THE USE OF ANIMAL BY-PRODUCTS AVAILABLE IN PORTUGAL IN DIET FORMULATIONS FOR RAINBOW TROUT

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by

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ERRATA

Throughout this thesis please read apparent dry matter digestibility instead of apparent organic matter digestibility.

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ABSTRACT

The chemical composition and the potential nutritive value of two Portuguese Animal by-products, Poultry by-product and hydrolysed feather meal and Meat and bone meal were evaluated in diets for rainbow trout replacing fish meal protein at levels of up to 100%. In addition six Portuguese Brown fish meals produced by different fish meal processing plants were also evaluated. Their effects on growth performance, feed utilization efficiency, liver somatic index, blood parameters, and carcass composition of fish fed diets containing these products were assessed. Based on the results of these experiments a further trial was carried out in order to evaluate the three Animal by-products in a compound diet containing different combinations of these products. A commercial trout ration produced on a small scale in Portugal was used as an additional control.

In conjunction with these feeding trials monthly samples of the Poultry by-product and hydrolysed feather meal, Meat and bone meal and the six Brown fish meals were analysed over a one year period in order to evaluate their degree of variability.

The Poultry by-product and hydrolysed feather meal successfully replaced up to 80% to 90% of the fish meal protein without any loss of performance and feed utilization efficiency although a significant alteration in fish carcass composition was indicated for fish fed diets containing this by-product. It was apparent however that the fish meal used in this initial trial was not of a particularly

high quality. Nevertheless, in a subsequent trial the performance of fish fed a ration where Poultry by-product and hydrolysed feather meal replaced half of the fish meal protein was slightly better than that of fish fed a control ration based on a good quality fish meal. Furthermore the year long survey indicated that the chemical composition of this by-product was consistent.

Five of the six Brown fish meals evaluated proved to be of low quality and only one gave similar results compared with a control ration based on the bacterial SCP "Pruteen". Furthermore a wide variation in the chemical composition of all six Brown fish meals was also indicated during the one year survey. A good quality fish meal was eventually located which contained a locally produced meal but which was blended with an imported high quality fish meal. This meal was subsequently used to produce control rations in the last two feeding trials.

The Meat and bone meal successfully replaced up to 80% of a good quality fish meal protein without any significant loss of growth performance and feed utilization efficiency although the partial removal of the bone material during pretreatment produced a meal with the characteristics of a meat meal rather than a meat and bone meal. In the final feeding trial where this pretreatment was not carried out, it was apparent that the maximum inclusion level of this product was less than 30% of the protein component. As had been the case with the Poultry by-product and hydrolysed feather meal the chemical composition of different batches of the Meat and bone meal was consistent.

The evaluation of the three Animal by-products alone or in different combinations indicated that Poultry by-product and hydrolysed feather meal is a good protein source that can replace a large proportion of a good quality brown fish meal without any loss of growth performance and feed utilization efficiency. By contrast growth performances and feed utilization efficiencies decreased with increasing inclusion levels of Meat and bone meal and therefore inclusion levels below 30% of the protein are recommended in diets for rainbow trout. The best growth performance and feed utilization efficiency where the three Animal by-products were used in different combinations was indicated for a diet containing these products in the ratio 1.3 Brown fish meal: 1 Poultry by-product and hydrolysed feather meal: 1 Meat and bone meal.

An economical appraisal of the diets produced in the four growth trials using the three Animal by-products was carried out. The costs of the three protein sources, the diets containing these products and finally the cost of fish production using these rations were compared. The Poultry by-product and hydrolysed feather meal and the Meat and bone meal were 19% and 35% , respectively, less expensive than Portuguese Brown fish meals and therefore significant savings could be expected where these products are included in diet formulations. However, based on the cost of a Kilogramme of fish production only certain formulations containing Poultry by-product and hydrolysed feather meal, Meat and bone meal or combinations of these animal by-products were cheaper than the fish meal control rations. The implications of the results obtained in this study for commercial production of trout rations in Portugal are discussed.

The work presented in this thesis is the result of my own investigations and has neither been accepted nor is being submitted for any other degree.

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...Barbara...Ross..... Supervisor

7th. October..1986. Date

CHAPTER 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

Portugal is the most eastern country in Europe situated between 37° and 42° North of the Equator. It has a temperate climate with water temperatures ranging from 5° to 11°C (mean 8°C) during winter and from 12° to 22°C (mean 17°C) in the summer. Exceptionally, water temperatures may rise to 27°C. Thus, although water temperatures are often close to the upper tolerance limit for rainbow trout (Hokanson et al., 1977; Elliot, 1982), environmental conditions are nevertheless suitable for trout farming, particularly in the North of Portugal where most of the reservoirs and rivers are located and where temperatures are somewhat lower than the average for the country as a whole.

Fish consumption in Portugal is one of the highest in Europe with an annual per capita intake of around 48.4 Kilogrammes (Coull, 1972). This level of fish consumption accounts for almost half of the total intake of animal protein and represents 18% of dietary calories (Coull, 1972). Currently most of the fish consumed in Portugal is of marine origin, thus fishing is a major Portuguese industry. Since the market price of trout in Portugal is lower than that of marine fish the future for rainbow trout farming is promising.

Trout farming in Portugal began in the late 1960's and by 1976 Portugal had only seven private farms culturing both rainbow trout and brown trout (Brown, 1977). The total production from these farms was 250 tons per year, nearly all of which was rainbow trout.

Currently Portugal has five private and 11 state trout farms (Fig. 1.1) which together produce around 1,000 tons per year (trout farm managers' personal communications). Portugal also has a single state carp farm and there have been attempts to cultivate eels in the centre and the south of the country.

The rainbow trout spawning season in Portugal is between November and February (Brown, 1977). Fish are raised in concrete ponds and raceways to a market size of 200-250 grammes when they are sold on ice for the table. The average on-growing period from hatchery to market size is between 12 and 15 months (Brown, 1977) although market size is attained within 8-10 months on certain farms as a result of favourable environmental conditions.

Rainbow trout in Portugal are fed pelleted diets all of which are imported with the exception of one private trout farm that produces its own feeds. In view of the current level of farming and its likely expansion it is highly desirable that Portugal develops a fish feed industry not only to create jobs and to save foreign currency, but also to avoid delays in supply.

Animal by-products are important in salmonid rations not only nutritionally but also economically since they represent a large proportion of their cost. Nowadays a nutritionally balanced diet is provided at an economically competitive price relying upon the cost and nutritional value of the range of ingredients available. As a result the dietary protein requirement of commercial trout rations is generally supplied using several ingredients of animal

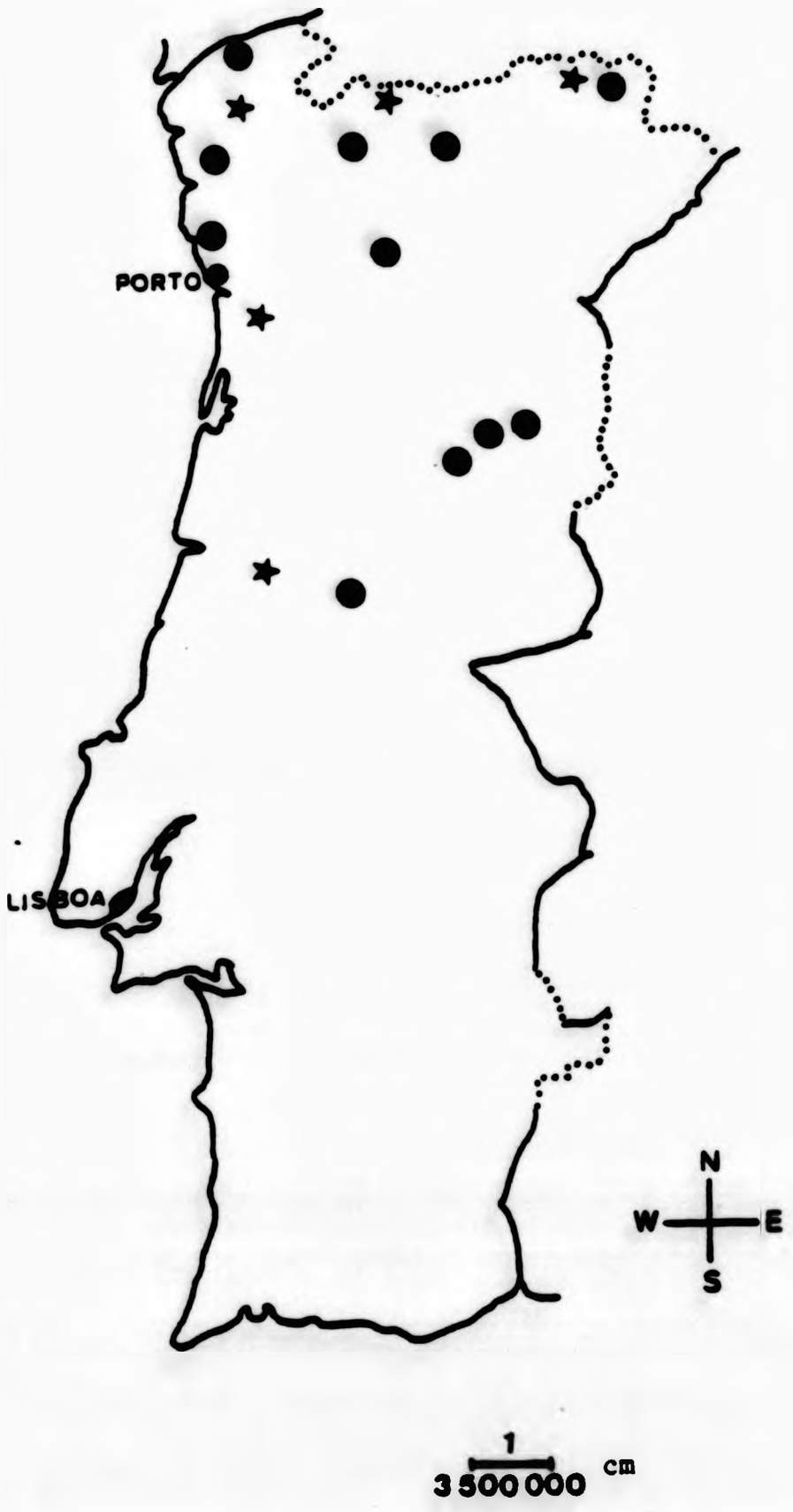

FIG. 1.1. Location of private and state trout farms in Portugal

origin. The unique value of feeds of animal origin in upgrading the nutritional qualities of diets for monogastric animals is well recognized. In diets based on cereal grains and other plant products it is difficult to avoid a deficiency of essential amino acids and some vitamins. Animal products, even when used in small amounts, can vastly improve the nutritional value of the entire diet (Cohl, 1981).

Fish meals have been traditionally used as the major protein source in rations for rainbow trout (Ketola, 1975; Fowler and Banks, 1976; Reinitz et al., 1978a; Nose, 1979; Spinelli et al., 1979; Watanabe et al., 1979; Dabrowski et al., 1980; Reinitz and Hitzel, 1980; Cowey, 1981; Tacon and Jackson, 1985). The nutritive value of a dietary protein source is governed principally by the extent to which its content of essential amino acids simulate the needs of the animal in question. The closer the composition of the protein to the pattern of requirements the greater will be its nutritive value (Cowey and Tacon, 1983), thus fish meal can be considered as an almost ideal protein source for fish in terms of its balanced amino acid profile (Reinitz, unpublished). Unfortunately, with the exception of fish meal, there is no single animal or plant protein source available to the fish feed compounder with an essential amino acid profile approximating to the dietary essential amino acid requirement of farmed fish. Alternatives to fish meal often have unbalanced amino acid profiles, particularly in the case of plant protein sources. Furthermore variation in the bio-availability of nutrients and problems associated with antinutritional factors

can also restrict the use of many potential protein sources (Tacon, 1981; Chubb, 1982; Tacon et al., 1984; Tacon and Jackson, 1985).

For a number of reasons many attempts have been made over the past decade to reduce the dependence of fish feed manufacturers on fish meals. As a result of the growing world demand for animal protein and as a consequence of fish shortages such as the 1972/73 Peruvian anchovy crisis, it is becoming increasingly difficult to obtain good quality fish meal for inclusion in fish rations in many parts of the world (Fowler and Banks, 1976; Dabrowska and Wojno, 1977; Reinitz et al., 1978a; Nose, 1979; Spinelli et al., 1979; Dabrowski et al., 1980; Reinitz and Hitzel, 1980; Tacon, 1981; Jackson et al., 1982). One response to the world demand for animal protein has been an increase in production from aquaculture. World aquaculture production doubled between 1971 and 1975 to more than 5 million tons per year (Milne, 1976). Furthermore the Food and Agriculture Organization of the United Nations has predicted that in order to provide food for the world's population the requirement will be 40 million tons per year by the year 2000 (Milne, 1976). This increased demand for fish production from aquaculture will also have the effect of increasing the requirement for fish meal as a component of compounded rations.

Fish meal is usually the most expensive ingredient in complete feeds for fish (Dabrowska and Wojno, 1977; Reinitz and Hitzel, 1980; Hilton, 1983; Tacon and Jackson, 1985). According to Dabrowski et al. (1980) it is expensive and is also often short in supply as many industrial fisheries are overworked and the cost, in fossil

fuel terms, of catching fish is gradually increasing. These authors claim that ninety-three percent of rainbow trout production costs are involved in feeding and 70% of this is due to fish meal. Tacon and Jackson (1985) further pointed out that in view of the high cost of good quality fish meals of relatively constant chemical composition, it is not surprising that feed costs can amount to 40%-60% of total operating costs in intensive aquaculture enterprises. In addition to variations in availability of fish meal there can also be wide variation in the quality. Such limited information as exists indicates that this is the case in Portugal.

In view of the increasing shortage of fish meal on the world market for fish feed formulation, the inflated prices, and the higher world demand for animal protein, alternative protein sources need to be assessed in an effort to reduce the dependence of the fish farming industry on this single commodity (Halver, 1976). Plausible alternatives to fish meal should be locally and readily available, both physically and economically. They should also be non toxic and cost effective. In addition they must be acceptable and palatable to fish and have a good nutritional quality (Tusé, 1984). Much work has been carried out on fish meal free feeds over the last ten years but so far none has been produced commercially since in general they do not perform as well as those based on fish meal (Tacon and Jackson, 1985).

The aim of this study is to review the work carried out to date on the use of animal by-products in diets for rainbow trout. This will then act as a basis for selecting the most promising animal

by-products available in Portugal for evaluation as major protein sources in diets for rainbow trout. In addition, the quality of these products and that of the available fish meals will be monitored over a period of a year in order to establish the degree of variation in chemical composition. Finally an economic evaluation of the findings will be carried out with a view to recommending diet formulations for the production of rainbow trout rations in Portugal.

CHAPTER 2

REVIEW OF ANIMAL BY-PRODUCTS

A wide range of materials are produced as by-products from animal processing industries. Some are currently used in rations both for terrestrial animals and for fish, although many need further evaluation as potential protein sources in dietsfor fish. In this review of the use of animal by-products in diets for rainbow trout, the range of products has been divided into the following categories: slaughterhouse by-products, poultry by-products, dairy by-products, fish products, invertebrates and bacterial single cell protein.

2.1 SLAUGHTERHOUSE BY-PRODUCTS

The raw materials of slaughterhouse by-products are animals that have died from disease, carcasses or parts of carcasses that have not passed meat inspections, technical blood, inedible parts of the digestive tract, (e.g. bibles), reproductive organs, bones and other trimmings that are not acceptable as food for aesthetic reasons (Göhl, 1981). From these raw materials a wide range of animal by-products such as bone meal, meat meal, meat and bone meal, blood meal, leather by-products and animal fat are produced.

2.1.1 Bone Meal

Bones suitable for processing can come not only from slaughterhouses but also from municipal dumps, hotels and restaurants. They are cooked under steam pressure and finally ground to produce a meal (Göhl, 1981).

Bone meal has a very low crude protein content of between 6% and 10% (Table 2.1), mainly in the form of collagen (Eastoe and Long, 1960), and an amino acid profile deficient in almost all essential amino acids with the exception of arginine (Table 2.2). It is however a rich source of minerals and trace elements (Eastoe and Long, 1960; Lall, 1979; Göhl, 1981) and, as such has been included at very low dietary levels (2%) but is generally mixed with other slaughterhouse by-products for fish feeds (King and Campbell, 1978).

2.1.2 Meat Meal

Meat meal is defined by the Fertilizers and Feeding Stuffs Regulations (HMSO, 1973) as:

> "the product containing not less than 55% of protein and not more than 4% of salt, obtained by drying and grinding animal carcasses or portions thereof (excluding hoof and horn) to which no other matter has been added, but which may have been preliminarily treated for the removal of fat."

Meat meal has a protein content, varying between 57% and 72% (Table 2.1) but it is deficient in both the essential amino acids methionine and tryptophan (Table 2.2; Summers et al., 1964; McDonald et al., 1981). It has a lipid content of between 13% and 30%, and it is this high lipid content which precludes its inclusion at high dietary levels. It is a good source of vitamins of the B complex, particularly riboflavin, choline, nicotinamide, and B_{12}.

Meat meals have been successfully included in rations for salmonids in conjunction with other animal by-products at levels not exceeding 10% by weight (Tacon et al., 1983a).

TABLE 2.1 Proximate composition of Bone meal, Meat and bone meals and Meat meals (% dry weight)

Proximate composition (% dry weight)	Bone meal 1	Meat and bone meal 1	2	3	4	Meat meal 1	5
Moisture (%)	7-8	9.35	6.5	8.6	6.1	9.02	3.57
Crude protein (%)	6-10	51.5	50.1	50.0	50.4	72.2	57.8
Lipid (%)	1-7	11.2	11.9	2.5	10.8	13.2	30.3
Ash (%)	78-92	27.5	32.0	33.2	30.7	3.8	7.0

TABLE 2.2 Amino acid profile of Bone meal, Meat and bone meals and Meat meals and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt.)	Bone meal 5	Meat and bone meal 1	3	2	Meat meal 1	5	Requirement of rainbow trout (% diet)
Arginine	4.0	4.15	4.1	3.01	5.25	3.83	1.40, 40% (Ogino, 1980)
Histidine	0.4	1.19	1.0	2.55	0.94	0.89	0.64, 40% (Ogino, 1980)
Isoleucine	0.3	1.35	1.3	1.40	3.94	1.45	0.96, 40% (Ogino, 1980)
Leucine	0.7	3.69	2.4	2.75	6.88	3.13	1.76, 40% (Ogino, 1980)
Lysine	1.3	3.07	2.8	2.05	2.57	2.78	2.12, 40% (Ogino, 1980)
Methionine	0.05	0.68	0.6	0.70	0.74	0.56	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	0.4	2.02	1.4	1.50	3.95	1.98	1.24, 40% (Ogino, 1980)
Threonine	1.3	1.84	1.5	1.55		1.91	1.36, 40% (Ogino, 1980)
Tryptophan		0.35	0.3	0.25	0.57		0.20, 40% (Ogino, 1980)
Valine	0.6	2.41	2.1	2.10	6.17	2.26	1.24, 40% (Ogino, 1980)
Alanine	2.4			3.56		4.64	
Aspartic acid	1.1			3.36	3.88	4.12	
Cystine		0.44		1.44	3.18		0.30, 35% (Rumsey et al. 1983)
Glutamic acid	1.6			5.31	6.26		
Glycine	6.3	7.82		6.71	6.87	9.11	
Proline	2.4	7.0		4.26	5.74		
Serine	0.8			2.25	2.32		
Tyrosine	0.2	1.39		0.95	2.23	1.04	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After McDonald et al. (1981)
2) After Skrede et al. (1980)
3) After Tacon et al. (1984)
4) After Fowler and Banks (1976)
5) After Eastoe and Long (1960)

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Moisture (%)	7-8	9.35	6.5	8.6	6.1	9.02	3.57
Crude protein (%)	6-10	51.5	50.1	50.0	50.4	72.2	57.8
Lipid (%)	1-7	11.2	11.9	2.5	10.8	13.2	30.3
Ash (%)	78-92	27.5	32.0	33.2	30.7	3.8	7.0

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Histidine	0.4	1.19	1.0	2.55	0.94	0.89	0.64, 40% (Ogino, 1980)
Isoleucine	0.3	1.35	1.3	1.40	3.94	1.45	0.96, 40% (Ogino, 1980)
Leucine	0.7	3.69	2.4	2.75	6.88	3.13	1.76, 40% (Ogino, 1980)
Lysine	1.3	3.07	2.8	2.05	2.57	2.78	2.12, 40% (Ogino, 1980)
Methionine	0.05	0.68	0.6	0.70	0.74	0.56	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	0.4	2.02	1.4	1.50	3.95	1.98	1.24, 40% (Ogino, 1980)
Threonine	1.3	1.84	1.5	1.55		1.91	1.36, 40% (Ogino, 1980)
Tryptophan		0.35	0.3	0.25	0.57		0.20, 40% (Ogino, 1980)
Valine	0.6	2.41	2.1	2.10	6.17	2.26	1.24, 40% (Ogino, 1980)
Alanine	2.4			3.56		4.64	
Aspartic acid	1.1			3.36	3.88	4.12	
Cystine		0.44		1.44	3.18		0.30, 35% (Rumsey et al. 1983)
Glutamic acid	1.6			5.31	6.26		
Glycine	6.3	7.82		6.71	6.87	9.11	
Proline	2.4	7.0		4.26	5.74		
Serine	0.8			2.25	2.32		
Tyrosine	0.2	1.39		0.95	2.23	1.04	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After McDonald et al. (1981)
2) After Skrede et al. (1980)
3) After Tacon et al. (1984)
4) After Fowler and Banks (1976)
5) After Eastoe and Long (1960)

2.1.3 Meat and Bone Meal

Meat and bone meal has for many years been included in combination with meat meal in rations for terrestrial animals (Henson et al., 1954; Eastoe and Long, 1960; Burgos et al., 1974). According to the Fertilizers and Feeding Stuffs Regulations (HMSO, 1973) meat and bone meal is defined as:

> "the product, containing not less than 40% of protein and not more than 4% of salt, obtained by drying and grinding animals carcasses or portions thereof (excluding hoof and horn) and bone, to which no other matter has been added but which may have been preliminarily treated for the removal of fat."

Meat and bone meal has a crude protein content of around 50% (Table 2.1) with an amino acid profile deficient in methionine, tryptophan and lysine (Table 2.2; Bloss et al., 1953; Henson et al., 1954; Mead and Teter, 1957; Luce et al., 1970; Stockland et al., 1978; McDonald et al., 1981). Methionine is the first limiting amino acid (Tacon and Jackson, 1985). It has a lipid content varying between 2.5% and 12% and an ash content of between 27% and 33%. Meat and bone meal is an excellent source of Ca, P, and Mg and of the vitamins of the B complex, particularly of riboflavin, choline, nicotinamide and B_{12}, (McDonald et al., 1981).

Fowler and Banks (1976) evaluated the nutritional quality of a meat and bone meal for chinook salmon (Oncorhynchus tshawytscha) replacing 77% and 88% of herring meal protein. The growth rate of fish fed these experimental rations was significantly lower than that of those fed a herring meal based control diet. Furthermore some fish fed diets containing the highest meat and bone meal level

exhibited a poorer protein and lipid deposition than those fed the control diet and in addition developed a mild nephrocalcinosis.

