texas, USA)
Oral presentation: The evaluation of quality animal proteins as a partial replacement for high quality LT-fishmeal in diets for rainbow trout (*Oncorhynchus mykiss*) a growth and digestibility study.

Poster presentation: Digestibility evaluation of selected animal proteins in diets for rainbow trout (*Oncorhynchus mykiss*).

Poster presentation: The potential use of polychaete worms (*Nereis virens*) and vegetable protein concentrates in diets for rainbow trout (*Oncorhynchus mykiss*)

Nutritional evaluation of rendered animal by-products and blends as suitable partial alternatives for fish meal in diets for rainbow trout

Robert D Serwata

Abstract

The current European legislation states that animal by-products should not be used due to the problems associated with BSE in the 1990's. Consequently, a ban was imposed in 2000/01 to prohibit the potential for BSE to be transferred to other species. One of the problems associated with older abattoirs and particularly working practices within this industry at this time was the potential for contaminated meat to come into contact with meat fit for human consumption or by products which were bound for rendering for animal feed. Since the first restriction with regard to feeding mammalian animal protein in 1994 within the UK to ruminants, the occurrence of BSE within Britain has reduced significantly. Since the EU curtailment in 2001, the rendering industry has improved drastically with HACCP principles applied to all UK facilities. Animals must be fully traceable and are indeed tested for BSE before being declared fit for human consumption. As a result of these significant changes there does seem to be a certain degree of scope to at least start including some of these products back into certain parts of agriculture. As such, aquaculture has probably the least risk associated due to the species barrier not being crossed with respect to cultured fish. As such the suitability of products derived from this industry is evaluated within this thesis for rainbow trout as an important farmed species of salmonid.

The potential of replacing high quality fishmeal with terrestrial animal by-products on a digestible protein and energy basis was established using preliminary digestibility trials, followed by a comprehensive 12-week nutrition trial with sub-adult class rainbow trout. The use of high quality fishmeal was an important standard when comparing terrestrial animal protein sources for their replacement value. The digestibility of these terrestrial animal protein sources was established by substituting 40% of the reference protein and adding an inert marker (chromic oxide) to the feeds. Diets were manufactured using a California pellet mill to produce pellets of suitable size. A series of test diets were fed at 1.5% bodyweight to rainbow trout (in triplicate groups) for three weeks prior to the fish being stripped for faecal material, this ensured the fish were well acclimated to their respective feeds prior to assessment.

The fishmeal control diet performed well and values for Crude Protein (CP), Energy, (E), and Essential Amino Acids (EAAs) were all around >90%. The best performing test terrestrial animal protein source in terms of digestibility was Spray Dried Haem (SDH) with values for (CP), (E), and (EAAs) all above 95% whilst, Poultry Meat Meal (PMM), Steam Hydrolysed Feathermeal (SHF), and Enzyme Treated Feathermeal (ETF) all provided good CP and E digestibility values (>66% digestibility). EAA digestibility values ranged from PMM (82-93%), SHF (52%-98%), ETF (71-91%) and blends of these ingredients PMM/SDH ranged from 73-100% and SHF/SDH (87-98%). The values obtained from the digestibility trials were then utilised to improve the diet formulations for the 12 week growth trial. All diets were formulated on a digestible CP

and E basis so that each ingredient could be compared on an equal basis. The growth trial included a digestibility evaluation towards the end of the investigation. Haematocrit and haemoglobin levels were also established as a health indicator for each treatment.

At the end of twelve week feed trial, there were no significant differences ($p>0.05$) with respect to growth performance, however it should be noted that the fishmeal control feed, SDH, and the PMM/SDH feeds all numerically out performed the other test diets in relation to growth performance, Specific Growth Rate ranged between (1.71-1.82). Diet composition did have a significant effect with respect to Protein Efficiency Ratio (PER) ($p<0.05$) with the control (2.00), SDH (1.94), PMM/SDH (1.87) and SHF (1.91) out performing other test ingredients. Blood indices such as haematocrit and haemoglobin indicated significant effects on rainbow trout ($p>0.05$) when fed the test diets containing SDH as an ingredient, though these values were within the normal range expected for rainbow trout. At the end of the feeding trial a further digestibility trial was undertaken; on this occasion fish were fed to apparent satiation (circa 4% body weight) to assess the efficiency of the test feeds compared to the control fishmeal diet. These digestibility results indicated that the fishmeal diet was less efficiently utilised in terms of (CP) digestibility (86%) when compared to the initial digestibility trials (92%), though this was to be expected due to the excessive feeding regime. The SDH diet indeed followed a similar pattern to the control feed with a digestibility (CP) value of 87% for the growth trial and a value of 95% for CP in the earlier trials. This pattern was not reflected for the other test diets which actually showed a marked improvement with respect to CP digestibility ranging from 75% for PMM to 82% for ETF. The EAA digestibility in the feed trial also displayed marked improvement over the initial digestibility trials for SHF (59% for tryptophan, all other EAAs 74%-92%), whilst all other test feeds had a similar digestibility pattern compared to the control feed (77-89%).

In summary, the digestibility trials demonstrated the importance of establishing the value of test feed ingredient inclusion within aquafeeds prior to incorporation into balanced diets. It can be elucidated from the test data that generally an ingredient with a high digestibility for CP, E and EAAs generally will allow for a higher inclusion within a practical diet with no loss of performance or indeed the potential to exceed a control feed formulation. The findings of this thesis verify that terrestrial animal proteins are a sustainable source of protein that can be effectively included in aquafeeds for rainbow trout without loss of growth performance or any apparent adverse effects on feed utilisation and health.

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List of Abbreviations

Amino Acid	(AA)
Animal By-product Regulation	(ABR)
Apparent Digestibility Coefficient	(ADC)
Acid Insoluble Ash	(AIA)
Anti Nutritional Factors	(ANF)
Apparent Net Energy Utilisation	(ANEU)
Apparent Net Protein Utilisation	(ANPU)
Association Of Analytical Chemists	(AOAC)
Bovine Spongiform Encephalopathy	(BSE)
Crude Protein	(CP)
California Pellet Mill	(CPM)
Department for Environmental and Rural Affairs	(DEFRA)
Docosahexaenoic Acid	(DHA)
Dry Matter	(DM)
Deoxyribonucleic Acid	(DNA)
Energy	(E)
Essential Amino Acids	(EAA)
Essential Fatty Acids	(EFA)
Enzyme Linked Immuno Specific Assay	(ELISA)
Eicosapentaenoic Acid	(EPA)
Enzyme Treated Feathermeal	(ETF)

European Union	(EU)
Food and Agriculture Organisation	(FAO)
Feed Conversion Efficiency	(FCE)
Feed Conversion Ratio	(FCR)
Fisheries Global Information System	(FIGIS)
Fish Meal	(FM)
Fats and Proteins Research Foundation	(FPRF)
Hazard Analysis and Critical Control Points	(HACCP)
High Performance Liquid Chromatography	(HPLC)
Highly Unsaturated Fatty Acids	(HUFA)
Linear Least Cost Formulation	(LLCF)
Low Temperature	(LT)
Metric tonnes	(Mt)
National Oceanic & Atmospheric Administration	(NOAA)
National Renders Association	(NRA)
National Research Council, United States	(NRC)
Nitrogen Free Extract	(NFE)
Processed Animal Protein	(PAP)
Polychlorinated Bi-phenyls	(PCB)
Polymerisation Chain Reaction	(PCR)
Protein Efficiency Ratio	(PER)
Poultry Meat Meal	(PMM)

Spray Dried Haem	(SDH)
Standard Environmental Temperature	(SET)
Specific Growth rate	(SGR)
Steam Hydrolysed Feathermeal	(SHF)
Transmissible Spongiform Encephalopathy	(TSE)
World Health Organisation	(WHO)

Chapter 1

General introduction

1.1 Aquaculture overview trends and developments

Aquaculture is the most rapidly expanding sector of agri-business today and has shown continuous growth for the last 20 years, with an average increase of 9.6% per annum, contributing an important input with regards to global fisheries production. This steady growth is mainly driven by the fact that commercial capture fisheries have been in progressive decline over the same period; demand for seafood for human consumption has increased significantly, the latter being due to economic growth especially in the Asian Pacific region. In the same time period the world population has grown significantly from 1970 to date and the projected increase for the next forty three years would suggest that the problem of supplying enough fish to meet demand is going to become an increasingly more difficult problem. The population figures illustrated in Figure 1 clearly demonstrate the growth in world population.

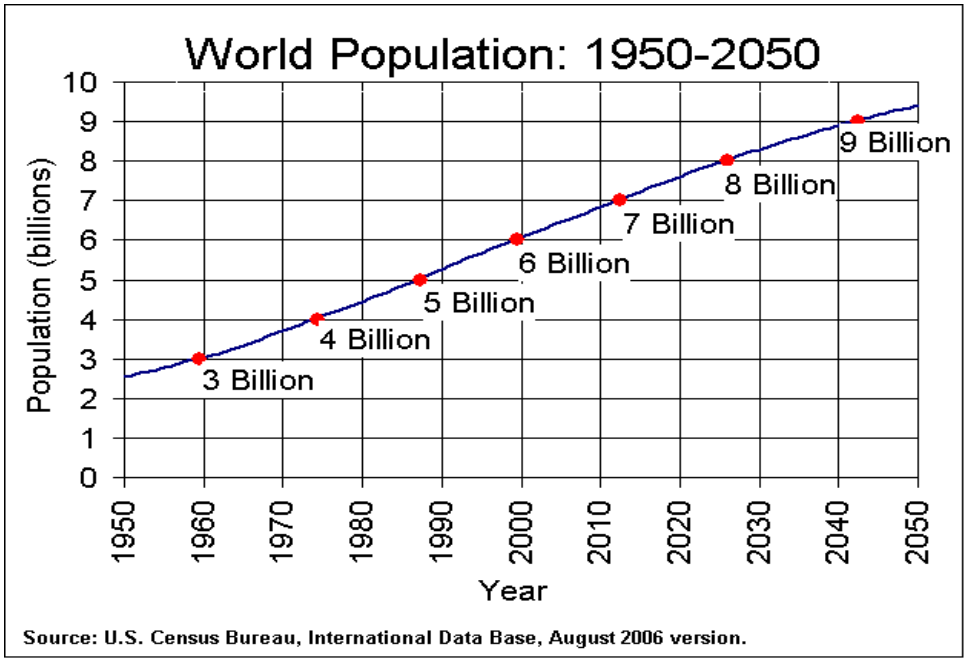

Figure 1.World population growth to date and projected, 1950-2050.

The linear growth in world population over the last 50 years seems set to continue (Figure 1) and as such world finfish aquaculture production would be expected to follow a similar trend. Figure 2 would suggest that currently, finfish aquaculture production is growing at a similar linear rate as world population growth.

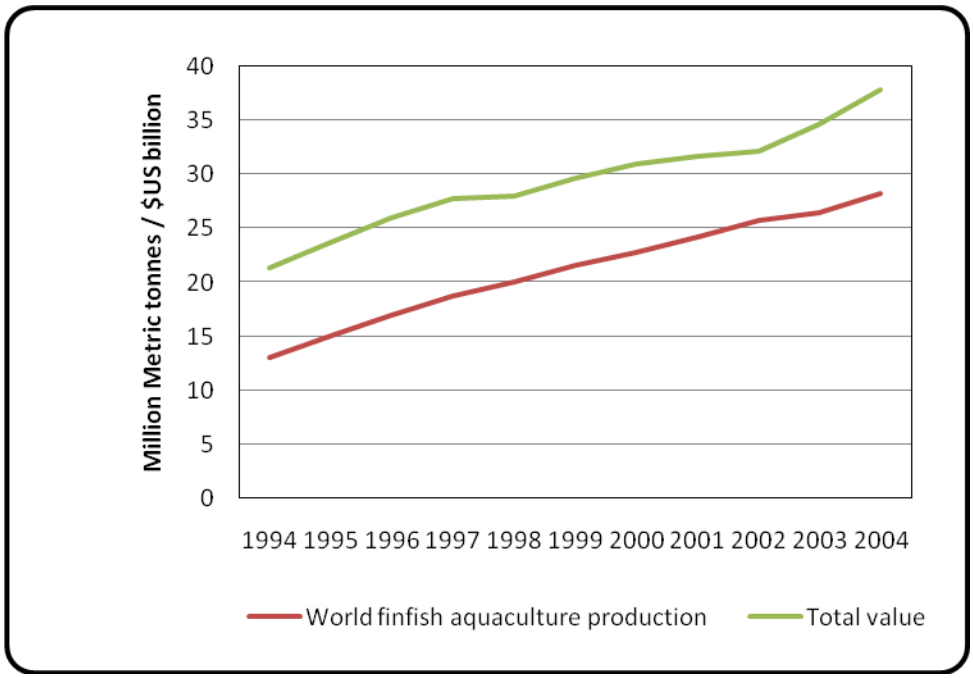

Figure 2.FAO 2006 (FIGIS data) World fish aquaculture production, 1994-2004.

Aquaculture in the early 1970's only accounted for around 3% of total world global fisheries. Today, aquaculture production is responsible for just over 27% of global fisheries and supplies over 36% of food fish to the consumer. This coincides with a period when conventional global capture fisheries have plateaued at about 95 million metric tonnes (Jia et al. 2001). Recent figures have indicated that the present harvest of exploitable fishery resources is unlikely to be surpassed and may even decline further in future years (Currie, 2000).

It is generally accepted that over-fishing has already caused devastating effects on fish stock abundance in many regions of the world, with a consequent decline in the supply of local seafood products (Naylor et al., 2000). This has caused major problems for coastal communities with a depression in the soci-economic status of personnel engaged in fishing and related industries. Consequently aquaculture is seen to be an important component of sustaining these economies and under specific circumstances may help to regenerate income development and wealth creation.

Aquaculture production of high value species has potential to further increase especially in marine locations where water is not as limiting as established freshwater sites employed for carp. The aquaculture sector is destined to become the main focus for future development with numerous candidate species being reared in different parts of the world (Ferlin & La Croix, 2000).

In terms of marine aquaculture development there is now considerable production of sea bream (*Sparus aurata),* sea bass (*Dicentrarchus labrax),* and turbot (*Scophthalmus maximus)* in Southern European regions close to the Mediterranean and North African States such as Tunisia, Algeria, Egypt and Libya. These have now become established aquaculture species making a valuable contribution for these nations, indeed comparing production of Southern European aquaculture in 1994 (21,347 Mt) to 2004 (85,968Mt) the expansion is evident. In terms of gross value to this region marine finfish aquaculture is worth circa 502,000 $US compared to 175,000 $US in 1994 (FAO, 2006).

In Japan and Australia, yellowtail (*S. quinqueradiata)* and tuna (*Scombridae*) are becoming important marine species and varieties of grouper (*Epinephelinae*),

barramundi (*L. calcarifer),* and Pacific sea bass and breams are now cultured routinely (Hong & Zhang, 2001). Such is the importance of marine aquaculture the US administration has recently announced a bill to allow deep water aquaculture development within US territorial waters (NOAA, 2005).

The range of commercially important freshwater and marine species produced intensively in Europe is shown in Table 1.

Table 1. European farmed species of marine & freshwater finfish of commercial importance in aquaculture

Marine Species	Freshwater species
Sea trout (*Salmo trutta*)	European eel (*Anguilla anguilla*)
Gilthead seabream (*Sparus Aurata*)	Carp (*Cyprinus carpio*)
Cod (*Gadus morhua*)	Tilapia (*Oreochromis spp.*)
Turbot (*Psetta maxima*)	Sturgeon (*Acipenser spp.*)
Halibut (*Hippoglossus hippoglossus*)	European catfish (*Silurus glanis*)
Sea bass (*Dicentrarchus labrax*)	Brown trout (*Salmo trutta*)
Atlantic salmon (*Salmo salar*)	Rainbow trout (*O.mykiss*)

Despite the tremendous potential for marine fish aquaculture, traditional production of salmonid species still prevails in cooler temperate countries such as North America, extreme South America (Chile) and Northern parts of Europe (Norway, Scotland, Sweden & Finland) as well as Tasmania and New Zealand in the southern hemisphere. Indeed, salmon, trout and Arctic char are regarded as very valuable fish for export and have a high market value due to their consistent quality and consumer acceptance.

Salmon of course contribute significantly to marine fish supply and hatchery production of Pacific salmon (*Oncorhynchus spp*.) contributes greatly to canning industries in North America when ranched fish (hatchery reared and then released) are captured returning to major rivers. However Atlantic salmon is the major species for cage net culture in most intensive aquaculture operations.

Current production of Atlantic salmon *(Salmo salar)* has been reported to be around 1.3 million metric tonnes (Tacon & Forster, 2003). Of this total, Norway accounts for almost 48%, Chile circa 30%, UK (Scotland) 12%, and other smaller producers accounting for the remainder. Total global value and production of Atlantic salmon are illustrated in Figure 3.

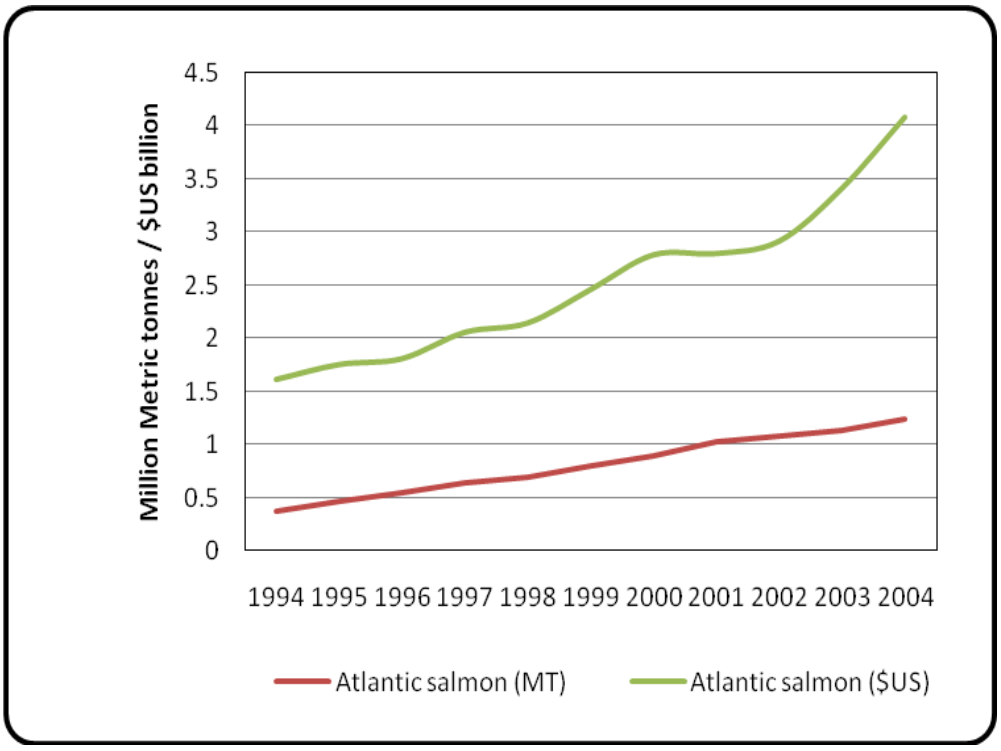

Figure 3.FAO 2006 (FIGIS data) Atlantic salmon production, 1994 - 2004

In terms of global salmon production (Atlantic/Pacific) Norway and Chile have the major market share. Apart from salmon, rainbow trout production provides the bulk of salmonid species reared in fresh water throughout Europe and North America as well as higher altitude regions in different parts of the world. Trout are very popular food fish and certain specialist aquaculture operations meet the demand for sports fisheries and recreation (Fuller, pers comm).

World wide, rainbow trout production is now estimated to be in excess of 550,000 tonnes, of this figure Chile produce >22%.

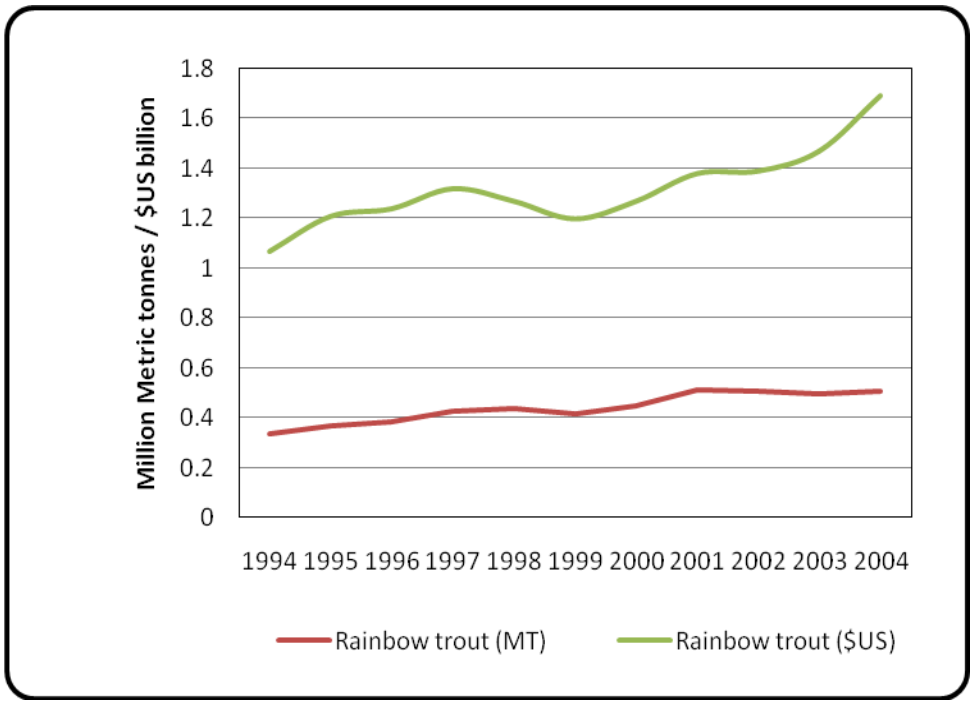

Figure 4.FAO 2006 (FIGIS data) Rainbow trout production, 1994-2004

European rainbow trout production accounts for over 60% of total world wide production, the biggest contributors to the European market being Norway, France, Denmark, and Italy. North America accounts for the rest of the total world production

and production is mainly confined to regions of Canada and the Northwest region of the USA. Growth of the trout sector is displayed in Figure 4; growth of this sector of aquaculture seems to have reached a plateau. This slowdown is most probably due to a culmination of economic, geographical reasons and reduced market value due to excessive supply to the market.

Intensive production of farmed aquatic species necessitates a full appreciation of the needs of the individual species taking into account the requirements for water quality, flow rates, containment design and health management.

However it is well known that once the main investment costs of a facility have been met, feed is by far the most important and costly input for the success of the enterprise, indeed feed cost effectively accounts for 50% of the total cost of fish production. These criteria have been very well established for salmon and trout.

For such systems, fish are supplied with expensive, balanced feeds throughout their production cycle based on quality raw materials and a range of ingredients (Fowler, 1991).

The relative costs of high quality feeds have been well documented for trout and salmon production, with the latter being the most costly due to the high density nutrient specifications (i.e. high protein, high oil) for this species and a dependency on marine protein and oil sources.

All modern commercial diets aim to maximise growth rates, minimise feed wastage and maintain optimum fish health at all stages of production. Recently for salmon and trout in Europe, United States and Canada this has become a major issue with respect to welfare and environmental organisations. One of the key areas of contention has been

directed towards the question of feed formulation and in particular the levels of marine proteins, such as fish meal, providing the bulk of the dietary protein (Powell et al., 2003; Naylor et al., 2000; Naylor et al., 1998). In response aqua feed companies have developed new formulations and feed management systems in an attempt to address these concerns. Additionally expansion of aquaculture globally has resulted in a drive towards the reduction of use of non sustainable marine derived proteins and oils with research to find alternatives (Seafeeds Workshop, 2003).

This is an urgent objective because global aquafeed production is projected to rise in accordance with growing fish farming and expected to reach a target of 95M metric tonnes by 2005 (Davis et al., 2005).

Indeed, it is not only the issue of obtaining sufficient marine proteins such as fish meals, that requires attention but also increasing use of fish oils in aquaculture due to formulation of high energy (high oil) diets for salmon, and more recently for trout, that is leading towards constraints (Torstensen et al., 2004; Fonseca-Madrigal et al., 2005). The drive towards supplying consumers with important omega-3 fish oil is seen as another major important requirement of the salmonid market. If fish oil from pelagic species such as mackerel, anchovy which are high in omega 3 fatty acids are replaced with plant oils such as palm oil, flax oil [sources without eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA)] these important fatty acids are not deposited within the carcass of the fish and the health benefits are not passed onto the consumer (Morris, 2001).

To more fully appreciate the dependency of finfish production on high quality feeds, it is first necessary to review certain aspects of the nutritional requirements of

domesticated fish for aquaculture in general. Table 2 displays the typical nutrient ranges within diet specifications for trout, salmon and marine fish of importance to aquaculture. All species of fish have requirements for the same basic nutrient components such as protein, lipid, carbohydrate as well as essential vitamins and minerals.

Table 2.Typical gross nutrient specifications feeds for popular European farmed species. (Ranges are for grower / production stages, expressed as % dry matter.)

Species	Protein	Lipid	Carbohydrate
Rainbow trout	36-47	15-35	<10
Salmon (Atlantic)	34-47	15-40	<10
Sea bass	45-55	10-20	15-20
Sea bream	45-55	10-20	15-20
Turbot	48-55	10-18	<10

1.2 Dietary proteins and Amino-acids

It is generally accepted that fish do not have a protein requirement as such; but require instead a balanced complement of the 10-essential amino acids (EEAs) to meet their nutritional requirements (NRC, 1993).

Dietary protein must supply the amino acid nitrogen required for synthesis and redevelopment of tissue and body protein during growth and different stages of development, especially reproductive demand (Desilva & Anderson, 1995).

The concept of an ‘ideal’ dietary protein that is perfectly balanced to meet the exact needs of the animal is now accepted in farm animal nutrition and has become the basis

for swine and poultry production (Cole & Van Lunen, 1994). This has also been confirmed for fish in a number of studies and is advocated for the formulation of advanced salmonid diets. It is now recommended that the minimum protein requirement where all the essential amino acids and vitamins meet the requirement for the species (typically the animals own carcass profile) are utilised and energy is supplied utilising plant / fish oil or well small quantities of well digested carbohydrate sources (Morris et al., 2005).

1.3 Lipids and essential fatty acids

Carnivorous fish have a definite requirement for the (n-3) series of fatty acids that are essential fatty acids (EFA's) for these species. Such EFA are long chain poly-unsaturates, including α linolenic acid; (18:3 n-3) and its highly unsaturated [HUFA] derivatives, eicosapentaenoic (EPA), 20:5 (n-3) and docosahexaenoic (DHA), 22:6 (n-3). These fatty acids are of special significance in the development and health of marine fish and are also abundant in the flesh of salmon and trout. Sargent et al. (1989) reviewed these requirements for most of the commonly cultured species. As well as satisfying EFA requirements, lipids provide the major source of non- protein dietary energy in the fish diet (NRC, 1993). Fish oils therefore serve an important protein-sparing role in modern commercial feed formulations where high-energy/ energy dense diets are produced for both temperate fresh water and marine species. This has become an accepted practice where rapid growth, optimum feed conversion and minimum environmental impact is desirable (Green et al., 2002). Within acceptable levels, various plant oils and certain animal fats may substitute in part for expensive marine

oils for some fish species. This is now a common practice in the formulation of diets for both trout and salmon (Morris et al., 2005). There is evidence that providing at least 1-2% of the diet in the form of EFA, maintains adequate growth, which can therefore be achieved by using alternative lipid sources such as plant oils for primary energy purposes (Bell et al., 2002; Morris et al., 2005).

1.4 Carbohydrates

Most commercial feeds contain small amounts of 'available' carbohydrate primarily as starch and a significant degree of assimilation can occur under conditions where adequate processing of cereals is utilised (Henrichfreise & Pfeffer, 1992; Hemre et al., 1996; Morris et al., 2005). The utilisation of carbohydrate as a protein sparing source of energy for rainbow trout has been reported by Hemre et al., (2002) and requires further validation for marine fish such as sea bream, sea bass and turbot which have similar dietary requirements to salmonid species. There is potential for using large amounts of carbohydrates in omnivorous/herbivorous fish but the scope is limited for strictly carnivorous fish such as salmonid species. Hemre et al., (2002) also investigated the effect of carbohydrate on glucose metabolism, hepatic enzymes and growth, and concluded that a small amount of dietary starch did not have any detrimental effects with respect to the general physiology status of rainbow trout.

For rainbow trout and salmon there is little scope for including carbohydrates in the diet as a major energy source although limited inclusions of cereals are made for the manufacture of extruded diets necessary to produce effective slow sinking high energy feeds. Tekinay, (1999) reported that high levels of carbohydrate in the form of purified

starch, dextrin or as a component of cereals could interfere with feed intake, digestion, as well as nutrient assimilation in rainbow trout.

1.5 Recent developments in fish feed technology

Commercial fish diets have changed markedly over the last decade with the development of extrusion, expansion, pellet technology, and fat coating (post vacuum). The drivers have largely been feed developments in the salmonid industry (Green et al., 2002).

These new technologies have enabled slow sinking and highly nutrient dense, high energy feeds to be manufactured. The resultant benefits include improvements in overall feed conversion efficiency (FCE) and reduced environmental impact, these include reduced nitrogen excretion and reduced phosphorus excretion. Green et al., (2002). Today, most salmon and trout diets contain approximately 20% to 40% lipid and 34-45% protein compared to an average of 10-17% lipid and 47-55% protein in the 1980's where feed was mainly steam pelleted using conventional technology (Tacon, 1996).

However, the dependency on quality feed ingredients and selected raw materials for inclusion in fish diets is important as ever and a vital area for consideration to meet increasing demand. The major influences on feed ingredient selection today are obviously cost and future availability. However other important factors in ingredient selection for farmed fed species are Genetically Modified Organisms (GMO), dioxin levels, poly chlorinated bi-phenols (PCBs) levels, and whether the marine fishery is sustainable (Bell et al., 2005; Robb, 2007).

In the 1970's, problems associated with the Peruvian fishmeal industry due to the decline of the anchovy fishery and the global energy crisis highlighted the need for alternative protein and oil sources for intensive culture of fish in regions dependent on fishmeal imports to meet the specification of aquafeeds. This prompted considerable research activity investigating the potential of different raw materials for use in aquafeeds.

To meet dietary formulation constraints and produce fish of premium quality in the world market today, fishmeal is still regarded as the first choice protein concentrate for aquafeeds destined for high value fish. Typically, diets contain 30-65% fishmeal within the formulation for the carnivorous fish species.

Although the scope for reducing the dependency on fish meals and fish oils is limited, much interest is now re-emerging on the possibilities of including alternative protein and energy rich ingredients. These include specific proteins originating from animals, or vegetable protein sources although they must compare favourably to the nutritional profile and biological value of fishmeal.

1.6 Fishmeal in aquafeeds

The fish feed industry is very still dependent on fishmeal and fish oil from the industrial fishing operations. These important ingredients provide a "balanced" dietary protein source, essential poly-unsaturates and primary energy sources for intensive fish production (Halver & Hardy, 2002).

Recently, manufacture of highly digestible, low temperature (LT) dried fishmeals (e.g. Norse milk Omega protein) with reduced biogenic amine contents (made from very

fresh fish) and improved palatability has become possible. Such fish meals are attractive materials for inclusion in a wide range of aquafeed pelleted products. The quality of fishmeal varies considerably from different countries due to seasonal fluctuations in species landed and changes in their compositional characteristics. These species can include anchovy, menhaden, and capelin all of which are oily pelagic shoaling fish species. The type of processing applied at the drying stage after oil extraction can markedly influence the nutritional value of the product for aquafeeds. Technological advances in fishmeal production such as low temperature drying eg; LT 94 (Norwegian fishmeal) have justified their extensive use, this is clearly demonstrated in many research papers which use a LT-94 grade fishmeal as a benchmark (Storebakken et al., 1998; Gouveia & Davies, 2000; Morris et al., 2005). This is mainly due to a high protein level (>70%) with good essential amino acid balance, good source of essential fatty acids, highly available vitamins and trace elements, and almost no anti nutritional factors, and essentially no carbohydrate.

Fishmeal quality is generally fairly consistent when obtained from a well defined source and manufactured under established standard conditions. Other key benefits when fishmeal is utilised is that it is has a perfect EEA balance, and most small marine pelagic fish EEA profiles are generally very similar regardless of species. Fishmeal is also a highly palatable protein source which is important with regards to feed intake and appetite in salmonids (Espe et al., 2006).

High value species such as salmon and trout are typically fed diets containing a majority of the protein as fishmeal. However there is increasing pressure to reduce the amount of fishmeal used within salmonid diets to reduce dependence on commercial

fisheries, and improve economic viability for the future of the global salmonid industry. There are also ethical, environmental considerations for a sustainable aquaculture industry. There is a broad range of alternative protein sources of potential value for inclusion in farmed fish diets. These are mainly derived from plant and animal by-products as well as yeast, bacteria, algae and single cell proteins. Tacon, (2006) has extensively reviewed the range of raw materials available for different fish species and regional practice.

1.7 Plant proteins

Generally plant and their derived by-products have been widely assessed for inclusion in feeds for numerous fish species (Akiyama, 1991; El Sayed, 1999). Although various legumes, pulses and cereals have been widely utilised, soyabean has by far been the subject of the most research interest. The success of soyabean meal in replacing fishmeal in diets for a number of teleost fishes is primarily due to a fairly good amino acid balance and generally high nutritional value (Mambrini et al,. 1999; Glenross et al., 2005; Morris et al., 2005). Other important factors are obviously market supply and cost.

However, there are a number of anti-nutritional factors (ANF's) present in these plant materials which may have considerable negative effects on fish health and production (Tacon, 1996). ANF's include oligosaccharide fractions such as stachyose and raffinose, and various proteins such as trypsin inhibitors. ANFs may often be present at appreciable levels due to inadequate processing of these thermo-labile components. Also in the case of soyabean meal, 30% is in the form of indigestible carbohydrate, also

present within soya. This represents another problem as carnivorous fish such as salmonids are not adapted to digesting large amounts of carbohydrate (Hemre et al., 2002).

Other ANF's are often present in appreciable levels, this is mainly due to inadequately processed meal; additionally unavailable phosphorus is also present in soyabean meal as phytate. The latter is of particular concern and the availability of phosphorus bound in soya bean is currently a major issue for research. Several authors have studied the phosphorus requirements of salmonid species in relation to soya bean substitution of fishmeal (Storebakken et al 1998; Sugiura et al, 1998). Generally the lower digestibility of protein in plant ingredients is attributable to the above anti-nutritional factors and also to the varying dietary fibre content in the whole meal. For many plant proteins one or more amino acids maybe deficient leading to impaired growth and feed utilisation, this problem is often countered by the increased processing of soya in the form of concentrates where large amounts of starch and ANFs are removed, whilst increasing the overall amino acid profile of the soyabean meal and increasing protein to around 60-66%, when this type of processing is applied soyabean meal digestibility can be around 90% in rainbow trout and salmon (Refstie et al., 2000). Inferior soyabean meal and corn gluten meal together with many other pulses and grain based proteins may demonstrate poor growth and digestibility in salmon and trout.

Additionally there have been some reports of the harmful effects of high inclusions of soya bean meal in trout and salmon causing histopathological changes to the hind gut region. Enteritis has been reported (Baeverfjord & Krogdahl, 1996; Krogdahl & Bakke-

McKellep, 2005) but conversely Morris *et al.,* (2005) showed no adverse effects of the use of soya bean meal in trout with no evidence of enteric lesions in the gut.

Soyabean is imported into Europe and to reduce the dependence on imports other plant protein sources (of European origin) such as lupins and pulses (peas and beans) and oilseed rape have been evaluated for fish species such as sea bass, turbot and rainbow trout (Gouveia and Davies, 1998; Gouveia & Davies, 2000; Burel et al., 2000). Pea meal and rapeseed meal have similar problems with regard to ANF's and high fibre content. For rapeseed meal glucosinolates, erucic acid, tannins and sinapine are present, and pea meal has considerable amounts of tannin, phytic acid and trypsin inhibitor present. These problems are being alleviated by plant breeding (by better genetic selection) and improved processing (de-hulling and more refined processing methods) of these materials. However, such improvements are frequently costly and require modern machinery and investment. This results in further elevating the cost of the ingredient and may limit scope for use. . Additionally the potential of using genetically modified forms of plant proteins has met with considerable resistance from buyers such as supermarket chains. The benefits of plant proteins in aqua feeds for carnivorous fish have been reviewed by Powell, (2003).

1.8 Animal proteins

There has always been an interest in meeting the protein requirements of salmonids and other species of fish through use of various terrestrial animal proteins. These have usually been obtained from the processing of offal and carcass remains from the rendering industry (Bureau et al., 1999). There were a number of investigations

throughout the 1970's and 1990's that demonstrated the feasibility of using terrestrial animal proteins in fish diets (Pfeffer & Henrichfreise, 1994).

Animal-derived proteins frequently possess a fairly good EAA balance and relatively high protein content. They may, however, vary in terms of their digestibility; amino acid profile and ash level but none the less provide a reasonable partial substitute for fishmeal in diets for farmed fish. Animal by-products such as poultry meat meal, steam hydrolysed / enzyme treated feathermeal and blood meals derived from abattoirs have considerable potential in fish and shrimp feeds. Williams et al. (1998) reviewed the applications of rendered protein meals in aquaculture. For most species it was reported that even above 30% inclusion, there were no detrimental effects on fish and prawns or adverse taste characteristics of the products for the consumer. Although these materials have proven to be effective substitutes and secondary protein sources to fishmeal in temperate, tropical and marine fish species, their role must be addressed in the light of new information and public confidence in commercial terrestrial animal based feeds.

One of the more promising ingredients available is poultry meat meal (PMM), the rendered product of poultry processing by-products, manufactured from inedible portions of poultry, excluding feathers.

PMM has been tested in extensively in diets for carnivorous fish such as Chinook salmon *Oncorhynchus tshawytscha*, (Brannon et al., 1976; Roley *et al*., 1977; Fowler, 1981a,b; 1990, 1991), Coho salmon *Oncorhynchus kisutch* (Markert et al., 1977; Higgs *et al*., 1979) and Atlantic salmon *Salmo salar* (Bergström, 1973). PMM has also been studied as a partial fishmeal replacement in the diets of channel catfish *Ictalurus*

punctatus (Brown *et al*., 1985) and rainbow trout *Oncorhynchus mykiss* (Alexis, *et al*., 1985; Bureau *et al*., 1999, 2000).

The technologies associated with the manufacture of terrestrial animal by products within the European Union to meet current legislative and quality criteria are now recognised to be state of the art with appreciable investment and engineering requirements (Woodgate, pers comm). For this purpose the following section outlines the current state of this industrial sector of agri-business.

1.9 Rendering of terrestrial animal by-products

Terrestrial animal protein and fats obtained from the rendering industry have an important role in many countries around the world. However usages within the European Union have been severely curtailed as a consequence of concerns relating to the BSE crisis in Europe. As a consequence, new legislation in the form of the EU Animal By-Products Regulation [ABPR 1774/2002] now requires that all raw animal by-products from either abattoirs or poultry processing plants within Europe have to be completely traceable. There are three categories of Animal By-Product (ABP). Category 1 relates to BSE risk material, category 2 concerns fallen stock on farms (including dead fish) and Category 3 relates to by-products from animals slaughtered fit for human consumption.

Using category 3 animal by-products allows the final protein product (Processed Animal Protein or PAP) to be utilised as a potential feed ingredient for use in aquaculture, or other areas of agriculture where species barrier is maintained with

regard to the original by-product, i.e. poultry could effectively be fed to porcine sources, or alternatively porcine to poultry.

Any products that are not deemed to be edible, Category 2 or 1 (i.e.not fit for human consumption) will be sent to specific rendering plants that will process these materials into fertilisers, chemicals or bio-energy. Full traceability is required even for these materials, to ensure that no cross contamination with Category 3 materials is possible.

It is also likely that in all rendering facilities within the European Union will become species specific, this is with the intention of meeting the intra species PAP feeding ban. These changes should help precipitate the re-opening of animal by-products to the animal feed markets.

Part of the process of re-introduction of these protein meals will require registration of all aspects of the rendering industry from farm and all intermediate steps to the animal feed producer (Woodgate & Van der Veen, 2004).

Most rendering plants now utilise a continuous rendering process facility. This is described in Figure 7 which demonstrates the sequence of processing methods employed. This is explained in more detail in the materials and methods section and is as described by Woodgate & van der Veen, (2004). The situation within Europe with regard to using these animal by products is explained in more detail in 1.9.1.

1.9.1 European legislation of animal by products

As previously mentioned, in the late 1980's and early 1990's Europe witnessed outbreaks of transmissible spongiform encephalopathies (TSE) diseases, of which scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle are the most

significant. This resulted in significant changes to, and the implementation of significant restrictions on use of animal proteins and in some cases complete withdrawal of animal proteins in terrestrial farm animal production and the aquaculture sector in Europe. Although there has been no proven link from feeding avian or porcine blood proteins to fish with regard to BSE, the EU passed a directive in 2000 which declared that any animal protein (except fishmeal) used in feedstuffs and exports for use in diets was prohibited by EU Commission Decision (2000/766). This effectively curtailed all usage of poultry by-product meals and porcine haem/blood meals in Europe despite a previous voluntary embargo by the industry.

However in the USA, Canada, South America, Asia and Australasia these products are still widely used in aquaculture with successful results and no legislative constraints so far.

The current situation in Europe is controlled by the ABPR (1774/2002) together with the TSE regulation (999/2001). Products which have currently been placed back onto the market primarily for use in aquaculture feeds are porcine blood processed to a category 3 standard. It is envisaged that poultry meat meal will also be included onto this list in the not to distant future pending review. There are very significant restrictions attached to the current and future use of processed animal proteins, including the use of stringent control tools. Suitable control tools are expected to include methods to determine the species identity in processed animal proteins and methods for marking certain categories of animal by-product (Categories 1 and 2). Species identification techniques under development include molecular biology systems such as PCR/DNA analysis, ELISA and chemo-immunofluorescence markers

and various tags are also being developed to prevent cross contamination and reduce the risk of fraud. All of these techniques will need to be validated by an independent scientific laboratory before the European Commission will accept that controls are in place.

The prospects for re-opening the market place in Europe to re-introduce poultry PAP therefore hinge upon adequate controls and political agreement on regionalisation of the EU with respect to BSE risk.

However, porcine blood products have been allowed again in aquafeeds in Europe since 2003 (Tacon, 2005) commission regulation 1234/2003 which is an amendment to the TSE regulation (999/2001). Not withstanding this change in legislation some effort will be required at technical and political levels to ensure that some products are able to return to the market place. Lack of success here could result in the temporary ban remaining permanent, which in turn could result in a complete loss of usage for these products in the European aquaculture market.

Given the urgent need to provide sustainable aquafeeds it is imperative that the nutritional advantages for modern processed animal by-products be demonstrated despite current legislation within Europe. Clearly the situation may change in the EU in relation to poultry by-products although the present outbreak of avian flu in South East Asia may compromise any decisions in favour of poultry products being used in aquaculture. Nonetheless poultry meat meal and feathermeal are widely used on a global basis and in North America and Australia are included in commercial diets for trout and salmon as well as other species and the pet food market. It is important to undertake further feasibility investigations to determine the quality and potential

nutritional value of modern animal by-products produced in Europe with the possibility of their re-introduction into salmon and trout diets in the near future.

1.9.2 Aims

The main aim of this investigation was to comprehensively assess a selection of animal by-products sourced from avian and porcine sources for inclusion in diets for salmonids and more specifically rainbow trout on the basis of nutritional experiments.

The principle avian-derived materials were poultry meat meal and feather meal, processed within the UK using modern technology and advanced methods.

The porcine protein product was essentially a haem concentrate manufactured in the United States from certified disease-free animals. The under lying philosophy was the aim to provide evidence that these materials could be safely introduced into compound diets for trout offering the potential to reduce our dependency on fish meal as the primary protein source.

An additional aim was to test the opportunities for creating new blended ingredients selected from the better performing ingredients in nutritional investigations in trout.

The final aim being to recommend suitable dietary inclusion levels for all the products tested with respect to nutritional characteristics whilst promoting consistent growth and health performance in the rainbow trout whilst reducing the overall cost of the feed. It is the main purpose of investigations to demonstrate that animal by-products could compete effectively with plant protein sources offering a wider basis for linear least cost formulation in the aqua feed industry.

1.9.3 Objectives

In order to achieve the stated aims, the major objectives of the research protocols are as follows:

1. To provide nutrient specification data for the target by-products and to formulate a succession of digestibility trials providing gross nutritional digestibility coefficients for the major nutrient components namely, crude protein, essential amino acids (EAA's) and gross energy.
2. Utilise this information to formulate balanced diets for the rainbow trout to assess the performance of animal by-products with respect to growth rate, feed conversion efficiency, nutrient retention/utilisation and several health indicators.
3. To refine diet formulations based on digestible nutrient data, which is not typical of other studies in the literature where gross substitution has been undertaken. The nutrition trial being designed to simulate as close as possible to a real aquaculture scenario with respect to fish production size and stocking density.
4. To evaluate the data obtained with the most relevant scientific literature for comparison with trout and salmon tested for other ingredients and to appraise the current selection with respect to their performance and with those obtained for other fish species that could benefit from these ingredients.
5. To assess the economic cost benefits of using animal by-products to replace fish meal in aqua feeds.