Tiews et al. (1976) also reported reduced growth performance and feed utilization efficiency when meat and bone meal protein replaced 38% of the herring meal protein in diets for rainbow trout. By contrast Tacon (1982b) reported that meat and bone meal (48.35% crude protein and 0.418% lipid) can successfully replace up to 40% of the herring meal protein in rainbow trout rations without any loss of growth performance or feed utilization efficiency. Furthermore the weight gain of fish fed diets in which 34% of the crude protein was supplied by meat and bone meal protein was better than that of those fed the herring meal based control diet.

Finally, Tiews et al. (1979) reported that the feed utilization efficiency of rainbow trout fed diets in which meat and bone meal was the sole source of dietary protein was only 6% to 15% less than that of those fed the fish meal based control diet.

Meat and bone meal has thus been successfully included in diets for salmonids at inclusion levels of up to 10% by weight in conjunction with other animal protein sources (Davis et al., 1976; Dabrowska and Wojno, 1977; Dabrowski et al., 1980; Tacon et al., 1983b, 1984; Dabroswka, 1984) and may occasionally be used at levels of up to 15% by weight (Stafford, 1984; Tacon and Rodrigues, 1984; Stafford and Tacon, in press).

2.1.4 Blood Meal

Dried blood meal is a dark chocolate-coloured powder with a characteristic smell. Blood is usually steam cooked at 100°C, then pressed to express occluded serum, and dried by steam heating and finally ground (McDonald et al., 1981). In the past blood meal was dried using vat-drying procedures, which according to Miller (1977) and King and Campbell (1978) reduced lysine availability and increased palatability problems. These problems have been somewhat alleviated however, by using newer flash drying procedures, including both the ring-drying and the rotatory steam-drying processes which result in improved lysine availability and improved platability (Miller, 1977; King and Campbell, 1978). It is important that the temperature should not exceed 120°C in any phase of the process otherwise the meal will be of inferior quality (Göhl, 1981).

Blood meal is a good source of dietary protein (Miller, 1977; Walker, 1977; McDonald et al., 1981; Tacon, 1983) containing as much as 95% crude protein (Table 2.3). It is a good source of almost all the amino acids (King and Campbell, 1978; Orme, 1978; Tacon, 1979a) containing particularly high levels of lysine (Miller, 1977; King and Campbell, 1978; Tacon, 1979a; McDonald et al., 1981) and tryptophan (Tacon, 1983). It is also rich in valine, leucine, and histidine (Tacon and Jackson, 1985) but is a poor source of both methionine and isoleucine (Walker, 1977; King and Campbell, 1978; Tacon, 1979a; Chvapil et al., 1980; McDonald et al., 1981; Tacon and Jackson, 1985). Unfortunately however, its amino acid profile (Table 2.4) is very unbalanced due to the disproportionate levels of specific amino acids, particularly isoleucine and leucine (Tacon

TABLE 2.3 Proximate composition of five Blood meals (% dry weight)

Proximate composition (% dry weight)	Blood meal 1	2	3	4	5
Moisture (%)	8.3	10.7	7.0-13.4	10.5	10.0
Crude protein (%)	85.8	80.2	90.7-94.5	88.5	80.0
Lipid (%)	1.5	1.4	N/A	3.9	0.8
Ash (%)	4.3	4.4	N/A	6.0	3.5

N/A = not available

TABLE 2.4 Amino acid profile of five Blood meals and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	Blood meal 3	2	6	4	5	Requirement of rainbow trout (% diet)
Arginine		3.2	3.58	3.7	3.37	1.40, 40% (Ogino, 1980)
Histidine	4.7-7.9	3.8	4.77	5.0	4.25	0.64, 40% (Ogino, 1980)
Isoleucine	1.0-1.3	0.9	1.15	2.0	0.99	0.96, 40% (Ogino, 1980)
Leucine	10.1-12.5	9.9	10.26	10.7	11.1	1.75, 40% (Ogino, 1980)
Lysine		5.4	7.64	7.1	3.87	2.12, 40% (Ogino, 1980)
Methionine	0.2-0.8	1.0	1.30	1.3	1.28	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	6.3-7.2	5.2	5.91	5.9	5.80	1.24, 40% (Ogino, 1980)
Threonine	3.7-4.9	3.9	4.57	4.0	4.34	1.36, 40% (Ogino, 1980)
Tryptophan	1.2-1.4	1.0	1.90	1.1	1.17	0.20, 40% (Ogino, 1980)
Valine	7.3-9.4	6.9	7.74	7.2	7.76	1.24, 40% (Ogino, 1980)
Cystine	1.5-1.7	1.4	1.23	0.5	1.50	0.30, 35% (Rumsey et al. 1983
Glycine		4.5		4.1	3.95	
Tyrosine	2.4-3.0		2.59	2.8	2.21	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After Fowler and Banks (1976)
2) After Miller (1977)
3) After Walker (1977)
4) After Cöhl (1981)
5) After McDonald et al. (1981)
6) After King and Campbell (1978)

and Jackson, 1985). Thus, due to the antagonistic effect of excess leucine on isoleucine (Reinitz, unpublished; Tacon and Jackson, 1985), animals fed high dietary levels of blood meal (>10%) suffer from an isoleucine deficiency caused by an excess of dietary leucine (King and Campbell, 1978; Tacon, 1979a; Tacon and Jackson, 1985).

Blood meal contains only small amounts of minerals (Göhl, 1981) although, it is a fairly good source of dietary selenium (Arthur, 1971). It is an excellent natural binder (Fowler and Banks, 1976; Orme, 1978; Tacon, 1983) and a flavouring agent (Orme, 1978).

The very unbalanced amino acid profile of blood meal together with low biological value, low digestibility and palatability problems (Miller, 1977; Chvapil et al., 1980; Göhl, 1981; McDonald et al., 1981) have resulted in it being restricted to inclusion levels not exceeding 5% by weight in diets for terrestrial animals (Göhl, 1981).

Blood meal is commonly used in diets for rainbow trout due to its high protein content and high content of the critically limiting amino acid, lysine (Miller, 1977; McDonald et al., 1981) but again its use is usually restricted to 2%-6% by weight of the diet (Fowler and Banks, 1976; Orme, 1978; McDonald et al., 1981).

Several authors have evaluated higher inclusion levels. Tiews et al. (1976) used blood meal at dietary inclusion levels of 8.5% and 25.5% by weight along with fish meal, and meat and bone meal with only a slight loss in fish performance. However, Schulz et al. (1982) reported a significant decrease in efficiency indices

when rainbow trout were fed a mixture of equal parts of blood meal, hydrolysed feather meal and poultry by-product meal (33:33:33% of the protein). Orme (1978) however, found that rainbow trout fed diets containing 25% by weight blood meal developed a dark flesh colouration and a meaty odour and flavour. Furthermore Fowler and Banks (1976) reported abnormalities in the livers of chinook salmon (Oncorhynchus tshawytscha) fed diets containing 17.5% blood meal. No abnormalities were observed when the inclusion level was restricted to only 5% of the diet.

Tacon (1983) recommended the use of spray dried blood meal up to an inclusion level of 12% in diets for rainbow trout as it has a high content of good quality protein. Nevertheless production costs will limit its inclusion level as blood meal is frequently more expensive than fish meal (Tacon, 1983).

2.1.5 Leather By-product Meal

Before hides are tanned the epidermis and any remaining flesh may be solvent extracted, dried and ground to produce a tannery by-product meal which is usually used as a fertiliser (Göhl, 1981). However it has a high nutritive value containing 85.1% crude protein, 9.0% lipid, and 5.3% ash and is very rich in both glycine (20.9%) and lysine (8.1%; Göhl, 1981). It has been used in poultry feeds to replace up to 25% of the soybean oil meal and although it does not affect feed efficiency it tends to depress growth (Göhl, 1981). To date this product has not been evaluated as a potential ingredient in diets for rainbow trout.

Scraps from the tanned leather industry can be hydrolysed to improve digestibility, cooked at a high temperature for partial removal of the fat and dried. Hydrolysed leather meal contains 55%-71% crude protein and 7%-21% lipid (Table 2.5) but has slight deficiencies in some essential amino acids (Table 2.6), in particular the sulphur amino acids (Cowey et al., 1979; Göhl, 1981).

Cowey et al. (1979) incorporated hydrolysed leather meal into moist rations for rainbow trout at levels ranging from 0% to 36.2% of the diet. Thus, up to 48% of the protein in a 50% protein diet was supplied by this by-product. Based on the performance of the fish fed the experimental rations Cowey et al. (1979) recommended that the maximum inclusion level of this material should be between 24% and 36% of the diet (13%-20% of the protein).

2.1.6 Animal Fat

A number of fats are produced from slaughterhouse wastes, the most important of which are lard and beef tallow. Cowey et al. (1979) consider that substantial amounts of animal fat can be used in diets for rainbow trout with no adverse effects providing they are included in nutritionally balanced diets. Their use reduces the risk of oxidation of diets since saturated animal fats are less susceptible to oxidation than polyunsaturated fats such as fish oils and certain vegetable oils (Reinitz and Yu, 1981; Chan et al., 1982; Hardy et al., 1983; Stansby, 1983; Watanabe et al., 1983). Furthermore, animal fats are readily available and the cost of both lard and beef tallow is significantly lower than that of marine

TABLE 2.5 Proximate composition of two Leather by-product meals (% dry weight)

Proximate composition (% dry weight)	Leather by-product meal 1	2
Moisture (%)	8.4	N/A
Crude protein (%)	71.4	55.0
Lipid (%)	7.1	21.0
Ash (%)	N/A	N/A

N/A = not available

TABLE 2.6 Amino acid profile of two Leather by-product meals and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	Leather by-product meal 1	2	Requirement of rainbow trout (% diet)
Arginine	9.1	3.99	1.40, 40% (Ogino, 1980)
Histidine	0.8	0.61	0.64, 40% (Ogino, 1980)
Isoleucine	2.6	1.34	0.96, 40% (Ogino, 1980)
Leucine	5.2	2.41	1.76, 40% (Ogino, 1980)
Lysine	4.3	2.32	2.12, 40% (Ogino, 1980)
Methionine	0.9	0.51	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	2.5	1.37	1.24, 40% (Ogino, 1980)
Threonine	1.8	3.03	1.36, 40% (Ogino, 1980)
Tryptophan	0.0		0.20, 40% (Ogino, 1980)
Valine	2.5	2.03	1.24, 40% (Ogino, 1980)
Cystine	5.4	0.14	0.30, 35% (Rumsey et al. 1983)
Glycine	25.5		
Tyrosine	1.2	0.96	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After Cowey et al. (1979)
2) After Cöhl. (1981)

oils (Yu et al., 1977; Yu and Sinnhuber, 1981). Reinitz (1980) and Reinitz and Yu (1981) have also suggested that animal fat may enhance the flavour of fish flesh.

Yu et al. (1977) evaluated the potential of lard as a partial replacement for herring oil in diets for rainbow trout. It was concluded that up to 50% of the lipid in a 22% lipid diet could be successfully replaced without any loss in performance, provided marine oils are used to supply adequate quantities of essential ω3 fatty acids.

Takeuchi et al. (1978c) reported that the best fish performance was achieved when 40%-60% of the lipid in a 10% lipid diet for rainbow trout was replaced by beef tallow. In similar trials conducted on coho salmon (Oncorhynchus kisutch) by Yu and Sinnhuber (1981) up to 8% of the diet could be successfully replaced by beef tallow provided that sufficient marine oils were incorporated to supply the necessary levels of essential fatty acids.

Reinitz and Yu (1981) reported that up to 30% of a mixture of marine and soy oils could be successfully replaced by animal fat (80% beef tallow and 20% hog fat) in a 15% lipid ration for rainbow trout. It was concluded that animal fat can be efficiently used by trout with no adverse effects when used in conjunction with marine oils to provide the essential ω3 fatty acids with the dual advantage of being less expensive and diets less susceptible to oxidation.

Fat containing bleaching earth is a waste product from the oil and fat industry. When oils and fats are refined for edible purposes, one stage of the process is treatment with an absorbent to remove colouring matters, soaps and proteins, etc. The absorbent most commonly used is activated Fuller's earth (Austreng, 1978b). This process results in considerable amounts of neutral fats being retained in the bleaching earth. According to Austreng (1978b) the Norwegian fat industry produces 3,000 tons per year of bleaching earth when treating marine oils. Thus, in addition to being a waste of valuable fat, the disposal of this material represents a potential pollution problem.

Fat containing bleaching earth has a fat content of 23% and is a good source of many minerals in particular Ca, Mg, K, and Fe that may be utilized by fish. However it also contains some heavy metals but according to Austreng (1978b) these are only present in small amounts.

Fat containing bleaching earth recovered from vegetable oils is used successfully in poultry feeds. Austreng (1978b) first evaluated the potential of a bleaching earth, used in the production of marine oils, as an ingredient in diets for rainbow trout. Up to 30% of the capelin oil in a 20% lipid diet was successfully replaced by this material without any adverse effects on fish performance or on the flavour of the flesh.

2.2 POULTRY BY-PRODUCTS

The development of intensive poultry industries, in which birds are produced in concentrated areas and processed in slaughterhouse plants, allows efficient conversion of the by-products into animal feeds (Göhl, 1981). The processed by-products coming from this industry are hydrolysed feather meal, poultry by-product meal, poultry offal meal, poultry by-product and hydrolysed feather meal, chick meal, chick shell meal and hatchery by-products meal.

2.2.1 Hydrolysed Feather Meal

Feathers are processed either under low pressure at 130°C for two and a half hours or under high pressure at 140°C for thirty minutes. After cooking the material is dried at about 60°C and ground (Göhl, 1981).

The nutritive value of hydrolysed feather meal is related to the physico-chemical breakdown of the feathers which depends upon the method and efficiency of hydrolysis (Naber and Morgan, 1956; Morris and Balloun, 1973a, b; Ichhponani and Lodhi, 1976). Hydrolysed feather meal has a high crude protein content (Table 2.7) which may be as high as 91% (Göhl, 1981). However 85% to 90% of the protein consists of keratin (Ichhponani and Lodhi, 1976) which has a high cystine content (Burgos et al., 1974; Ichhponani and Lodhi, 1976; Thomas and Beeson, 1977; Tacon, 1979a; Göhl, 1981; Bielorai et al., 1982). Due to the antagonistic effect of cystine upon methionine animals fed high dietary inclusion levels of hydrolysed feather meal may suffer from methionine deficiency caused by an excess of

TABLE 2.7 Proximate composition of three Hydrolysed feather meals (% dry weight)

Proximate composition (% dry weight)	1	2	3
Moisture (%)	9.0-12.1	1.2	3.5-7.9
Crude protein (%)	82.9-84.7	88.9	74.6-81.4
Lipid (%)	1.2-2.4	5.8	1.2-12.7
Ash (%)	3.6-4.2	4.1	2.7-5.0

TABLE 2.8 Amino acid profile of several Hydrolysed feather meals and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	4	Hydrolysed feather meals 1	5	2	3	Requirement of rainbow trout (% diet)
Arginine	4.6	3.64-7.30	3.60	5.9	5.8	1.40, 40% (Ogino, 1980)
Histidine	0.3	0.18-0.55	Trace	0.6	0.5	0.64, 40% (Ogino, 1980)
Isoleucine	3.2	3.78-5.17	2.46	2.6	4.0	0.96, 40% (Ogino, 1980)
Leucine	0.2	5.79-10.11	4.49	6.2	6.5	1.76, 40% (Ogino, 1980)
Lysine	1.2	1.39-2.72	1.89	1.9	1.5	2.12, 40% (Ogino, 1980)
Methionine	0.6	0.11-0.46	0.29	0.3	0.5	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	3.2	3.52-6.52	2.62	3.9	4.1	1.24, 40% (Ogino, 1980)
Threonine	3.3	1.42-1.86	2.08	3.6	3.7	1.36, 40% (Ogino, 1980)
Tryptophan	0.5				0.6	0.20, 40% (Ogino, 1980)
Valine	5.4	5.63-8.67	3.33	6.0	6.2	1.24, 40% (Ogino, 1980)
Alanine		1.85-3.40	2.19	4.0	3.9	
Aspartic acid		4.41-4.89	2.83	6.5	5.1	
Cystine	3.5	1.28-3.06	5.34	4.6	2.4	0.30, 35% (Rumsey et al. 1983)
Glutamic acid		5.11-7.37	5.69	8.8	8.2	
Glycine		4.54-7.49	3.50	6.4	6.3	
Proline		7.14-12.21	5.62		8.7	
Serine		6.53-7.67	4.71	8.7	9.2	
Tyrosine	2.7	2.43-2.68	1.54	2.3	2.2	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After Morris and Balloun (1973b)
2) After Thomas and Beeson (1977)
3) After Bielorai et al. (1982)
4) After Tacon (1982a)
5) After Wessels (1972)

dietary cystine (Ichhponani and Lodhi, 1976; Tacon and Jackson, 1985). Autoclaving reduces the cystine content from about 10% to 3.5% (Göhl, 1981). Hydrolysed feather meal also has a very unbalanced amino acid profile (Göhl, 1981; Bielorai et al., 1982) with low levels of lysine, methionine, tryptophan, and histidine (Lillie et al., 1956; Naber and Morgan, 1956; Naber, 1961; Moran and Summers, 1968; Thomas and Beeson, 1977; Potter and Shelton, 1978). Lysine is the first limiting amino acid (Jackson and Fulton, 1971; Tacon and Jackson, 1985 ; Table 2.8). Hydrolysed feather meal contains relatively low levels of lipid (1.2%-12.7%) and ash (2.7%-5.0%; Table 2.7).

Despite the limited improvement in nutritional quality produced by advances in processing techniques, hydrolysed feather meal is now frequently mixed with other animal by-products (mainly other poultry by-products) in order to obtain a product with a more balanced amino acid profile (Wilder, 1953). Its inclusion alone in fish feeds is usually restricted to 5% to 7% of the diet (Hung et al., 1980; Hilton et al., 1981; Beamish and Thomas, 1984) although it is occasionally used at levels up to 20% (Higgs et al., 1979; Koops et al., 1979; Hardy et al, 1984).

2.2.2 Poultry By-product Meal

Poultry wastes from slaughterhouses including offal, feet, necks, heads, and blood are cooked, dried and ground to produce a meal.

Poultry by-product meal has a crude protein content of between 56% and 59% (Table 2.9) with an amino acid profile deficient in the essential amino acids lysine (Tiews et al., 1976; Gropp et al., 1979), methionine (Tiews et al., 1976; Gropp et al., 1979; Higgs et al., 1979), and tryptophan (Tiews et al., 1979). Lysine has been reported to be the first limiting amino acid (Table 2.10; Tacon and Jackson, 1985). Poultry by-product meal usually has a high lipid content. Levels ranging from 15%-21% are common (Table 2.9) and values as high as 30% are also found (Göhl, 1981). Furthermore poultry fats contain low levels of ω3 fatty acids (Higgs et al., 1979). Thus the very high lipid content of some poultry by-product meals precludes their inclusion at high dietary levels.

Poultry by-product meal has an ash content of between 12% and 20% (Table 2.9). It is a good source of some minerals, Bielorai et al. (1983) reported values of 3.5-6.5g and 2.0-3.2g per 100 grammes dry weight of Ca and P respectively.

A limited number of studies have been carried out to assess the potential of poultry by-products as alternatives to fish meal in diets for fish (Jauncey and Ross, 1982). Tiews et al. (1976) found that 50% of the fish meal component of rainbow trout diets could be successfully replaced by a combination of poultry by-product meal and hydrolysed feather meal in the ratio 1.5:1. Furthermore, provided supplements of lysine, methionine and tryptophan were included, 100% fish meal replacement was possible without significant loss in performance.

TABLE 2.9 Proximate composition of some meals of Poultry by-products (% dry weight)

Proximate composition (% dry weight)	PBm 1	PBm 2	POHFm 3	POHFm 4	PBHFm 5
Moisture (%)	4.2-8.6	5.8	7-10	3.39-4.79	3.09-10.12
Crude protein (%)	56.6-59.4	59.9	56.1-61.5	62.70-72.18	70.30-76.60
Lipid (%)	15.3-17.2	17.1	19.8-21.5	N/A	7.93-18.27
Ash (%)	12.2-20.6	15.5	N/A	N/A	6.84-15.15

PBm = Poultry by-product meal
POHFm = Poultry offal and hydrolysed feather meal
PBHFm = Poultry by-product and hydrolysed feather meal
N/A = not available

TABLE 2.10 Amino acid profile of some meals of Poultry by-products and amino acid requirement of rainbow trout (g/100 g dry weight

Amino acid (g/100 g dry wt)	POHFm 3		POHFm 4	PBm 1	PBHFm 5	Requirement of rainbow trout (% diet)
Arginine	4.22	4.62	3.00-4.10	5.0	3.9	1.40, 40% (Ogino, 1980)
Histidine	0.63	0.82	0.80-0.90	1.2	0.71	0.64, 40% (Ogino, 1980)
Isoleucine	2.46	2.64	1.23-2.17	2.5	2.90	0.96, 40% (Ogino, 1980)
Leucine	3.98	4.86	2.97-4.50	4.2	5.15	1.75, 40% (Ogino, 1980)
Lysine	2.43	2.78	2.40-3.10	3.5	2.41	2.12, 40% (Ogino, 1980)
Methionine	0.59	0.62	0.81-1.17	1.2	1.22	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	2.64	2.78	1.43-2.37	2.6	3.16	1.24, 40% (Ogino, 1980)
Threonine	2.27	2.55	1.67-2.67	2.4	2.92	1.36, 40% (Ogino, 1980)
Tryptophan				0.5		0.20, 40% (Ogino, 1980)
Valine	3.13	3.68	1.87-3.23	3.1	3.83	1.24, 40% (Ogino, 1980)
Alanine	2.78	3.06	2.90-4.43	5.0	3.63	
Aspartic acid	3.71	4.16	3.57-5.17	5.3	4.56	
Cystine	3.92	3.98	0.30-1.10	1.0	2.24	0.30, 35% (Rumsey et al., 1983)
Glutamic acid	6.10	6.82	6.40-9.07	7.7	7.33	
Glycine	4.88	4.83	5.63-6.87	7.4	5.87	
Proline	4.80	5.34	3.67-5.53	5.2	6.18	
Serine	4.50	5.26	2.13-4.37	3.1	5.47	
Tyrosine	1.62	1.95	0.95-1.79	1.9	2.24	0.84, 40% (Ogino, 1980)

BPm - Poultry by-product meal
POHFm - Poultry offal and hydrolysed feather meal
PBHFm - Poultry by-product and hydrolysed feather meal
• Percentage of crude protein in the diet

1) After Bielorai et al. (1983)
2) After Göhl (1981)
3) After Jackson and Fulton (1971)
4) After Burgos et al. (1974)
5) After Bhargava and O'Neil (1975)

Higgs et al. (1979) evaluated a poultry by-product meal in combination with hydrolysed feather meal in the ratio of 2:1 in diets for coho salmon (Oncorhynchus kisutch). They concluded that poultry by-product meal and hydrolysed feather meal can successfully replace 75% of herring meal protein without any loss of performance and up to 100% replacement is possible if adequate methionine supplementation is provided. Furthermore it was evident that coho salmon utilize poultry fat as efficiently as fish oil for energy production. Histological and haematological examination revealed that fish were in excellent health even at 100% protein replacement.

Tiews et al. (1979) formulated 43 fish meal free diets for rainbow trout, some containing poultry by-product meal and hydrolysed feather meal. It was reported that these products could be included at up to 100% protein replacement (1:1.25 poultry by-product meal: hydrolysed feather meal) with only a slight fall in performance. Furthermore an economic appraisal of all diets tested demonstrated that the two cheapest acceptable diets were one containing 44% poultry by-product meal and 56% hydrolysed feather meal and another containing 25%, 19% and 56% poultry by-product meal, hydrolysed feather meal and soybean oil meal respectively.