For these reasons this thesis describes a sequence of investigations to evaluate selected avian and specialised by-products such as blood meal from swine sources as well as blends of these commodities for applications in salmonid diets. The rainbow trout was chosen as the model for these studies as a typical farmed salmonid. Rainbow trout have an established nutritional requirement profile and would serve as a reliable species providing detailed information that could also be relevant for the Atlantic salmon and possibly other fish with similar characteristics such as Pacific salmon and char.

Chapter 2

General Materials & Methods

2.1 DIGESTIBILITY EVALUATION OF TEST INGREDIENTS

Digestibility evaluations of animal by-products were based on standard protocols and based on the methodology advocated by Guelph researchers whose procedures are widely recognised as being suitable for salmonid species (Glencross et al., 2007).

Specific raw materials were carefully selected after consultation with the sponsoring organisation (Prosper de Mulder) as being representative of high quality animal by-products that met full quality control criteria for the animal feed industry. Current legislation allows pourcine blood material to be utilized for aquafeed purposes in Europe as well as many other parts of the world. These products are typically termed blood meal, drum dried blood meal, blood plasma, and spray dried haem (Tacon, 2006).

As stated before, fishmeal is the premier protein ingredient that serves as the major component in diets for carnivorous fish such as salmon and trout and has very well defined characteristics in terms of meeting the nutritional profile of fish in terms of protein balance (i.e. EAA sources) oil, energy and palatability properties. Low temperature fishmeals are almost always included in dietary investigations that assess novel feed ingredients (potential fishmeal replacers in practical diet formulations) as a control. A series of preliminary digestibility experiments were conducted to enable a comprehensive ingredient assessment to be undertaken under controlled laboratory conditions. It was decided to incorporate the test protein sources at a realistic inclusion level (40%) to replace fishmeal since this might be expected to have a significant effect on measurement of availability.

The digestibility experiments measure the nutritional availability of the major nutrients important for most feed formulation purposes namely crude protein, amino acids and

gross energy. The main objective being to present reliable digestibility coefficient values for these nutrients within each of the test proteins selected.

2.2 Fish stock

Rainbow trout of all female origin 150-180g in weight were obtained from Hatchlands Fisheries, Greyshoot Lane, Rattery, South Brent, UK. Trout were transported from the fish farm in a 1000L tank supplied with pure oxygen (British Oxygen Company, Plymouth, UK). Fish were acclimated to the experimental system over a period of two weeks during which they were fed Trouw Aquaculture Elite 4-5mm diet (now Skretting, UK) until the they reached a mean weight of 220-240g before being switched to the experimental feeds. All fish within the system were graded to ensure uniformity of fish size at the start of the trial period.

2.3 Fish holding system

The trials conducted utilized the following experimental system (Figure 5). The system comprised twenty 130 litre, square, with rounded edges (30°), tanks of fibreglass construction. Each tank was provided with 95% re-circulated freshwater at a rate of 8L h^{-1}, supplied by a 19mm inlet spray bar. The water was additionally aerated by means of a synthetic air diffuser. Air was supplied by a low pressure side channel blower (Rietschle, UK Ltd).

Water in the tanks flowed in a clockwise direction, waste water from the tank was removed through a central standpipe with an outer sleeve, thus ensuring an upwelling action removing detritus from the tank bottom/centre. The inner standpipe maintained

the water level within the tank. The waste water was first filtered through plastic scourers to trap large solids, and then pumped through a 60cm Lacron Hi Pressure sand filter to remove finer suspended solids from the water. Water was then passed over a porous clay filter medium (submerged biological filter bed) which was vigorously aerated to maintain good aerobic conditions for nitrifying bacteria, this process of heavy aeration also provides for some oxygen re-gassing. The process of nitrification (Figure 6) converts ammonia, produced by the fish into nitrite (NO_2) and then nitrate (NO_3), subsequent backwashing of the sand filter helped keep nitrate levels at a safe level (<300mg/l). This was mainly achieved by exchange of water from the Plymouth mains supply which has a very low level of nitrate (typically less than <0.1mg/l). The sand filter system has a self cleaning backwash mechanism for clearing detritus build up in the sand filter media. This is activated twice daily and also allowed a 2-5% water change per day using mains tap water to refill the system. System pH was monitored weekly and the system buffered with sodium hydrogen carbonate or MagnaSpheres-magnesium hydroxide (DrydenAqua Ltd, Butterfield, Bonnyrigg, Edinburgh, Scotland) as required.

Water temperature throughout the trial period was maintained at 15°C with the differential band set at 1°C, the photo period for the duration of the experiments were maintained to ensure 14hs light: 10h darkness.

Figure 5. Re-circulation system utilised for digestibility and growth evaluation for all materials tested.

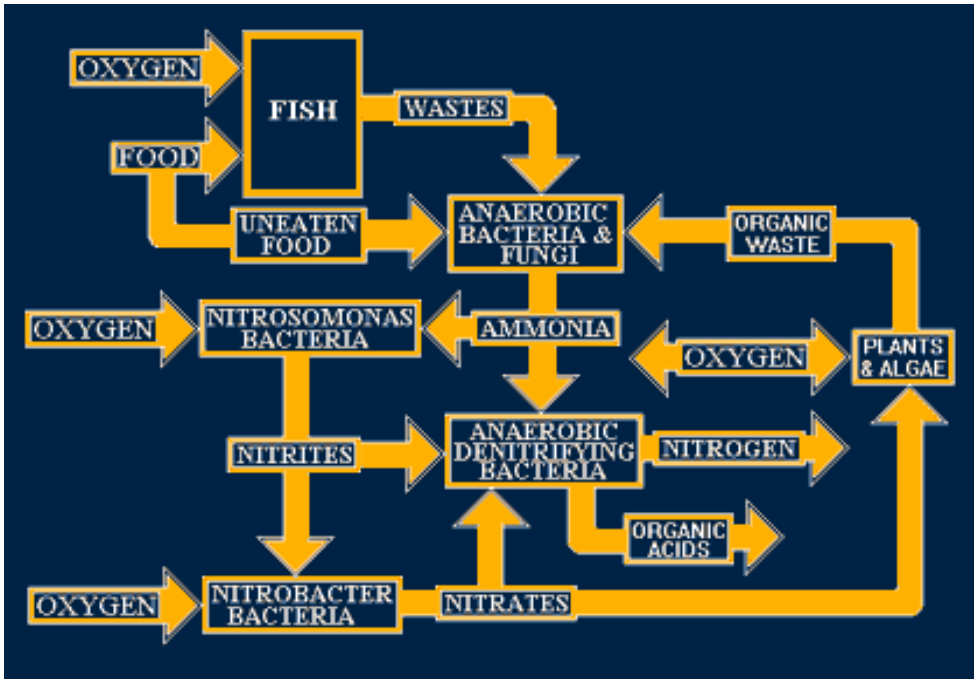

Figure 6. Nitrogen cycle in water for a freshwater aquarium system

2.4 Continuous rendering process utilised for test ingredients

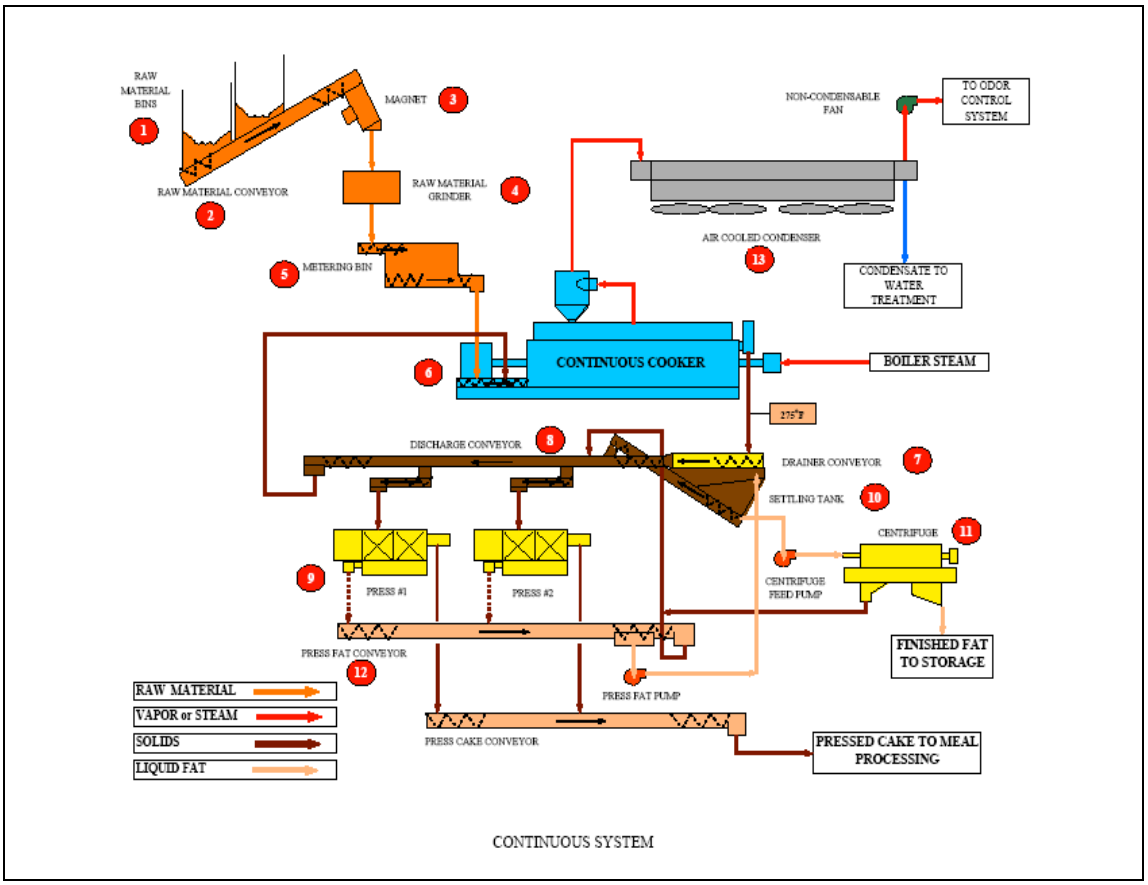

Figure 7.Continuous processing of animal by-product materials

Process explanation of Figure 7.

Raw material to be processed is kept within the temporary storage bins (1).

Material is moved from bins onto a conveyer belt (2) Material is then passed over a magnet (3) to remove any potential ferrous metal contaminants. All material is then subjected to grinding (4) this process ensures even particle size for ease of handling and allows more efficient heat transfer in the cooking step.

The ground material is fed at a controlled speed from the metering bin (5) into the continuous cooker (6). The cooker is a constantly agitated container heated to a temperature of 122-140°C this rapidly removes moisture and removes protein and fat from the bone of the raw material.

The de-watered slurry of proteins and solid material is slowly emptied from the cooker at a controlled rate. This slurry is then transported to the drain conveyor (7). This conveyor separates the fats from the solid material; this is then channeled to the discharge conveyor (8). In the discharge conveyor solid material from the drain conveyor are mixed with the solids material from the settling tank (10) and from the decanter type centrifuge (11).

The solid material from the discharge conveyor is sent to the screw presses (9) this process reduces the solid lipid content to circa 10-12%. Any solids that bypass the screw presses are re-distributed back to the continuous cooker. The solid materials that have successfully been pressed are discharged in the form of pressed cake and are sent to the pressed cake conveyor for transport to further processing to a meal.

The fat removed in the screw presses is channeled to the press fat conveyor (12), which removes large particles from the liquefied fat and returns them to the discharge

conveyor system. The lipid from the press fat conveyor is then sent to a settling tank (10). Fat discharged from the drainer conveyor (7) goes to the settling tank (10). The settling tank of lipids precipitates the heavier bone and protein particulates which settle in the bottom of the tank, this is then discharged by a screw conveyor into the discharge conveyor (8).

The liquid fat is then removed from the settling tank and pumped to the centrifuge (11) which removes all residual solids from the fat. The solid residuals from the centrifuge go to the discharge conveyor (8). The refined fat is then either further purified or transported to storage as finished fat.

Water vapour is discharged from the continuous cooker (6) through a vapour duct system that generally includes an entrainment trap to separate and return small particles to the cooker. The vapour duct system transports the vapour stream to an air cooled condensing system (13) which condenses the water vapour. (Other forms of air condensers maybe used such as direct contact or indirect shell and tube units may be used). Non condensable vapour gases are removed from the condenser by a non-condensable fan.

Redolent gases formed at differing points in the process are merged by a ductwork system and are pulled from the system along with non-condensable gases from the condenser to the odour control system (not shown) for neutralisation of the redolent components. The ingredients are defined in Table 3 (below). These materials form the basis for all investigations within this thesis for both the major digestibility trials and the successive growth study.

Table 3. Ingredient nutrient profile

Nutrient composition g kg^{-1}	Fishmeal [A]	SHF [B]	PMM [B]	SDH [C]	ETF [B]	SHF1 [B]	FDPM [B]	SPM 1 [B]	SPM 2 [B]
Dry matter	92	93	92	93	91	93	92	96	96
Protein	720	800	620	900	811	825	606	654	647
Lipid	120	30	130	10	30	32	131	121	123
Ash	90	30	130.5	30	32	33	130	132	131
Energy MJ kg^{-1} diet	20.6	20.5	20.2	21.3	20.67	21.38	18.75	19.68	19.34
Essential amino acid g / 100g	Fishmeal	SHF	PMM	SDH	ETF	SHF1	FDPM	SPM 1	SPM 2
Histidine	1.65	0.66	1.14	7.50	0.77	0.75	0.98	0.95	0.93
Arginine	4.54	6.13	4.17	4.00	3.63	3.66	3.30	3.29	3.30
Threonine	2.90	3.93	2.56	3.60	2.41	2.37	1.77	1.81	1.76
Valine	4.30	6.48	2.86	9.20	2.59	2.80	1.97	1.95	1.96
Methionine	2.08	0.50	1.00	0.80	0.81	0.82	0.98	0.98	0.98
Lysine	5.57	1.73	3.83	9.00	2.49	2.60	3.06	3.14	2.96
Isoleucine	3.13	3.98	1.82	0.60	1.83	1.95	1.60	1.57	1.45
Leucine	5.19	6.80	4.35	13.4	3.82	3.91	2.95	3.00	3.02
Phenylalanine	2.71	4.14	2.31	7.10	2.15	2.22	1.60	1.62	1.63
Tryptophan	0.77	0.35	0.55	1.20	n/a	n/a	n/a	n/a	n/a

[A] Fishmeal, Icelandic (LT-94), Skretting, Longridge, Preston, UK. [B] Steam hydrolysed feathermeal (SHF), PDM, Doncaster UK. [B] Poultry meat meal, [C]Spray Dried Haem (SDH), American Protein Corporation (APC) Des Moins, Iowa, USA. [B] ETF, Enzyme treated feathermeal (Allzyme), [B] SHF1, Standard hydrolysed feathermeal, [B]FDPM, Fast dried poultry meat meal [B] SPM 1-2, Standard poultry meal. N/A not available.

2.5 Definition and Specification of ingredients employed

LT Fishmeal Icelandic: The production of LT meal implies a reduced drying temperature (<90°C). The result of this lower drying temperature is reduced protein damage and lipid peroxidation. The standard specification for an LT grade fishmeal is minimum 72% crude protein and an oil content of 10%, ash 9% and total volatile nitrogen TVN in raw material 150mgN 100^{-1} .

Steam hydrolysed feathermeal (SHF): Poultry feathers are steam hydrolysed at up to 227 kpa for approximately 30 minutes in a continuous hydrolyser. The hydrolysed feathers are then dried in an indirect steam heated drier (Rota-disc drier) to ~5% moisture, cooled, and milled the protein content is typically around 80%. The pressure is critical as over pressurising (above 227kpa) can potentially reduce amino acid availability.

Poultry meat meal (PMM): mixed species poultry material is reduced in size by mincing to <30mm, introduced into a continuous process dryer (Rota-disc) that evaporates water in the presence of natural fat levels and sterilises the components. The residence time is approximately 90 minutes and the maximum temperature reached is 125ºC. On leaving the process, dried components are separated into a protein fraction, and excess fat is removed by an expeller press. The protein fraction (PMM) is cooled, milled and treated with an antioxidant.

Spray Dried Haemoglobin (SDH): the raw material is obtained from whole porcine blood. Blood is chilled and separated into plasma and red blood cells by centrifugation. The red blood cell fraction (haemoglobin) is spray dried to produce a

dry (< 5% moisture) powder with a protein content of around 90% and rich in lysine and histidine.

Enzyme treated feathermeal (ETF): mixed poultry feathers are heated to 50ºC in the presence of an enzyme (Allzyme, major enzyme activities include amylase, cellulase, phytase, xylanase, beta glucanase, pectinase and protease) this is continually mixed for 30 minutes. Following the enzyme hydrolysis, the feathers are pressure processed at 200 kpa for 15 minutes. The enzyme hydrolysed feather meal is dried in a Rota-disc drier to 5% moisture and cooled.

Standard hydrolysed feathermeal (SHF1): Mixed species poultry feathers are hydrolysed for approximately 30 minutes. The hydrolysed feathers are dried in an indirect steam heated drier (Rota-disc drier) to ~5% moisture, cooled, and milled. These particular feathers are hydrolysed at a higher pressure (450kpa) than steam hydrolysed feathermeal and at a higher temperature (141°C) as such maybe damaged as part of this process.

Fast dried poultry meat meal (FDPM): mixed species poultry material are reduced in size by mincing to < 30mm and introduced into a continuous process which contains high levels of poultry fat (1 part Raw Material: 5 Parts Fat). Water is evaporated and the components are sterilised during the process. The residence time is approximately 30 minutes and the maximum temperature attained is 135-140ºC. On leaving this process the dried components are separated into a protein fraction and fat by pressing in an expeller press. The protein fraction (PMM) is cooled, milled and treated with an antioxidant.

Standard poultry meal 1&2 (SPM1 & SPM2): mixed species poultry material are reduced in size by mincing to < 30mm and introduced into a continuous process (Rota-disc) that evaporates water in the presence of natural fat levels, this also sterilises the raw material. The residence time is approximately 90 minutes. Maximum temperatures attained are 125ºC. On leaving this process, the dried components are separated into protein/fat fractions by pressing in an expeller press. The protein fraction (PMM) is cooled, milled and treated with an antioxidant. These particular poultry meal products are from two different processing plants with differing species of poultry the purpose of testing these 2 differing materials was establish if avian species had any positive effect on digestibility.

Blended ingredients: Additionally, composite blends were made with ratios of 75/25 of the following ingredients: SHF/SDH, PMM/SDH, and ETF/SDH. These blends are subject to the same processing treatments on individual ingredients as stated in the previous text.

2.6 Diet preparation, manufacture and composition

All test diets for the digestibility studies were prepared in 2 kg batches. All of the raw materials were first individually weighed, and placed into a 5kg stainless steel food mixer bowl. Diets were then dry mixed for approximately twenty minutes in a Hobart A120 food processor (Hobart Manufacturing Company Ltd, London, England).

After initially blending the dry ingredients, marine fish oil was added very slowly in a continuous flow. After a period of five minutes further mixing, distilled water was added to the ingredients until the mixture within the bowl reached a dough-like consistency. A typical volume of supplementary water added was between 300-400ml per kg of dry mix.

Pelleting was achieved by use of the mincer attachment of the Hobart processor; the extruder was fitted with a 3mm-die to achieve the desired pellet. The 3mm strands produced by the mincer were carefully broken up, and spread onto trays lined with tinfoil; the trays were placed into a drying cabinet at 55°C where the diets were left to dry for 48 hours. The dried diets were then transferred into airtight opaque containers for storage in a cool dry place prior to feeding the diets to experimental fish. Small samples of fresh diets were also taken for proximate composition and amino acid analysis within seven days of their manufacture.

Table 4.Diet feed composition for digestibility trial 1 (g/kg).

Ingredient	**Fishmeal**	**(SHF)**	**(PMM)**	**(SDH)**	**(SHF/SDH)**	**(PMM/SDH)**
Fishmeal LT-94 [A]	600	360	360	360	360	360
Feathermeal [B]	0	380	0	0	285	0
Bloodmeal [C]	0	0	0	360	95	95
Poultry meat meal [B]	0	0	360	0	0	285
Cod liver oil [D]	60	100	60	100	100	75
Corn starch/Dextrin 2:1[E]	315	135	195	155	135	160
Vit & Min premix 50:50[A]	20	20	20	20	20	20
Chromic oxide[E]	5	5	5	5	5	5

[A]Skretting, Longridge Preston UK. [B]Prosper de Mulder, Doncaster, UK. [C]American Protein Corporation (APC) Des Moins, Iowa, USA.. [D]Seven Seas, Hull, UK. [E]Sigma Chemical Company, Poole, Dorset, UK.

Table 4a.Proximate composition of diets for digestibility trial 1 as fed.

Nutrient composition g kg^{-1}	**Fishmeal**	**(SHF)**	**(PMM)**	**(SDH)**	**(SHF/SDH)**	**(PMM/SDH)**
Moisture	81	63	82	84	76	83
Protein	454	560	523	613	593	578
Lipid	162	151	172	154	157	164
Ash	110	80	121	84	85	112
Gross Energy MJ kg^{-1} diet	21.3	20.1	20.3	22.1	22.2	21.4
Essential AA g / 100g	**Fishmeal**	**(SHF)**	**(PMM)**	**(SDH)**	**(SHF/SDH)**	**(PMM/SDH)**
Histidine	0.76	0.91	1	2.95	1.4	1.33
Arginine	3.77	3.26	3.56	3.13	3.36	3.53
Threonine	2.42	1.8	1.96	2.04	1.95	2.34
Valine	3	1.89	2.04	4.12	2.21	3.3
Methionine	0.8	1.03	1.05	0.85	0.92	0.79
Lysine	2.28	3.1	2.98	4.15	3.19	2.76
Isoleucine	2.17	1.49	1.65	1.13	1.33	1.94
Leucine	4.05	2.93	3.23	6.23	3.82	4.52
Phenylalanine	2.31	1.55	1.76	3.08	2.05	2.52

Table 5.Diet feed composition for digestibility trial 2 (g/kg).

Ingredient	**Fishmeal**	**ETF**	**SHF1**	**FDPM**	**SPM1**	**SPM2**
Fishmeal LT-94 [A]	600	360	360	360	360	360
ETF[B]	0	380	0	**0**	0	0
SHF1 [B]	0	0	380	0	0	0
FDPM[B]	0	0	0	380	0	0
SPM1 [B]	0	0	0	0	380	0
SPM2 [B]	0	0	0	0	0	380
Cod liver oil [C]	110	100	100	90	90	90
Corn starch/Dextrin (2:1)[D]	250	130	130	140	140	140
Vit & Min premix 50:50 [A]	20	20	20	20	20	20
Chromic oxide [D]	5	5	5	5	5	5
Alpha cellulose [D]	14	4	4	4	4	4

[A]Trouw Aquaculture, Longridge Preston UK. [B]ETF, Enzyme treated feathermeal, [B]Standard Hydrolysed SHF 1, [B]FDPM Fast dried poultry meat meal, [B]SPM 1 Poultry meat meal, [B]SPM 2 Poultry meat meal, Prosper de Mulder, Doncaster, UK. [C]Seven Seas, Hull, UK. [D]Sigma Chemical Company, Poole, Dorset, UK.

Table 5a.Proximate composition of feed for digestibility trial 2 as fed.

Nutrient composition g kg^{-1}	**Fishmeal**	**ETF**	**SHF1**	**FDPM**	**SPM 1**	**SPM 2**
Moisture	82	81	85	85	83	87
Protein	432	564	565	481	486	484
Lipid	173	159	157	171	180	184
Ash	103	121	123	77	95	83
Gross Energy MJ kg^{-1} diet	20.3	20.3	20.4	20.1	20.4	20.4
Essential AA g / 100g	**Fishmeal**	**ETF**	**SHF1**	**FDPM**	**SPM 1**	**SPM 2**
Histidine	0.87	0.77	0.75	0.98	0.95	0.93
Arginine	2.79	3.63	3.66	3.3	3.29	3.3
Threonine	1.69	2.41	2.37	1.77	1.81	1.76
Valine	1.81	2.59	2.8	1.97	1.95	1.96
Methionine	1.01	0.81	0.82	0.98	0.98	0.98
Lysine	3.14	2.49	2.6	3.06	3.14	2.96
Isoleucine	1.36	1.83	1.95	1.6	1.57	1.45
Leucine	2.79	3.82	3.91	2.95	3	3.02
Phenylalanine	1.44	2.15	2.22	1.6	1.62	1.63

2.7 Feeding protocol

Fish were randomly stocked into triplicate groups comprising 15 fish per tank, the fish were then fed on the test diets for a period of 4-5 days to acclimate them to the change from the commercial feed. Fish were then fed to 1.5% of their body weight, this level of feeding was appropriate for the temperature (15°C) and size of fish used (215g). Fish were fed the daily ration over a period of 30 minutes between 4pm-5pm daily. The fish were fed the test diets for a further 14 days and then stripped (see Figure 8) of their faecal material under anaesthetic (MS222). Fish were then recovered using well aerated water. This procedure was carried out at an approved Home Office licenced facility.

Figure 8.Faecal stripping of rainbow trout, as adovocated by Austreng, 1978.

2.8 Proximate chemical composition

2.9 Crude protein

The protein contents of fish feeds and carcasses were determined by the Kjeldahl method. Typically 50-100mg of the dried feed, faeces or carcass was weighed into a weighing boat and then transferred to the borosilicate digestion tube. Prior to digesting of the samples, the Gerhardt Turbosog unit was switched on to neutralize acid fumes by means of bubbling through 15% NaOH, the resulting fumes then condense in a neutralisation chamber.

Digestion was performed on 40 position Gerhardt Kjeldatherm digestion block (C. Gerhardt Laboratory Instruments, Bonn, Germany) for 30 minutes at 220°C. The temperature was then increased to 380°C for a further 60 minutes and at this stage samples were completely digested. The borosilicate tube rack was then removed from the heating block and allowed to cool, ensuring the Turbosog scrubber unit was left on for at least a further 30 minutes after the tubes were removed from the heating block.

The Gerhardt Vapodest 40 (Vap 40 Distillation Unit) was prepared by priming the NaOH, distilled water, and orthoboric acid pipelines. The Vapodest unit dilutes the sample with distilled water (typically 6 times sample volume) and neutralizes the sample with 37% NaOH.

The ammonia in the sample is then collected in a conical flask containing 50cm^3 boric acid with '4.5' BDH indicator by steam distillation. The resulting distillate is then titrated against 0.1 M HCl and the percentage protein in the dry sample calculated by the following equation:

$$\% \text{ Crude protein} = \frac{(\text{Sample titre (ml)} - \text{Blank titre (ml)}) \times 0.10 \times 14 \times 6.25}{\text{Sample weight (mg)}} \times 100$$

Equation values:
0.10= HCL in moles
14= Relative atomic mass of nitrogen (N)
6.25= Constant relationship between N and animal protein of sample

2.9.1 Determination of total lipid

Lipid contents of feed and carcass were determined by a modification of the Folch method. Modifications included using 6-7 N HCl to perform acid hydrolysis prior to extraction to yield the extra lipid bound to the protein in the feed. 2g of feed (0.5g carcass) were placed in a 50ml polypropylene centrifuge tube to which 10ml of 6M HCl was added with 10ml of methanol, the tubes were capped and then placed in the oven for 30 minutes at 70°C.

After cooling of samples to room temperature 18ml of dichloromethane (DCM) were added to each tubes, tubes were then recapped and shaken vigorously and left to stand for a further hour. Tubes were then re-shaken and centrifuged for 10 minutes at 3000 RPM (MSE Mistral 3000 refrigerated centrifuge).

After centrifugation the upper-layer was carefully decanted with a 10 ml pipette leaving the lower layer and the sample of feed. A 5ml Hamilton gas tight syringe was carefully inserted through the sample and up to 3 ml of extract collected. 2ml of this was accurately pipetted into an empty pre-weighed 4ml vial and then the syringe washed with a further 1ml of clean DCM. All vials were evaporated using a gentle stream of nitrogen until all solvent was removed, vials were then placed into a drying oven (Pickstone E 70F oven, R.E. Pickstone Ltd., Thetford, Norfolk, U.K) at 105°C for 1 hour. Vials were then transferred to a dessicator to cool and were reweighed.

Lipid content was calculated using the following formula:

$\% \text{ Lipid} = \frac{\text{Weight gain of vial in (g)}}{\text{Sample weight in (g)}} \times \text{dilution} \times 100$	Equation values: Dilution amount in ml

2.9.2 Moisture determination

Moisture determinations for feed and carcass were carried out according to A.O.A.C (1990). Weighed samples were dried to constant weight at 105°C in a fan assisted/exhaust extracted drying oven. (Pickstone E 70F oven, R.E. Pickstone Ltd., Thetford, Norfolk, U.K).

The percentage moisture content in the sample was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{\text{Change in weight (g)}}{\text{Initial weight (g)}} \times 100$$

2.9.3 Determination of ash content

Ash content of the previously dried feed or carcass was determined in accordance with A.O.A.C (1990). 450-550mg of sample was weighed accurately into pre-weighed ceramic crucibles and incinerated at 550°C for 12 hours in a Carbolite GLM 11/7 muffle furnace (Carbolite Furnaces Ltd., Bamford, Sheffield, U.K). The resulting residue in the crucible was the ash of the sample. The residue was then re-weighed (including crucible weight) and a comparison made with the starting weight of the sample, (pre-combustion) this difference in weight was the ash content of the sample.

The calculation for ash content in samples is as follows:

$$\text{\% Ash Content} \quad \frac{\text{(Weight of crucible + ash) – weight of crucible (g)}}{\text{sample weight (g)}} \times 100$$

2.9.4 Gross energy by Adiabatic Bomb Calorimetry

All samples were analysed using the Parr Adiabatic Bomb Calorimeter model No.6200 (Parr Instrument Company, Moline, Illinois).

Samples were first pelleted using a tablet press, the tablet was weighed. The tablet was placed into a nickel crucible which in turn was placed into the bomb crucible carrier. A piece of 10cm fuse wire was then cut and attached to each electrode (+ and - terminals), the fuse wire was carefully adjusted so the wire just touched the top of the sample in the crucible. The lid of the bomb was then attached to the main body and carefully screwed hand tight. A pure oxygen line was then attached to the bomb and the pressure in the bomb was increased to 3100 kpa. The stainless steel water bath in which the bomb is located was first tared on a balance (accuracy to 0.1g) and then filled with approximately with 2-litres of distilled water.

The bomb vessel was then placed into the water bath and the electrical terminals attached to the bomb vessel. The bomb was allowed to equilibrate the water jacket temperature to the bucket temperature. When this was achieved the bomb was fired and the temperature increase of the water bath recorded until no further increase in bucket temperature was detected. The resulting increase in temperature was used to calculate the energy content of the feed, faecal material, and carcass. Integrated software within the calorimeter automatically calculated the result in MJ/kg.

2.9.5 Amino acid analysis

75-100mg samples were weighed into plastic weighing boats and the weighed sample transferred to a 4ml sealable glass ampoule. To this sample 3ml of 6N HCl was added to digest the sample. For tryptophan 50µl 4N methane sulfonic acid was used). Before sealing the ampoules 0.25ml of Norleucine (40mM) of internal standard was added to

each vial. The ampoules were sealed using a fine torch Bunsen burner. Ampoules were placed into a drying oven at 110ºC for 24 hours (for tryptophan 22h at 110ºC). Ampoules were removed, allowed to cool to room temperature and refrigerated until analysis. Before analysis samples were diluted with HPLC grade water (40ml) after which they were transferred to 1ml HPLC vials and sealed. The vials were then loaded onto the auto sampler tray ready for analysis.

Amino acid analyzer conditions:

All samples were subjected to Pre-column analysis using automatic O-Phthaldildehyde OPA derivatisation with gradient HPLC and fluorescence detection.

Instruments required: Gradient pump; Low pressure mixing, solvent degasser: auto injector: auto sampler: detector type: Fluorescence Detector. Column type: Nucleosil C18. 5µm, 60 x 4 mm. Knauer, pre-column: Nucleosil C18. 5µm, 5 x 4 mm. Knauer.

Mobile phase conditions: Mobile phase A: 0.1 M sodium acetate, pH 6.95: methanol: tetrahydrofuran (92.5: 5: 2.5)

Mobile phase B: methanol: tetrahydrofuran (97.5: 2.5), Flow rate: 1.2 ml/minute

Detection: excitation, 330 - 365 nm; emission, 440 - 530 nm.

Derivatisation: OPA / 2-mercaptoethanol, temperature: Auto sampler: +60ºC, Column conditions: Ambient

Reagents: Acetate buffer, 100 mmol/L, pH 6.95, Sodium acetate, Mw: 82.03 ; 8.2 g, distilled water 900 ml, Adjust pH to 6.95 with acetic acid, distilled water 1000 ml, filter through a 0.45 µm filter.

2. Mobile phase A: Acetate buffer 925 ml, methanol 50ml, tetrahydrofuran, 25 ml.

3...Mobile phase B: methanol: 975 ml, tetrahydrofuran: 25 ml

4. Borate buffer, 0.4 mol/L, pH 9.3, boric acid, H3BO3: 2.47 g, distilled water to: 90

ml Adjust pH to 9.3 with sodium hydroxide, distilled water to: 100 ml. 5. Derivatisation reagent, OPA 40 mmol/L, o-phthaldialdehyde, C8H6O2, Mw: 134.1 Sigma P-1378: 27 mg, ethanol, 99 %: 500 µl, 2-mercaptoethanol, SHCH2CH2OH, Fluka: 20µl borate buffer (4): 4.5 ml. Store all buffers in refrigerator for 24 hours before use. Add 5 µl 2-mercaptoethanol once a week, can be used for up to two weeks.

2.9.6 Chromic oxide determination

The chromic oxide contents of fish feeds and faecal samples were determined by the method of Furukawa & Tsukahara, (1966). Typically 50-100mg of the dried feed, faeces or carcass was weighed into a weighing boat and then transferred to a borosilicate digestion tube to which was added 6ml of nitric acid (Aristar grade). Samples were digested for approximately one hour by which time all of the carbon was oxidised. Samples were allowed to cool to room temperature.

To each tube 3ml of 70% perchloric acid (Analar grade) was added. Samples were then digested for 75mins at 220°C. Once cooled samples showed a strong red colour indicating that all of the chromic (III) oxide has been oxidised to hexavalent chromium (which is very toxic). Samples were carefully decanted from each tube into a 50ml volumetric flask. The digestion tubes were washed with distilled water and any residue poured into the volumetric flask which was made up to 50ml with distilled water and then poured into a 50ml polypropylene tube.

Calibration standards of varying concentrations were then made within the tolerance of the machine to produce a standard curve. Finally, samples were run on a Varian (model no.SpectrAA600) Atomic Absorption Spectrophotometer at a wavelength of 425.4nm.

2.9.7 Digestibility calculations

Digestibilities of the nutrient components in diets were calculated according to equation 1 and the respective ingredient by the ratio of test ingredient contribution and reference diet as stated in equation 2. These are described by Lupatsch et al., (1997) as applied to sea bream.

Equation (1)

$$ADC\ (\%) = 100-[100x\ (Cr_2\ O_3\ feed / Cr_2\ O_3\ faeces) * (Nutrient\ faeces / Nutrient\ food)]$$

Apparent digestibility coefficient (ADC)
$Cr_2\ O_3$ and nutrient in g kg

Equation (2)

Partial digestibility coefficients were calculated using:

$$DC_T = [DC_D - (DC_R * r) / t$$

Formula described below

Where DC_D is the digestibility coefficient of the nutrient in the test diet (%); DC_R is the digestibility coefficient of the nutrient in the diet which is fishmeal (%); DC_T is the digestibility coefficient of the nutrient in the test (%); r is the contribution of the nutrient of the reference ingredient to the diet (%); and t is the contribution of the nutrient of the test ingredient to the diet (%).

Chapter 3

Results
(Digestibility, trial 1 & 2)

3 Results, trial 1 and 2

3. 1 Digestibility evaluation

The reference diets for both experiments (trial 1 and 2) correlated well in terms of proximate composition, and protein/energy digestibility. This was an important consideration with respect to comparisons between separate experiments using different fish stocks.

Rainbow trout fed well on each of the experimental diets evaluated and there were no observed palatability problems associated with inclusion of high levels of test ingredients.

Growth was not assessed during the three-week period as the time frame was not expected to yield significant growth. In addition, the diets were not designed to be balanced with respect to protein and energy (neither isonitrogenous nor isoenergetic).

3.2 Dry matter, crude protein and energy

The digestibilities of Dry Matter (DM), Crude Protein (CP), and Energy (E) for the diets (Trial 1) are displayed in Table 6.

Similar data for (Trial 2) are presented in Table 7. These values represent mixtures of the fishmeal diet and each test ingredient and reflect possible interactions between these two components of the diet fed to rainbow trout. Mean values for DM ranged from 59-73% for both trials.

CP digestibility coefficients for mixtures are displayed in Tables 6 & 7, these values ranged from 73.6-93.4%.

E was also highly digestible and all values reported were above 72% for the complete mixtures.

Table 6.Apparent digestibility profile of combination test diets trial 1, Fishmeal + test ingredient mixture, (N=3 ± SEM).

Digestibility %	LT-94 Fishmeal	SHF	PMM	SDH	PMM/SDH	SHF/SDH
Dry matter	72.56 ± 1.29	71.32 ± 1.63	69.10 ± 0.26	77.95 ± 1.43	73.45 ± 0.67	71.02 ± 2.16
Dietary Protein	90.49 ± 0.78	77.87 ± 1.60	82.18 ± 0.89	93.41 ± 0.29	86.72 ± 0.86	83.63 ± 0.39
Energy	79.81 ± 1.73	74.08 ± 1.47	72.09 ± 2.58	83.68 ± 0.96	77.69 ± 1.13	76.53 ± 2.36

Table 7.Apparent digestibility profile of combination test diets trial 2, Fishmeal + test ingredient mixture, (N=3 ± SEM).

Digestibility %	LT-94 Fishmeal	ETF	SHF1	FDPM	SPM 1	SPM 2
Dry matter	75.40 ± 1.36	72.78 ± 1.01	65.67 ± 0.59	59.79 ± 0.55	67.44 ± 1.88	62.69 ± 1.29
Dietary Protein	91.78 ± 0.66	82.12 ± 1.01	73.57 ± 1.21	75.36 ± 1.09	80.77 ± 0.53	76.80 ± 0.93
Energy	83.37 ± 1.31	80.10 ± 0.68	72.89 ± 0.52	73.66 ± 0.72	77.67 ± 1.19	73.07 ± 1.33

3.3 Test ingredients dry matter, crude protein and energy

The Apparent Digestibility Coefficients (ADC) of the test ingredients are displayed in tables 8 & 9. These values relate to the separate ingredients and assume non-interaction with the basal reference diet. Highest values were obtained for fishmeal within the reference diet and Spray Dried Haem (SDH). The lowest CP digestibility was recorded for the FDPM (Table 9) which resulted in a digestible crude protein (DCP) of 59.13%. The trends and values reported in the various tables are presented in histogram format in Figs 9 & 10.

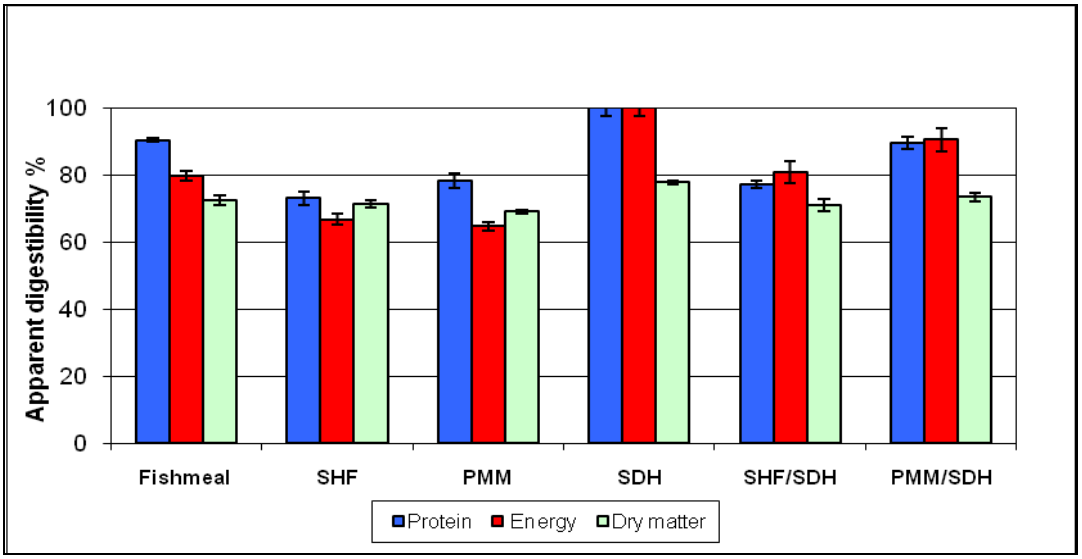

Figure 9.Digestibility of test ingredients as fed to rainbow trout (1) (n=3, ±SEM)

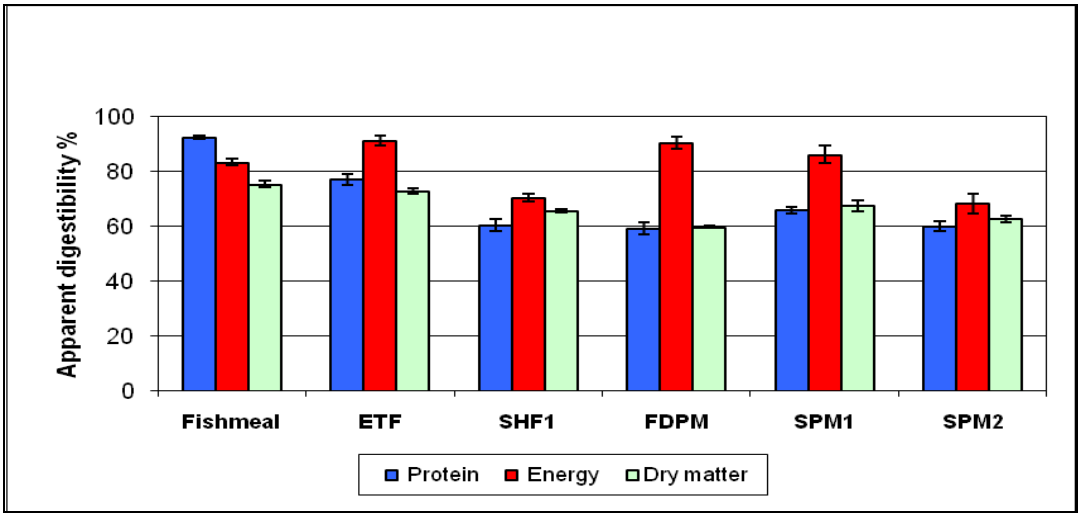

Figure 10.Digestibility of test ingredients as fed to rainbow trout (2) (n=3, ±SEM)

Table 8.Apparent digestibility of test ingredients trial 1 (N=3 ± SEM).

Digestibility %	LT-94 Fishmeal	SHF	PMM	SDH	PMM/SDH	SHF/SDH
Dry matter	72.56 ± 1.29	71.32 ± 1.63	69.10 ± 0.26	77.95 ± 1.43	73.45 ± 0.67	71.02 ± 2.16
Dietary Protein	90.49 ± 0.78	73.05 ± 5.29	78.35 ± 3.30	100 ± 0.90	89.66 ± 3.00	77.21 ± 1.22
Energy	79.81 ± 1.73	66.85 ± 3.86	64.72 ± 6.89	100 ± 4.43	90.52 ± 10.81	80.88 ± 10.83

Table 9.Apparent digestibility of test ingredients trial 2 (N=3 ± SEM).

Digestibility %	LT-94 Fishmeal	ETF	SHF1	FDPM	SPM 1	SPM 2
Dry matter	75.40 ± 1.36	72.78 ± 1.01	65.67 ± 0.59	59.79 ± 0.55	67.44 ± 1.88	62.69 ± 1.29
Dietary Protein	91.78 ± 0.66	77.04 ± 1.86	60.26 ± 2.18	59.13 ± 2.28	65.82 ± 1.02	60.13 ± 1.84
Energy	83.37 ± 1.31	91.36 ± 1.77	70.44 ± 1.32	90.43 ± 2.24	86.07 ± 5.58	68.31 ± 3.53

3.4 Amino acid test mixtures and ingredients

The apparent Amino Acid (AA) digestibility data displayed in Tables 10 & 11 for the fishmeal diet and in combination with each test ingredient shows very high digestibility for all amino acids determined. These were all generally above 70% with most in the region of 80-88%. Many AA were actually more than 90% digestible principally for the fishmeal protein constituting the reference diet and the SDH protein sources. High amino acid digestibilities for all other products indicated uniform availability suggesting little interaction between the fishmeal diet and test ingredient.