Gropp et al. (1979) replaced up to 100% of the fish meal in diets for rainbow trout with a combination of poultry by-product meal and hydrolysed feather meal in the ratio 1:0.5-1.3. It was concluded that these products could replace between 75% and 100% of the fish meal protein without any loss in growth performance and feed utilization efficiency provided the diets were supplemented with methionine.

2.2.3 Poultry Offal Meal

Poultry offal meal contains 68% crude protein and 23% lipid (Göhl, 1981) and, as with the previous poultry by-products its main disadvantage is its high lipid content.

Wojno and Dabrowska (1984a) evaluated a poultry offal meal for rainbow trout and concluded that it can successfully replace fish meal protein at levels of up to 50% without any loss of fish performance.

Markert et al. (1977) evaluated this animal by-product for chinook salmon (Oncorhynchus kisutch) and arrived at the conclusion that fish fed a moist diet in which 67% of the protein was provided by poultry offal meal protein performed slightly worse than those fed an Oregon moist diet. An unbalanced amino acid profile and a deficiency of essential fatty acids were proposed as possible causes of the poorer performance. Furthermore it was noted that carcasses of fish fed the poultry offal meal protein had a significantly higher lipid content and a lower protein content.

2.2.4 Poultry Offal and Hydrolysed Feather Meal

Offals and feathers can be combined and processed to produce a meal. This poultry by-product has a crude protein content of between 56% and 72% and contains between 20% and 22% lipid (Table 2.9) and an amino acid profile deficient only in lysine and methionine (Table 2.10). Again the high lipid content is its main disadvantage.

for inclusion at high dietary levels in rations for rainbow trout. To date this product has not been evaluated in fish feeds.

2.2.5 Poultry By-product and Hydrolysed Feather Meal

Feathers, offals, blood, feet, necks, and heads in their natural proportions when ground, cooked, and dried produce a meal with a good proximate composition (70%-77% crude protein and 8%-18% lipid; Table 2.9) and with an amino acid profile low in lysine (Table 2.10). Bhargava and O'Neil (1975) reported values of 1.47-4.93g and of 1.14-2.69g per 100 grammes dry weight for Ca and P respectively.

Bhargava and O'Neil (1975) evaluated this poultry by-product in diets for chickens. They reported that growth was slightly depressed when poultry by-product and hydrolysed feather meal was incorporated at levels of up to 15% of the diet replacing soybean meal protein. To date this product has not been evaluated in fish feeds.

2.2.6 Hatchery By-products Meal

Hatchery by-products meal is produced from all the waste materials from hatcheries. Infertile eggs, dead embryos, shells of hatched eggs and unsaleable chickens are cooked, dried, and then ground to produce a meal. The meal has a crude protein content of 37% (Table 2.11) with a reasonable amino acid profile although it is deficient in lysine, methionine, and tryptophan (Table 2.12). It has a high lipid content of around 21% and a particularly high ash content (36%) of which 22% is calcium which restricts its

TABLE 2.11 Proximate composition of Hatchery by-products meal, Chick meal and Chick shell meal (% dry weight)

Proximate composition (% dry weight)	Hatchery by-products meal 1	Chick meal 2	Chick shell meal 2
Moisture (%)	6.3	4.89	1.46
Crude protein (%)	37.2	55.39	14.03
Lipid (%)	21.7	31.98	0.10
Ash (%)	36.0	7.61	86.83

TABLE 2.12 Amino acid profile of Hatchery by-products meal, Chick meal, Chick shell meal and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	Hatchery by-products meal 1	Chick meal 2	Chick shell meal 2	Requirement of rainbow trout (% diet)
Arginine	1.8	3.3	0.67	1.40, 40% (Ogino, 1980)
Histidine	0.7	1.1	0.28	0.64, 40% (Ogino, 1980)
Isoleucine	1.3	2.3	0.33	0.96, 40% (Ogino, 1980)
Leucine	2.3	3.3	0.45	1.76, 40% (Ogino, 1980)
Lysine	1.5	4.0	0.32	2.12, 40% (Ogino, 1980)
Methionine	0.7	1.2	0.23	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	1.3	2.5	0.20	1.24, 40% (Ogino, 1980)
Threonine	1.3	2.1	0.44	1.36, 40% (Ogino, 1980)
Tryptophan	0.5			0.20, 40% (Ogino, 1980)
Valine	1.9	2.8	0.51	1.24, 40% (Ogino, 1980)
Alanine		3.3	0.36	
Aspartic acid		3.9	0.69	
Cystine	0.4	2.3	0.89	0.30, 35% (Rumsey et al. 1983)
Glutamic acid		6.5	1.04	
Glycine	2.0	3.7	0.86	
Proline		3.9	0.76	
Serine		2.7	0.40	
Tyrosine	0.9	1.9	0.22	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After Göhl (1981) 2) After Tacon (1982a)

inclusion at high dietary levels (Göhl, 1981). It has been used in broiler diets at 4% by weight with excellent results (Göhl, 1981) but to date has not been evaluated in fish diets.

2.2.7 Chick Meal

Poultry hatcheries produce considerable amounts of dead embryos and culled chicks, particularly the male chicks of laying strains, which have little or no commercial value (Ichhponani and Lodhi, 1976; Tacon, 1982a).

Tacon (1982a) evaluated the potential nutritive value of day-old chicks as an animal foodstuff. Deep frozen day-old chicks were homogenized, air dried at 60°C and ground to produce a meal which was subsequently analysed to assess its potential as a feed ingredient for salmonids.

The chick meal was found to be a reasonable source of crude protein (55.39%; Table 2.11) and also of the amino acids methionine, lysine, and particularly of cystine (Table 2.12). However it has a high lipid content (31.98%) which will prevent its inclusion at high dietary levels, particularly since the lipids are predominantly composed of saturated and monounsaturated fatty acids (mainly oleic and palmitic acids; Tacon, 1982a).

Chick meal is a good source of the major elements, Ca, Mg, Fe, P, Na, K, NaCl and of the microelements Cu, Ni, Cr, but a poor source of Mn. It contains however extremely high levels of Zn (1676 ppm

by weight) and to a lesser extent Pb (14.23 ppm by weight) which are known to depress growth and induce Cu deficiency in animals (McDonald et al., 1981). Finally, it is a good source of niacin and riboflavin but a poor source of thiamine and does not contain the fat soluble vitamins A and D (Tacon, 1982a).

Chick meal also contains traces of the protein avidin which is a biotin antagonist. However avidin is a heat labile anti-nutritional factor and can be destroyed by moist heat (Chubb, 1982; Tacon, 1982a).

It is known that poultry waste by-products carry substantial amounts of contaminating micro-organisms such as microbial pathogens and parasites (Ichhponani and Lodhi, 1976; Tacon, 1982a). To eliminate the risk of disease transmission Tacon (1982a) recommended that this by-product should be heat processed in such a manner as to ensure partial or complete destruction of these micro-organisms.

Based on the findings above Tacon (1982a) concluded that chick meal could only be incorporated in animal feeds at low dietary inclusion levels, although this recommendation was not tested in feeding trials.

2.2.8 Chick Shell Meal

In addition to culled chickens, wastes from poultry hatcheries include shells of hatched eggs and also unhatched eggs which are obviously unsuitable for sale. When processed they can be regarded

as potential fish foodstuffs (Ichhponani and Lodhi, 1976; Göhl, 1981; Tacon, 1982a).

Tacon (1982a) evaluated the nutritional quality of egg shells as a potential ingredient in rainbow trout diets. Shells were ground, air dried at 60°C and then finely ground in a rotary mill. Chick shell meal consists of 86% ash and it is thus a good source of many minerals, in particular $CaCo_3$, Mg, Fe, Cu, Ni, and Cr although it is deficient in P and Zn. Furthermore a high proportion of the total nitrogen within chick shell meal is nonprotein nitrogen and is in the form of pclymeric (N-acetyl) hexosamines (Tacon, 1982a). It is deficient in all essential amino acids and it has a high cystine content (Table 2.12). It also contains negligible amounts of lipid (Table 2.11).

Like chick meal, chick shell meal may be contaminated with micro-organisms. However Rai and Netke (1973) reported that heating egg shells at 110°C for 15 hours produces a foodstuff free from these organisms.

Chick shell meal is therefore a valuable source of Ca, Mg, Cr, and trace elements, and thus Tacon (1982a) suggested that it could be incorporated in fish feeds at low inclusion levels.

In contrast to chick shell meal, whole egg meal has a higher protein (60.9%) and lipid (22.8%) content but contains substantially less ash (10.2%). In addition it has a very low market price. Davis et al. (1976) incorporated this foodstuff in a ration for

lake trout (Salvelinus namaycush) at a level of 23.3% of the diet replacing 50% of the fish meal in a 52% crude protein diet. The fish fed the ration containing whole egg meal performed slightly better than those fed the fish meal control diet.

Thus the high protein content, low cost and evidence that this animal by-product can be successfully included at relatively high levels in salmonid diets, indicates that it is a promising ingredient for inclusion in diets for rainbow trout.

2.3 DAIRY BY-PRODUCTS

There is a wide range of dairy by-products that could be recycled into animal feeds with the advantage of an economic return. These include skimmed milk, buttermilk, whey, casein and dairy processing wastes.

Skimmed milk is milk from which most of the lipid has been removed but in which all the protein remains (Göhl, 1981). It has a crude protein content of 37.5% of which 97%-98% is digestible by rainbow trout (Pfeffer, 1982) and it is a good source of the amino acids isoleucine, leucine, and valine, but a poor source of arginine (Table 2.13). It has a very low lipid content of 1.6% and is a good source of vitamin B but is deficient in both the fat soluble vitamins A and D. It is usually used for first feeding of calves (Göhl, 1981).

Buttermilk consists of the residue from churned whole milk (Göhl, 1981) and has a crude protein content of about 33% and a reasonable amino acid profile although it contains low levels of arginine and methionine (Table 2.13). It has a lipid content of around 12% and has been used in conjunction with skimmed milk for pigs and poultry.

Whey is the dairy product residue from the manufacture of cheese. It has a crude protein content of only 10.8% of which 91%-98% is digestible by rainbow trout (Pfeffer, 1982) and it is deficient

TABLE 2.13 Amino acid profile of five Dairy by-products and amino acid requirement of rainbow trout (g/100g dry weight)

Amino acid (g/100g dry wt)	Skimmed milk	Buttermilk	Whey	Casein	Dairy processing waste	Requirement of rainbow trout (% diet)
Arginine	1.3	1.3	0.3	3.78	1.23	1.40, 40% (Ogino, 1980)
Histidine	0.9	1.0	0.2	2.78	0.39	0.64, 40% (Ogino, 1980)
Isoleucine	2.3	2.1	0.6	6.33	1.04	0.96, 40% (Ogino, 1980)
Leucine	3.5	3.6	1.0	9.55	1.78	1.76, 40% (Ogino, 1980)
Lysine	2.7	3.3	0.9	7.78	1.04	2.12, 40% (Ogino, 1980)
Methionine	1.0	0.8	0.2	3.00	0.32	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	1.8	2.1	0.3	5.11	1.11	1.24, 40% (Ogino, 1980)
Threonine	1.6	1.7	0.7	4.22	1.43	1.36, 40% (Ogino, 1980)
Tryptophan	0.5	0.4	0.2	1.11		0.20, 40% (Ogino, 1980)
Valine	2.3	2.6	0.6	7.55	1.42	1.24, 40% (Ogino, 1980)
Alanine					2.08	
Aspartic acid					2.81	
Cystine	0.4	0.3		0.33	0.46	0.30, 35% (Rumsey et al. 1983)
Glutamic acid					3.47	
Glycine	1.4			1.67	1.54	
Proline					1.26	
Serine					1.33	
Tyrosine	1.7	1.7		5.22	0.73	0.84, 40% (Ogino, 1980)

* Percentage of crude protein in the diet

1) After Göhl (1981) 2) After Crampton and Harris (1969) 3) AFter Rumsey et al. (1981)

in all the essential amino acids (Table 2.13). It also has a very low lipid content of around 0.4%.

All three of these dairy by-products have been used at very low levels in feeds for terrestrial animals since their cost prohibits extensive use (Göhl, 1981). Their use in fish feeds has also been restricted to very low levels of between 5%-10% by weight in experimental diets for rainbow trout (Austreng, 1979; Austreng and Refstie, 1979; Hung et al., 1980, 1981; Reinitz, 1980; Reinitz and Hitzel, 1980; Bromley and Smart, 1981; Reinitz and Yu, 1981; Hardy et al., 1983).

Casein is the dried protein precipitated from milk after acid or rennin has been added (Crampton and Harris, 1969; Cullison, 1979). Casein has a very high crude protein content of about 88% (Åsgård and Austreng, 1985) of which 93%-97% is digestible by salmonids (Lall and Bishop, 1977; Rychly and Spannhof, 1979; Pfeffer, 1982). It has a very balanced amino acid profile with only slight deficiencies in arginine and methionine (Table 2.13; Åsgård and Austreng, 1985). Casein is successfully used in experimental diets for salmonids by nutritionists (Lee and Wales, 1973; Åsgård and Austreng, 1985) but, like the dairy by-products considered above, cost prevents its utilization in commercial diets.

Wastewaters from dairy processing plants are usually digested aerobically before being released into running water. The remaining semi-solid wastes are sources of protein, carbohydrate, and minerals that can be recycled into diets for animals (Rumsey et al., 1981).

Spray dried processing waste from a plant which processes a variety of dairy products was evaluated by Rumsey et al. (1981) as a foodstuff for fish. It had a crude protein content of 29.9% but contained relatively low levels of almost all essential amino acids (Table 2.13). It also had a high ash content of 26.51% and was particularly rich in Ca, Na, P, Cl, P and Mg (42.500, 29.000, 26.750, 26.000, 4.800, 4.783, and 3.800 ppm dry weight respectively). This dairy processing waste was used by Rumsey et al. (1981) to partially replace dairy whey and wheat middlings in diets for rainbow trout. They reported that the performance of fish fed rations containing 10% of dairy processing wastes was comparable to that of those fed a fish meal control. Furthermore the growth of fish fed diets containing 20% dairy processing wastes was only 10% less than of those fed the control diet. Cost analysis revealed that dairy processing wastes replacing 10% by weight dried whey represented a saving of $11.00 per metric ton of fish feed costs (Rumsey et al., 1981).

2.4 FISH PRODUCTS

Waste fish can be fed to fish without any processing. However, although it is generally relatively inexpensive to feed unprocessed fish there can be problems with supply and spoilage (Johnsen and Skrede, 1981; Windsor and Barlow, 1981; Jackson et al., 1984a). Ideally fish farms should be located near to a fishing port, and even so there is no guarantee of a continuous supply. Raw fish is a highly perishable commodity particularly at high environmental temperatures (Windsor and Barlow, 1981). Thus the action of bacteria present in the gut, enzyme digestion and the tendency for poly-unsaturated fish oils to become rancid can result in serious spoilage problems within a very short time (Johnsen and Skrede, 1981; Windsor and Barlow, 1981). Cold storage of raw fish to prevent spoilage is often prohibitively expensive (Rungruangsak and Utne, 1981).

The most common processing techniques for waste fish products is the production of meals. A further option is preservation by ensiling.

2.4.1 Fish Meal

Properly processed fish products are among the very best source of high quality protein for animals and also contain relatively large quantities of lysine, methionine, and tryptophan, the amino acids most often deficient in cereal based diets. They are rich in minerals and vitamins and are reported to supply unidentified growth and hatchability factors (Göhl, 1981).

The dependence on fish meal in fish feeds is due largely to its nutritional quality. Firstly it has a high crude protein content of between 60% and 72% (Table 2.14) with protein and lipid digestibilities ranging from 60% to 95% and 82% to 97.3% respectively (Smith and Rumsey, 1976; Atack and Matty, 1979; Cho and Slinger, 1979; Lovell, 1981; Pfeffer, 1982; Watanabe et al., 1983). It has an excellent amino acid profile and is particularly rich in the essential amino acids lysine and methionine, the two amino acids most often deficient in feedstuffs (Lovell, 1981; Windsor and Barlow, 1981). In addition fish meal is the richest natural source of available lysine (Table 2.15; Windsor and Barlow, 1981). Tacon and Jackson (1985) reported that its first limiting amino acid is threonine. It is also one of the best sources of minerals, particularly of Ca, P, Fe, Mn, and Se (Phillips and Podoliak, 1957; Arthur, 1971; Cowey, 1976; Lovell, 1979, 1981; McDonald et al., 1981; Soevick et al., 1981; Windsor and Barlow, 1981), of several vitamins, particularly choline (Lovell, 1981; Windsor and Barlow, 1981), and of ω3 essential fatty acids (Lovell, 1981; Watanabe et al., 1983). Fish meals also have an appealing flavour to most fish species (Lovell, 1981).

Fish meals have traditionally been used in commercial fish feeds for many years (Ketola, 1975; Nose, 1979; Dabrowski et al., 1980; Lovell, 1981; Tacon and Jackson, 1985). Currently good quality fish meals supply the major proportion of the protein component (30-40% by weight) within commercial fish feed rations and this is particularly the case in diets for salmonids (Nose, 1974; Orme, 1978; Kaushik and Luquet, 1980; McDonald et al., 1981; Tacon, 1981; Tacon and Jackson, 1985).

TABLE 2.14 Proximate composition of six Fish meals (% dry weight)

Proximate composition (% dry weight)	Kushino 1	Choski 1	Peruvian 1	Peruvian 2	Herring 3	Brown 4
Moisture (%)	8.9	9.5	9.0	9.9	7.7-10.5	9.9
Crude protein (%)	63.5	59.5	65.8	64.5	60.4-72.0	6 8
Lipid (%)	10.0	8.2	11.3	9.9	6.8-9.7	7.6
Ash (%)	15.4	20.2	17.8	15.4	N/A	13.2

N/A = not available

TABLE 2.15 Amino acid profile of five Fish meals and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 dry wet)	Peruvian 2	Herring 5	Brown 4	Tuna 3	Sardine 6	Requirement of rainbow trout (% diet)
Arginine	6.47	3.90	5.2	3.42	0.66	1.40, 40% (Ogino, 1980)
Histidine	2.09	1.53	2.1	1.81	2.42	0.64, 40% (Ogino, 1980)
Isoleucine	4.80	3.40	2.8	2.37	2.90	0.96, 40% (Ogino, 1980)
Leucine	8.04	5.40	4.4	3.83	5.74	1.76, 40% (Ogino, 1980)
Lysine	7.05	5.88	5.9	3.89	5.67	2.12, 40% (Ogino, 1980)
Methionine	2.95	2.19	2.0	1.46	2.08	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	5.74	2.95	2.3	2.18	2.84	1.24, 40% (Ogino, 1980)
Threonine		8.00		2.31	2.83	1.36, 40% (Ogino, 1980)
Tryptophan			0.5	0.56	4.18	0.20, 40% (Ogino, 1980)
Valine	5.38	4.10	3.7	2.83	3.50	1.24, 40% (Ogino, 1980)
Alanine				3.60	4.18	
Aspartic acid	4.70	3.14	2.9	4.95	2.02	
Cystine		0.69		0.42	0.64	0.30, 35% (Rumsey et al. 1983)
Glutamic acid				6.35	8.58	
Glycine		4.50		4.34	3.48	
Proline				2.89	2.76	
Serine				2.22	2.63	
Tyrosine		2.19		1.75	2.21	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After Watanabe et al. (1983)
2) After Tacon et al. (1983a)
3) After Windsor and Barlow (1981)
4) After Tacon et al. (1984)
5) After McDonald et al. (1981)
6) After Toyama et al. (1983)

2.4.2 Fish Silage

Fish silage as a means of preserving fish products was developed in northern Europe and has been used in Scandinavia since 1930 (Tacon, 1981) and commercially since 1948 (Tatterson and Windsor, 1974; Hardy et al., 1984).

Fish silage is a liquid product which develops when whole fish or parts of fish are treated with an acid, usually either formic or a mineral acid. Liquefaction is caused by enzymes present in the fish, and is accelerated by the acid which in addition to creating the right conditions for the enzymes to work, helps to break down bone and limits the growth of spoilage bacteria (Tatterson, 1982).

The two main methods of producing silage are by chemical acid preservation and by bacterial fermentation. Acid preserved silage is obtained by the addition of both inorganic and organic acids to give a final pH of 2 or 4 respectively (Tatterson, 1982; Tacon and Jackson, 1985). The methods involved in processing have been fully described by Windsor and Barlow (1981) and reviewed by MacKie (1982). Fermented silages are produced by storing the raw materials in airtight containers and preserved by lactic acid bacterial fermentation by the addition of a carbohydrate source (Tacon and Jackson, 1985).

In comparison with fish meal there are several advantages to be gained from the production of fish silage. The capital cost of a fish meal plant is much higher than one for fish silage

(Göhl, 1981; Rungruangsak and Utne, 1981; Tacon, 1981; Windsor and Barlow, 1981; Tatterson, 1982; Jackson et al., 1984a). In addition, processing fish meal requires specialised staff, whereas silage can be made by relatively unskilled workers (Windsor and Barlow, 1981; Tatterson, 1982). The production of fish silage is also a practical way of preserving wastes from canning and filleting industries, low grade industrial fish species and fish by-catches into a high quality foodstuff (Johnsen and Skrede, 1981; MacKie, 1982; Tatterson, 1982; Hardy et al., 1984; Tacon and Jackson, 1985). It is particularly useful where it is economically impractical to transport relatively small amounts of fish to the nearest fish meal plant (Åsgård and Austreng, 1981; Tatterson, 1982; Jackson et al., 1984a, b; Tacon and Jackson, 1985). Finally, provided fish is treated correctly, silage has good storage characteristics (Tacon, 1981; Jackson et al., 1984a, b; Tacon and Jackson, 1985).

There are however a number of disadvantages associated with the use of fish silage. It is usually deficient in tryptophan since it is a labile amino acid when free and under acid conditions (Jackson et al., 1984b; Tacon and Jackson, 1985). Nevertheless Tacon and Jackson (1985) reported that tryptophan loss rarely exceeds 50%. Since fish silage contains unsaturated lipids it is prone to oxidation during storage if not adequately protected (Johnsen and Skrede, 1981; Windsor and Barlow, 1981; Jackson et al., 1984a,b). The addition of ethoxyquin to give a final concentration of 250 ppm has been found to give the best protection (Tacon, 1981; Jackson et al., 1984a, b; Tacon and Jackson, 1985).

The antinutritional factor thiaminase is present in a wide range of both freshwater and marine fish species (Tacon and Jackson, 1985). It is inactivated by heat, but since the production of silage does not involve heating, the enzyme remains active. Furthermore while fish meal can be processed within hours fish silage normally takes longer and proper storage is always required (Windsor and Barlow, 1981). Another disadvantage when ensiling with inorganic acids is that it is necessary to neutralize the silage prior to feeding (Göhl, 1981; Tatterson, 1982; Tacon and Jackson, 1985). Finally, it is not easy to incorporate fish silage into dry pelleted diets (Hardy et al., 1984).

The proximate composition of some fish silages is presented in Table 2.16. Fish silage has more or less the same proximate composition as that of the raw material from which it is made (Göhl, 1981; Windsor and Barlow, 1981) however digestibility of the protein is higher and the availability of the amino acids is better (Göhl, 1981; Skrede et al., 1980). The crude protein content varies between 14% and 17% (Tacon, 1981; Windsor and Barlow, 1981). It has an excellent amino acid profile (Table 2.17) similar to that of the fish from which the silage is made (Göhl, 1981) but with relatively higher lysine levels, slightly lower levels of the sulphur amino acids and particularly low levels of tryptophan (Johnsen and Skrede, 1981; Windsor and Barlow, 1981).