Table 10.Apparent digestibility coefficients (%) of essential amino acids in combined fishmeal and test diets, (trial 1)

Amino Acid	LT-94 Fishmeal	SHF	PMM	SDH	PMM/SDH	SHF/SDH
Histidine	93.14	87.91	86.81	96.97	93.15	90.22
Arginine	95.26	83.39	87.12	92.22	88.24	87.76
Threonine	96.10	74.61	86.06	94.19	83.37	89.47
Valine	97.52	76.72	87.53	96.97	83.68	92.87
Methionine	94.10	91.24	88.93	93.88	91.81	87.03
Lysine	96.19	91.45	90.30	97.19	94.06	90.37
Isoleucine	97.14	82.02	87.44	92.45	83.86	90.44
Leucine	97.30	79.69	88.14	97.43	88.58	92.28
Phenylalanine	96.78	77.98	87.15	96.96	87.97	91.87
Tryptophan	N/a	N/a	N/a	N/a	N/a	N/a

N/a Not available

Table 11.Apparent digestibility coefficients % of essential amino acids in combined fishmeal and test diets (trial 2)

Amino Acid	LT-94 Fishmeal	ETF	SHF1	FDPM	SPM 1	SPM 2
Histidine	91.43	84.27	75.91	74.33	76.67	74.20
Arginine	95.32	87.66	80.91	83.01	85.31	84.98
Threonine	92.12	80.48	70.95	73.67	75.71	73.63
Valine	93.23	80.32	71.88	75.51	79.51	78.53
Methionine	92.88	86.44	82.54	78.12	80.72	79.53
Lysine	95.68	89.48	86.37	80.06	84.73	83.79
Isoleucine	92.76	82.92	76.58	77.84	81.02	75.59
Leucine	94.18	84.08	75.77	76.38	79.78	79.16
Phenylalanine	92.60	84.80	75.28	74.74	77.80	75.63
Tryptophan	N/a	N/a	N/a	N/a	N/a	N/a

N/a Not available

Apparent digestibility coefficients for EAA in test ingredients are presented in Tables 12 & 13. These values relate to test ingredients weighted to their proportion within the mixture that includes the fishmeal diet. It assumes minimum interaction between the proteins and should reflect the actual amino acid availability of the separate protein components of these ingredients. All values were consistent with expected trends for protein digestibility and the amino acid digestibility previously reported for the mixtures.

Table 12.Apparent digestibility (%) of essential amino acids for test ingredients (trial 1)

Amino Acid	SHF	PMM	SDH	PMM/SDH	SHF/SDH
Histidine	91.63	88.74	100	100	97.70
Arginine	76.55	86.38	99.80	89.33	88.07
Threonine	52.20	82.32	100	75.25	91.29
Valine	55.61	84.07	100	73.93	98.11
Methionine	98.94	92.88	100	100	87.87
Lysine	96.37	93.36	100	100	93.53
Isoleucine	70.12	84.39	97.58	74.98	92.28
Leucine	63.75	85.99	100	87.16	96.89
Phenylalanine	60.05	84.19	100	86.34	96.60
Tryptophan	N/a	N/a	N/a	N/a	N/a

Table 13.Apparent digestibility (%) of essential amino acids for test ingredients (trial 2)

Amino Acid	ETF	SHF	FDPM	SPM 1	SPM 2
Histidine	84.62	62.63	58.46	64.62	58.12
Arginine	87.71	69.93	75.45	81.52	80.65
Threonine	73.62	48.53	55.69	61.06	55.57
Valine	71.51	49.31	58.86	69.38	66.80
Methionine	88.15	77.89	66.25	73.10	69.97
Lysine	91.96	83.77	67.16	79.45	76.98
Isoleucine	79.07	62.39	65.59	74.09	59.78
Leucine	79.98	58.12	59.72	68.69	67.05
Phenylalanine	84.26	59.20	57.78	65.82	60.11

The LT fishmeal within the reference diet for both trials 1 and 2 clearly demonstrated high digestibility coefficients for all EAAs with a mean of about 95%. The other ingredients showed distinct variations that were more noticeable when test ingredients are demarcated with respect to the basal component.

It was obvious that SDH was highly available with all amino acid digestibility coefficients values close to, or at, 100%. SHF was markedly inferior for specific amino acids especially threonine, valine, isoleucine, leucine and phenylalanine. In trial 2 a similar type of feathermeal SHF1 also showed inferior performance. In contrast an enzyme treated feathermeal ETF yielded better amino acid digestibility compared to a standard grade material obtained from the same source and manufacturer.

Interestingly, PMM alone demonstrated good and consistent performance for each amino acid evaluated, all within the 82-93% range. The derived combination of PMM and SDH resulted in noticeable improvements for certain amino acids (up to 100% digestibility) but seemed to cause a reduction in the digestibility of other amino acids. The feathermeal/SDH combination was actually very well digested in terms of amino acids for rainbow trout and this suggested a synergistic effect which raised the quality of the combined ingredients compared to feathermeal alone.

Overall the data supports evidence that variations can be expected for different ingredients included in a test mixture fed to rainbow trout and that selected nutrient digestibility coefficients can be obtained from the weighting of each test ingredient in association with the reference diet-test ingredient blend. The poultry meat meal products evaluated in the second trial FDPM, SPM1 & SPM2 were all inferior to the poultry meat meal used in trial 1. The amino acid digestibility ranged as follows: FDPM (55-75%), SPM 1 (61-81%), and SPM 2

(58-80%) respectively. All values reported are displayed in histogram format in Figures 11 & 12 for visual comparisons.

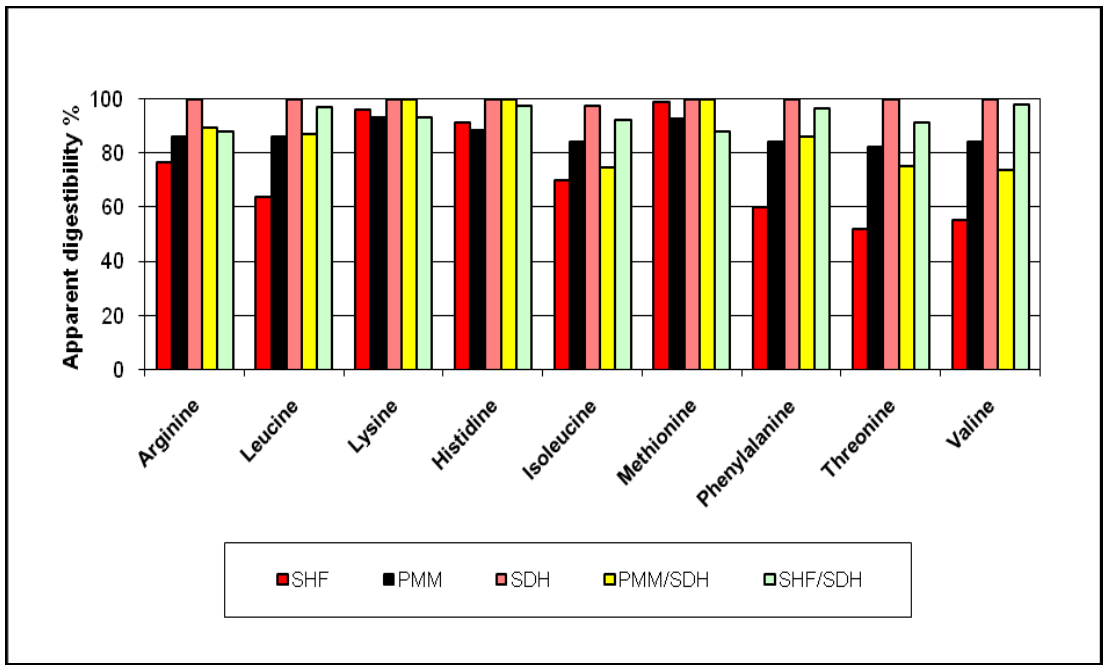

Figure 11.Amino acid digestibility of test ingredients as fed to rainbow trout in trial 1.

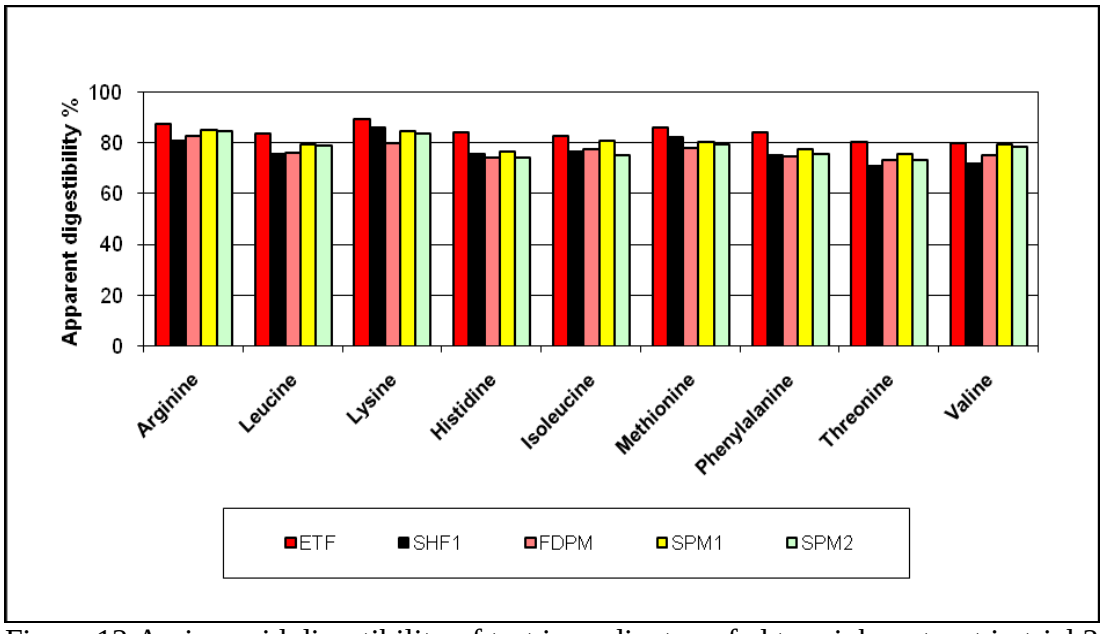

Figure 12.Amino acid digestibility of test ingredients as fed to rainbow trout in trial 2.

Chapter 4

Materials and methods (2)
(Growth evaluation & Health)

4.1 FEEDING AND GROWTH EVALUATION

The digestibility evaluation as described in the previous section forms the basis for the final evaluation of test ingredients. Test ingredients selected for growth evaluation performed well when large substitutions of fishmeal were made in previous experiments.

Diets were formulated using apparent digestible protein/energy values obtained from the previous digestibility trials so that realistic amounts of fishmeal protein were substituted in diets for rainbow trout. There were 7 diets in total, 6 tested in triplicate and the control in duplicate. The trial duration was for 12 weeks.

4.2 Fish stock

Rainbow trout of all female origin 20-25g in weight were obtained from Hatchlands Fisheries, Greyshoot Lane, Rattery, South Brent, U.K. Trout were transported from the trout farm in a 1000L tank supplied with pure oxygen (British Oxygen Company, Plymouth, U.K).

Fish were acclimated to the experimental system over a period of four weeks. Fish were fed Trouw Aquaculture Elite 2mm diet (now Skretting, UK) until they reached a mean weight of 35-40g. All fish within each tank (15) were individually weighed under anaesthetic (MS222) prior to the start of the trial to ensure uniformity of fish size before being fed the experimental feeds. All fish procedures were carried out in accordance with to the 1986 scientific procedures act.

4.3 Fish holding system

The twenty tank fish holding facility was as previously described in the general materials and methods (2.3).

4.4 Diet preparation and manufacture

Test diets for the growth evaluation trial were prepared in 6kg batches, formulas were as described in Table 14. All raw materials were first individually weighed and placed into a 136L mini-mix, cement mixer (Belle Group, Sheen, NR Buxton, Derbyshire, SK17 OEU), and diets were dry mixed for approximately twenty minutes.

After the initial blending period marine fish oil was added very slowly in a continuous flow. After a period of ten minutes further mixing, distilled water was added to the ingredients until a dough-like consistency was achieved. A typical volume of supplementary water added was between 200-300ml per kg of dry mix.

Pelleting was achieved using a California Pellet Mill 1114 E. Wabash Avenue Crawfordsville, IN (USA) 47933 (CPM) and a 3mm-die was utilized to achieve the desired pellet size. The 3mm pellets produced by the CPM were carefully collected in a large stainless bowl and then spread onto plastic trays that were placed into a drying cabinet at 55°C for 48 hours to less than 10% moisture. Dried diets after cooling (to stop pellet sweating) were then transferred into airtight opaque containers for storage in a cool dry place prior to feeding to experimental fish. Small samples of fresh diets were also taken for proximate composition and amino acid analysis within seven days of their manufacture.

4.5 Feeding protocol (growth trials)

Fish were fed by hand twice daily utilizing an established chart feeding rate (based on a similar Skretting protein/oil ratio trout diet) for fish of 35-40g. Feed input was increased daily, assuming a Feed Conversion Ratio (FCR) of 1, and fish were re-weighed on a fortnightly basis

to ensure that growth performance of the fish was as expected; at this point any corrections to feed input were made for the next 2 week period of feeding. Feed input was recalculated in the event of a mortality, the individual weight was removed from the bulk weight and feed input recalculated) on a daily basis.

All diets were assigned to tanks randomly; (i.e. not starting at tank 1 and finishing with tank 20) to maintain unbiased feeding of experimental fish and to cancel possible tank effects due to routine practices. Fish were fed to satiation (around 4) % for the digestibility evaluation at the end of the experiment to establish extreme effects of feeding the terrestrial animal proteins

4.6 Test ingredients

All test ingredients used are described in the general materials and methods (2.4)

Table 14.Formulation of the test diets for trial 3 (g/kg).

Ingredient composition (g kg $^{-1}$)	Fishmeal LT-94	ETF	PMM	SDH	ETF/SDH	PMM/SDH	SHF
Icelandic LT [A]	718.19	574.55	574.55	646.37	574.55	574.55	574.55
Cod liver oil [B]	128.2	133.54	113.6	135.4	134.06	127.9	132.17
Starch/dextrin (2:1) [C]	146.11	135.16	81.26	160.1	143.3	142.9	113.55
Vit & min premix (1:1) [A]	5	5	5	5	5	5	5
Chromic oxide [C]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Enzyme Treated Feathermeal [D]	*	149.25	*	*	*	*	*
Poultry Meat Meal [D]	*	*	223.09	*	*	*	*
(SDH) [E]	*	*	*	50.63	*	*	*
ETF/SDH 75:25 [D]	*	*	*	*	140.59	*	*
Poultry Meat M eal/ SDH 75:25 [D,E]	*	*	*	*	*	147.15	*
Steam Hydrolysed Feathermeal [D]	*	*	*	*	*	*	172.23
Ingredient total (g)	1000	1000	1000	1000	1000	1000	1000

[A] Skretting, Longridge Preston UK

[B] Seven Seas, Hull, UK.

[C] Sigma Chemical Company, Poole, Dorset, UK

[D]Prosper de Mulder, Doncaster, UK

[E] American Protein Corporation (APC) Des Moins, Iowa, USA

Table 14a.Nutrient analysis of trial 3 test diets as fed.

Nutrient composition g kg^{-1}	Fishmeal	ETF	PMM	SDH	ETF/SDH	PMM/SDH	SHF
Moisture	98	95	91	93	93	97	91
Protein	516	564	545	517	542	492	542
Lipid	178	167	180	179	181	181	182
Ash	112	93	128	103	92	110	92
Energy MJ $kg^{-1\ diet}$	21.5	21.9	21.8	21.9	21.8	22.5	22.5
Essential amino acid g / 100g	Fishmeal	ETF	PMM	SDH	ETF/SDH	PMM/SDH	SHF
Histidine	1.3	1.02	1.18	1.38	1.17	1.17	1.03
Arginine	3.72	3.73	3.8	3.37	3.48	3.51	3.73
Threonine	2.33	2.48	2.24	2.08	2.31	2.25	2.36
Valine	2.32	2.84	2.7	2.78	2.79	2.63	3.07
Methionine	1.36	1.17	1.24	1.16	1.11	1.1	1.1
Lysine	3.99	3.59	4.13	4.07	3.56	3.88	3.32
Isoleucine	1.93	2.19	2.26	2.08	2	1.97	2.4
Leucine	3.6	4.03	3.88	3.94	4.01	3.91	4.07
Phenylalanine	2.21	2.28	2.11	2.1	2.22	2.11	2.29
Tryptophan	0.47	0.48	0.56	0.45	0.54	0.37	0.34

4.7 Proximate composition of feed & carcass

Feed and carcass proximate analyses were performed by methods described within the general materials and methods section (2.8).

4.8 General haematology

Rainbow trout were anaesthetised using MS222 (Alpharma) and fish were bled from the caudal vein using a 0.5ml lithium heparinised 2ml plastic syringe (19G needle). Heparinisation prevented clotting, the lithium salt being used to prevent contamination of blood with sodium. The blood was used to determine haemoglobin content and haematocrit.

4.8.1 Haematocrit

Haematocrits were determined using sodium heparinised haematocrit tubes filled with blood by capillary action, the tubes were then sealed using a gas flame. Tubes were centrifuged at 13000rpm for 2 minutes. The length of the packed cell layer was then expressed as a percentage of the total length of the tube occupied by the blood.

4.8.2 Haemoglobin

This was determined using a Sigma diagnostic kit (No.525 A) (Sigma Ltd. Poole, Dorset, UK). 20µl of fresh whole blood was added to 5ml of Drabkins reagent and vortex mixed immediately. The absorbance was read at 540nm on a Jasco Spectrophotometer. De-ionised water (20µl) was used for the blank cuvette in place of blood. A standard curve was constructed using four dilutions of the commercial haemoglobin standard in Drabkins reagent. The regression equation of the standard curve was used to calculate the haemoglobin concentrations of samples in (g/dl) from the absorbance reading. (Standard curve equation: Hb g / dl =36.517 x Absorbance. (R^2 = 0.9996).

4.9 Growth performance and feed utilisation indicators

Specific Growth Rate (SGR)	(Ln weight final g –ln weight initial g * 100)/time
Feed Conversion Ratio (FCR)	(Live weight gain g / Feed intake g)
Protein Efficiency Ratio (PER)	(Live weight gain g / Total protein intake g)
Apparent Net Protein Utilisation (ANPU)	Carcass protein retained g / Total protein intake g *100
Apparent Net Energy Utilisation (ANEU)	Carcass energy retained MJ/kg / Total energy intake MJ/kg *100

4.9.1 Digestibility evaluation of diets

The formula used to determine digestibility of test feeds can be found in the general materials and methods (2.9.7).

Chapter 5

Results
(Growth Evaluation & Health)
Trial 3

5 Results: (Trial 3)

5.1 Performance evaluation of selected animal by-products for rainbow trout

The growth trial was slightly compromised within the first 4 weeks of feeding due to a minor outbreak of white spot (*Ichthyophthirius multifilis*) after week 2, it should be noted this infection was uniform across all tanks. The infection was quickly treated with formalin (Fisher Scientific chemicals, U.K.) at a dosing rate of (15mg/l) for 7 days and fish responded well to this treatment. White spot levels dropped to undetectable levels after this treatment period. Conditions were restored to normal feeding and growth status rapidly. Fish responded actively to the test feeds and the nutrition trial was successfully completed within three months.

After 84 days of feeding the effects of each of the experimental diets can clearly be seen in Figure 13 which displays the live weight gain for each treatment over the twelve week study. Table 15 presents growth performance parameters for the respective experimental treatments with values reported for Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Apparent Net Retention values for protein and energy respectively (ANPU & ANEU).

SGRs of rainbow trout were very similar for the control diet (LT-94 fishmeal as the major protein source) and Spray Dried Haem (SDH), together with Hydrolysed Feathermeal (SHF). The remaining test diets produced slightly reduced growth performance in rainbow trout, namely Poultry Meat Meal (PMM), Enzyme Treated Feathermeal (ETF), PMM & SDH and ETF & SDH blends, however none of the values for any diet were significantly different ($P>0.05$).

FCRs ranged between 0.82 (fishmeal diet) and 0.90 for the ETF/SDH blend. All FCRs were considered to be very good suggesting that fish consumed all of the feed offered

with little waste, again no significant differences ($P>0.05$) were found between these treatments.

PER is a measure of protein utilisation efficiency that does not take into account protein retention per se and values ranged between 2.00 and 1.77 (PMM and ETF/SDH). The best value was for the reference diet (2.00). Significant differences ($P<0.05$) were found between the reference diet when compared to PMM, ETF, ETF/SDH, however the SHF fed fish were not significantly different from the control or the test diets mentioned previously.

ANPU is a direct measurement of protein retention efficiency and revealed interesting trends that were generally in accordance with the growth performance of rainbow trout in this study. The fishmeal reference diet gave an ANPU of 46.82% with high values (>40%) for SHF, SDH, ETF, and ETF / SDH. The PMM / SDH blend had a slightly inferior value of 39.85%, no significant differences were found.

ANEU is a measure of energy retention efficiency and also revealed trends that reflected growth performance of rainbow trout. The fishmeal reference diet produced an ANEU of 53.91% followed by 50.38% for SHF fed trout. Values above 49% were obtained for ETF, SDH groups, with the lowest value of 47.12 determined for the ETF/SDH treatment.

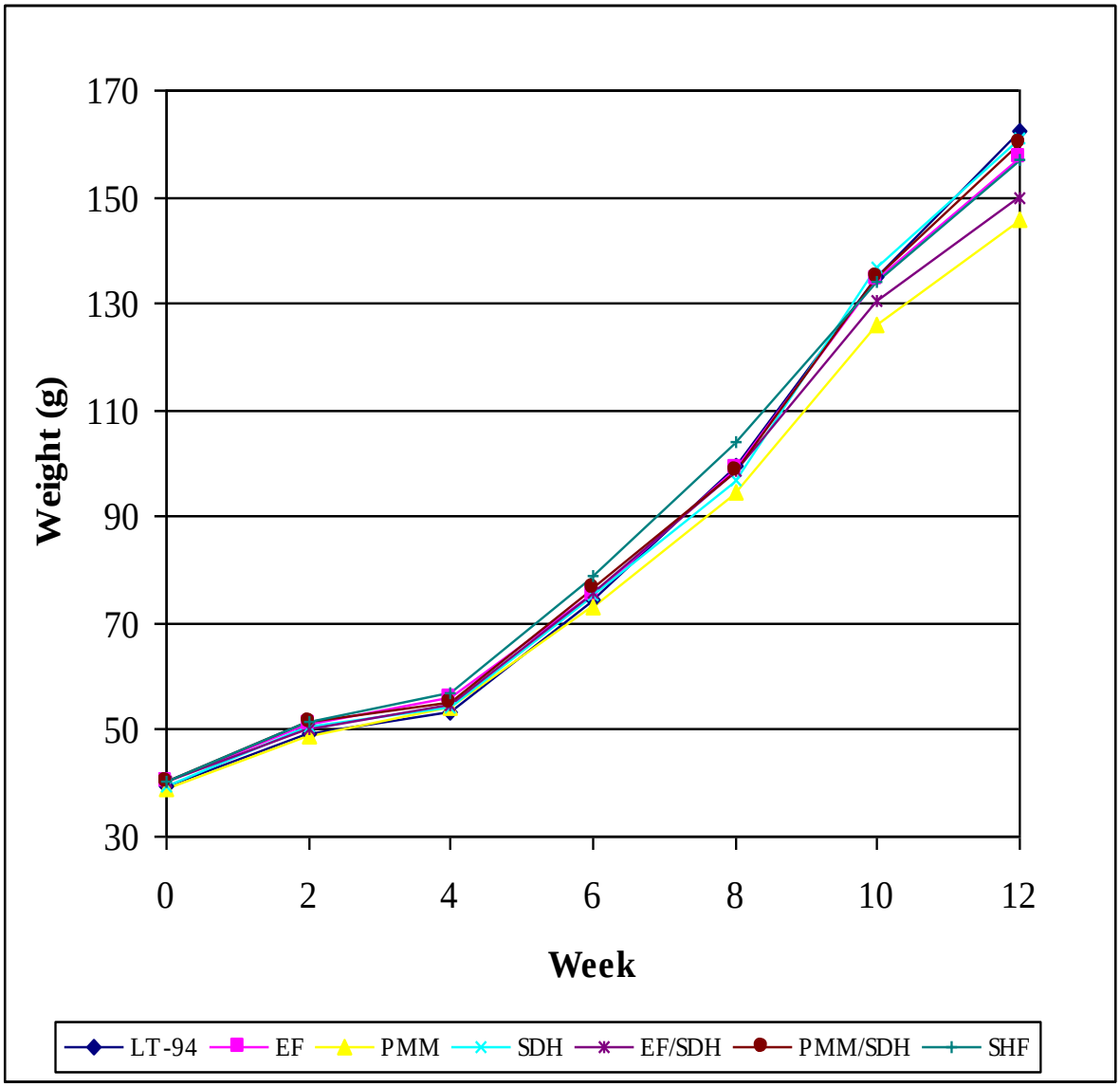

Figure 13.Growth performance of rainbow trout fed experimental diets.

LT-94 (Control)

PMM (Poultry Meat Meal)

EF (Enzyme Treated Feathermeal);

SDH (Spray Dried Haem)

EF/SDH (Enzyme Treated Feathermeal/Spray Dried Haem)

PMM/SDH (Poultry Meat Meal / Spray Dried Haem)

SHF (Steam Hydrolysed Feathermeal)

Table 15.Growth performance data for 12 week trial period (Mean ± SEM, n=2 for Fishmeal diet, n=3 for test diets).

Treatment	Mean Int. weight (g)	Fin. mean weight (g)	SGR	FCR	PER	ANPU	ANEU
Fishmeal (LT-94)	39.37 ± 0.06	162.57 ± 0.71	1.82 ± 0.01	0.82 ± 0.01	2.00 ± 0.02[b]	46.82 ± 3.73	53.91 ± 2.71
ETF	40.50 ± 0.37	157.29 ± 4.54	1.74 ± 0.06	0.88 ± 0.02	1.84 ± 0.05[a]	42.75 ± 1.11	49.85 ± 1.73
PMM	38.94 ± 0.47	145.57 ± 4.44	1.72 ± 0.06	0.90 ± 0.03	1.77 ± 0.07[a]	40.50 ± 1.22	47.14 ± 1.22
SDH	39.47 ± 0.65	161.21 ± 2.11	1.81 ± 0.03	0.85 ± 0.01	1.94 ± 0.02[b]	43.00 ± 1.42	49.58 ± 0.93
ETF / SDH	40.28 ± 0.49	149.64 ± 3.58	1.71 ± 0.09	0.90 ± 0.03	1.77 ± 0.08[a]	40.96 ± 2.27	47.12 ± 1.95
PMM / SDH	40.38 ± 0.37	160.21 ± 2.54	1.77 ± 0.02	0.87 ± 0.01	1.87 ± 0.02[b]	39.85 ± 1.96	48.60 ± 1.05
SHF	40.39 ± 0.18	157.03 ± 3.64	1.80 ± 0.06	0.84 ± 0.00	1.91 ± 0.01[a,b]	43.89 ± 2.39	50.38 ± 0.81

Different superscripts within a column indicate significant (P< 0.05) differences between means

Table 16.Proximate analysis of initial and final fish carcasses (Mean ± SEM, n=2 for Fishmeal diet, n=3 for test diets).

Proximate analysis %	Moisture	Protein	Lipid	Ash	NFE*	Total	Energy
Initial fish	74.18 ± 1.55	14.92 ± 0.62	5.42 ± 1.09	1.92 ± 0.12	3.56	100	4.86 ± 0.50
Final carcass analysis %	**Moisture**	**Protein**	**Lipid**	**Ash**	**NFE***	**Total**	**Energy**
Fishmeal (LT-94)	68.94 ± 1.01	17.04 ± 0.91	11.61 ± 0.81	2.20 ± 0.11	0.21	100	8.24 ± 0.46
ETF	68.99 ± 0.19	16.41 ± 0.54	10.88 ± 0.13	2.31 ± 0.01	1.41	100	8.08 ± 0.16
PMM	69.73 ± 0.42	16.38 ± 0.55	10.39 ± 0.04	2.37 ± 0.04	1.13	100	7.81 ± 0.02
SDH	68.96 ± 0.35	16.58 ± 0.52	10.88 ± 0.09	2.31 ± 0.01	1.28	100	7.99 ± 0.10
ETF / SDH	69.74 ± 0.23	16.77 ± 0.34	10.53 ± 0.10	2.24 ± 0.01	0.72	100	7.79 ± 4.50
PMM / SDH	69.23 ± 0.22	15.45 ± 0.43	10.81 ± 0.27	2.39 ± 0.01	2.12	100	7.88 ± 0.12
SHF	69.37 ± 0.44	16.61 ± 0.97	10.71 ± 0.06	2.28 ± 0.01	1.04	100	7.89 ± 0.12

* NFE by difference

Different superscripts within a column indicate significant (P< 0.05) differences between means

5. 2 Carcass composition

The carcass composition for fish sampled at the end of the trial is reported in Table 16. There were no significant differences ($P>0.05$) in the relative distribution of protein, lipid, ash and moisture components between groups of fish fed the experimental diets. However there was an indication of higher protein deposition in the fishmeal reference group together with elevated lipid. This was also evident for the total energy value per gram of fish tissue.

Interestingly there was no apparent reduction in the moisture content of the reference diet fed fish in relation to higher lipid. Carcass composition was used to determine the previously stated values of ANPU and ANEU.

5.3 Digestibility

Digestibility coefficients were determined for Dry Matter (DM), Crude Protein (CP) and Energy (E). These values (Table 17) varied considerably between protein sources. The values confirm the expected differences in gross nutrient digestibilities predicted from the results of trials 1 & 2 previously undertaken. The lowest DM, CP, and E values were obtained for PMM and were significantly different to the reference diet ($P<0.05$). The PMM / SDH blend was slightly better in terms of DM, CP and E but not significantly different to PMM ($P>0.05$). The SDH diet showed digestibility of 70.15% for DM, 87.15% CP and 80.70% for E these compared favourably to DM, CP and E values for the reference fishmeal diet and again were not significantly different from the SDH diet. It should be noted that the target protein levels in the diet were based on 46% digestible crude protein and digestible energy at 21MJ/kg.

The digestibilities of essential amino acids (EAA) are presented in Table 18 and these are also displayed in histogram format for clarity (Figure 14).

It is obvious that in terms of amino acid availability all diets were highly digested with most values over 80%. High digestibility values were determined for the EAA SDH product however, there were some inferior values for tryptophan in ETF and PMM. The lowest value for tryptophan was found for the SHF diet (59.86%).

Table 17.Digestibility of trial diets (Mean ± SEM, n=2 for reference, n=3 for test diets).

Treatment	Dry matter %	Protein %	Energy %
Fishmeal (LT-94)	67.99 ± 0.35[a,b]	86.82 ± 0.30[b]	79.86 ± 0.02
ETF	65.51 ± 3.24[a,b]	82.49 ± 2.05[a,b]	77.25 ± 2.38
PMM	55.19 ± 3.62[a]	75.44 ± 2.95[a]	74.48 ± 2.40
SDH	70.15 ± 3.31[b]	87.15 ± 1.57[b]	80.70 ± 1.96
ETF & SDH	60.19 ± 2.96[a,b]	79.16 ± 1.86[a,b]	73.03 ± 2.01
PMM & SDH	57.09 ± 0.85[a,b]	78.18 ± 0.07[a,b]	73.45 ± 0.38
SHF	63.77 ± 3.09[a,b]	77.08 ± 2.02[a]	76.03 ± 2.01

Different superscripts within a column indicate significant (P< 0.05) differences between means

Table 18. Digestibility of essential amino acids of experimental diets (pooled samples).

Amino acid	Fishmeal	ETF	PMM	SDH	ETF/SDH	PMM/SDH	SHF
Arginine	84.71	83.32	81.56	89.57	77.59	81.01	75.12
Leucine	74.66	87.74	85.71	93.12	85.39	92.46	84.00
Lysine	87.34	91.43	90.84	94.83	91.26	95.70	92.01
Histidine	83.13	84.07	81.42	88.51	80.32	79.55	76.90
Isoleucine	83.20	87.85	85.17	90.30	82.42	88.94	86.03
Methionine	89.72	88.18	87.18	90.06	84.35	84.85	83.34
Phenylalanine	77.85	84.69	82.07	89.57	79.25	77.92	75.99
Threonine	84.18	83.69	81.00	88.78	79.01	82.72	74.06
Valine	82.47	85.64	83.60	90.98	80.74	83.91	82.47
Tryptophan	86.48	79.84	79.28	94.26	79.05	69.97	59.86

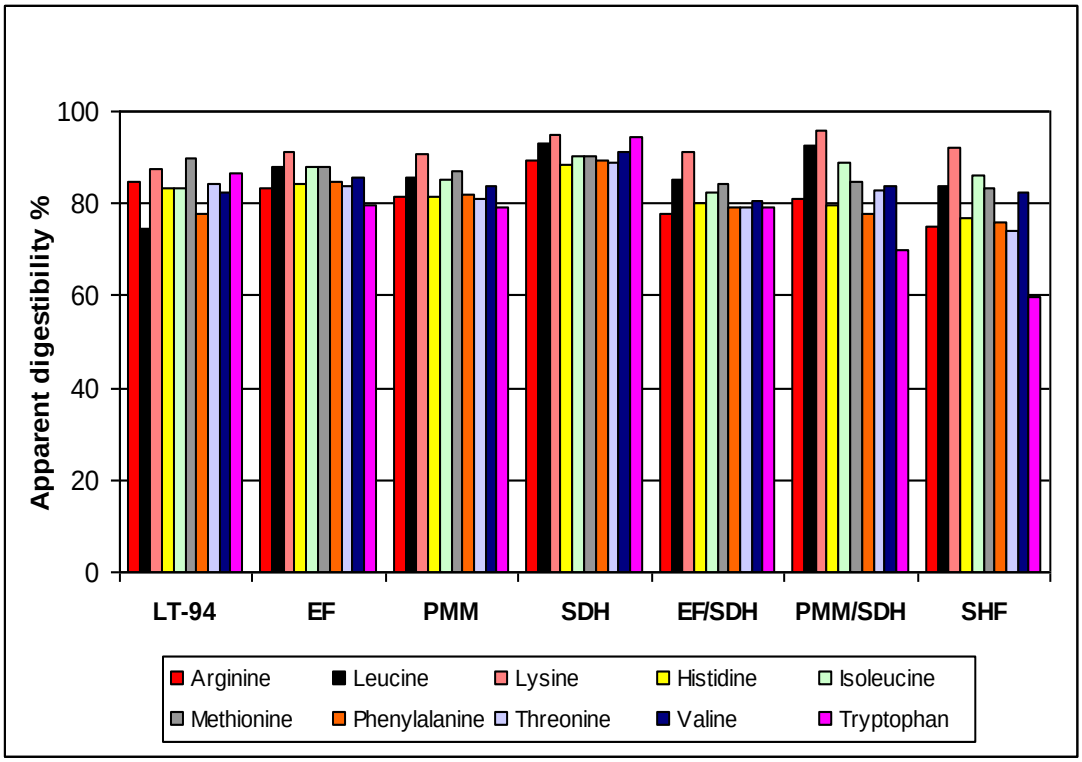

Figure 14.Apparent essential amino acid digestibility of test diets as fed to rainbow trout

5.4 Haematological and health assessment

At the end of the feeding trial there were no treatment related mortalities. Rainbow trout were in excellent condition displaying uniform conformation and complete fin and caudal tail morphology. Haematological assessment of blood samples obtained at the end of the trial did show some significant differences ($P<0.05$) in respect to haematocrit values, with the SDH, ETF/SDH and PMM/SDH diets all displaying lower packed cell volume. The values for haemoglobin and haematocrit are displayed in Table 19. No other significant differences were observed for the other test diets. SDH and PMM/SDH diet values for haemoglobin were significantly different from the reference diet ($P<0.05$). All other test diets were comparable to the control. It should be noted however that all of the haematocrit and haemoglobin values are within the normal range for this species.

Table 19.Blood indices (Mean ± SEM, n=2 for reference diet, n=3 for test diets)

Treatment	Haematocrit %	Haemoglobin (g/dL)
Fishmeal (LT-94)	34.10 ± 1.50 [b]	6.30 ± 0.04 [b]
ETF	33.20 ± 1.33 [b]	5.86 ± 0.39 [b]
PMM	32.53 ± 0.48 [b]	6.13 ± 0.13 [b]
SDH	27.67 ± 3.25 [a]	4.99 ± 0.51[a]
ETF / SDH	25.33 ± 3.93 [a]	5.55 ± 0.63 [b]
PMM / SDH	28.33 ± 0.73 [a]	5.32 ± 0.32 [a]
SHF	30.73 ± 0.87 [a]	5.90 ± 0.33 [b]

Different superscripts within a column indicate significant (P< 0.05) differences between means

Chapter 6

General Discussion

General Discussion

Numerous advances have been made in developing compound diets for aquaculture and with many new species emerging there is a clear need to formulate specific diets for each species being cultured (Craig et al., 2006; Wang et al., 2006). Although there are now many species of fish being successfully cultured both in freshwater and marine environments there remains considerable scope for further development of salmonid species notably rainbow trout, salmon and Arctic char (Tacon, 2005).

Established intensive production technologies for salmon and trout still provide an economically important contribution to global fish production amounting to approximately 2 MT per annum with a global production value amounting to US$ 6 billion (FAO, 2006). Due to their importance salmonids have been fairly widely researched in terms of their nutritional requirements and there is consequently a wealth of information that provides a strong database for the formulation of balanced diets for these species (Pfeffer & Henrichfreise, 1994; Davies & Morris, 1997a; Davies et al., 1997b; Suguira et al., 1998; Mambrini et al., 1999; Bureau et al., 1999-2000; Francesco et al., 2004; Glenross et al., 2005).

The growing demand for safer and reliable farmed seafood necessitates full trace-ability of the end product. This is particularly relevant to the manufacture of diets from raw materials and ingredients. This is a main criterion for new legislation as directed by DEFRA (UK), and the EU Feed Standards Committee (EU, 2000; EU, 2001; EU 2002; EU, 2003).

Traditional use of high quality fish meals in salmonid feeds has been seen to be consistent with quality and in terms of providing an effective protein source. However, demand for sustainable fish production has been at the forefront of recent debate and has necessitated a re-evaluation of the use of fish meal from increasingly

pressurised marine resources (Hardy & Tacon, 2002; Huntington et al., 2004). The Marine Aquaculture Task Force in the United States (Report of the Marine Aquaculture Task Force: Sustainable Marine Aquaculture, 2007) has promoted salmon and trout as high value products that must meet strict criteria for production with emphasis on feed quality and composition. In such respects fish meal derived from well managed stocks is still regarded by many as a sustainable commodity supplying high quality protein for carnivorous fish species (Hardy & Tacon, 2002; Tacon, 2004).

Fish meal is well established as a high quality protein with a complete complement of essential amino acids (EAAs) meeting the known requirements of fish. It is not surprising that the EAA pattern of fish meal (mostly derived from muscle proteins) matches the requirement pattern of growing fish (where growth is mostly fish muscle protein deposition) given that EAA are selectively conserved for anabolism. The advent of Low Temperature (LT) processed fish meals over the last fifteen years has provided an improved resource for production of nutrient dense salmonid diets that produce less nitrogenous waste and lead to improved feed efficiency (low FCR) and minimised pollution (Cho et al., 1994). Such modern high quality fish meals have been a major factor in the expansion of salmon and trout production compared to inferior fish meals used in previous years (FAO, 1971). Considerable variation exists in fish meal quality throughout the world ranging from the high quality brown fish meals to the white fish meals resulting from processed frame and discards from the fishing industry. A comprehensive review on current fish meal processing and quality control has been undertaken by Ariyawansa, (2000). It is imperative that a high standard fish meal is employed in modern salmonid production to maintain uniform quality of fish and feeding strategies.

Expansion of global aquaculture necessitates the use of sustainable alternatives to fish meal based on plant as well as animal derived materials. This need was recently highlighted at the 13th International Symposium on Fish Nutrition and Feeding held in Biarritz, France (May, 2006). The head of EWOS (A major global fish feed manufacturer) Innovation (Skjervold, 2006) provided a prospectus for future trends in world aquaculture production with reliance on reducing our dependence on marine proteins and oils presently mainly derived from pelagic fish species obtained from Iceland, Norway, Denmark and Scotland as well as the major producers in Chile and Peru.

Therefore the primary purpose of this thesis was to assess the potential to reduce the fish meal demands of salmonid species using rainbow trout as a suitable model for investigation. To date, the alternative strategy of using plant protein ingredients as partial substitutes for fish meal in diets for salmonid species has been the main focus of research and continues to offer promising results (Cheng et al. 2003a,b; Morris et al., 2005; Davies & Serwata, unpublished). Although some potential plant ingredients have met with variable success for salmonids, processed soybean and maize gluten meals are now consistently present in modern feeds at moderate inclusion levels (Morris, 2001). In this respect Morris et al., (2005) have demonstrated the feasibility of soybean meal substitution in trout diets without obvious detriment to growth, feed efficiency or health. However, contrasting results are presented by Baeverfjord & Krogdahl, (1996) suggesting that both salmon and trout display evidence of enteric lesions in the intestinal tract at higher soybean inclusion levels. In addition, the negative effects of Anti Nutritional Factors (ANF's) are known to constrain the inclusion of soybean for many fish species. In particular the presence of protease inhibitors and phytate bound phosphorus are especially important (Tacon, 1996;

Storebakken et al., 1998; Suguira et al., 1998; Thiessen et al., 2004). Environmental discharge of phosphorus is also of serious concern and is one of the major areas for increased legislation. Several authors have reported the problems associated with phosphorus availability in studies with rainbow trout (Storebakken et al., 1998; Sugiura et al., 1998; Richie & Brown, 1996). Indeed the present author (Serwata & Davies unpublished data) has investigated phosphorus availability in different plant protein sources. Plant proteins may also be limiting with regards to specific essential amino acids such as methionine and lysine and occasionally tryptophan. As such, diets containing vegetable-derived protein must either be carefully balanced with complementary protein sources or supplemented with crystalline amino acids (Cheng et al., 2003b; Francesco et al., 2004).

Due to a variety of issues including those mentioned above there is renewed interest in the use of rendered animal proteins in aquafeeds for fish, and in particular salmonids. The use of terrestrial animal proteins in aquaculture feeds is not a recent concept since it was well established in the 1970s. A variety of animal proteins have been utilised from the 1970s to the present day (Brannon et al.. 1976; Roley et al. 1977; Higgs et al.. 1979; Fowler 1990,1991; Gouveia, 1992, Bureau et al., 1999,2000). These researchers evaluated a range of protein sources including meat and bone meals, blood meals (mainly from ruminant sources as well as porcine derivatives) and avian by-products such as poultry offal, poultry by-product meal and feathermeals that offered protein concentrates that could effectively substitute for fish meal at appreciable levels in feeds. Such interests were particularly acute during the global energy crisis of the 1970's, indeed these interests were further enhanced by the collapse of the anchovy fish meal harvest which was caused by a severe El nino effect, thus resulting in high prices of fish meal from South America (Idyll, 1973).

However, stable supplies and relatively low costs of fish meals towards the end of the 20th century resulted in reduced demand for alternatives and interest in animal by-products waned. Additional concerns associated with the BSE 'episode' in Europe greatly limited potential for use of terrestrial animal by-products in any animal feed. Since BSE there have been a number of problems associated with the foot and mouth outbreak in the U.K. which also severely dented public confidence with regard to the use of these terrestrial animal proteins in feedstuffs generally. Within the last twelve months the problem of avian flu (H5N1 strain) has also made the issue of using poultry-derived products even more difficult. At the time of writing the U.K has experienced a case of where a leading turkey producer has faced a serious problem of infection with this particular strain of virus. This has resulted in the culling of the flock and removal of dead animals through controlled operations such as incineration. DEFRA the U.K Ministry for agriculture has allayed fears that this can pose a threat to consumers. It is stated that effective heat treatment (70°C for 30 minutes) completely destroys the pathogen. This is further documented by WHO, (2005) guidelines on bird flu and associated risks. The question that these avian pathogens can contribute to the poultry by product meal do not arise since only animals certified to be completely healthy and suitable for human consumption can be processed into animal feed ingredients (EU, 2002). These factors were discussed in more detail within the introduction section. Such issues have led to the concept of developing a new generation of animal-derived raw materials based on improved processing technologies and certified animal sources (Woodgate, pers comm).

There is a paucity of information with regards to these new terrestrial animal by-products and their application in aquafeeds and this was a key factor influencing the theme of this thesis. The results of the investigations described in this thesis relate to a

selection of by-products recommended by the sponsoring manufacturer and the technical knowledge gained from other terrestrial animal species. Instrumental in selection of test materials were Mr Stephen Woodgate of Prosper de Mulder U.K. and Mr Gary Pearl from the American Fats and Proteins Research Foundation (FPRF).

As stated in the introductory section of this thesis there is increasing support for the controlled use of poultry / porcine derived products in aquafeeds as opposed to material of ruminant origin (Tacon, 2006). Despite some continuing concerns it is now deemed feasible to allow modest partial inclusion of pure blood products from porcine sources in fish and crustacean diets in Europe (NRA, 2003).

The present investigations describe a sequential series of experiments using rainbow trout as a model salmonid the results from which could be extrapolated more widely to other carnivorous fish species. On the basis of scientific literature the methodology employed here with trout conformed to that described by Bureau & Viana, (2003). In most cases it is essential that a full digestibility evaluation is undertaken before any growth study to define nutrient availabilities for the major components including protein, amino acids, energy and specific macro elements such as phosphorus (P) and calcium (Ca).

Many authors (Bureau et al., 1999; Burel et al., 2000; Glencross et al., 2005) have reported data for digestibility coefficients for a wide range of feed ingredients for different fish species including salmonids. Such an approach provides a good foundation that can lead to further and more refined investigations to test raw materials and ingredients in complete diets for growth and feed performance evaluation in fish. Use of a high quality reference protein in both digestibility and growth performance trials is vital for a definitive and quantitative assessment of

alternative protein sources. As mentioned previously, LT fish meals are consistent in physical characteristics and nutritional quality and are widely used in commercial feed formulations for salmon and trout. The first experiments in the present programme of work successfully provided digestibility coefficients for the major nutrient classes (protein and energy) in selected animal protein sources as compared to LT fish meal.