Tacon and Jackson (1985) reported that preliminary feeding trials with fish indicate that fermented fish silages are nutritionally equivalent to fish meal. A number of workers have evaluated

TABLE 2.16 Proximate composition of some Fish silages (% dry weight)

Proximate composition (% dry weight)	White fish offal 1	Herring offal 1	Sprats 1	Brown fish offal 2
Moisture (%)	78.9	75.4	69.4	84.9-86.9
Crude protein (%)	15.0	13.5	15.5	9.3-11.1
Lipid (%)	0.5	8.7	13.0	0.5-2.1
Ash (%)	4.2	2.6	2.2	1.3-1.5

TABLE 2.17 Amino acid profile of some Fish silages and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	White fish offal 2	Fish offal 3	White fish 1	Herring 1	Requirement of rainbow trout (% diet)
Arginine	1.1	3.2-4.1	5.1	3.7	1.40, 40% (Ogino, 1980)
Histidine	0.4	1.2-1.9	1.8	1.2	0.64, 40% (Ogino, 1980)
Isoleucine	0.6	2.4-2.8	2.8	1.9	0.96, 40% (Ogino, 1980)
Leucine	1.0	4.6-4.9	4.5	3.7	1.75, 40% (Ogino, 1980)
Lysine	1.1	4.3-5.2	4.7	4.2	2.12, 40% (Ogino, 1980)
Methionine	0.4	1.4-1.8	1.8	0.8	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	0.5	2.1-2.7	2.3	2.4	1.24, 40. (Ogino, 1980)
Threonine	0.7	2.7-3.0	3.1	2.2	1.36, 40% (Ogino, 1980)
Tryptophan	0.4	0.1-0.3			0.20, 40% (Ogino, 1980)
Valine	0.6	3.0-3.4	3.3	2.4	1.24, 40% (Ogino, 1980)
Alaline	1.2	3.9-4.1	5.4	3.0	
Aspartic acid	1.5	5.0-5.6	6.6	3.7	
Cystine	0.1	0.7-0.9	0.5	0.3	0.30, 35% (Rumsey et al. 1983)
Glutamic acid	2.2	8.1-8.4	9.7	5.7	
Glycine	1.4	4.7-5.6	6.1	3.1	
Hydroxyproline	0.7	0.4-0.8			
Proline		2.8-3.3			
Serine	0.8	3.1-3.5	3.7	2.0	
Tyrosine	0.6	1.7-2.0	2.1	1.1	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After Windsor and Barlow (1981) 2) After Johnsen and Skrede (1981)
2) After Tatterson (1982)

acidified silages in diets for salmonids (Rungruangsak and Utne, 1981; Hardy et al., 1984; Jackson et al., 1984a). Rungruangsak and Utne (1981) evaluated silages produced using HCl, formic acid, or H_2SO_4 by replacing 50% of a minced raw fish feed with each of these silages. They concluded that rainbow trout fed the HCl acidified silage performed as well as fish fed the control feed, whereas those fed the formic acid and the H_2SO_4 acidified silages had a poorer growth performance.

Jackson et al. (1984a) evaluated a silage mixed with an equal weight of a binder meal which was extruded to form a moist pelleted diet and fed to Atlantic salmon (Salmo salar). They reported that fish fed these moist diets had a growth performance comparable to that of those fed a commercial dry diet proving that silage is an acceptable ingredient for Atlantic salmon.

To overcome the difficulty of producing dry diets when fish silage is to be used, Hardy et al. (1984) blended fish silage with a small percentage of other dry foodstuffs and co-dried this mixture in conventional fish meal drying equipment. Silage produced using a mixture of sulphuric and proprionic acids was co-dried with soybean meal and hydrolysed feather meal. The resulting co-dried fish silage was incorporated into diets for rainbow trout at levels up to 50% by weight in rations containing 60% crude protein. It was concluded that co-dried fish silage gives satisfactory results, similar to those of the fish silage control diet but slightly poorer than those of the fish meal control diet. The lower digestibility of soybean meal and hydrolysed feather meal were proposed as possible causes of the poorer fish performance.

2.5 INVERTEBRATES

A number of invertebrate species have been evaluated as potential ingredients in diets for fish. These include fly larvae, worms and a variety of crustaceans.

2.5.1 Fly Larvae

Many flies breed in a variety of substrates including decaying fruits and vegetables, damp grains, decomposing organic matter and animal wastes (Bondari and Sheppard, 1981). They are efficient convertors of waste materials into usable proteins, thus upgrading the feed value of these waste products. Large amounts of silkworm pupae (Bombyx mori), a by-product of the silk industry, are also available without any production costs involved.

Several species of fly larvae and pupae have been used successfully in diets for terrestrial animals and fish. Silkworm pupae have been used to raise carp in Japan and China (Hickling, 1962); face fly larvae for channel catfish (Loyacano Jr., 1974); house fly larvae for chickens (Calvert et al., 1969), and dried ground soldier fly larvae have been fed to pigs (Newton et al., 1977).

Most fly larvae have a fairly good crude protein content varying between 38% and 48%. Solvent extracted silkworm pupae have a particularly high crude protein content of 77.6% (Table 2.18). Spinelli et al. (1979) and Göhl (1981) reported that only around 75% of the crude protein of fly larvae is true protein, the remaining 25% is

TABLE 2.18 Proximate composition of a Fly and of two Fly meals (% dry weight)

Proximate composition (% dry weight)	Musca domestica 1	Hermetia illucens 2	Bombyx mori (solvent extracted) 3
Moisture (%)	8.0	N/A	7.5
Crude protein (%)	45.0	38-40	77.6
Lipid (%)	15.0	18.29	1.0
Ash (%)	9.0	N/A	7.3
Chitin (%)	25.0	N/A	N/A

N/A = not available

TABLE 2.19 Amino acid profile of two Fly meals and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	Musca domestica 1	Bombyx mori 3	Requirement of rainbow trout (% diet)
Arginine	2.44	5.1	1.40, 40%* (Ogino, 1980)
Histidine	1.57	2.2	0.64, 40% (Ogino, 1980)
Isoleucine	1.35		0.96, 40% (Ogino, 1980)
Leucine	3.13		1.75, 40% (Ogino, 1980)
Lysine	3.32	7.8	2.12, 40% (Ogino, 1980)
Methionine	1.01	1.5	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	3.13	1.9	1.24, 40% (Ogino, 1980)
Threonine	2.04		1.36, 40% (Ogino, 1980)
Tryptophan	0.65	1.2	0.20, 40% (Ogino, 1980)
Valine	2.62		1.24, 40% (Ogino, 1980)
Alanine	2.77		
Aspartic acid	4.86		
Cystine	0.37	1.5	0.30, 35% (Rumsey et al. 1983)
Glutamic acid	5.49		
Glycine	2.43		
Proline	1.65		
Serine	2.03		
Tyrosine	3.64	4.9	0.34, 40% (Ogino, 1980)

* Percentage of crude protein in the diet

1) After Spinelli et al. (1979)
2) After Sandari and Sheppard (1981)
3) After Göhl (1981)

chitin. Fly larvae have an excellent amino acid profile (Table 2.19). Methionine is reported to be the first limiting amino acid (Tacon and Jackson, 1985). Fly larvae have a relatively high lipid content of between 15% and 28% and an ash content of around 8%.

Spinelli et al. (1979) evaluated house fly larvae (Musca domestica) in diets for rainbow trout. The larvae were grown on a substrate composed of two parts wheat middlings, one part alfalfa and three parts water at 30°C for 3-4 days. After this incubation period the larvae and substrate were dried in a rotary vacuum dryer (735 mm Hg) at 85°-100°C. Finally, the larvae were separated from the substrate by screening and were then ground to produce a meal. However Ichhponani and Lodhi (1976) reported that the feasibility of its commercial production is not promising since they reported that house fly larval protein yields are quite low due to an inability to utilize nonprotein nitrogen from manures. Rainbow trout fed diets in which up to 100% of the fish meal was replaced by fly larvae meal protein exhibited a growth performance and feed utilization efficiency as good as those fed the fish meal control diet. According to Spinelli et al. (1979) the value of the fly larvae was equivalent to that of the whole fish. Furthermore they reported that the larvae contained no antinutritional or toxic factors.

Bondari and Sheppard (1981) assessed the potential quality of soldier fly larvae (Hermetia illucens) as a foodstuff for channel catfish (Ictalurus punctatus) and blue tilapia (Oreochromis aureus). Larvae were collected from poultry manure by washing it over a screen. Chopped, frozen soldier fly larvae were then fed to fish as the

sole source of dietary protein and also in combination with a commercial diet. Both channel catfish and tilapia fed just larvae and those fed half larvae and half commercial diet performed as well as the control fish fed conventional diets. Furthermore no aromas, flavours or differences in texture in either fish species were detected.

One of the major disadvantages associated with the use of fly larvae and pupae in fish feeds is the potential hazard from the accumulation of toxins and heavy metals which are often associated with waste materials used as substrates for larval production (Tacon, 1981). The cost effectiveness of the process is also a significant constraint in the commercial production of fly larvae and pupae. Bondari and Sheppard (1981) pointed out that newer and more efficient methods of producing fly larvae from waste materials are necessary. Even so they reported that extrapolations from experimental units indicate that a self harvesting technique could result in the production of two metric tons of larvae per month from a 20,000 chicken layer house. Furthermore they consider that this figure may be increased substantially if improved management or harvesting techniques can be developed.

2.5.2 Worms

There has been considerable interest in the use of detritivorous terrestrial and aquatic oligochaete worms as a means of breaking down and utilizing human and animal wastes (Tacon *et al.*, 1983b; Stafford and Tacon, in press). Earthworm activity converts waste

materials effectively into a loose friable compost with potential value as a plant growth medium (Burrows, 1984). As a by-product of this waste management the earthworms themselves represent a potential source of valuable protein for animal feeds.

Earthworms have a crude protein content varying between 50% and 67% (Table 2.20) and a good amino acid profile (Table 2.21) with lysine as the first limiting amino acid (Tacon and Jackson, 1985). They have a lipid content of between 7% and 12% and an ash content of around 10% to 15%.

In view of the promising potential of worms as a protein source, a number of investigations have been carried out on a range of worm species to determine both their acceptability and suitability as an alternative to fish meal protein in fish rations.

Tacon et al. (1983b) and Stafford (1984) evaluated three species of terrestrial lumbricid worms Eisenia foetida, Allolobophora longa, and Lumbricus terrestris in diets for rainbow trout. These worm species were tested fresh as the sole source of dietary protein and E. foetida was also assessed in combination with a commercial trout diet. Fish fed fresh A. longa and A. terrestris performed as well as fish fed a commercial trout diet. By contrast fish fed fresh E. foetida alone or in combination with a commercial trout diet showed very little or no growth. Tacon et al. (1983b) and Stafford (1984) noted that E. foetida appears to be unpalatable to trout and suggested that this could possibly be due to a foetid or garlic smell or taste. In addition E. foetida contains haemolysin

TABLE 2.20 Proximate composition of some Worms and Worm meals (% dry weight)

Proximate composition (% dry weight)	*A.longa* 1	*A.terrestris* 1	*E.foetida* 1	*D.veneta* 2	*D.subrubicundus* 2	*D.subrubicundus* meal 2	*E.eugenige* meal 3
Moisture (%)	78.29	81.09	83.26	81.67	84.26	9.07	14.7
Crude protein (%)	50.73	56.10	58.78	56.98	67.79	65.13	60.4
Lipid (%)	1.44	2.13	9.04	19.15	6.66	9.62	12.0
Ash (%)	35.20	28.72	17.24	19.63	13.14	13.05	10.5

1) After Tacon *et al.* (1983b); Stafford (1984)
2) After Stafford (1984)
3) After Hilton (1983)

TABLE 2.21 Amino acid profile of some Worms and Worm meals and amino acid requirement of rainbow trout (g/100g dry weight)

Amino acid (g/100g dry wt.)	A.longa 1	A.terrestris 1	E.foetida 1	D.veneta 2	D.subrubicundus 2	D.subrubicundus meal 2	E.eugenige meal 3	Requirement of rainbow trout (% diet)
Arginine	7.17	6.54	5.62	6.31	6.50	3.50	1.73	1.40, 40% (Ogino, 1980)
Histidine	2.30	2.85	2.96	2.68	2.93	0.40	0.64	0.64, 40% (Ogino, 1980)
Isoleucine	5.10	4.53	4.14	4.34	4.48	2.21	0.99	0.96, 40% (Ogino, 1980)
Leucine	8.13	8.48	8.29	7.46	7.90	4.35		1.76, 40% (Ogino, 1980)
Lysine	7.81	7.25	6.52	6.77	7.45	4.95	1.83	2.12, 40% (Ogino, 1980)
Methionine	1.14	2.29	2.80	2.78	2.58	0.42	0.77	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	6.04	4.17	3.97	4.54	5.04	2.17	1.19	1.24, 40% (Ogino, 1980)
Threonine	4.80	5.12	5.60	4.67	5.14	2.77	1.37	1.36, 40% (Ogino, 1980)
Tryptophan		0.91	0.72					0.20, 40% (Ogino, 1980)
Valine	5.60	4.75	4.65	6.59	6.51	2.29	1.15	1.24, 40% (Ogino, 1980)
Alanine	5.97	6.03	6.50	5.62	5.48	2.86		
α-aminobotiric acid		0.93	0.66					
Aspartic acid	11.36	9.35	9.30	9.58	10.37	5.36		
Cystine	0.68	0.66	0.70	3.15	1.95	0.45	0.23	0.30, 35% (Rumsey et al. 1983)
Glutamic acid	13.66	17.56	17.70	15.07	13.67	7.17		
Glycine	6.26	5.70	6.44	6.04	5.20	2.48		
Proline	3.96	3.73	4.75	3.30	3.73	2.04		
Serine	5.47	5.47	5.19	5.15	5.11	2.66		
Tyrosine	4.53	3.67	3.46	4.54	3.81	1.83	1.01	0.84, 40% (Ogino, 1980)

* Percentage of crude protein in the diet

1) After Tacon et al. (1983b); Stafford (1984)
2) After Stafford (1984)
3) After Hilton (1983)

a heat labile haemolytic factor. Thus in view of the potential noxious quality of both factors, which appear to be present in the coelomic fluid of E. foetida, Tacon (1983) suggested that treatment processes may be required to remove or inactivate the antinutritional factors present before this species of worm can be fed to fish. Stafford (1984) reported that blanching E. foetida by immersing live earthworms in boiling water for five minutes prior to being dried and ground to produce a meal, significantly improved its acceptability by rainbow trout. This was considered to be due to the partial removal of the noxious coelomic fluid fraction and also to the possible denaturation of heat labile antinutritional factors.

Stafford (1984) also tested two further worm species for rainbow trout, namely Dendrobaena veneta and Dendrodrilus subrubicundus (Tables 2.20, 2.21). The performance of fish fed D. veneta as the sole source of dietary protein was slightly reduced when compared with that of those fed a herring meal control diet. Fish fed fresh D. subrubicundus however performed as well as those fed the control diet.

Hilton (1983) evaluated the African night crawler (Eudrilus eugenige) as a protein source for rainbow trout. Worms were freeze dried and ground to produce a meal with a crude protein content of 60.4% (Table 2.20) and with a protein digestibility coefficient of almost 95%. The worm meal was reported to be slightly deficient in the essential amino acids histidine, lysine, methionine, and threonine (Table 2.21). It also had a lipid content of 12%. The E. eugenige meal was used to provide up to 100% of the protein in

diets for rainbow trout, replacing capelin meal. There was a significant decrease in growth rate with increasing levels of dietary worm meal, although there were no significant differences in feed utilization efficiency or in fish carcass composition. Hilton (1983) attributed the poor growth to amino acid imbalances and concluded that this worm meal is not an adequate or satisfactory replacement for rainbow trout in practical trout diet formulations.

Tacon et al. (1983b) and Stafford (1984) utilized freeze dried E. foetida to produce a meal which contained 61.71% crude protein, 9.33% lipid and 4.85% ash. This worm meal was evaluated replacing herring meal protein at levels up to 100%. Fish fed E. foetida meal at 50% protein replacement exhibited a very good growth response which was only slightly less than that of fish fed the control diet in which herring meal was the sole source of dietary protein. Furthermore fish carcass composition was not significantly affected. However fish fed 100% worm meal displayed a poor growth response and the carcass composition was significantly affected, with a lower lipid and a higher moisture content, compared with fish fed the herring meal control ration.

In view of these results Stafford (1984) conducted further trials in which E. foetida and D. veneta meal (56.98% crude protein, 19.20% lipid) replaced only 30% and 50% by weight of the herring meal protein respectively in diets for rainbow trout. It was concluded that E. foetida meal can successfully replace dietary herring meal at this level without adversely affecting either growth performance or feed utilization efficiency. Furthermore there were

no significant alterations in fish carcass composition and no detectable accumulation of heavy metals. When D. veneta meal was used to replace 50% of the herring meal protein, the fish performed poorly with a significant reduction in both growth rate and feed utilization efficiency. There was also evidence of accumulation of the heavy metals Zn and Pb. Finally the same author evaluated D. subrubicundus meal (Tables 2.20, 2.21) replacing herring meal protein at levels of up to 100%. Based on growth performance and feed utilization efficiency it was concluded that D. subrubicundus meal can successfully replace only 10% of herring meal protein without adverse effects on performance. However at all levels fish carcass composition was greatly modified and at inclusion levels greater than 50% protein replacement there was evidence of accumulation of the heavy metals Fe, Zn, Cu, and Pb.

The main constraint on the utilization of worms in commercial rations for rainbow trout is the current high costs of production (Tacon, 1981; Hilton, 1983; Stafford, 1984) and problems associated with antinutritional factors and palatability have also been encountered. In view of the generally high quality of worm protein it is worth investigating methods of eliminating antinutritional factors and also evaluating other worm species which may be more acceptable to fish. Finally production techniques must be improved if earthworms are to become an economically feasible proposition to fish feed manufacturers (Ichhponani and Lodhi, 1976; Tacon, 1981; Hilton, 1983; Stafford, 1984).

2.5.3 Crustaceans

A variety of crustaceans including shrimps, crabs, krill, and amphipods have been evaluated as foodstuffs for salmonids. In addition to being a potential protein source they also impart colour to flesh which is highly desirable in the culture of salmonids (Schmidt and Baker, 1969; Ellis, 1979; Simpson and Kamata, 1979).

Carotenoids are the main pigments of many aquatic animals and in salmonids astaxanthin is responsible for the typically red colour of the flesh. Most shellfish such as shrimps, crab, krill, lobsters, crayfish, copepods, amphipods and isopods contain astaxanthin (Ellis, 1979). Fish are unable to synthetise carotenoids de novo and thus these compounds must be supplied in the diet (Ellis, 1979; Simpson and Kamata, 1979).

Of the 300 known carotenoids only astaxanthin, which exists in three forms (astaxanthin monoester, astaxanthin diester and astacene) and canthaxanthin produce red pigmentation (Ellis, 1979; Simpson and Kamata, 1979; Torrissen and Braekkan, 1979; Torrissen et al., 1981/1982). In addition a synthetic astaxanthin has recently been developed. However, these artificial pigments are expensive and are also unacceptable in some countries. Consequently a variety of crustaceans have been included in diets for salmonids to provide both a source of nutrients and a source of pigments (Schmidt and Baker, 1969; Spinelli et al., 1974; Ellis, 1979).

2.5.3.1 Shrimp Meal

Shrimp meal can be made from freezing plant waste (heads and scales) or from whole shrimps in areas where the quality of the shrimps is not good enough for human consumption (Göhl, 1981). Before 1978 very little of the by-products from shrimp processing plants was used to produce meals. Indeed, since up to 70% of the biomass of processed shrimp is waste, disposal was a problem (Ellis, 1979).

Meals produced from whole shrimps have around 74% crude protein (Table 2.22) with an excellent amino acid profile, particularly rich in both the essential amino acids arginine and leucine (Table 2.23). Shrimp waste meal has a significantly lower crude protein content (41.0%-48.9%) and a higher ash content (31.7%-36.53%). The high ash content is mainly composed of chitin and $CaCo_3$ (Meyers and Rutledge, 1971). Shrimp meal contains 25.26 mg per Kilogramme dry weight astaxanthin of which 88.5% is in the diester form and 11.5% is the monoester.

Saito and Regier (1971) evaluated the pink shrimp Pandalus borealis in diets for brook trout (Salvelinus fontinalis). Carapaces and abdominal shells were vacuum dried (20-30 mg Hg) at 50°C for 24 hours and then ground. The astaxanthin content of the resulting meal was 100 mg per Kilogramme dry weight. The shrimp waste meal was included at 20% and 30% by weight in diets for brook trout. It was concluded that a dietary inclusion level of 20% by weight imparted the desired colour to fish flesh. Furthermore no marked growth inhibiting effect was observed.

TABLE 2.22 Proximate composition of some Shrimp wastes and Whole shrimp (% dry weight)

Proximate composition (% dry weight)	Waste 1	Waste 2	Whole 2
Moisture (%)	10.04	10.2	N/A
Crude protein (%)	41.0	48.9	73.6
Lipid (%)	4.92	0.1	16.6
Ash (%)	36.53	31.9	18.6

N/A = not available

TABLE 2.23 Amino acid profile of a Shrimp meal and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	Shrimp meal (whole) 2	Requirement of rainbow trout (% diet)
Arginine	6.0	1.40, 40% (Ogino, 1980)
Histidine	1.4	0.64, 40% (Ogino, 1980)
Isoleucine	3.2	0.96, 40% (Ogino, 1980)
Leucine	5.6	1.76, 40% (Ogino, 1980)
Lysine	5.6	2.12, 40% (Ogino, 1980)
Methionine	2.3	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	3.5	1.24, 40% (Ogino, 1980)
Threonine	3.4	1.36, 40% (Ogino, 1980)
Tryptophan	0.7	0.20, 40% (Ogino, 1980)
Valine	3.6	1.24, 40% (Ogino, 1980)
Cystine	0.9	0.30, 35%(Rumsey et al. 1983)
Glycine	5.9	
Tyrosine	2.7	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After Choubert and Luquet (1983)
2) After Cöhl (1981)

In feeding trials by Choubert and Luquet (1983) it was found that the amount of astaxanthin fixed by rainbow trout is low with regard to the ingested quantity not exceeding 3%. In an attempt to reduce the high dietary inclusion level required to enhance flesh colouration Torrissen et al. (1981/1982) investigated the suitability of three forms of shrimp products, namely shrimp waste meal, shrimp waste silage and shrimp meal. The digestibility of astaxanthin from ensiled shrimp waste was 71% compared with only 45% from both the meals. Although the digestibility of astaxanthin from shrimp waste meal was only 45%, the concentration was 10 times greater than in the shrimp meal. It was therefore concluded that shrimp waste silage and shrimp waste meal can be used efficiently as pigment sources in rainbow trout diets at dietary inclusion levels of 10.5% and 11.0% by weight respectively.

2.5.3.2 Crab Meal

Crabs like the red crab, Pleuroncodes planipes, and the snow crab, Chinoecetes opilio, are very abundant in certain areas. Spinelli et al. (1974) reported that there is a potential annual catch of 30-300 thousand tons of red crab off the coasts of lower California and Mexico, although the economic feasibility of these crabs as a fishery resource has not been assessed.