The LT fish meal was always provided from the same source and was well defined in terms of nutritional profile. This is not always the situation in other investigations where it is frequently apparent that inferior fish meal such as NorseSeaMink quality and Provimi 66 compared to NorseSeaMink LT-94 produced inferior feed efficiency in fish (0.58 to 0.66) and in true Mink digestibility (84% for inferior fish meals compared to 94% in LT-94 fish meal (Asknes et al., 1997; Pike, Pers comm). In our investigations diets were designed to evaluate animal protein by-products in a matrix representing typical rainbow trout diets appropriate for this species on the basis of their nutritional requirements and feeding physiology.

The results obtained from the present studies were consistent with values obtained by other investigators based on similar techniques and methodology. In this respect Austreng, (1978); Choubert et al., (1982) and Cho & Slinger, (1979) performed digestibility trials using a variety of methodologies but mainly the Guelph protocol in which faeces were obtained by natural voidance and subsequent settling in faecal traps. Alternative 'advanced' mechanical faecal collection systems (based on collecting solids on screens), such as that advocated by Choubert et al., (1982), provide a similar basis for collection of faeces but might be expected to minimise leaching losses of protein and lipids through bacterial degradation, with water soluble trace elements and water soluble vitamins not being subjected to water exchange

within the collection chamber (as in Guelph system) which would otherwise dilute their concentration in faecal matter.

Storebakken et al., (1998) compared faecal stripping with natural faecal voidance in Atlantic salmon. It was concluded that both methods were equally effective methods of assessing nutrient digestibility and both had minor advantages and disadvantages. It was nonetheless decided by the present author that there were particular advantages to the faecal stripping method (Austreng, 1978) as applied to salmonids. In comparison to methods where there could be significant nutrient leaching (naturally voided faecal material being immersed for some time prior to collection/separation) the stripping technique would ensure no losses of nutrients but could be criticised for underestimation of digestibility due to the possibility of incomplete digestibility and nutrient absorption (Austreng, 1978). As the hindgut of fish is not differentiated into 'large intestine' digestion and absorption are presumed to occur up to the vent (Austreng, 1978).

Another important consideration pertinent to these investigations is the choice of inert marker for digestibility assessment. Several authors Davies & Gouveia, (2006) and papers cited there in have questioned the validity of using chromic oxide (Cr_2O_3) as an inert marker due to the potential for differential passage rates in the digesta (Vandenberg and Noue, 2001; Carter et al., 2003). Other concerns relate to possible interactions with other dietary components especially micronutrients such as trace elements.

There have been numerous research publications that have compared various markers for application in fish nutrition research. Many of the more recent are based on rare earth elements such as yttrium, ytterbium, and titanium oxide. However, many of these markers require complex analysis and are particularly expensive. Austreng et

al., (2000) investigated several of the rare earth metals and found the majority of these made suitable inert markers with minimal interactions between themselves and the nutrient being assessed. Although yttrium oxide has now gained wide acceptance as a marker for many species including salmon and trout the author decided that for the present work with rainbow trout, chromic oxide should provide the reference marker of choice in order to make comparisons with the existing and past literature for this species.

It should be noted that digestibility studies often use a specified ratio of test ingredient to a defined fishmeal based diet for inclusion in mixed experimental diet for feeding trout prior to faecal collection. The original Guelph recommendation was for a 30:70 ratio with 30% of the test ingredient included with the remainder being the fishmeal based diet. However, there maybe benefits to increasing this ratio to encompass the expected higher inclusion levels of animal protein concentrates in complete aquafeeds. In this study a fixed 40% inclusion of each test ingredient (namely Poultry Meat Meal, (PMM) Feather Meal (FM), Enzyme treated Feather Meal (ETF) and Spray Dried Haem (SDH) was employed. Similarly the blended ingredients were also substituted at the same level of 40% for evaluation.

Previous work by other investigators may be criticised with respect to the acclimation period necessary for fish to be conditioned to the change in diet and feeding regime as well as temperature and photoperiod involved in transfer from a farm situation to experimental. Siguira et al., (1998) and Glenross et al., (2004) have only fed fish test diets for a seven day period whilst initiating faecal collection. It is advisable that a period of adaptation be allowed prior to digestibility evaluation of diets to enable the digestive system of fish to adapt to changes in protein and energy concentration as

well as the feeding rate and, in many cases pellet size and texture (Gomes et al., 1995; Bureau et al., 1999).

Additionally specific digestive enzymes maybe induced by the presence of increased nutrient levels (for example starch/amylase; lipid/lipase/bile secretion). The need for such caution has been reported by Venou et al., (2003) who investigated the effect of diet composition on nutrient digestibility and digestive enzyme levels in gilthead sea bream. These workers stated that digestive enzyme activities and nutrient digestibility values were affected by dietary composition, with carbohydrate and fat changes eliciting the strongest effect.

To avoid this possibility it was decided to condition rainbow trout for a minimum period of one month to each of the test diet combinations. This was to ensure valid outcomes to the subsequent measurements of nutrient digestibility in rainbow trout.

The reference diet for trials 1 and 2 produced good repeatability with respect to crude protein (~91%), gross energy (~81%) and dry matter digestibility (~74%) and these values compare well with earlier studies on rainbow trout by Bureau et al., (1999) with digestibility values crude protein (91%), gross energy (88%), and for dry matter of (84%). and. Allen et al., (2000) also used a high quality fishmeal (Peruvian and Danish) again the values for protein digestibility (~91%), gross energy (88%) and dry matter digestibility(~85%). and found in Australian silver perch (*Bidyanus bidyanus***)** were similar or better than values obtained in the digestibility component of this thesis confirming the fishmeal control diet to be of a high quality protein source.

The Apparent Digestibility Coefficients (ADCs) for steam hydrolysed feathermeal with respect to dry matter (71%), protein (78%) and gross energy (74%) were slightly inferior to those obtained by Sugiura et al., (1998) for dry matter (81%), and protein

(85%). These values are also similar to protein and dry matter values for rainbow trout as reported by Bureau et al., (1999).

However the method of faecal collection in the latter two studies were based on the Guelph system in contrast to the present study in which fish were manually stripped of faecal material.

Enzymatically treated feathermeal in trial 2 displayed a small increase in digestible crude protein (82%) and digestible gross energy (80%) although not significant compared to values obtained for steam hydrolysed feathermeal for rainbow trout. This is possibly attributable to the much lower processing temperatures used for the enzyme treated feathermeal (Woodgate, pers comm). The digestibility of essential amino acids in enzyme treated feathermeal displayed a significant increase for threonine, valine, leucine and phenylalanine over steam hydrolysed feathermeal. The improvement in digestibility of these specific amino acids maybe due to less complexing or bridging of the protein due to a milder effect of treatment at the processing stage (Wang & Parsons, 1998). The influence of heat treatment on protein quality with respect to digestibility in animals and in particular complex associations between lysine and other nutrient components is described by D'Mello, (1994).

Standard Hydrolysed Feathermeal (ETF) was a very similar product to the steam hydrolysed feather-meal used in trial 1 and gave very similar protein digestibility values (70%). This provided some evidence that close agreement existed between separate trials using different fish stock but standardised methodologies.

The spray dried haem utilised for this study displayed a very high ADC (in most cases producing complete digestion) with respect to protein, energy and dry matter digestibility. This is not really surprising given the nature of the product. It is manufactured to be an exclusively spray dried blood cell concentrate, with very high

protein content (mainly haemoglobin). These results are supported by those of other workers (Hajen et al., 1993; Bureau et al., 1999) who reported values for apparent protein dry matter digestibility of (~91%), protein (~94%) and gross energy (~90%) for trout and salmon. These new spray dried haem products offer significant digestibility improvements over conventional drum dried blood meals (Bureau et al., 1999) in which digestibility coefficients for protein and amino acids were often lower compared to fish meal.

The poultry meat meals assessed in trial 1 resulted in ADCs for dry matter of (69%), protein (82%) and gross energy (72%). These values are again similar to those reported by Hajen et al., (1993), Sigiura et al., (1998) and Bureau et al., (1999) who all reported values over 70% for dry matter 75% for ADC of crude protein and 70% for gross energy, in poultry meat meals fed to salmonids. These higher values may be attributed to the methodology employed by these workers which comprised the Guelph technique for faecal collection. As described previously, this approach often results in leaching losses of nutrients resulting in elevated coefficients of digestibility that may vary depending on the type of protein within the feed.

The combination of poultry meat meal with spray dried haem (75:25) improved the ADC for dry matter (73%), protein (87%) and gross energy (78%). This improvement in apparent protein digestibility was also generally reflected for the essential amino acid digestibility within this blend. The blend of poultry meat meal and spray dried haem complemented each other well and the improvement in digestibility is clearly reflected in dry matter, protein, gross energy and essential amino acid digestibility. The use of complementary proteins may generally produce a more balanced amino acid profile and increase the protein biological value. These results are particularly interesting in our studies for rainbow trout and are clearly demonstrated when you

compare the essential amino acid profile of the blended materials (p36 Table 4a) to the control diet amino acid profile and then to the respective test ingredients on there own. An example of the improved essential amino acid profile when blending proteins such as PMM and SDH compared to the PMM diet alone maybe demonstrated by the following example: the Leucine level in the PMM diet amounted to 3.23g/100g. For the blended PMM/SDH diet this was elevated to 4.52g/100g which exceeds the value of 4.05 for the control (fishmeal based diet). This complementary use of different proteins achieves the objective of meeting specific requirements for essential amino acids. This pattern is repeated in almost all other essential amino acids with the exception of methionine, lysine and arginine.

This approach is more preferable and cost effective than supplementing the diets with crystalline amino acids. However as shown in this situation the use of crystalline amino acids is still recommend when specific amino acids become limiting.

Olsen et al. (2006) replaced fish meal with Antarctic Krill (*Euphausia superba*) and achieved high apparent dry matter (95%), protein (87%), and lipid (94%) digestibility whilst maintaining the essential amino acid profile of the test feeds compared to the control. (Serwata, unpublished data) A similar trend when replacing fish meal with plant protein and other marine protein sources would suggest that the essential amino acid profile is an important factor for growth and digestibility studies (Espe et al. 2006).

The ‘fast dried’ Poultry Meat Meal (FDPM) resulted in the poorest ADC values of all ingredients tested in this study, dry matter digestibility (59%) and protein (59%). There is no scientific literature to compare/support this data, and as such makes it impossible evaluate the reasons behind its poor performance given that its essential

amino acid profile is actually superior than some of the other test materials evaluated in the digestibility component of this study . It is likely that excessive thermal damage to the protein may have occurred during processing (135-140°C) which is a minimum of 10°C higher than all of the other test materials at the processing stage. This type of heating can result in cross-linking and reductions in the availability of specific key amino acids as described by Wang & Parsons, (1998) and as such has a negative impact when used as a feed ingredient.

In general it should be noted that the increased values obtained for digestibility of similar raw materials by other workers are largely based on the Guelph system. In the Guelph (faecal sedimentation) method, there is a possibility of small losses of nitrogen through leaching and bacterial breakdown in the faecal collection chamber, sometimes resulting in elevated values. However the Guelph system can collect faeces continuously over the period of a feeding trial and is a recognised method for many fish species. Vandenberg and Noue, (2001) compared the collection of faecal material using three different methods and using chromic oxide (marker used for this study). It is clear from their findings that faecal stripping gave a 8-10% reduction in values compared to the Guelph method for digestible protein; this was also compared to mechanical faecal collection of material after feeding from the water exiting the tank.
The Table 20 summarises the findings of Vandenberg and Noue, (2001) and is compared to the control diet for the digestibility study for this thesis and the control diet from Bureau et al., (1999).

Table 20.Different methods of faecal collection, comparing apparent digestible dry matter, apparent digestible crude protein, and apparent gross energy. All diets based on a quality fishmeal source.

Diet	ADC Dry matter %	ADC Protein %	ADC Gross Energy %
A Stripping	73	91	80
B Stripping	48	80	60
C Column	75	91	80
D Column	83	92	86
E Collection	68	87	75

(A)Present thesis, (B, C & E) Vandenberg and Noue, (2001). (D) Bureau et al., (1999).

All diets used chromic oxide as the inert marker with the exception of Diet D which utilised Acid Insoluble Ash (AIA). All diets were fish meal based. Although only a true comparison could be made if the control feeds were all the same, tested at the same time with same stock of rainbow trout and similar water conditions. This table is to simply demonstrate the differences between alternative methods used for digestibility evaluations.

The opportunity for leaching of certain nutrient components from the faeces during prolonged immersion is the probably the main explanation for slightly higher reported digestibility values reported by researchers using the Guelph method for salmonids (Hajen et al., 1993; Suguira et al., 1998; Bureau et al., 1999).

However, the faecal stripping method employed here also has some disadvantages, the first being the potential for incomplete digestion of the diet (Vandenberg and Noue, 2001). This has the possibility of producing decreased ADC values for any ingredient tested. Other associated problems with faecal stripping are; increased susceptibility to handling stress that could lead to lower assimilation of test diets; the use of large numbers of fish and frequent stripping sessions are also required to give adequate amounts of faecal material for analysis.

Most studies, including the present investigation, report values as 'Apparent Digestibility' Coefficients (ADC) that do not discriminate between dietary and endogenous losses of protein, amino acids and energy related nutrients. The determination of 'true digestibility' values for the ingredients would require a separate diet designed to be essentially 'protein free' in order to generate absolute amino acid, protein and energy digestibility data for rainbow trout – so-called 'true' digestibility values (Halver & Hardy, 2002). This would have necessitated use of a special 'purified' diet formulation based on refined ingredients such as starch and dextrin and alpha cellulose that would have probably been unpalatable and difficult to feed. It is notoriously difficult to get carnivores to feed on purified protein-free diets. However, a semi-purified diet with 2% albumin masked with various attractants was successfully used by Davies (unpublished data) for determining endogenous protein and amino acid losses in rainbow trout. Most researchers are in agreement that for practical purposes apparent digestibility coefficients provide valid and realistic values (Cho & Slinger, 1979; Cowey, 1988). However given the relatively high dietary protein levels within the experimental diets evaluated in the present study with animal by-products, the small contribution of endogenous faecal protein would have been a minor factor in relation to the digestibility coefficients measured.

This study highlighted a number of products that demonstrated good acceptability and digestibility for rainbow trout under experimental conditions. The digestibility trials proved effective as a prerequisite before full growth trials were implemented to test the optimum inclusion levels of animal by-products in practical diets for trout.

The next phase of the programme addressed the growth trial and reported the nutritional parameters associated with growth performance, feed intake, and nutrient utilisation.

A fairly novel approach was employed that utilised the digestibility profile obtained previously for each test ingredient, especially with respect to protein and energy, to formulate feeds for the growth study. It should be noted that Bureau et al., (1999) also adopted this methodology with some success. This approach is in contrast to that of most other workers who routinely formulate diets based on gross nutrient levels which does not allow for the availability of such nutrients for the fish species of concern (Hajen et al.. 1993; Suguira et al., 1998). Many studies on rainbow trout and salmon fail to address this issue and whilst such diets maybe iso-nitrogenous and iso-energetic in gross terms they vary with respect to digestible protein and energy contents. In effect this might underestimate the potential value of test ingredients where digestibility rather than nutrient balance per se is the issue. Such underestimation may result in ingredients being downgraded on the basis of performance assessed by growth and nutrient utilisation. In fact, formulation of diets on the basis of digestible protein, digestible essential amino acids and digestible energy data for raw materials is the accepted protocol for feed formulation in ruminant, swine and poultry nutrition (Schalble & Homer, 1981).

The designs of the growth trials undertaken in the present study are based on protocols that have been well established for trout by many investigators (Glencross et al., 2007).

From the outset it was decided that the growth trial should provide at least a minimum three-fold increase in biomass (of best performing treatments) in order to make meaningful evaluation of diets and to maintain a high feeding rate appropriate to the species. It was decided that after evaluating results from the most relevant work to date on terrestrial animal by-products by Bureau et al., (2000) that a three-fold increase was important to show any major differences in growth performance. A re-

circulating system was selected to maintain a stable environment at an optimum temperature of 15°C and regulated photoperiod ensured uniform and uninterrupted growth during the course of the assessment (Glencross et al., 2007).

The growth trial performed in this study produced a feed conversion ratio of 0.82 to 0.9 this compared favourably to trials undertaken by other workers in either outdoor locations where feed conversion values of (0.79 to 0.85) were attained (Morris et al., 2005) or facilities with open flow systems (Glencross et al., 2006) where feed conversion ratios (0.81 to 0.86) were achieved for rainbow trout.

Protein retention, as measured by Apparent Net Protein Utilisation (ANPU), was high (39-46%, Table 15, p91) which indicates that protein deposition rates were consistently high during the rapid growth phase of trout in this nutrition trial. Other workers such as Glencross et al., (2006) reported ANPU values for rainbow trout of 34-42% which is comparable to the data for the growth study. High ANPUs are associated with a balanced amino acid profile, optimal dietary protein level and an adequate intake and use of non-protein energy in the form of oil or available carbohydrate (Young et al., 2005).

In this study, net energy retention was also determined for rainbow trout receiving each diet and it was found that almost 47-54% of the energy intake was retained by the fish. Again this correlates well to Glencross et al., (2006) who obtained values between 44-52%. Bureau et al., (2000) and Francesco et al., (2004) for example have reported both the protein and energy balance in fish as affected by variations in diet formulation. Similarly, carcass composition of trout at the end of the growth trial provided evidence that the inclusion of animal by products did not adversely affect the ratios or levels of moisture (69%) protein (16%), lipid (11%), and ash (2.3%) within whole fish and correlated well with data for rainbow trout reported by Bureau et al.,

(2000) for who found values in the final carcass for moisture of 71%, protein 15.7%, lipid 9.5% and ash 2.2%. Nutrient digestibility in the test diets was found to be in agreement with the previous experiments reflecting the high DCP values of poultry meat meal, blood meal and specific amino acids within feathermeal (both standard and enzyme-treated type). These correlations were also true for the specific blended ingredients evaluated. The digestibility coefficients of the ten EAAs were of particular relevance as predictors of protein performance when ingredients substitute fish meal at increasing levels. In the present investigation, consistently high EAA digestibility values (Table 17, p93) confirmed that the animal by products tested should have met requirement thresholds at the levels tested. The amino acid digestibility values are consistent with the findings of Cheng & Hardy., (2002). These workers presented the only definitive recent study on rainbow trout in relation to poultry meat meal. In that study the ADCs of nutrients including (amino acids) were reported for herring meal, menhaden meal, feed grade poultry by product meal, prime poultry by-product meal (similar to the poultry meat meal material used in this study) and a refined poultry by product meal. Interestingly in respect of the similar grades of poultry by-product from the USA and the UK product tested in this thesis, there was a remarkable agreement and consistency for amino acid availability coefficients. For instance the ADC for arginine was 86.7% compared to our value of 86.4%, lysine 91.6% compared to 93.3%, methionine 94.8% compared to 93.3% and threonine 84.6% compared to 82.3%. Additionally Cheng and Hardy provided a value for 97% for tryptophan which was not measured in the present study. All remaining ADCs for EAAs were of similar magnitude compared to the profile of the poultry meat meal tested in this study. Similarly, all the major EAAs agreed closely for the two separate investigations with trout with respect to high grade fish meals. Values were found to be between 90-95%.

These results are interesting because Cheng and Hardy based their methodology on direct faecal recovery on settlement compared to the stripping method employed in the present investigation. Unfortunately there is a paucity of information for rainbow trout for the other types of terrestrial by-products and derived materials. However more detailed examination has been reported by other workers, Gaylord et al., (2004) on juvenile hybrid striped bass (*Morone chrysops x M.saxitalis*) and Allen et al., (2000) on silver perch (*Bidanyus bidyanus*) who reported the digestibility of a variety of terrestrial animal by-products with extensive information for amino acids. Despite the differences between fish species and there respective metabolic rates compared to rainbow trout held at 15°C there were nonetheless close agreements for the apparent digestibility coefficients for the major EAAs. In particular for poultry meat meal the values correlated well with the coefficients determined for rainbow trout in the present study. However it should be noted that values for feathermeal were all surprising high (in excess of 90% for most essential amino acids) compared to values obtained for rainbow trout here. Also more variation was observed for rainbow trout in this study, although lower protein digestibility for feathermeal is generally reported for most fish species (Nengas et al., 1999). These higher values for silver perch may reflect differences in metabolic rate and gut morphology.

Allen et al. (2000) also determined amino acid availabilities for spray dried blood meal for silver perch. These values also validated the similar high coefficients of 97-100% for trout compared to a mean of 92% for silver perch.

Dietary mineral levels and the availability of calcium, phosphorus, magnesium and iron maybe expected to vary for each of the tested ingredients. These were not adjusted for in the present formulations with respect to the mineral premixes. Protein utilisation and energy status are also vital factors for maintaining the health and

production of blood and component cells such as the erythrocytes and macrophages important for the immune system (Steffens et al., 1999). It was therefore decided to monitor the principal haematological indices to provide information on fish health in a long term nutrition trial. Other workers have reported the effects of diet on blood physiology (Davies & Gouveia, 2004, Rehulka et al., 2004) and biochemical profile for a variety of fish species. In the present study with rainbow trout blood haematocrit was found to be within normal reported levels, 24% to 43% (for haematocrit) ascribed to clinically healthy rainbow trout (Wedemeyer, 1996; North et al., 2006), which agree well with levels found (25-34%) in the present study. This would indicate there were no significant effects due to the dietary regime. However there were significant differences between the test diets containing SDH when compared to the control feed.

Haemoglobin levels in the present study of 5.55 to 6.30 g/dl were also found to be well within levels reported by other workers such as Benfey & Biron (1999) who obtained levels nearer 6.70 g/dl for rainbow trout and brook trout (*Salvelinus fontinalis)* whilst Rehulka et al. (2004) found levels nearer 5.4g/dl for rainbow trout.

There is obviously a large drop in haematocrit (25%) and haemoglobin (5.32g/dl) in fish that have been fed diets that contain SDH (an iron rich ingredient) when compared to the control fish which had values of 34% for haematocrit, and 6.3g/dl for haemoglobin. A possible explanation could be due to the very high iron levels in haem protein concentrate that could influence the homeostatic mechanisms involved in the regulation of iron metabolism in fish. The elevated intake of iron may have resulted in a negative feed back effect on erythrocyte production affecting the levels in blood. Physiological modulation of iron metabolism in rainbow trout fed low and high iron rich diets were investigated by Carriquiriborde et al. (2004) in which

detailed assessment of the biochemical and metabolic effects of varying iron intake was made. A number of parameters were affected including haematological indices. This hypothesis needs to be tested further in order to substantiate these changes in trout fed iron rich ingredients.

In the present study no histopathology was undertaken on key organs or tissues and on reflection perhaps it would have been interesting to have compared gastro-intestinal, liver, pancreas and kidney morphology and ultra structure resulting from the long term feeding of terrestrial animal proteins to trout.