The proximate composition of the red crab is given in Table 2.24. It has a very low protein content (5.5%-10.2%) and a relatively high chitin content (1.3%-1.8%). Spinelli et al. (1974) pointed out that the essential amino acid profile of the total protein is

TABLE 2.24 Proximate composition of Red Crab and Red Crab meal (*P. planipes*) (% dry weight

Proximate composition (% dry weight)	*P. planipes*	Red crab meal (whole)	Red crab pulp meal
Moisture (%)	75.0-86.0	N/A	N/A
Crude protein (%)	5.5-10.2	41.5	46.0
Lipid (%)	0.9-3.5	11.0	13.4
Ash (%)	3.2-4.8	34.3	28.3
Chitin (%)	1.3-1.8	11.3	7.1

N/A = not available

TABLE 2.25 Amino acid profile of red crab (*P. planipes*) and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	*P.planipes*	Requirement of rainbow trout (% diet)
Arginine	0.42	1.40, 40%* (Ogino, 1980)
Histidine	0.14	0.64, 40% (Ogino, 1980)
Isoleucine	0.20	0.96, 40% (Ogino, 1980)
Leucine	0.33	1.76, 40% (Ogino, 1980)
Lysine	0.37	2.12, 40% (Ogino, 1980)
Methionine	0.14	0.55-0.75, 35% (Rumsey *et al*. 1983)
Phenylalanine	0.28	1.24, 40% (Ogino, 1980)
Threonine	0.21	1.36, 40% (Ogino, 1980)
Tryptophan	0.09	0.20, 40% (Ogino, 1980)
Valine	0.43	1.24, 40% (Ogino, 1980)
Alanine	0.32	
Aspartic acid	0.51	
Cystine	0.05	0.30, 35% (Rumsey *et al*. 1983)
Glutamic acid	0.68	
Glycine	0.33	
Proline	0.23	
Serine	0.22	
Tyrosine	0.50	0.84, 40% (Ogino, 1980)

* Percentage of crude protein in the diet

After Spinelli *et al*. (1974)

similar to that of fish except for the tryptophan content which is higher (Table 2.25). It has a lipid content varying between 4.92% and 7.9%, 40% of which comprises linolenic-type fatty acids (Spinelli et al., 1974). The carotenoid content of red crab meal varies between 0.10 mg and 0.16 mg per 100 grammes dry weight while that of snow crab meal is 0.47 mg per 100 grammes dry weight (Saito and Regier, 1971; Spinelli et al., 1974).

Spinelli et al. (1974) tested both whole red crab meal and crab pulp meal in diets for rainbow trout. The red crab whole meal was incorporated at 10% and 25% by weight and the pulp meal at 25% by weight into Oregon moist pelleted diets. No significant differences were noted in the flesh colouration of fish fed the whole and the pulp meals and only at the 25% inclusion level was there distinct pigmentation.

Saito and Regier (1971) evaluated snow crab wastes from processing plants as a potential colouring agent for brook trout (Salvelinus fontinalis). Carapaces and leg shells were vacuum dried (20-30 mg Hg) at 55°-60°C for 24 hours and ground. The snow crab waste meal was then included in brook trout diets at a level of 20% by weight. Saito and Regier (1971) reported that at the level tested snow crab waste meal had only a slight effect on the pigmentation of the trout, but no marked growth inhibiting effect was observed.

2.5.3.3 Krill Meal

Krill is extremely abundant in both the Antarctic Sea and in Norwegian and Mediterranean waters in spring and early summer (Ellis, 1979; Lukowicz, 1979). Euphausia superba are found in the former and Megancyctiphanes norwegica in the latter. Lyubimova et al. (1973) estimated that the total annual production of Antarctic krill was 0.8 to 5 billion tons which represents a considerable resource.

Antarctic krill has a crude protein content of around 56% (Table 2.26) with an excellent amino acid profile (Table 2.27) reported to be equivalent to fish meal by Lukowicz (1979) and only slightly inferior to fish protein by Koops et al. (1979) and Wojno and Dabrowska (1984b). It contains 9%-11% lipid, 48% of which is phospholipids and 36% tryglycerides (Shibata, 1983). Yamaguchi et al. (1983) reported that the carotenoid content of Antarctic krill meal was 15-20 mg per 100 grammes, of which 65%-75% was in the form of astaxanthin diester, 15%-25% as astaxanthin monoester, and 5%-15% of unidentified carotenoids.

Lukowicz (1979) evaluated Antarctic krill meal in diets for carp (Cyprinus carpio) replacing fish meal protein as the sole source of dietary protein or a mixture of several animal by-products at levels of up to 100%. It was concluded that Antarctic krill meal can successfully replace all the fish meal protein with gain of growth rate and feed utilization efficiency. The replacement of all the animal protein sources in the test diets by Antarctic krill meal was less successful than replacement of the fish meal component only.

TABLE 2.26 Proximate composition of two Antarctic krill meals (% dry weight)

Proximate composition (% dry weight)	Antarctic krill meal 1	2
Moisture (%)	9.2	8.8
Crude protein (%)	56.5	56.2
Lipid (%)	10.7	9.2
Ash (%)	15.0	15.9

TABLE 2.27 Amino acid profile of Antarctic krill meal and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	Antarctic krill meal 1	2	Requirement of rainbow trout (% diet)
Arginine	3.6	2.7	1.40, 40% (Ogino, 1980)
Histidine		0.7	0.64, 40% (Ogino, 1980)
Isoleucine	2.9	2.5	0.96, 40% (Ogino, 1980)
Leucine	4.5	3.6	1.76, 40% (Ogino, 1980)
Lysine	4.4	3.3	2.12, 40% (Ogino, 1980)
Methionine	1.4	1.9	0.55-0.75, 35 % (Rumsey et al. 1983)
Phenylalanine	2.4	2.1	1.24, 40% (Ogino, 1980)
Threonine	2.6	2.0	1.36, 40% (Ogino, 1980)
Valine	3.0	2.5	1.24, 40% (Ogino, 1980)
Alanine	3.4	2.6	
Aspartic acid	6.6	5.3	
Cystine	0.8	1.2	0.30, 35 % (Rumsey et al. 1983)
Glutamic acid	8.3	6.5	
Glycine	2.9	6.5	
Proline	2.1		
Serine	2.4	2.2	
Tyrosine		1.5	0.84, 40% (Ogino, 1980)

• Percentage of crude protein in the diet

1) After Lukowicz (1979)
2) After Koops et al. (1979) after Hoffman-La Roche

Koops et al. (1979) evaluated Antarctic krill meal in diets for rainbow trout by replacing 50% and 100% of fish meal as the sole source of dietary protein and at levels of up to 100% replacing equal amounts of fish meal and a mixture of poultry by-product meal and hydrolysed feather meal (4:3). They found that the total replacement of fish meal protein and of all the animal protein sources by Antarctic krill meal improved growth rate and feed utilization efficiency. In addition pigments from the krill imparted a desirable colouration to the flesh. Thus, from the limited work to date it appears that Antarctic krill meal can successfully replace fish meal protein without adverse effects on growth or feed utilization efficiency. Furthermore the inclusion of Antarctic krill meal in diets for rainbow trout confers the additional advantage of imparting a desirable flesh colouration. However the current costs of harvesting and processing krill render its large scale production impractical at present (Ellis, 1979).

2.5.3.4 Amphipods

Crustaceans such as the freshwater amphipod Gammarus lacustris are very abundant in lakes and Mathias et al. (1982) reported that it is possible to harvest between 500 and 1,000 Kilogrammes of G. lacustris per hectare.

Fresh G. lacustris has a fairly high crude protein content of around 40% (Table 2.28) with an amino acid profile (Table 2.29) considered to be sufficient to meet the requirements of rainbow trout (Mathias et al., 1982). It contains between 3.5% and 14%

TABLE 2.28 Proximate composition of the Amphipod G. lacustris (% dry weight)

Proximate composition (% dry weight)	Gammarus lacustris 1	2	3
Moisture (%)	84.5-87.1	85.6 ± 1.8	7.3-8.7
Crude protein (%)	39.2-41.6	39.6 ± 3.7	42.0-49.0
Lipid (%)	8.7-13.8	10.5 ± 3.5	6.0
Ash (%)	27.4-31.8	27.9 ± 3.6	21.0-31.0

TABLE 2.29 Amino acid profile of the Amphipod G. lacustris and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	Gammarus lacustris 1	2	Requirement of rainbow trout (% diet)
Arginine	2.3-3.1	2.6-2.9	1.40, 40% (Ogino, 1980)
Histidine	0.7-1.2	0.9-1.1	0.64, 40% (Ogino, 1980)
Isoleucine	1.8-1.9	1.7-1.7	0.96, 40% (Ogino, 1980)
Leucine	3.23-3.3	3.0-3.2	1.76, 40% (Ogino, 1980)
Lysine	2.8-2.8	2.6-2.7	2.12, 40% (Ogino, 1980)
Methionine	0.7-1.0	0.8-0.9	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	2.1-2.2	2.0-2.1	1.24, 40% (Ogino, 1980)
Threonine	2.0-2.1	2.0-2.0	1.36, 40% (Ogino, 1980)
Valine	2.2-2.3	2.0-2.1	1.24, 40% (Ogino, 1980)
Alanine	2.6-2.6	2.4-2.5	
Aspartic acid	4.7-4.8	4.5-4.6	
Cystine	0.1-0.4	0.2-0.8	0.30, 35% (Rumsey et al. 1983)
Glutamic acid	6.4-6.4	6.0-6.2	
Glycine	2.1-2.2	2.0-2.1	
Proline	1.8-1.9	1.8-3.0	
Serine	2.2-2.3	2.3-2.4	
Tyrosine	1.8-1.3	1.7-1.3	0.84, 40% (Ogino, 1980)

* Percentage of crude protein in the diet

1) After Mathias et al. (1982)
2) After Yurkowski and Tabachek (1979)
3) After Salonen et al. (1976)

lipid but also has a relatively high ash content varying between 27% and 32% (Table 2.28).

Mathias et al. (1982) evaluated the potential of live G. lacustris as the sole source of dietary protein for rainbow trout. After a 27 day feeding trial it was apparent that both the growth rate and feed utilization were significantly lower than that of fish fed a commercial trout diet. Furthermore, although the pigment from the amphipods enhanced the external colouration of the fish there was no effect on flesh colouration but it is likely that the experimental feeding period was too short for it to show. The evaluation of a meal produced from G. lacustris in compounded diets was not attempted, although the feasibility and cost of harvesting and processing amphipods to produce a meal may again limit its potential as a foodstuff for salmonid diets.

Shrimps, crabs, krill, and amphipods represent a considerable resource but harvesting and processing of crustaceans into a meal is still a formidable challenge (Ellis, 1979). Another important disadvantage of some crustacean meals is their low protein and high ash content (Spinelli et al., 1974; Lukowicz, 1979; Choubert and Luquet, 1983) which may have deleterious effects on overall nutrition. Meyers and Rutledge (1971) have suggested that the use of meals made from crustaceans may jeopardize pellet integrity. In addition the high inclusion level of certain crustacean meals necessary to impart the desirable colouration may result in a nutritionally unbalanced diet (Ellis, 1979).

2.6 BACTERIAL SINGLE CELL PROTEIN

Single cell protein (SCP) is a term applied to a wide range of algae, fungi (including yeasts), and bacteria which are produced by fermentation processes for use as an animal feed (Tacon and Jackson, 1985). Bacterial SCP's are nonphotosynthetic bacteria and consequently can be regarded as ingredients of animal origin.

Bacterial SCP has a number of advantages over more conventional protein sources. It can be cultivated from relatively inexpensive substrates such as methanol, petroleum, aldehydes and organic acids (Schulz and Oslage, 1976; Atack and Matty, 1979; Beck et al., 1979; Kaushik and Luquet, 1980; Tusé, 1984; Tacon and Jackson, 1985). The bacterial SCP produced is highly proteinaceous ranging from 67% to 82% of the dry weight depending on the species (Atack and Matty, 1979; Beck et al., 1979; Tacon, 1981; Tacon and Jackson, 1985). Bacteria have a very short generation period. Under optimum culture conditions they can double their cell mass in between half an hour to four hours (Schulz and Oslage, 1976; McDonald et al., 1981; Tacon, 1981; Tacon and Jackson, 1985). Thus despite the limited area occupied by a fermentation plant significant amounts of bacterial protein can be yielded continuously (Tacon, 1981; Tusé, 1984; Tacon and Jackson, 1985). Bacterial SCP can also be genetically manipulated relatively easily to specific requirements (Schulz and Oslage, 1976; Tacon, 1981; Tusé, 1984; Tacon and Jackson, 1985).

Although SCP has many advantages over more conventional protein sources there are also problems associated with its use. Probably the most important disadvantage is its high nucleic acid content

of between 8% and 16% by weight (Kihlberg, 1972; Matty and Smith, 1978; Tacon and Jackson, 1985). High levels of nucleic acids in humans lead to increased levels of uric acid in the blood and urine, and since the capacity of the excretion mechanism is limited, gout and renal problems may arise (Schulz and Oslage, 1976; Tusé, 1984). By contrast, Tacon and Cooke (1980) maintain that fish are able to assimilate and metabolize nucleic acids to end products although they have no nutritive value, thus there is no gain in nitrogen. Certain purine and pyrimidoxine bases however have growth depressing properties, although no pathological symptoms are observed. Nucleic acid levels can be reduced by some autolytic methods, such as heat shock which may follow processing (Tusé, 1984).

The second major disadvantage of SCP is the cost of production. Not only is the cost of the plant required to produce SCP expensive, but there has also been a significant increase in the cost of certain substrates. Methanol for example, was at one time considered to be mainly an industrial waste by-product. However, it is now used increasingly and it has been predicted that its use will rise to 23 million tons by 1990 (Tusé, 1984). As a consequence the law of supply and demand has caused its price to increase which has subsequently resulted in increased production costs of SCP based on methanol.

Methanol and to a slightly lesser extent methane, are the substrates most commonly used to produce SCP since they are both economical and readily available (Tusé, 1984). A wide number of bacterial SCP are known to grow well in these substrates including

Methylophilus methylotrophus, Methylococcus sp., Methylomonas sp., Pseudomonas extorquens, and P. methylotropha in methanol and Pseudomonas methanitrificans and P. methanica in methane. Of these bacterial species M. methylotrophus and to a lesser extent Pseudomonas sp., are currently most commonly produced.

Single cell protein production using M. methylotrophus is the result of 14 years of research and development by British Petroleum Ltd. (BP) and Imperial Chemical Industries (ICI). It is currently marketed under the trade name of "Pruteen" by ICI (Tacon, 1981; Tusé, 1984). The preliminary research work with M. methylotrophus began in 1968. In 1973 ICI built a pressure-cycle fermentor with a production capacity of 1,000 tons per year. In 1979 as a result of improvements in processing techniques, a new plant was built with a production capacity of 50,000 tons per year (Tusé, 1984) and in 1981 commercial "Pruteen" production within the United Kingdom was around 36,000 tons per year which was mostly used in diets for terrestrial animals (Tacon, 1981).

M. methylotrophus is a good source of dietary protein (67%-80%; Table 2.30) and it has an excellent amino acid profile (Table 2.31) although it is deficient in the sulphur amino acids (Schulz and Oslage, 1976; Atack and Matty, 1979; Beck et al., 1979; Kaushik and Luquet, 1980). Lysine is its first limiting amino acid (Tacon and Jackson, 1985). It has a lipid content of between 8% and 13% and it has an ash content of between 9% and 10% and is a good source of both P and Ca, 2.2% and 1.3% by weight, respectively (Tacon, 1981).

TABLE 2.30 Proximate composition of five bacterial Single cell proteins and Activated sludge single cell protein (ASCP) (% dry weight)

Proximate composition (% dry weight)	M. methylotrophus ("Pruteen") 1	2	3	Pseudomonoas sp. 4	5	ASCP 6
Moisture (%)	11.4	N/A	N/A	3.4	3.3	2.76-7.36
Crude protein (%)	67.3	72.0	80.2	77.7	81.9	39.0-46.0
True protein (%)	N/A	N/A	N/A	N/A	N/A	17.0-28.0
Lipid (%)	11.1	8.5	12.6	1.6	7.9	1.0-6.0
Ash (%)	9.5	10.0	N/A	16.3	9.7	19.0-27.0
Crude fibre (%)	0.4	N/A	N/A	N/A	5.0	10.0-14.0

N/A = not available

TABLE 2.31 Amino acid profile of three bacterial Single cell protein and Activated sludge single cell protein (ASCP), and essential amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100 g dry wt)	M. methylotrophus ("Pruteen") 3	1	Pseudomonas sp. 7	ASCP 8	Requirement of rainbow trout (% diet)
Arginine	3.7	5.50	3.7	1.9	1.40, 40% (Ogino, 1980)
Histidine	1.4	1.75	1.4	0.8	0.64, 40% (Ogino, 1980)
Isoleucine	3.3	5.41	3.6	0.3	0.96, 40% (Ogino, 1980)
Leucine	5.1	8.81	5.7	2.9	1.75, 40% (Ogino, 1980)
Lysine	4.6	6.79		2.0	2.12, 40% (Ogino, 1980)
Methionine	2.1	1.89	2.9**	0.3	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	2.7	7.51		2.4	1.24, 40% (Ogino, 1980)
Threonine	3.3	6.05		2.2	1.36, 40% (Ogino, 1980)
Tryptophan	0.6		3.4		0.20, 40% (Ogino, 1980)
Valine	3.9	6.73	4.2	2.6	1.24, 40% (Ogino, 1980)
Cystine	0.5		2.9**	0.3	0.30, 35%(Rumsey et al. 1983)
Glycine			4.1		
Tyrosine	0.2			0.8	0.84, 40% (Ogino, 1980)

* Percentage of crude protein in the diet

** Cystine + methionine

1) After Tacon et al. (1983a)
2) After Tacon (1981)
3) After Kaushik and Luquet (1980)
4) After Matty and Smith (1978)
5) After McDonald et al. (1981)
6) After Tacon and Ferna (1978/1979)
7) After Schulz and Oelage (1976)
8) After Tacon (1978/1979)

Beck et al. (1979), Spinelli et al. (1979), Tiews et al. (1979) and Kaushik and Luquet (1980) evaluated "Pruteen" for rainbow trout by replacing fish meal protein at levels of up to 100% with this bacterial SCP. They concluded that "Pruteen" can successfully replace fish meal protein at levels of up to 75% protein replacement without any loss of growth rate and feed utilization efficiency and up to 100% protein replacement with only a slight loss of fish performance. Tacon et al. (1983a) reported similar findings. Rainbow trout fed a diet in which 75% of the fish meal protein was replaced by "Pruteen" had a better growth performance and feed utilization efficiency when compared with those fed a herring meal based control diet.

Atack and Matty (1979) evaluated the nutritional quality of "Pruteen" for rainbow trout as the sole source of dietary protein. They reported a true digestibility value of 93.5% which was slightly better than that obtained with herring meal (91.2%). However the growth performance of the fish fed the SCP based diet was again slightly lower than that of the fish fed the fish meal control.

The major effect of M. methylotrophus ("Pruteen") on fish fed high dietary inclusion levels is an alteration in carcass composition. Atack and Matty (1979) reported that carcasses of fish fed 100% "Pruteen" had a lower lipid content (5.91%) when compared with those of fish fed the control diet (9.17%). These findings are however in contrast to those reported by Kaushik and Luquet (1980) who found a slight increase in body fat with increasing dietary inclusion levels of bacterial SCP. Finally, Beck et al. (1979) noted that fish fed high dietary inclusion levels of bacterial SCP had very

pale livers which was attributed to extremely high liver glycogen levels.

Single cell protein produced from Pseudomonas sp., grown on methanol, has a very high protein content of between 77% and 82% (Table 2.30). It has an excellent amino acid profile (Table 2.31) and it is particularly rich in leucine and valine (McDonald et al., 1981). Matty and Smith (1978, 1979) evaluated the nutritional quality of Pseudomonas sp. for rainbow trout at four dietary protein levels of 20%, 25%, 30%, and 35%. The bacterial SCP was the sole source of crude protein and replaced glucose on a weight for weight basis. Based on growth performance and feed utilization efficiency it was concluded that 25% crude protein level was the optimum inclusion for this SCP.

In addition to M. methylotrophus ("Pruteen") and Pseudomonas sp. other bacterial SCP's have been produced although none have been evaluated in diets for fish. The European company Hoechst in association with Uhde have produced an SCP under the trade name "Probion" from Methylomonas clara an obligate methylotrophic bacterium (Tusé, 1984). Finally in the United States of America ITT Rayonier Inc. has been producing an SCP called "Raypro" at its for est products mill with the aim of reducing the biochemical oxygen demand of the plant's effluents. "Raypro" has a crude protein content of 53% and a crude fibre content of 16% (Tusé, 1984).

The bio-oxidation of crude domestic sewage is another alternative and advantageous method of producing SCP. Nowadays the disposal

of waste sewage is one of the problems that industrial plants are obliged to face. An alternative method of sludge disposal would be to use it as an animal foodstuff with the added benefit of fiscal return (Tacon and Ferns, 1978/1979). Since the largest portion of the biomass is made up of micro-organisms (bacteria and protozoa) it is usually considered to be an SCP. Tacon (1978/1979) and Tacon and Ferns (1978/1979) evaluated the suitability of activated sludge single cell protein (ASCP) as an animal feed. A one year survey was conducted with bi-weekly analysis since these authors considered that a product such as ASCP needs to be of fairly constant chemical composition throughout the year if its full nutritional value is to be realized by commercial animal feed formulators.

The proximate composition is given in Table 2.30. It has a crude protein content of between 39% and 46% although between 18% and 40% of the total nitrogen was in the form of non-amino acid N-containing compounds, mainly in the form of purine and pyrimidine bases of nucleic acids and nucleotides (Tacon, 1978/1979). The amino acid profile (Table 2.31) reveals that it is deficient in the amino acids methionine, cystine, tyrosine, and to a lesser extent isoleucine (Tacon, 1978/1979). ASCP has a low lipid content (1%-6%) but high fibre (10%-14%) and ash (19%-27%) contents (Tacon, 1978/1979). It is a good source of Ca, P, Mg, Mn, Sr, Mo, N, Cr, Cu, and Co but a poor source of K. Tacon (1978/1979) also reported that ASCP contains many potentially hazardous elements at high levels such as Fe, Al, Zn, Cu, Bo, Sn, Pb, and Cd. However, Tacon (1979b) pointed out that there is some evidence that a large proportion of these elements are biologically unavailable. In addition to

possible problems associated with heavy metal accumulations, there is also a potential health hazard arising from the presence of faecal parasites and pathogens. However ASCP processing involves temperatures of 60°C for at least 48 hours thus practically eliminating the risk of disease transmission (Tacon, 1978/1979; 1979b). Another major problem related to ASCP production is its harvesting cost which is predicted to be high (Tacon, 1979b).

Tacon and Ferns (1976) and Tacon (1979b) evaluated the nutritional quality of ASCP for rainbow trout by replacing wheat middlings and soybean meal at levels of up to 33% by weight. Based on growth performance and feed utilization efficiency it was concluded that ASCP can be successfully incorporated into diets for rainbow trout at dietary inclusion levels of up to 33%. Considerable effects were noted however on fish carcass composition, in particular there was a significant increase in fat deposition (Tacon, 1979b). Provided problems associated with heavy metal accumulation and micro-organism contamination can be overcome economically and also possible problems associated with consumer acceptability, the potential of ASCP as a feed ingredient for fish is promising.

CHAPTER 3

ANIMAL BY-PRODUCTS AVAILABLE IN PORTUGAL

3.1 ANIMAL BY-PRODUCTS AVAILABLE IN PORTUGAL

If Portugal is to embark on large scale commercial production of feeds for use in intensive culture of trout, then a suitable range of animal by-products must be available in sufficient quantities and on a reliable basis. A survey was therefore carried out to investigate the availability of animal by-products in Portugal, and the results of this are presented in Table 3.1. Based on this survey poultry by-product and hydrolysed feather meal and meat and bone meal were selected as potential candidates to supply a significant proportion of the protein in rations for rainbow trout in Portugal. This selection was based on the large quantities of these feedstuffs available and also on information from the preceding review (Chapter 2) concerning their nutritional status.

The aim of this thesis was to determine the optimum inclusion level for each of these products with a view to maintaining dietary costs as low as possible without jeopardizing the nutritional quality required for rainbow trout. During the course of the survey it also became apparent that there were many fish meal producers in Portugal, with production capacities varying between approximately 400 tons and 2,800 tons per year (managers' personal communications). Therefore a further aim was to compare the performance of a selection of these in rations for trout.

Based on the results from these investigations the aim was to prepare and assess the performance of diets containing all three of these animal by-products. The degree of variability in chemical

composition of the ingredients is an important factor when considering the use of a product in fish feeds (Tacon and Ferns, 1978/1979). Finally, an evaluation of the costs of diets using these ingredients was carried out.

TABLE 3.1 Animal by-products available in Portugal

By-product	Available	Not available
Bone meal	*	
Meat meal	*	
Meat and bone meal	*	
Blood meal		*
Hydrolysed feather meal		*
Poultry by-product meal		*
Poultry by-product and hydrolysed feather meal	*	
Brown fish meal	*	
White fish meal		*
Fish silage		*
Bacterial SCP	*	

CHAPTER 4

GENERAL MATERIALS AND METHODS

4. GENERAL MATERIALS AND METHODS

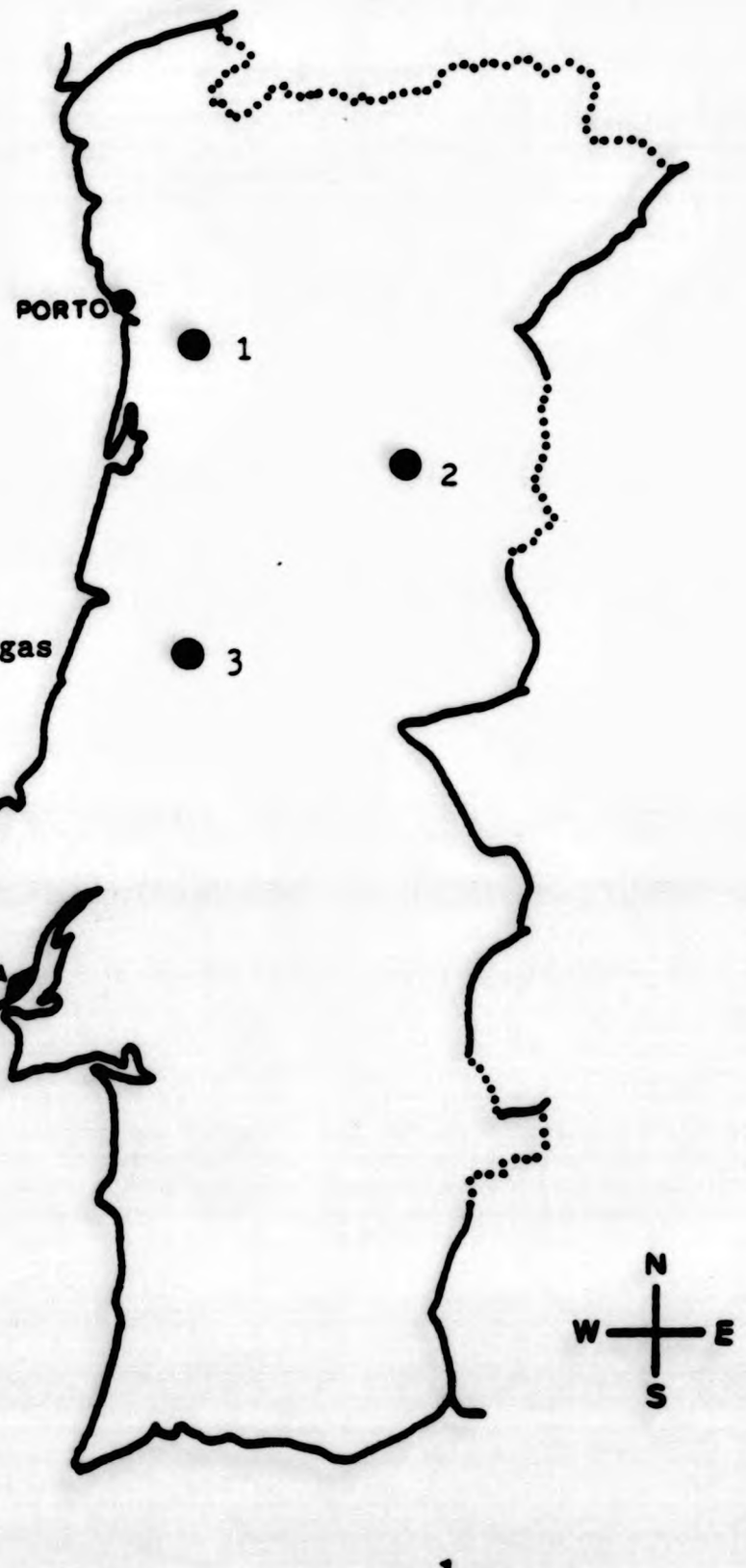
1. Fish

Rainbow trout in the size range 19-36g were obtained from the following three trout farms: Truturão, Cernache, Coimbra; Posto Aquícola de Manteigas, Serra da Estrela and Inha, S. João da Madeira (Fig. 4.1).

Fish were starved for 24 hours prior to collection to reduce stress during handling and subsequent transportation. 600 randomly selected fish were distributed between two $0.8m^3$ plastic buckets. The water was aerated with two portable air pumps fitted with diffusers to supply very small air bubbles. Fish were then transported to the Experimental System where they were acclimatized to the new environment for a period of one to two weeks in accordance with the recommendations of Singh and Nose (1967); Smith (1971); Kaushik and Luquet (1976); Windell *et al*. (1978); Braaten (1979); and Fauconneau *et al*. (1983). During this acclimatization period the fish were fed a commercial trout ration at a total rate of 1% body weight twice daily.

2. Experimental System

All growth trials were carried out in net cages located at the University of Porto Experimental Station at Rio Caldo in the north of Portugal (Figs. 4.2a, 4.2b; Plate 4.1).



1. Inha
2. Posto Aquícola de Manteigas
3. Truturão

1 / 3 500 000 cm

FIGURE 4.1 Location of trout farms in Portugal

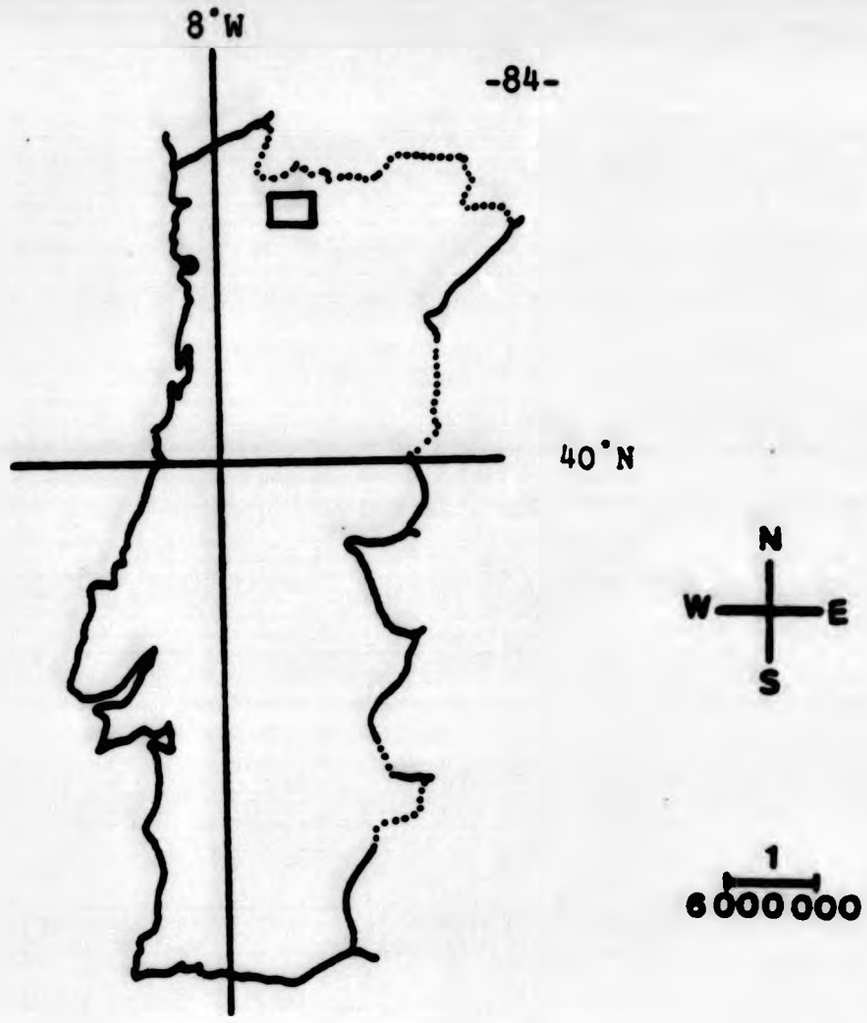


FIGURE 4.2a Location of Caniçada reservoir

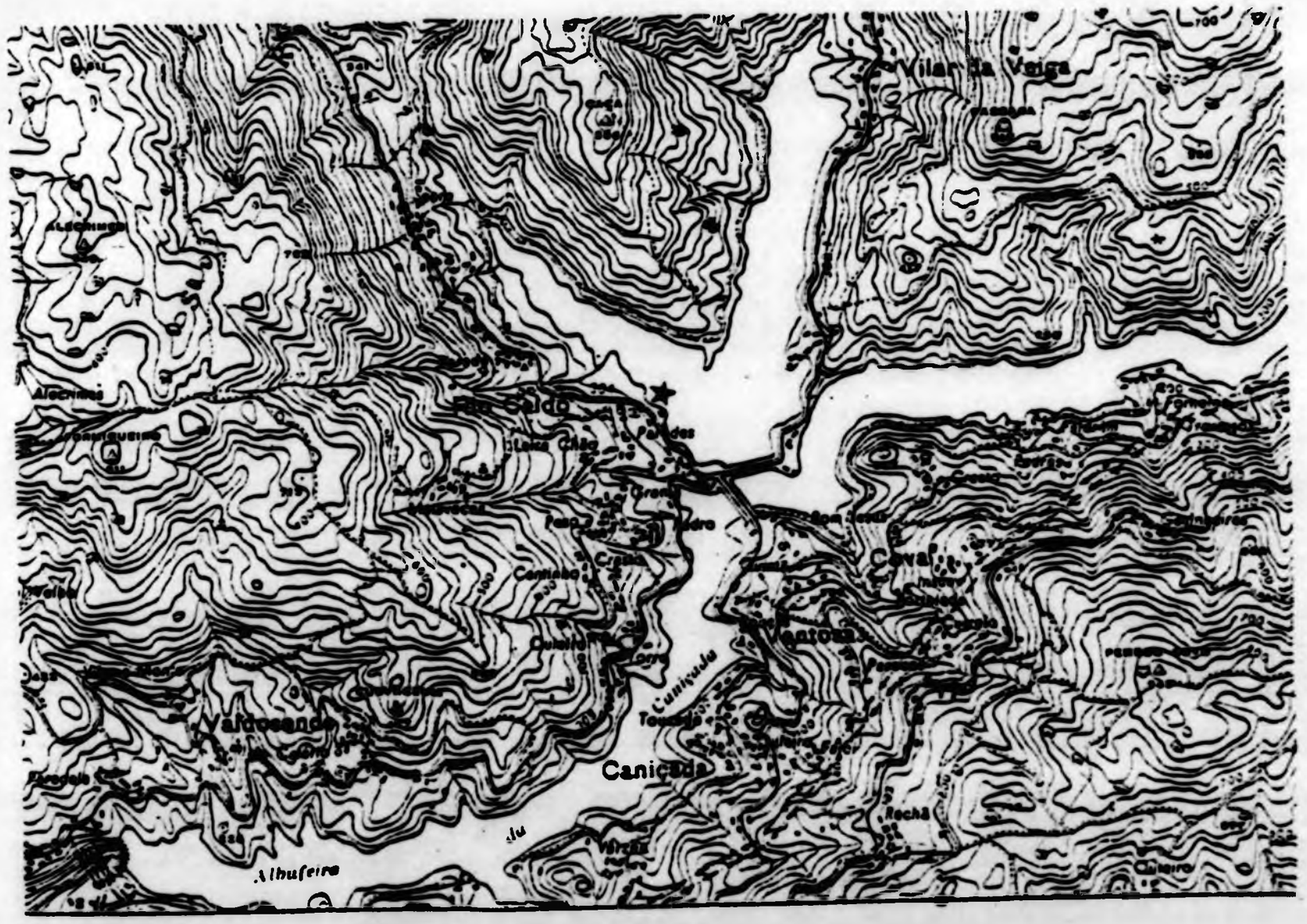


FIGURE 4.2b Location of the Experimental Aquaculture Station at Rio Caldo (★siting of net cages; Scale 1:25,000)

PLATE 4.1 Experimental Aquaculture Station at Rio Caldo

PLATE 4.1 Experimental Aquaculture Station at Rio Caldo

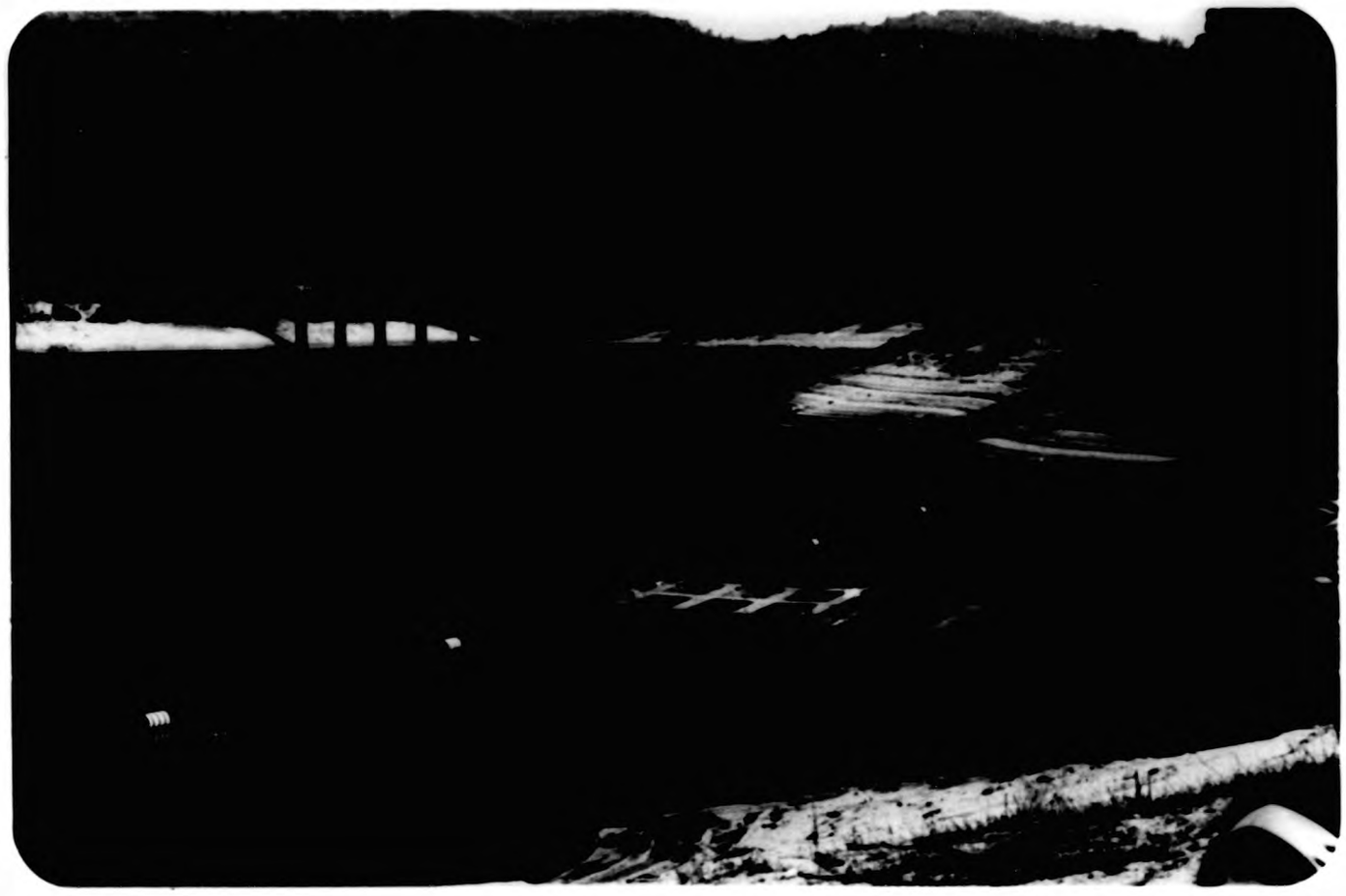


PLATE 4.1 Experimental Aquaculture Station at Rio Caldo

The Aquaculture Station was constructed in 1981 on the reservoir formed by the Caniçada Dam on the outskirts of the Peneda-Gerês National Park. The reservoir is located in a deep narrow valley and is 5 Km long and 0.5 Km wide. The dam was constructed as part of an hydroelectric programme and consequently water levels in the reservoir are subjected to fairly wide fluctuations, especially during the summer. Nevertheless the water depth near the Experimental System was almost always greater than 10 metres and only once was it necessary to move the cages into deeper water towards the centre of the reservoir.

The cages were moored 100 metres offshore in the middle of a sheltered inlet protected from the prevailing northern winds by hills (Fig. 4.2b). The working platform was held in place by a single point mooring and by two cables extending to the shore. Access to the cages was by boat.

The Experimental System consisted of 15 0.3m^3 net cages. The side panels and lockable lid were of 1 cm mesh plastic net and the bottom panel was of 2 mm mesh to prevent food loss. Each cage was braced by a wooden frame to maintain its shape (Fig. 4.3). Every two weeks following batch weighings of the fish the nets were cleaned and replaced if necessary.

The cages were attached by chains to a floating superstructure in three rows of five (Fig. 4.4). The fish were fed through a plastic funnel inserted through a 2 cm x 2 cm hole in the centre of each lid. Electrical supply to the experimental system was available by means of a 200 metres submersible cable.

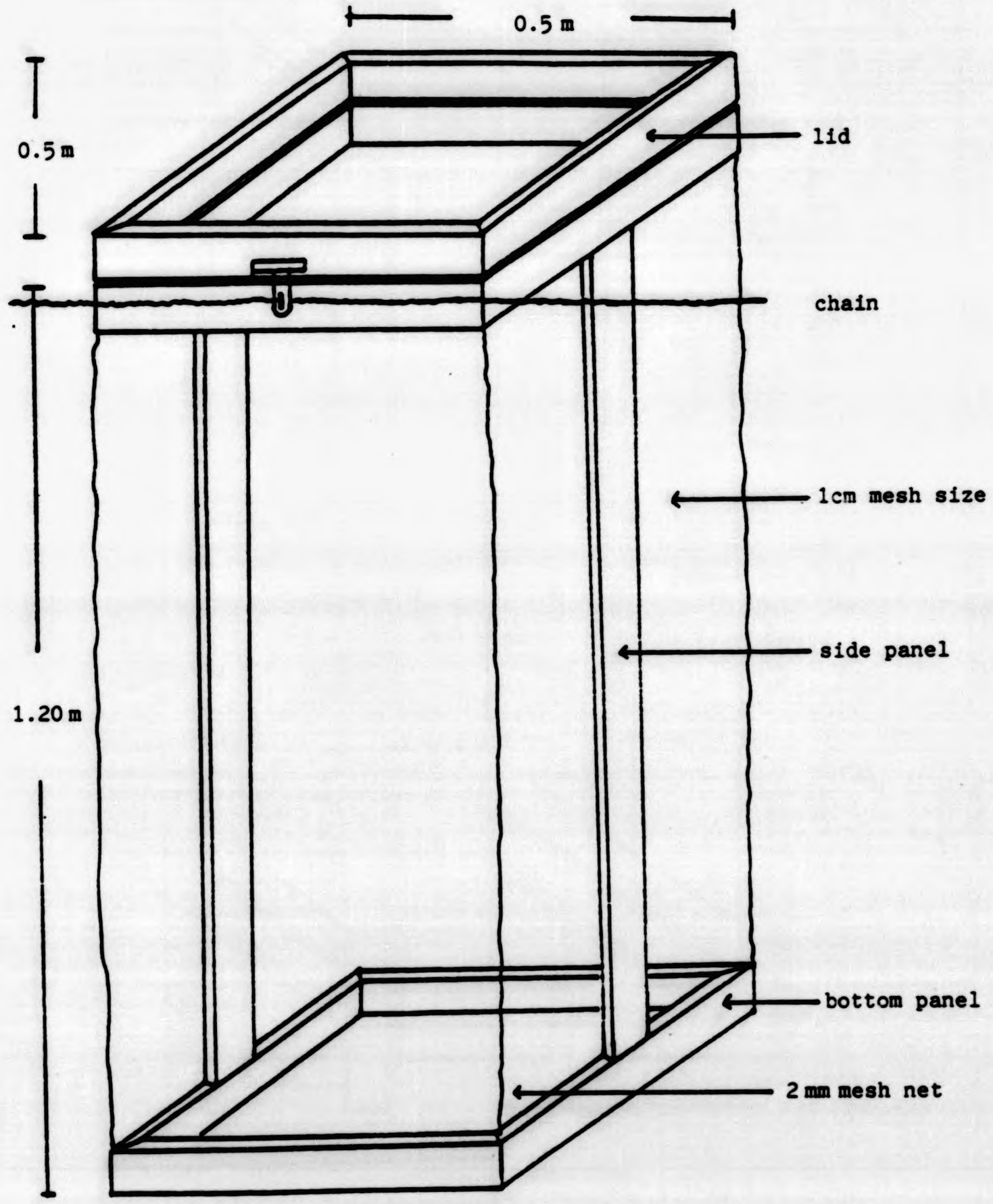


FIGURE 4.3 Schematic diagram of a single net cage (Scale 1:8)

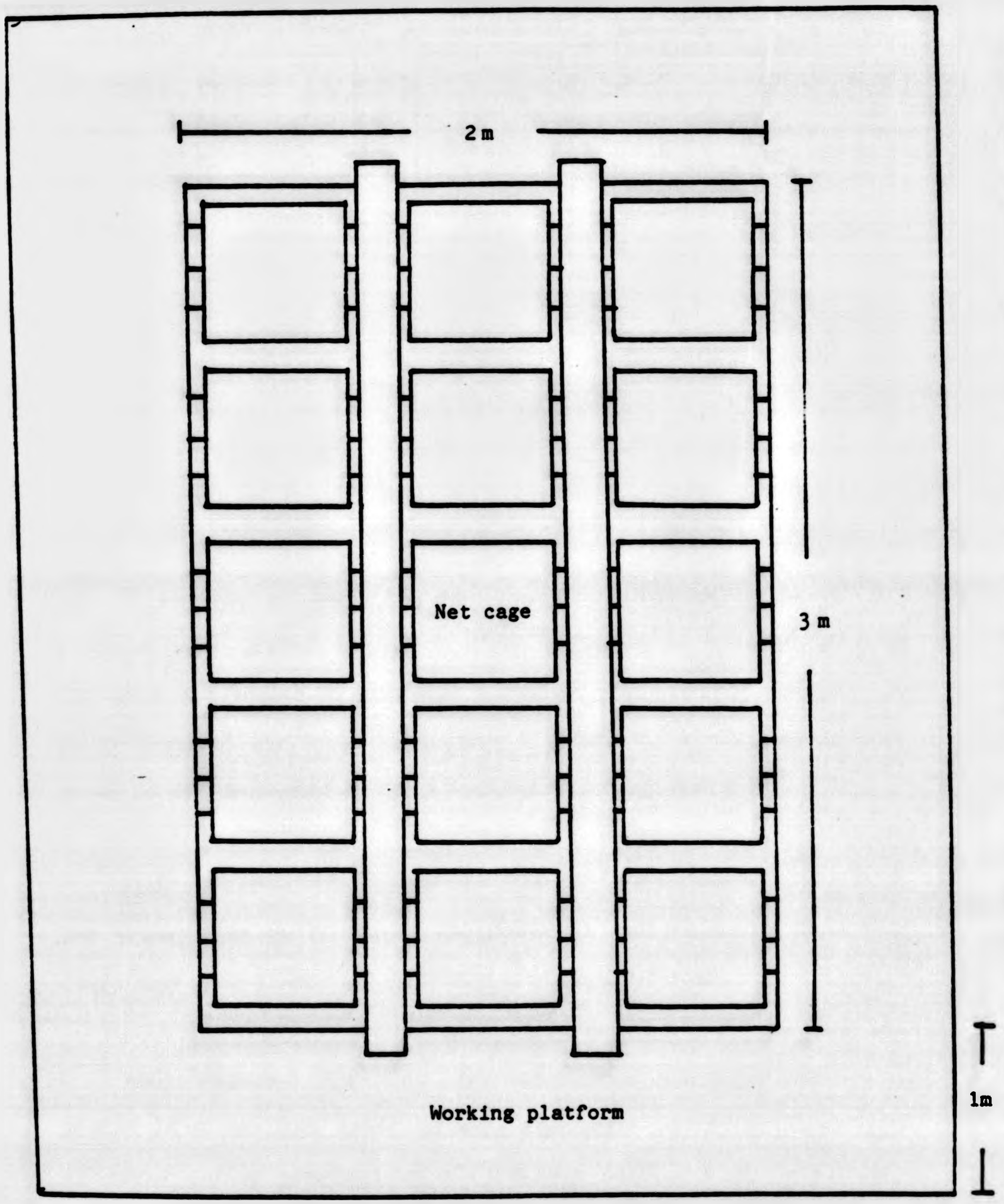


FIGURE 4.4 Schematic diagram of the Experimental System (Scale 1:20)

During the experimental growth trials several water quality parameters were monitored at monthly intervals (Table 4.1). All the values recorded were within acceptable limits for rainbow trout culture. Water temperatures were also monitored every week during each feeding trial and the data are presented in the relevant Chapters.