In the present programme of work the terrestrial animal by-products were all 'category 3'. These materials are not allowed to be sited near a category 1 site (slaughter house) or near a category 2 building (processed material from a slaughter house) this is to ensure that no cross contamination of unprocessed material with processed material occurs (category 3 material-processed material is cooked at 130°C at 225kpa for at least 20 minutes). In addition no equipment is to be moved between any of these designated sites. All rendering plants wanting to process terrestrial by-products for feedstuffs must have Hazard Analysis and Critical Control Points (HACCP) principles to guarantee the safety of the material (EU, 2002). The designated materials were fully processed to meet strict protocols and standards outlined above. Indeed new technologies are constantly being developed to process such materials including use of mechanical and solvent extraction processes as well as enzymatic treatment of rendered material and discards (Woodgate, pers comm) Extraction of fats and oils leads to the possibility of elevating the protein concentrations of meals as well as providing animal fat as a commodity. Animal fats may have some use in aquafeed for certain species of fish, in particular tropical

species (Fasikin et al., 2004). Alternatively defatted poultry meat meal, for example, has recently been tested by (Laporte, unpublished data) with very promising results for gilthead seabream (*Sparus aurata*), especially in respect to carcass lipid ratios and being able to reduce the amount of poultry meal added due to the increase in protein content (circa 10% increase) and subsequent elevation in amino acid values due to over 60% of the lipid fraction of the poultry meat meal being removed. The meal in question was initially 13% fat and after solvent extraction the fat content was significantly reduced to 5%. This resulted in a significant reduction of saturated fat and did influence the final carcass content of the gilthead seabream (Laporte, unpublished data).

Improved processing technologies to increase the effectiveness of animal by-products such as poultry meat meal (PMM) and feathermeals include the addition of enzymes to pre-hydrolyse protein thus enhancing product quality and consistency (Woodgate, pers comm.). It is also possible to consider the supplementation of fish diets with crystalline amino acids to correct any minor essential amino acid imbalances when high inclusions of animal proteins such as PMM are used. Rawles et al. (2006) were able to greatly improve the quality of poultry by-product meal (PBM) for use in hybrid striped bass (*Morone chrysops C x Morone saxatilis F*) by supplementing diets containing 40% digestible protein with a mixture of amino acids based on the ideal amino acid profile of striped bass muscle. 100% replacement of fish meal with PBM was feasible with the addition of lysine, methionine, threonine, and leucine. However, lysine alone failed to elicit the same response. Similar work has been conducted by Davies and Morris (1997) and Davies et al. (1997) on rainbow trout fed maize gluten and wheat gluten fortified with multiple amino acids to otherwise balanced diets. These investigators demonstrated the importance of maintaining a balanced amino

acid profile for trout in accordance with their nutritional requirements and relative EAA tissue profile.

However, crystalline amino acids are individually quite expensive but may be cost effective at low inclusion levels when they can allow moderate or even considerable use of cheaper raw materials in formulated rations for fish. One disadvantage of crystalline amino acids is that many workers have reported differential utilisation due to their rapid absorption reaching sites of protein biosynthesis prior to the main assimilation of dietary protein (Cowey, 1988). This appears to be particularly acute for warm water species compared to temperate fish such as trout and salmon, due to their higher metabolic rate and difference in gut morphology (Cowey, 1988). There are indications that coating amino acids with a stable starch matrix or casein may delay absorption and improve their efficacy (Wilson et al., 1986).

As well as standard avian proteins evaluated in the present study, a porcine source of spray dried haem (SDH) protein concentrate was tested. These spray dried blood cells resulting from 'high tech' processes were considered to be a unique quality product composed of highly digestible and palatable protein. The designated product AP301 (SDH) (American Protein Corporation, USA) was evaluated for trout and found to be well digested in terms of protein, amino acids and energy.

The product was also a rich source of leucine and lysine but low in isoleucine. The exceptionally high iron concentrations in SDH (2700ppm) may pose a serious limitation preventing its use at high levels in feeds due to the active pro-oxidant effect of iron on high unsaturated oils causing irreversible lipid peroxidation and rancidity (Baker et al., 1997; Carriquiriborde et al., 2004).

However, low levels of SDH may be useful in enhancing the palatability and binding qualities of diets containing high levels of bland ingredients that could replace fish

meal. The work of Carriquiriborde et al., (2004) would suggest that if iron levels were adjusted accordingly a slight reformulation of the trace element premix to remove supplemented iron to acceptable levels (circa 300mg/kg) could allow more moderate inclusion of SDH products perhaps approaching 10% without sub-lethal toxicological effects. A renewed interest in blood-based proteins for aquaculture was presented by Tacon (2006) in which it was reported that blood proteins of the standard blood meal variety may make an invaluable contribution to the range of ingredients available for effective diet formulation in fish.

The formulation of diets necessitates consideration of the relative cost and availability of different ingredients as well as their nutritional value. Replacement of fish meal with alternative plant and animal by-products offers the scope to produce flexible solutions whilst minimising the final cost of the diet. The prices of raw materials and feed ingredients varies regionally and can be appreciably different between countries depending on importation tariffs, energy costs, seasonal factors and the economic status of the country in question. However prices are based on fluctuating global markets and are considered to be major commodities for trading (Feedstuffs.com).

For example, fish meals and fish oils are the most costly at approximately £890/Mt (fish meal) and £550/Mt fish oil (Globefish.com). Other ingredients are relatively cheaper and the lower cost of terrestrial plant and animal-based proteins would be beneficial in reducing feed costs and the fish meal burden.

It is important that a cost benefit analysis is undertaken for the animal by-products specifically evaluated in the course of the present programme of work. Linear Least Cost Formulation (LLCF) software (Winfeed 2.8) was employed to formulate a series of model diets for rainbow trout based on the latest market price listings for fish meal, fish oil, poultry meat meal, steam/enzyme hydrolysed feathermeals, and the spray

dried haem protein concentrate. These were presented to the ration formulation to replicate the experimental diets this was purely to demonstrate that when applied to the experimental data (based on FCR) the feeds do demonstrate a cost saving to the feed manufacturer. This data is however only based on the cost per metric tonne of protein. The cost benefit (Table 21) analysis clearly demonstrated that fish meal could be reduced in the diet at the expense of the afore-mentioned ingredients and that animal by-products, especially steam hydrolysed feathermeal, enzyme-treated feathermeal and the spray-dried haem/poultry meat meal blend could make a valuable contribution in reducing feed costs and help to reduced the burden of fish meal.

The data displayed in Table 21 below clearly demonstrates the cost savings per tonne of protein (no other supplementary ingredients were evaluated) when based on cheaper terrestrial proteins that were utilised in the final experiment at the expense of LT-fish meal (control feed).

Table 21.Cost benefit analysis of terrestrial animal proteins in aquafeeds

Diet	Test protein (fish meal) inclusion (kg/MT)	£MT of test protein	FCR for trial	Cost per MT of protein	Cost per MT of protein fed / MT live weight gain £	Cost saving per MT of protein, per MT of feed produced in £
Control[a]	718	890	0.82	639	526	0
Enzyme Treated Feathermeal (ETF)[b]	149+575	280	0.88	554	486	40
Poultry Meat Meal (PMM)[b]	223+575	300	0.90	579	519	7
Spray Dried Haem (SDH)[b]	51+646	550	0.85	613	510	16
ETF/SDH[b]	141+575	348	0.90	561	501	25
PMM/SDH[b]	147+575	363	0.87	565	493	33
Steam Hydrolysed Feathermeal[b]	172+575	220	0.84	550	464	62

[a]LT-Fish meal cost / per MT (Nash & Son, pers comm) [b]All other test protein costs / per MT (Steve Woodgate, pers comm)

It maybe concluded that the series of trials reported in this thesis was able to satisfactorily provide evidence that selected animal proteins of high quality could effectively provide good nutrition for rainbow trout and be effectively used to partially reduce the level of fish meal in compound diets for salmonids in aquaculture. Rainbow trout was employed as a model salmonid fish that could be reliably used throughout the study to provide digestibility data and nutritional performance indicators using a longer term feeding trial.

The conclusions from rainbow trout suggest that these products may also be suitable for Atlantic salmon as well as Arctic char and other related species although digestibility/feeding trials would be required to substantiate their suitability. They may even be applicable to non-related species of fish such as sea bass (*Dicentrachus labrax*) and sea bream (*Sparus aurata*) and emerging candidate species for aquaculture including cobia (*Rachycentron canadum*) and barramundi (*Lates calcarifer*).

The investigations with trout were conducted on the juvenile fast growing phase (40g), with fish being grown onto just under market size (160g). The data may be extrapolated to other stages of development but it should be cautioned that relative nutrient requirements vary considerably with respect to fish size, feed intake, and feed frequency. Abiotic factors such as salinity, photoperiod and water quality also need to be considered. Indeed temperature plays a dominant role with respect to fish metabolism, physiology and growth (Jobling, 1994). In this study the Standard Environmental Temperature (SET), for rainbow trout (15°C) was employed for all investigations. Although this was useful for making accurate comparisons between the digestibility and growth trial data salmonid aquaculture is often practised over a wide temperature range from 4-20°C (Edwards, 1978).

Clearly further work is required to assess the efficacy of novel proteins at these extremes. Salmonid aquaculture, and especially the growth of Atlantic salmon, is mainly conducted

in seawater and studies with rainbow trout may only be applicable to the fresh water phase of the production cycle. Therefore the effect of seawater transfer and exposure to a variety of salinities need to be fully elucidated with respect to feed composition.

Most investigations to assess alternative proteins have focused on fish reaching edible size and there is a lack of information regarding the formulation of brood stock diets and the effects on reproduction and subsequent production of ova, milt, fecundity, hatchability, and fry quality. The use of terrestrial animal by-products needs to be fully investigated for this important sector of aquaculture as well as the main production phases.

Increasingly the consumer has become an important factor in the direction of aquaculture research and fish nutrition is no exception. Although the present investigation has demonstrated the feasibility of using animal proteins to meet the nutritional requirements to support growth and health of intensively reared rainbow trout, it has not evaluated the effects of fish meal replacement with terrestrial animal proteins in respect of fish texture, dress out weight, taste, colour or visual appearance of the final product. Therefore it would be pertinent to include sensory evaluation of fish subjected to dietary formulations containing terrestrial animal derived proteins compared to standard marine protein based feeds to evaluate their effects on taste and quality. These studies require extensive training of personnel (Nick Joy, Pers Comm) and although costly to undertake provide invaluable information concerning the acceptability of the products in relation to market requirements.

An example of an important criterion in the European production of rainbow trout is the use of astaxanthin as a pigmenting carotenoid added to the feed (Sinnott, 1989). A number of nutritional factors may influence the retention of astaxanthin and its stability in fish fillets over time which includes the degree of oxidation and the level and quality of

fat in the carcass. Evidently this needs to be assessed with respect to major changes in diet formulation in the final and critical phases of production prior to harvest. Moderate inclusions of animal proteins are likely to influence the taste, texture and possibly the colour of rainbow trout at market (Sinnott, 1989). These factors need to be explored further for a range of fish species.

The focal point of this research was aimed at optimising diet formulations using terrestrial animal by-products based on their protein and energy contribution. It should be emphasised that other nutrient components are also important in a full assessment.

The fat content of these terrestrial proteins differ considerably in their overall fatty acid profile to marine fish oil. Although the essential fatty acids were satisfied by the supplementation of marine oil in the growth study it is well known that the fatty acid profile of the tissues and particularly the edible flesh would be influenced markedly by dietary fatty acid ratios (Subhadra et al. 2006; Liu et al., 2004). These need to be validated in future studies since it may affect fish health due to modulation of the immune system and may even affect the fatty acid profile of gill tissue and liver (Bureau et al., 1997). Indeed the health implications of major changes in dietary formulation have not been well documented and such information would be very useful in the context of the present study.

Additionally changes in macro ingredient formulation will greatly affect mineral availability and this is of significance in providing balanced trace elements for fish and the design of trace element premixes. For salmonid fish the bioavailability of macro elements such as magnesium (Mg), calcium (Ca) and in particular phosphorus (P) are important (Watanabe et al., 1997). Phosphorus is particularly relevant due to its high levels in fishmeal-based feeds and of increasing concern from an environmental perspective (Tacon & Forster, 2003). This element is necessary to meet an extensive

range of metabolic processes in fish and is vital for bone mineralisation and overall health (Watanabe et al., 1997). It would be interesting to assess mineral availability and especially that of Ca and P from different terrestrial animal by-products such as those in this thesis for rainbow trout and salmon in more detail.

This would necessitate experiments on mineral digestibility and carcass retention using the methods described in this thesis.

In terms of quantifying the influence of terrestrial animal proteins on fish health, it is envisaged that future research involving feeding trials should terminate with investigations on full haematological profiling to include immunological data and possibly a challenge study with a known pathogen to assess disease resistance as well a comprehensive histological appraisal of the major organs and tissues e.g. liver, kidney, spleen, intestine and muscle (Blazer & Wolke, 1984; Bransden et al. 2001).

Finally, legislative and consumer constraints need to be addressed to gain acceptability for these materials to be used as credible and reliable safe alternatives for use in aquafeed formulations.

The selection of raw materials and degree of processing clearly has a major impact on the quality and final composition of poultry meals, feather meals and haem proteins. These commercial products are useful commodities and should be further evaluated. It would be advisable to refine the processing methods and review in particular the temperature levels and duration of exposure to heat to optimise the correct processing methods suitable for there applications in aquafeeds.

Fortunately these by-products are strongly advocated in many parts of the world including North America, South America, Asia Australia and New Zealand (Tacon, 2006). Unless consumer confidence is restored in Europe with respect to the use of terrestrial animal proteins there will be a serious deficit of effective high quality protein and energy rich

ingredients that can be used to offset the demands for and high cost of fish meal and fish oil. It is hoped that the findings of the programme of work presented in this thesis have made an important contribution towards advocating the use of terrestrial animal proteins for salmonid species and for more general use in other important commercially farmed fish globally.

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Appendix

WinFeed 2.8 (Least Cost Feed Formulation)					
Batch Name	Control LT-94				
Feed Store	Integrated Feed Store				
Formulation Method	Linear Formulation				
Formulation Basis	As-Fed Basis				
Batch Quantity Required g/kg	1000				
Cost per kg £	641				
INGREDIENTS	Dry Matter	As Fed	Nutrient g/100g	Dry Matter	As Fed
LT Fishmeal	69.2	70.0	Dry Matter	96.1	96.1
Wheat filler	15.2	15.0	Protein (CP)	55.1	52.9
Methionine	0.9	0.9	Lipid	19.8	19.0
Lysine	0.0	0.0	Carbohydrate	10.9	10.5
mineral premix	2.1	2.0	Ash	8.3	8.0
vitamin premix	2.1	2.0	Ca	1.6	1.6
Marine fish oil	9.4	9.0	P	1.4	1.3
Vegetable oil	1.2	1.2	Mg	0.2	0.1
			Methionine	2.0	1.9
			Lysine	4.2	4.0
			Arginine	3.5	3.4
			Threonine	2.2	2.1
			Histidine	1.3	1.2
			Tryptophan	0.6	0.6
			Phenylalanine	2.1	2.0
			Leucine	4.0	3.8
			Isoleucine	2.4	2.3
			Valine	3.3	3.1

WinFeed 2.8 (Least Cost Feed Formulation)

Batch Name	Enzyme Treated Feathermeal
Feed Store	Integrated Feed Store
Formulation Method	Linear Formulation
Formulation Basis	As-Fed Basis
Batch Quantity Required g/kg	1000
Cost per kg £	582

INGREDIENTS	Dry Matter	As Fed	Nutrient g/100g	Dry Matter	As Fed
LT Fishmeal	58.3	58.5	Dry Matter	95.3	95.3
Enzyme feathermeal	14.3	15.0	Protein (CP)	59.2	56.5
Wheat filler	13.3	13.1	Lipid	17.7	16.9
Methionine	0.4	0.4	Carbohydrate	9.7	9.3
Lysine	0.0	0.0	Ash	7.5	7.2
mineral premix	2.1	2.0	Ca	1.4	1.3
vitamin premix	2.1	2.0	P	1.2	1.1
Marine fish oil	9.4	9.0	Mg	0.1	0.1
			Methionine	1.6	1.6
			Lysine	3.9	3.7
			Arginine	3.5	3.4
			Threonine	2.2	2.1
			Histidine	1.2	1.1
			Tryptophan	0.6	0.5
			Phenylalanine	2.1	2.0
			Leucine	3.9	3.7
			Isoleucine	2.3	2.2
			Valine	3.2	3.0

WinFeed 2.8 (Least Cost Feed Formulation)

Batch Name	Poultry meat meal
Feed Store	Integrated Feed Store
Formulation Method	Linear Formulation
Formulation Basis	As-Fed Basis
Batch Quantity Required g/kg	1000
Cost per kg £	560

INGREDIENTS	Dry Matter	As Fed	Nutrient g/100g	Dry Matter	As Fed
LT Fishmeal	51.1	51.5	Dry Matter	95.7	95.7
Poultry Meat Meal (PDM)	21.9	22.3	Protein (CP)	56.4	53.9
Wheat filler	12.9	12.7	Lipid	19.3	18.5
Methionine	0.5	0.5	Carbohydrate	9.5	9.1
Lysine	0.0	0.0	Ash	9.4	9.0
mineral premix	2.1	2.0	Ca	2.3	2.2
vitamin premix	2.1	2.0	P	1.6	1.5
Marine fish oil	9.4	9.0	Mg	0.2	0.1
			Methionine	1.6	1.6
			Lysine	4.0	3.8
			Arginine	3.6	3.4
			Threonine	2.2	2.1
			Histidine	1.2	1.2
			Tryptophan	0.6	0.5
			Phenylalanine	2.1	2.0
			Leucine	4.0	3.8
			Isoleucine	2.2	2.1
			Valine	3.1	2.9

WinFeed 2.8 (Least Cost Feed Formulation)

Batch Name	Spray Dried Haem
Feed Store	Integrated Feed Store
Formulation Method	Linear Formulation
Formulation Basis	As-Fed Basis
Batch Quantity Required g/kg	1000
Cost per kg £	615

INGREDIENTS	Dry Matter	As Fed	Nutrient g/100g	Dry Matter	As Fed
LT Fishmeal	59.1	59.7	Dry Matter	96.0	96.0
Spray Dried Haem (APC)	5.8	6.0	Protein (CP)	53.7	51.5
Wheat filler	18.2	18.0	Lipid	19.8	19.0
Methionine	1.1	1.1	Carbohydrate	13.2	12.6
Lysine	0.0	0.0	Ash	7.6	7.3
mineral premix	2.1	2.0	Ca	1.4	1.3
vitamin premix	2.1	2.0	P	1.2	1.2
Marine fish oil	9.4	9.0	Mg	0.2	0.1
Vegetable oil	2.33	2.24	Methionine	2.0	1.9
			Lysine	4.2	4.0
			Arginine	3.3	3.2
			Threonine	2.1	2.1
			Histidine	1.6	1.5
			Tryptophan	0.6	0.6
			Phenylalanine	2.3	2.2
			Leucine	4.3	4.1
			Isoleucine	2.1	2.0
			Valine	3.4	3.3

WinFeed 2.8 (Least Cost Feed Formulation)

Batch Name	Poultry Meat Meal-Spray Dried Haem
Feed Store	Integrated Feed Store
Formulation Method	Linear Formulation
Formulation Basis	As-Fed Basis
Batch Quantity Required g/kg	1000
Cost per kg £	572

INGREDIENTS	Dry Matter	As Fed	Nutrient g/100g	Dry Matter	As Fed
LT Fishmeal	51.9	52.4	Dry Matter	95.9	95.9
Poultry Meat Meal (PDM)	10.8	11.0	Protein (CP)	53.2	51.0
Spray Dried Haem (APC)	3.2	3.3	Lipid	19.8	19.0
Wheat filler	18.2	18.0	Carbohydrate	13.3	12.7
Methionine	0.6	0.6	Ash	8.2	7.9
Lysine	0.0	0.0	Ca	1.7	1.7
mineral premix	2.1	2.0	P	1.4	1.3
vitamin premix	2.1	2.0	Mg	0.2	0.2
Marine fish oil	9.38	9	Methionine	1.6	1.6
Vegetable oil	1.78	1.71	Lysine	3.9	3.8
			Arginine	3.3	3.2
			Threonine	2.1	2.0
			Histidine	1.4	1.3
			Tryptophan	0.6	0.5
			Phenylalanine	2.1	2.0
			Leucine	4.0	3.8
			Isoleucine	2.0	2.0
			Valine	3.1	3.0

WinFeed 2.8 (Least Cost Feed Formulation)

Batch Name	Enzyme Treated Feathermeal-Spray Dried Haem
Feed Store	Integrated Feed Store
Formulation Method	Linear Formulation
Formulation Basis	As-Fed Basis
Batch Quantity Required g/kg	1000
Cost per kg £	586

INGREDIENTS	Dry Matter	As Fed	Nutrient g/100g	Dry Matter	As Fed
LT Fishmeal	55.5	56.0	Dry Matter	95.8	95.8
Enzyme feathermeal	6.4	6.7	Protein (CP)	53.8	51.5
Spray Dried Haem (APC)	2.9	3.0	Lipid	19.8	19.0
Wheat filler	18.2	18.0	Carbohydrate	13.2	12.6
Methionine	0.8	0.8	Ash	7.3	7.0
Lysine	0.0	0.0	Ca	1.3	1.3
mineral premix	2.1	2.0	P	1.2	1.1
vitamin premix	2.1	2.0	Mg	0.2	0.1
Marine fish oil	9.39	9	Methionine	1.8	1.7
Vegetable oil	2.62	2.51	Lysine	3.8	3.7
			Arginine	3.3	3.1
			Threonine	2.1	2.0
			Histidine	1.3	1.3
			Tryptophan	0.6	0.5
			Phenylalanine	2.1	2.0
			Leucine	3.9	3.8
			Isoleucine	2.1	2.0
			Valine	3.1	3.0

WinFeed 2.8 (Least Cost Feed Formulation)

Batch Name	Steam Hydrolysed Feathermeal
Feed Store	Integrated Feed Store
Formulation Method	Linear Formulation
Formulation Basis	As-Fed Basis
Batch Quantity Required g/kg	1000
Cost per kg £	540

INGREDIENTS	Dry Matter	As Fed	Nutrient g/100g	Dry Matter	As Fed
LT Fishmeal	51.6	52.0	Dry Matter	95.7	95.7
Steam hydrolysed FM	16.4	17.0	Protein (CP)	56.0	53.5
Wheat filler	15.2	15.0	Lipid	19.9	19.0
Methionine	0.7	0.7	Carbohydrate	11.2	10.7
Lysine	0.0	0.0	Ash	8.8	8.4
mineral premix	2.1	2.0	Ca	1.3	1.2
vitamin premix	2.1	2.0	P	1.2	1.1
Marine fish oil	9.4	9.0	Mg	0.2	0.2
Vegetable oil	2.39	2.29	Methionine	1.6	1.6
			Lysine	3.4	3.3
			Arginine	3.8	3.6
			Threonine	2.4	2.3
			Histidine	1.1	1.0
			Tryptophan	0.5	0.5
			Phenylalanine	2.3	2.2
			Leucine	4.2	4.0
			Isoleucine	2.5	2.4
			Valine	3.6	3.4