3. Analytical Methods

The chemical analysis carried out on samples of by-products, experimental diets, fish carcasses, faeces and blood are summarised in Table 4.2.

On arrival at the nutrition laboratory samples of animal by-products were ground through a 1 mm die plate on an Alexanderwerk GKM mill and stored in airtight containers at 3°-5°C in a cold room until required for diet preparation. 100g samples of both the by-products and experimental diets were retained for analysis.

Fish carcasses and faeces were frozen at the Aquaculture Station and transported in insulated containers to the nutrition laboratory where they were stored at -20°C for subsequent analysis.

When required the fish carcasses were defrosted naturally, ground in a Moulinex grinder, and dried. The proximate composition of three pooled samples of two fish per treatment was determined. Following total lipid extraction, the samples were stored in airtight glass flasks until required for protein and ash determinations.

TABLE 4.1 Water quality parameters monitored during the experimental trials (means of monthly values recorded over a 2 and a half years period between March 1983 and September 1985)

Depth (m)	Nitrate (μgat N-NO_3 l^{-1})	Nitrite (μgat N-HO_2 l^{-1})	Silicate (μgat Si l^{-1})	Oxygen (mgl^{-1})	Hardness (ppm $CaCO_3$)	Ammonia (mg N-NH_4 l^{-1})	Phosphate (μgat P-PO_4 l^{-1})
0.0	7.52	0.11	58.20	10.57	2.61	1.18	
0.5	7.61	0.14	65.50	10.64	2.76	1.10	
1.0	7.91	0.11	65.14	10.55	2.55	1.21	
1.5	7.56	0.10	66.03	10.62	2.61	1.15	
3.0	7.77	0.10	68.35	10.62	2.65	0.71	
5.0	7.92	0.09	64.08	10.92	2.57	0.48	
10.0	7.99	0.10	59.27	10.95	2.84	0.83	
15.0	8.45	0.08	57.49	10.29	2.85	0.20	
Maximum	16.87	0.30	101.45	13.08	6.00	7.27	0.12
Minimum	0.66	0.02	9.96	8.16	1.50	0.01	0.03

TABLE 4.2 Summary of chemical analysis carried out on samples of by-products, experimental diets, fish carcasses, faeces and blood

Parameters	By-products 1	Diets 1	Fish carcass 2	Faeces 3	Blood 4
Moisture	x	x	x	x	
Crude protein	x	x	x	x	
True protein	x	x			
Nonprotein nitrogen	x	x			
Amino acids	x	x			
Lipid	x	x	x	x	
Peroxide value	x	x			
Crude fibre	x	x			
Ash	x	x	x	x	
Acid insoluble ash	x	x			
Chromic oxide		x		x	
Minerals	x				
Energy	x	x			
Haematocrit					x
Haemoglobin					x

1) Average of 4 replicates
2) Average of 12 replicates
3) Average of 2-4 replicates
4) Average of 4-8 replicates

Faecal samples were dried, ground, and stored in airtight glass flasks for subsequent analysis.

I. Moisture

Preweighed samples were dried at 105°C (AOAC, 1980) in a Memmert drying oven to constant dry weight and reweighed to the nearest 0.1 mg on a Mettler H10 balance. Fish carcasses were dried for 48 hours and samples of by-products for 24 hours.

II. Protein

(a) Crude Protein

Total nitrogen was determined by the semi-microkjeldahl technique (Munro and Fleck, 1969) using a Tecator 1016 digestion unit and a Tecator 1002 distillation unit. The ammonia liberated was determined by back titration with 0.2 N HCl in a Methrom micro-burette and the normality of the acid was corrected by running an urea standard. The percentage of crude protein was calculated by multiplying the nitrogen content by the empirical value of 6.25.

(b) True protein

True protein was determined by the phenol method of Lowry et al. (1951). The optical densities of the final solutions were measured at a wavelength of 750 nm on a Kontron-Uvikon 810 spectrophotometer.

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(b) True protein

True protein was determined by the phenol method of Lowry et al. (1951). The optical densities of the final solutions were measured at a wavelength of 750 nm on a Kontron-Uvikon 810 spectrophotometer.

(c) Nonprotein nitrogen

Nonprotein nitrogen was determined by the trichloroacetic acid extraction method of Mezincescu and Szabo (1936). About 500 mg of finely ground sample was weighed to the nearest 0.01 mg. 500 cm^3 of distilled water and 50 cm^3 of 20% tricholoroacetic acid were added, shaken vigorously for 10 minutes and allowed to react for three hours in a refrigerator at 3°-5°C. Samples were then filtered through a Whatman 111 filter paper and the nitrogen content of 4 cm^3 of the resulting filtrate was determined by the Kjeldahl method.

III. Amino acids

Amino acids were assayed using an LKB Biochrom 4151 Alpha Plus amino acid analyser following hydrolysis with 5 cm^3 of 5.7N HCl (Roach et al., 1967). All essential amino acids were analysed with the exception of tryptophan.

IV. Lipid

Crude lipid was determined by the method of Bligh and Dyer (1959). However, due to the very high lipid content of some fish carcasses the more economical trichlorofluoromethane method of Korn and Macedo (1973) was adopted for these samples.

V. Peroxide value

Lipid was extracted from about 5g of finely ground sample with a 3:2 v/v mixture of acetic acid and chloroform. The peroxide value of the extracted lipid was determined by the method given by AOAC (1980).

VI. Crude fibre

Crude fibre was measured as the loss on ignition of the dried residues remaining after the digestion of samples with 0.255N H_2SO_4 followed by 0.313N NaOH (AOAC, 1980).

VII. Ash

Ash was determined by incinerating samples in porcelain crucibles at 450°C for 16 hours in a Heraeus M-110 muffle furnace (AOAC, 1980).

VIII. Acid insoluble ash

Acid insoluble ash was determined on ashed samples according to the method of Pearson (1970).

IX. Chromic oxide

Chromic oxide was measured using the wet acid oxidation method of Furukawa and Tsukahara (1966). The optical densities of the resulting solutions were measured at a wavelength of 350 nm with a Spectronic 70 spectrophotometer.

X. Minerals

Levels of Ca, Mg, Na, K, and Zn were determined using a Perkin Elmer 2280 atomic absorption spectrophotometer following digestion of samples with concentrated HNO_3 (APHA AWWA WPCF, 1985).

Phosphorus levels were measured by predigesting the samples with a mixture of concentrated HNO_3 and $HClO_4$ according to the method of Eisenreich et al. (1975). The optical densities of the resultant solutions were measured at a wavelength of 690 nm on a Kantron-Uvikon 810 spectrophotometer.

XI. Energy

Energy was measured by direct combustion in a Newham-Electronics Model AH9 micro-bomb calorimeter. This is a ballistic calorimeter with electronic ignition and is a development of the apparatus described by Phillipson (1964). 10-30 mg samples were pelleted in a bench press and weighed to the nearest 0.1 µg on a Chan electro-balance. The micro-bomb was calibrated with benzoic acid (BDH Chemicals Ltd., Poole, Dorset, England).

XII. Blood

Approximately 3 cm^3 blood samples were obtained by severing the caudal peduncule and bleeding into a 10 cm^3 heparinised vial. Blood parameters were determined immediately at the Aquaculture Station since Soivio and Nyholm (1973) reported rapid alteration of blood characteristics with time.

Haematocrit was determined using heparinised microhaematocrit tubes (Blaxhall and Daisley, 1973). Haemoglobin was determined by the cyancmethaemoglobin method of Larsen and Snieszko (1961).

4. Diet Preparation

In view of the wide range of diets used throughout this thesis, their formulation and detailed composition is considered in the relevant Chapters. The method of preparation however, is common to all and is described here. In addition to the animal by-products being evaluated as protein sources certain ingredients were common to all diets and these are summarised in Table 4.3.

Cod liver oil was used to provide a secondary source of dietary lipid and as it is a good source of essential fatty acids, particularly the ω3 series required by rainbow trout (Castell et al., 1972; Castell, 1979; Watanabe et al., 1974a, b; Takeuchi and Watanabe, 1976, 1977; Yu and Sinnhuber, 1976; Castledine and Buckley, 1980; Reinitz and Yu, 1981; Henderson and Sargent, 1985) and also of vitamins A and D (Phillips and Brockway, 1957; Castell, 1979; Watanabe, 1982). Lipid peroxidation of the diets was inhibited by the addition of the antioxidant butylated hydroxytoluene (BHT) to the cod liver oil to give a final concentration of 150 mg per Kg of diet. 0.2 percent of potassium sorbate was added to diets to inhibit fungal development (Jauncey and Ross, 1982).

A mixture of corn starch and yellow dextrin in the ratio 2:1 was used as a carbohydrate source. This mixture was employed since certain diets were formulated to certain relatively high carbohydrate levels and Spannhof and Plantikow (1983) have reported that no depression of starch digestion is observed if diets contain dextrin.

TABLE 4.3 Ingredients used in diet preparation

Corn starch	Drogaria Castilho, Porto, Portugal
Yellow dextrin	Drogaria Castilho, Porto, Portugal
Cod liver oil	Drogaria Moura, Porto, Portugal
Butylated hydroxytoluene	BDH Chemicals Ltd., Poole, Dorset, England
Vitamin premix	After the formula of Tacon and Ferns (1976)
Mineral premix	After the formula of Tacon and Beveridge (1982)
Carboxymethylcellulose disodium salt, high viscosity	BDH Chemicals Ltd., Poole, Dorset, England
Chromic oxide	BDH Chemicals Ltd., Poole, Dorset, England
Potassium sorbate	BDH Chemicals Ltd., Poole, Dorset, England

All diets contained 2% vitamin premix (Table 4.4) and over 1% mineral premix (Table 4.5). In addition 1% carboxymethylcellulose was added as a binding agent and 0.5% chromic oxide (Cr_2O_3) was used as an inert marker to determine apparent digestibility.

In order to obtain a consistent pellet with good binding properties diets were prepared using ingredients with a particle size of less than 1 mm. Thus when necessary, ingredients were ground through a 1 mm die plate on an Alexanderwerk GKM mill.

The dry ingredients, with the exception of the mineral premix, were weighed to the nearest 0.01g on a Mettler PC 4400 delta range balance and were thoroughly mixed in an Alexanderwerk GKM mixer. The minerals were mixed with 500 cm^3 of distilled water to ensure an even distribution of the mineral elements throughout the diet, and were then added with the oil and sufficient water to produce a paste. In practice approximately 200 cm^3 of water per Kilogramme of diet was added. The mixture was then passed under pressure through a 2 mm die plate on an Alexanderwerk GKM pelletiser. Cutters were set to produce 10 mm long pellets and these were dried in a convection air dryer at 35°C for 16 hours. When dry the pellets were sieved through a 1 mm sieve to remove dust and stored in airtight plastic containers away from light to inhibit vitamin loss (Reinitz, unpublished; Goldblatt et al., 1979; Slinger et al., 1979; Cowey and Sargent, 1979).

Approximately 100g samples of each diet were retained for chemical analysis.

TABLE 4.4 Vitamin premix (after Tacon and Ferns, 1976)

Vitamin	mg per Kg of diet
Thiamine HCl	.50
Riboflavin	.50
Ca pentothenate	1.00
Niacin	2.00
Pyridoxine HCl	.40
Biotin	.60
Folic acid	.15
B_{12}	.10
Inositol	20.00
Ascorbic acid	10.00
Choline chloride	40.00
Menadione (K3)	.40
ɣ -aminobenzoic acid	.50
α-tocopherol acetate	.40
Vitamin A	2000 IU
Vitamin D_3	1000 IU

TABLE 4.5 Mineral premix (after Tacon and Beveridge, 1982)

Mineral	g per Kg of diet
$MgSO_4 . 7H_2O$	5.1
KCl	2.0
NaCl	2.4
$FeSO_4 . 7H_2O$	1.0
$ZnSO_4 . 7H_2O$	0.22
$CuSO_4 . 5H_2O$	0.0314
$MnSO_4 . 4H_2O$	0.1015
$CoSO_4 . 4H_2O$	0.0191
$Ca(IO_3)_2 . 6H_2O$	0.0118
$CrCl_3 . 6H_2O$	0.051

5. Feeding Trials

After acclimatisation to the experimental system 20 fish were allocated to each net cage. Fish were anaesthetised in a 3 ppm solution of ethylenoglycolmonophenylether and individually weighed to the nearest 0.01g on a Mettler PC 4400 data range balance. Total fish weights in each cage were balanced to ensure that there were no significant differences ($P < 0.05$) at the start of each trial (Castell and Tiews, 1980).

Each experimental diet was allocated randomly to two net cages giving one replicate of each treatment and allowing statistical evaluation of the results (Phillips and Balzer, 1957; Castell and Tiews, 1980; Roberts, 1983). In each trial a further cage of 20 fish was unfed for the duration of the experimental period to assess the possible effect of natural production on growth performance.

At the start of each experimental feeding trial 20 fish were killed by a sharp blow on the head, immediately frozen and stored at -20°C for subsequent analysis of chemical composition.

Trials began the next day. The fish were fed at a fixed rate of 2% body weight per day by hand as it is considered that hand feeding optimises the food conversion ratio and growth rates (Pfeffer, 1977). The daily ration was divided into two equal portions and fed at dawn and late afternoon in accordance with the natural photo-period. Phillips (1956); Cowey (1981) and Fauconneau and Luquet (1984) consider that maximal growth rates are attained by feeding rainbow trout twice daily and Grayton and Beamish (1977) reported

that food conversion efficiency decreases when rainbow trout are fed four or more meals daily.

Fish were bulk weighed every two weeks to the nearest 5g on an Omega PP 50 balance, and daily feed levels were adjusted accordingly.

During the last month of each trial, faecal samples were collected after routine bi-weekly weighings to determine apparent digestibility. Fish were anaesthetised in a 3 ppm solution of ethylenoglycolmonophenylether, and faeces were collected by the hand stripping method of Austreng (1978a). Following stripping fish were allowed to recover fully in a bucket of well-aerated water before being returned to the net cages.

At the end of each trial fish were anaesthetised, individually weighed to the nearest 0.01g and faecal samples were again collected. Ten fish from each cage were then sacrificed, and immediately frozen for subsequent carcass analysis. The remaining fish were then sacrificed and used to collect blood samples and to determine the liver somatic index.

6. Feed Trial Analysis

I. Weight gain

Weight gain is expressed as a) the mean increase in weight as a percentage of the initial weight, and b) the mean daily increase in weight.

$$\text{a) Weight gain (\%)} = \frac{\text{Final wt. - Initial wt.}}{\text{Initial wt.}} \times 100$$

$$\text{b) Weight gain (mg/day)} = \frac{\text{Final wt.(mg) - Initial wt.(mg)}}{\text{Time (days)}}$$

II. Specific growth rate (SGR)

The specific growth rate (SGR) defines the growth rate in terms of change in weight of the fish expressed as percentage per day.

$$\text{SGR (\%/day)} = \frac{\log_e \text{Final wt.} - \log_e \text{Initial wt.}}{\text{Time (days)}} \times 100$$

III. Food intake

Food intake is expressed as the food consumption per day per fish.

$$\text{Food intake (mg/day)} = \frac{\text{Total food fed (mg)}}{\text{Time (days) x no. fish}}$$

IV. Food conversion ratio (FCR)

The food conversion ratio (FCR) is expressed as the proportion of dry food fed per unit live weight gain of fish.

$$FCR = \frac{\text{dry feed fed (g)}}{\text{live weight gain (g)}}$$

V. Protein efficiency ratio (PER)

The protein efficiency ratio (PER) gives a measure of the efficiency of dietary protein utilization by fish. PER is calculated as the live weight gain of fish per gram of crude protein fed.

$$PER = \frac{\text{live weight gain (g)}}{\text{crude protein fed (g)}}$$

VI. Nitrogen intake

The nitrogen intake is expressed as the nitrogen consumed per day per fish.

$$\text{Nitrogen intake (mg/day)} = \frac{\text{Protein intake (mg)}}{6.25}$$

VII. Nitrogen deposition

Nitrogen deposition is expressed as the nitrogen retained in the fish body per day per fish.

$$\text{Nitrogen deposition (mg/day)} = \frac{\text{Live weight gain (mg)}}{\text{Nitrogen intake (mg)}}$$

VIII. Apparent net protein utilization

Apparent net protein utilization (NPU) is expressed as the percentage of nitrogen retained in the fish body of the total nitrogen ingested.

$$\text{Apparent NPU (\%)} = \frac{\text{Nitrogen deposition}}{\text{Nitrogen intake}} \times 100$$

IX. Apparent food digestibility

Apparent digestibility coefficients were calculated using the formula of Maynard and Loosli (1969) employing an inert marker (Cr_2O_3) at a known level in the food and then measuring the nutrient level in food and faeces relative to that inert indicator.

$$\text{Apparent digestibility coefficient (\%)} = 100 - \left(100 \times \frac{\text{\% indicator in feed}}{\text{\% indicator in faeces}} \times \frac{\text{\% nutrient in faeces}}{\text{\% nutrient in feed}}\right)$$

$$\text{Apparent organic matter digestibility (\%)} =$$

$$100\left(1 - \frac{\text{wt.indicator/g dry matter in diet}}{\text{wt.indicator/g dry matter in faeces}} \times \frac{\text{1-wt.indicator/g dry matter in faeces}}{\text{1-wt.indicator/g dry matter in diet}}\right)$$

X. Liver somatic index (LSI)

The liver somatic index (LSI) is expressed as a ratio between liver weight and live fish weight.

$$\text{LSI} = \frac{\text{liver weight (g)}}{\text{live fish weight (g)}} \times 100$$

XI. Statistical analysis

Statistical evaluation of the data was carried out by analysis of variance and mean differences were determined using the Duncan's Multiple Range Test (Duncan, 1955). Standard errors (± SE) and standard deviation (± SD) were also calculated to identify the range of means.

XII. Graphics

Graphs were produced using a Sinclair QL computer and an Epson FX80 + printer.

CHAPTER 5

THE NUTRITIONAL EVALUATION OF POULTRY BY-PRODUCT AND HYDROLYSED FEATHER MEAL AS A FEED FOR RAINBOW TROUT

5.1 INTRODUCTION

A number of poultry by-products have been used successfully in fish feeds, in some cases at relatively high dietary inclusion levels. The range of products available and their use in diets for rainbow trout was reviewed in Chapter 2.

Poultry by-products and hydrolysed feather meal consists of a mixture of feathers, offals, feet, blood, necks and heads in their naturally occurring proportions. Production of this by-product can be by one of two methods. The feathers can be processed separately and then blended with the poultry by-product meal, or alternatively all of the waste products can be processed together. In the latter case a meal with a lower protein quality is obtained (Burgos et al., 1974). In Portugal the two poultry by-products are processed together.

Poultry by-product and hydrolysed feather meal is the commonest by-product from Portuguese chicken slaughterhouses with a production cost 19% lower than that of Portuguese fish meals. The production of this product by one of the principle slaughterhouse plants in Portugal amounts to 3,000 tons per year (manager, personal communication). The total production in Portugal as a whole is unknown, but there are at least 100 plants with a similar capacity scattered all over the country although most are concentrated in the North.

To date this commodity has not been evaluated in diets for fish. In view of the availability, relatively low cost and promising

nutritional quality of poultry by-product and hydrolysed feather meal its potential as a protein source in diets for rainbow trout trout was evaluated.

5.2 MATERIALS AND METHODS

5.2.1 Diets

25 Kilogrammes of poultry by-product and hydrolysed feather meal (PBHFm) were obtained from the Soaves, Pomarelho, Guimarães, chicken slaughterhouse processing plant located in the North of Portugal (Fig. 9.1). The meal was produced on the day following slaughter from feathers, offals, feet, blood, necks and heads in their naturally occurring proportions. 25 Kilogrammes of brown fish meal were obtained from Olfaixe-Produtos de Óleos e Farinhas de Peixe, Ltd., Portas Fronhas, Póvoa do Varzim. This was produced mainly from the scraps of the sardine canning industry. On arrival at the laboratory the PBHFm and the brown fish meal were immediately ground and their crude protein and lipid content were determined according to the methods given in Chapter 4.

Seven experimental diets were formulated using the PBHFm and brown fish meal as the principle protein sources. The PBHFm contained 60.16% crude protein and 23.80% lipid and was included in the diets at levels of up to 100% of the protein, replacing brown fish meal (53.96% crude protein and 9.80% lipid). Thus the protein component of the diets consisted of:

Diet 1: control, 100% brown fish meal protein

Diet 2: 20% PBHFm protein + 80% brown fish meal protein

Diet 3: 40% PBHFm protein + 60% brown fish meal protein

Diet 4: 60% PBHFm protein + 40% brown fish meal protein

Diet 5: 80% PBHFm protein + 20% brown fish meal protein

Diet 6: 90% PBHFm protein + 10% brown fish meal protein

Diet 7: 100% PBHFm protein

The full dietary formulations are presented in Table 5.1. All seven diets were formulated on an isonitrogenous and isocaloric basis to contain 40% crude protein and 17% lipid (Table 5.3). Seven Kilogrammes of each diet were manufactured as described in Chapter 4.

Formulation of the diets to contain a high lipid level was necessary due to the high lipid content of the PBHFm (23.80%). However, diets did contain sufficient fish oil to supply the necessary essential fatty acids. In addition, due to the lipid sparing effect on dietary protein the crude protein content of diets was fixed at 40% (Satia, 1974; Halver, 1976; Gulbrandsen and Utne, 1977; Reinitz et al., 1978a, b; Takeuchi et al., 1978a, b, d, 1981; Austreng, 1979; Buckley and Groves, 1979; Watanabe et al., 1979; Rychly, 1980; Millikin, 1982; Watanabe, 1982). These authors reported that the percentage of dietary protein can be lowered to 35%-40% if the level of dietary lipid is as high as 15%-20%. Furthermore improved growth rates and feed conversion efficiencies have been reported for rainbow trout fed this combination of protein and lipid levels (Takeuchi et al., 1978a, b; Austreng, 1979; Watanabe et al., 1979; Watanabe, 1982).

The percentage of dietary carbohydrate in the diets varied from 4.5% to 16.5% and 2.3% to 8.2% for corn starch and yellow dextrin respectively (Table 5.1). Thus the overall carbohydrate content of Diets 5, 6 and 7 was slightly higher than the recommended maximum

Diet 6: 90% PBHFm protein + 10% brown fish meal protein

Diet 7: 100% PBHFm protein

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TABLE 5.1 Formulation of the experimental diets (% by weight)

Diet No.	1	2	3	4	5	6	7
% inclusion PBHFm protein	0%	20%	40%	60%	80%	90%	100%
Brown Fish meal	79.10	63.20	47.40	31.60	15.80	7.90	-
PBHFm	-	14.10	28.10	42.20	56.20	63.20	70.20
Corn starch	4.50	6.90	9.30	11.70	14.10	15.30	16.50
Yellow dextrin	2.30	3.50	4.70	5.80	7.00	7.60	8.20
Cod liver oil[1]	9.31	7.51	5.71	3.91	2.11	1.21	0.31
Vitamin premix[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[3]	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Binder[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Potassium sorbate	0.20	0.20	0.20	0.20	0.20	0.20	0.20

1 Containing 150 mg/Kg diet of butylated hydroxytoluene (BDH Chemicals Ltd., Poole, Dorset, England)

2 According to Table 4.4

3 According to Table 4.5

4 Carboxymethylcellulose, dissodium salt, high viscosity (BDH Chemicals Ltd., Poole, Dorset, England)

level of 20% (Phillips et al., 1948; Hilton and Dixon, 1982; Spannhof and Plantikow, 1983). Nevertheless Edwards et al. (1977) and Hilton et al. (1982) reported that rainbow trout can tolerate carbohydrate levels of up to 25% without any adverse effects on growth performance and Bergot (1979) considered that up to 30% is acceptable.

The full chemical analysis carried out on both the foodstuffs and the diets is summarised in Table 4.2 and the methods are described in Chapter 4.

5.2.2 Growth Trial

300 rainbow trout of mean weight 35g (34.64g-35.88g; Appendix I) were obtained from the Truturão fish farm at Cernache, Coimbra, and were allocated to 15 net cages as described in Chapter 4. Each experimental diet was allocated randomly to two net cages and a further cage of 20 fish was unfed for the duration of the experimental period. Fish were fed at a fixed rate of 2% body weight per day throughout the 18 week trial. The full protocol of the trial is described in Chapter 4. In addition, the glucose in plasma was determined by the o-toluidine method of Hyvarinen and Nikkila (1962). Furthermore, at the end of the trial, a histological examination was carried out on four fish from each treatment to determine whether there were differences in tissue structure between fish fed the experimental diets. Gills, liver, spleen, and kidney were removed and immediately fixed in Bouin's solution. Tissue slices were embedded in paraffin wax, 5 µm sections were cut and these were stained with haematoxylin and eosin and examined under light microscopy. Haemoglobin was not determined.

The mean water temperature during the experimental period was 20.2°C (11°-23°C ± 3.42) and the daily variation never exceeded 2°C (Table 5.2).

Statistical methods and the production of graphs were carried out as described in Chapter 4.

TABLE 5.2 Water temperature (°C) and standard deviation over the feeding period

Weeks	May	June	July	August	September	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0	11.0					11.0	0.00		
1		11.5							
2		17.0							
3		23.0				18.5	4.58		
4		19.0							
5		22.0							
6			22.0						
7			22.0						
8			22.5			22.0	0.35	20.2	3.42
9			22.0						
10			21.5						
11				21.0					
12				21.5					
13				21.5		21.4	0.22		
14				21.5					
15				21.5					
16					21.5				
17					21.0	21.2	0.29		
18					21.0				

5.3 RESULTS

5.3.1 Diets

The crude protein content of the PBHFm was 60.16% (Table 5.3) which is within the normal range of reported values for poultry by-products (56%-76%; Table 2.9). Two slightly different values were obtained for the nonprotein nitrogen component. Subtraction of true protein determined by the Lowry method from crude protein gave a nonprotein value of 7.26%. However, the direct approach of precipitating nonprotein nitrogen using trichloroacetic acid gave a value of 9.73% (Table 5.3). The discrepancy in results was possibly due to the use of 6.25 as the Kjeldahl factor which may not have been appropriate since it is known that the nitrogen content of protein from different sources is not the same (Jobling, 1983). Tacon (1979a) reported that a variation of between 12% and 19% is possible.

The brown fish meal had a crude protein content of only 53.96% which is significantly lower than the value of 60% which is considered to be the minimum protein content of a good quality fish meal (Windsor and Barlow, 1981). The nonprotein nitrogen component calculated from the difference between crude protein and true protein was 2.46% which again is slightly different from the value of 1.60% determined using the direct approach. Spinelli and Dassow (1979) reported values of 0.5% and 1% for nonprotein nitrogen in fish muscles and Jobling (1983) reported a value of 2% for the nonprotein nitrogen content of fish skeletal muscle.

TABLE 5.3 Proximate composition, Cr_2O_3 and energy content of Brown fish meal, Poultry by-product and hydrolysed feather meal (PBHFm) and experimental diets (% dry weight)

Nutrient content (% dry weight)	Products		Dietary treatments						
	Brown fish meal	PBHFm	1	2	3	4	5	6	7
Moisture (%)	12.51(0.23)[1]	11.16(0.05)	4.32(0.08)	4.24(0.01)	4.69(0.03)	4.46(0.02)	4.01(0.08)	4.01(0.05)	4.59(0.02)
Crude protein	53.96(0.17)	60.16(0.21)	40.83(0.55)	40.95(0.71)	41.25(0.29)	40.87(0.86)	41.69(0.42)	40.81(0.18)	40.58(0.17)
True protein (%)	51.50(0.51)	52.90(0.32)	40.74(0.71)	39.91(0.83)	39.18(0.27)	38.61(0.75)	36.50(0.57)	35.55(0.49)	33.99(0.52)
Nonprotein nitrogen (%)	1.60(0.21)	9.73(0.35)	1.26(0.47)	1.73(0.31)	2.39(0.25)	3.58(0.17)	4.51(0.23)	5.83(0.31)	6.57(0.24)
Lipid (%)	9.80(0.21)	23.80(0.15)	17.67(0.05)	17.65(0.06)	17.83(0.02)	17.81(0.02)	17.69(0.03)	17.72(0.06)	17.70(0.06)
Ash (%)	12.51(0.21)	11.26(0.15)	15.95(1.05)	19.46(0.12)	18.28(0.15)	15.37(0.19)	14.23(0.11)	9.30(0.16)	6.14(0.03)
Nitrogen free extract[2](%)	11.49	-	19.33	16.24	16.73	19.18	21.87	26.27	29.07
Peroxide value (mEq/Kg oil)	1.51(0.08)	1.87(0.21)	2.64(0.02)	2.37(0.00)	2.08(0.02)	1.20(0.01)	1.56(0.03)	1.82(0.08)	1.88(0.21)
Acid insoluble ash (%)	0.75(0.36)	0.45(0.01)	0.80(0.07)	0.78 (0.15)	0.76(0.03)	0.67(0.02)	0.51(0.13)	0.38(0.04)	0.75(0.24)
Crude fibre (%)	0.59(0.22)	0.71(0.51)	0.73(0.21)	0.77(0.21)	0.90(0.25)	0.99(0.40)	1.19(0.31)	1.32(0.31)	1.94(0.42)
Cr_2O_3 (%)	-	-	0.47(0.01)	0.49(0.01)	0.49(0.01)	0.50(0.01)	0.51(0.01)	0.52(0.00)	0.41(0.01)
Energy-ash free (Kcal/g)	6.81(0.40)	6.03(0.33)	5.78(0.21)	5.78(0.23)	5.64(0.14)	5.57(0.30)	5.56(0.07)	5.41(0.19)	5.47(0.08)

[1] Standard deviation

[2] Nitrogen free extract = 100-(% moisture + % true protein + % nonprotein nitrogen + % lipid + % ash + % crude fibre)

The amino acid profile shows that the PBHFm tested was a good source of glycine, proline, glutamic acid and aspartic acid and of the essential amino acid isoleucine, but a poor source of alanine and cystine and of the essential amino acids leucine and phenylalanine. Leucine was its first limiting amino acid (Table 5.4). The cystine content of the PBHFm was also relatively low, and since this amino acid is normally present at high levels in feather meals (Burgos et al., 1974; Bielorai et al., 1982; Tacon, 1982a), it indicates that this component was included at a low level. The amino acid profile of the brown fish meal indicates that it was a good source of certain nonessential amino acids including both proline and glycine, but a poor source of the essential amino acids leucine, lysine, and phenylalanine. Leucine was its first limiting amino acid (Table 5.4). The sum of the analysed amino acids in fish meal did not equal the true protein content and therefore 6.24% of amino acids were not accounted for. The sum of the analysed amino acids in the PBHFm was close to the true protein content of 52.90%.

The PBHFm had a high lipid content (23.80%; Table 5.3) and a high ash content (11.26%), although both values are within the normal ranges reported for other poultry by-products. The lipid and ash content of the brown fish meal, 9.80% and 12.51% respectively, were also within the normal range of reported values.

The peroxide value, acid insoluble ash, and crude fibre levels of both meals were all very low (< 2 mEq/Kg oil and 1% respectively, (Table 5.3).

TABLE 5.4 Amino acid profile of Brown fish meal and Poultry by-product and hydrolysed feather meal (PBHFm), experimental diets and amino acid requirement of rainbow trout (g/100g dry weight)

Amino acid (g/100g dry wt)	Fish meal	PBHFm	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Requirement of rainbow trout (% diet)
Arginine	1.68	1.63	1.27	1.24	1.22	1.17	1.16	1.12	1.10	1.40, 40% (Ogino, 1980)
Histidine	2.33	2.33	1.76	1.73	1.71	1.65	1.65	1.60	1.57	0.64, 40% (Ogino, 1980)
Isoleucine	3.12	3.95	2.36	2.43	2.51	2.55	2.67	2.64	2.66	0.96, 40% (Ogino, 1980)
Leucine	1.20	1.25	0.91	0.90	0.89	0.87	0.87	0.85	0.84	1.76, 40% (Ogino, 1980)
Lysine	1.68	2.98	1.27	1.42	1.59	1.72	1.91	1.95	2.01	2.12, 40% (Ogino, 1980)
Methionine	1.62	1.70	1.22	1.21	1.21	1.18	1.19	1.16	1.15	0.55-0.75, 35% Rumsey et al. 1983)
Phenylalanine	0.74	0.80	0.56	0.56	0.56	0.55	0.55	0.54	0.54	1.24, 40% (Ogino, 1980)
Threonine	2.34	2.89	1.74	1.81	1.86	1.89	1.96	1.94	1.95	1.36, 40% (Ogino, 1980)
Valine	1.48	1.74	1.12	1.14	1.16	1.16	1.18	1.17	1.17	1.24, 40% (Ogino, 1980)
Alanine	0.47	1.17	0.35	0.44	0.53	0.61	0.72	0.74	0.79	
Aspartic acid	4.19	4.24	3.17	3.12	3.08	2.99	3.00	2.91	2.86	
Cystine	0.64	0.40	0.48	0.44	0.40	0.35	0.32	0.29	0.27	0.30, 35% (Rumsey et al. 1983)
Glutamic acid	4.86	5.08	3.67	3.64	3.62	3.54	3.57	3.47	3.43	
Glycine	7.13	7.97	5.39	5.41	5.45	5.41	5.52	5.40	5.38	
Proline	7.26	7.23	5.49	5.39	5.31	5.15	5.13	4.96	4.88	
Serine	3.47	3.70	2.62	2.60	2.60	2.56	2.59	2.48	2.49	
Tyrosine	1.05	1.25	0.79	0.81	0.82	0.83	0.85	0.84	0.84	0.84, 40% (Ogino, 1980)
TOTAL	45.26	50.31	34.17	34.29	34.52	34.18	34.84	34.06	33.93	

* Percentage of crude protein in the diet

The PBHFm and the brown fish meal were both a good source of all the minerals analysed, in particular K, Ca, and Zn for the former and Ca and Na for the latter (Table 5.5).

The experimental diets had a crude protein content varying between 40.58% and 41.69% which is close to the formulated level of 40% (Table 5.3). However, due to the relatively high nonprotein nitrogen content of the PBHFm, the true protein content of the diets decreased as the PBHFm dietary inclusion level increased. Thus, Diet 1 (fish meal control) had a true protein content of 40.74% while Diet 7 (100% protein replacement) had a true protein content of only 33.99% (Table 5.3). All experimental diets had to a greater or lesser extent essential amino acid deficiencies. Thus, Diets 1 to 4 were slightly deficient in both the essential amino acids arginine and valine and significantly deficient in leucine, lysine and phenylalanine. Diets 5 to 7 were deficient in both leucine and phenylalanine and slightly deficient in arginine, lysine, and valine (Table 5.4). Arginine, leucine, and phenylalanine deficiencies increased with increasing inclusion levels of PBHFm while the converse applied for lysine and valine.

The lipid content of the experimental diets varied between 17.65% and 17.83% and were thus close to the formulated value of 17% (Table 5.3). The energy content of the diets varied between 5.41 and 5.78 Kcalories per gramme dry weight (Table 5.3).

TABLE 5.5 Concentration of mineral elements in Brown fish meal and Poultry by-product and hydrolysed feather meal (BPHFm) and mineral requirement of rainbow trout

Element	Brown fish meal	PBHFm	Requirement of rainbow trout (mg/100g dry wt.)
Ca (g/100g)	5.58	5.89	650-750 (Ogino and Takeda, 1978)
Mg (g/100g)	0.15	0.15	50-70 (Ogino *et al*., 1978) (Knox *et al*., 1981)
K (g/100g)	0.57	3.47	160 (Frenzel and Pfeffer, 1982)
Na (g/100g)	1.39	1.28	220 (Frenzel and Pfeffer, 1982)
P (g/100g)	1.19	1.39	700-800 (Ogino and Takeda, 1978) 650 (Nose and Arai, 1979)
Zn (mg/100g)	6.11	19.44	1.5-3.0 (Ogino and Yang,1978)

5.3.2 Growth Response and Feed Utilization Efficiency

Fish accepted the experimental diets readily within a few days and thereafter they were consumed quite aggressively. Hence no palatability problems were encountered even by fish fed Diet 7 in which all the brown fish meal was replaced by PBHFm. A slight loss of appetite was noted during weeks 6 to 10 which was thought to be due to high water temperatures (21.5° to 22.5°C) experienced at this time (Table 5.2). Nevertheless the daily rations were all consumed although the fish took longer to finish them.

At the start of the trial there were no significant ($P < 0.05$) differences in mean fish weights of around 35g (34.47g-35.88g) between treatments (Table 5.6). However, by the end of the 18 week growth trial the mean fish weights varied between 62.39g for fish fed Diet 1 and 136.60g for those fed Diet 6 (Table 5.6; Fig. 5.1). Thus, the overall weight gain of the fish fed the diet where 90% of the brown fish meal was replaced by PBHFm was more than twice that of the fish fed the diet based on brown fish meal alone. There was a trend of improved growth with increasing levels of PBHFm, with maximum weight gain achieved by the fish fed Diet 6 (Table 5.6; Fig. 5.2). Although the final weight of fish fed Diet 7 (127.35g) was slightly lower than that of fish fed Diet 6 it was still comparable with those of fish fed Diets 4 and 5 (118.47g and 121.38g, respectively).

The mortality rate of fish fed the seven experimental diets ranged from 5% to 20% (Table 5.7). No correlation was found between mortality rate and dietary treatment. The mortality rate of the unfed

TABLE 5.6 Growth performance, feed utilization efficiency, liver somatic index, and blood parameters of rainbow trout (280 fish, 35g) fed the experimental diets after 18 weeks

Mean values	Dietary treatments							± S.E.[1]
	1	2	3	4	5	6	7	
Mean initial weight (g)	34.68[a]	35.53[a]	34.64[a]	34.47[a]	35.33[a]	34.81[a]	35.88[a]	0.761
Mean final weight (g)	62.39[a]	79.65[b]	89.85[bc]	118.47[d]	121.38[d]	136.60[e]	127.35[d]	4.952
Weight gain (%)	79.92	124.19	159.63	243.65	243.48	292.45	255.11	
Specific growth rate (%/day)	0.47	0.64	0.76	0.99	0.99	1.09	1.02	
Food intake (mg/day)	1032	1070	1149	1300	1352	1424	1398	
Protein intake (mg/day)	421	438	474	531	564	581	567	
Weight gain (mg/day)	222	353	442	672	689	815	732	
Food conversion ratio	4.64	3.03	2.59	1.93	1.96	1.74	1.91	
Protein efficiency ratio	0.53	0.81	0.93	1.26	1.22	1.40	1.29	
Nitrogen intake (mg/day)	67.36	70.08	75.84	84.96	90.24	92.96	90.72	
Nitrogen deposition (mg/day)	3.30	5.04	5.83	7.91	7.64	8.77	8.07	
Apparent net protein utilization (%)	4.90	7.18	7.69	8.51	7.64	9.43	8.07	
Apparent protein digestibility (%)	83.67[bc]	81.22[a]	83.03[b]	84.46[bcd]	85.83[d]	85.26[cd]	85.50[d]	0.565
Apparent lipid digestibility (%)	74.73[bc]	71.14[a]	74.48[b]	76.21[d]	76.46[de]	77.13[e]	79.96[f]	0.270
Apparent organic matter digestibility (%)	57.76[a]	59.68[ab]	60.89[bc]	67.62[f]	67.50[e]	64.32[de]	63.33[cd]	0.941
Liver somatic index	1.01[a]	1.07[a]	1.09[a]	1.07[a]	1.06[a]	1.13[a]	1.23[b]	0.028
Haematocrit (%)	27.50[a]	31.76[ab]	34.82[b]	35.38[b]	36.07[b]	35.44[b]	34.82[b]	2.333
Glucose in plasma (mg/100 cm^3)	75.33[a]	95.57[b]	98.76[b]	101.54[b]	91.94[b]	98.54[b]	97.44[b]	5.128

[1] Standard error; calculated from residual mean square in the analysis of variance

abcdef Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

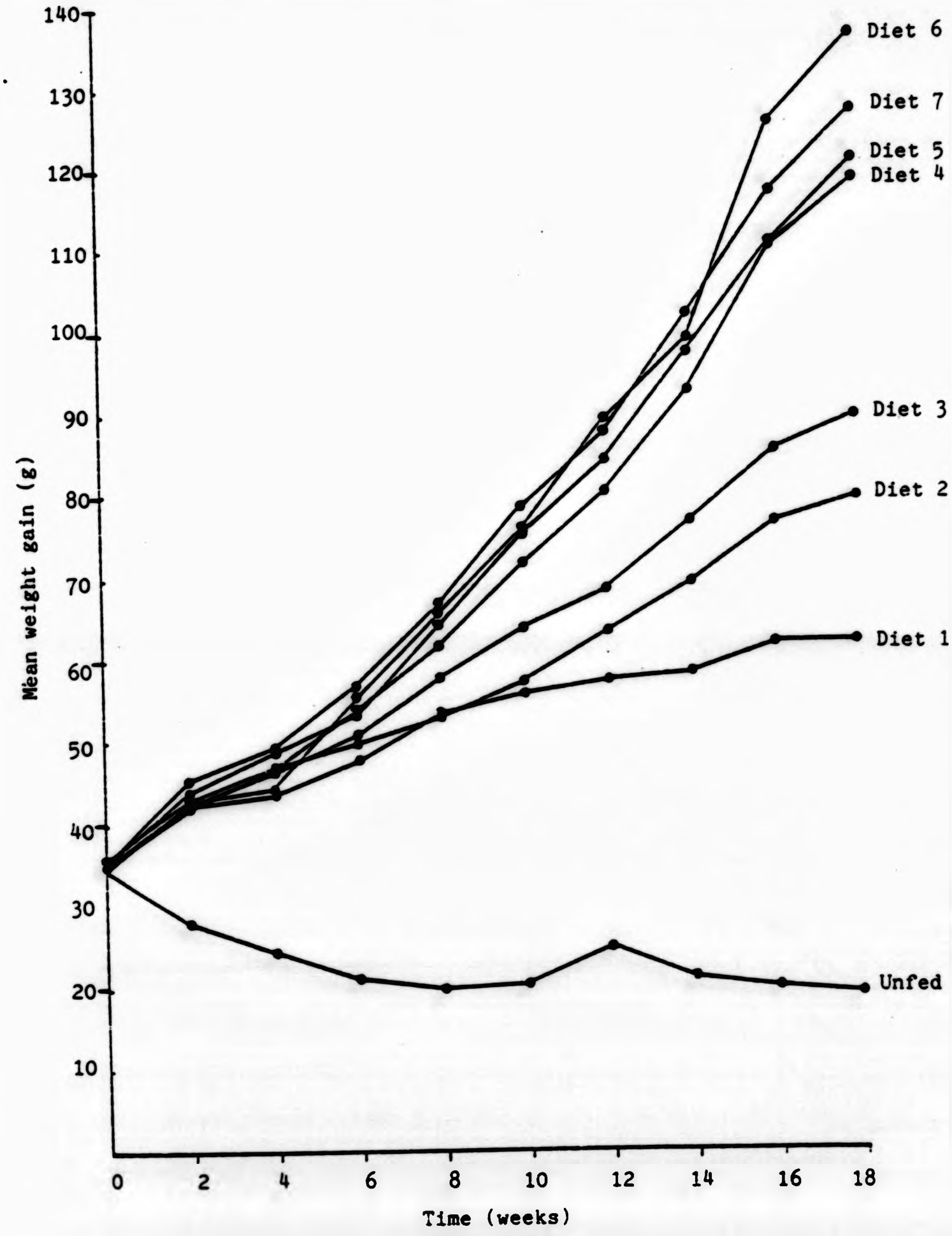

FIGURE 5.1 Overall mean weight gain (g) of rainbow trout at successive fortnightly intervals over the experimental test period

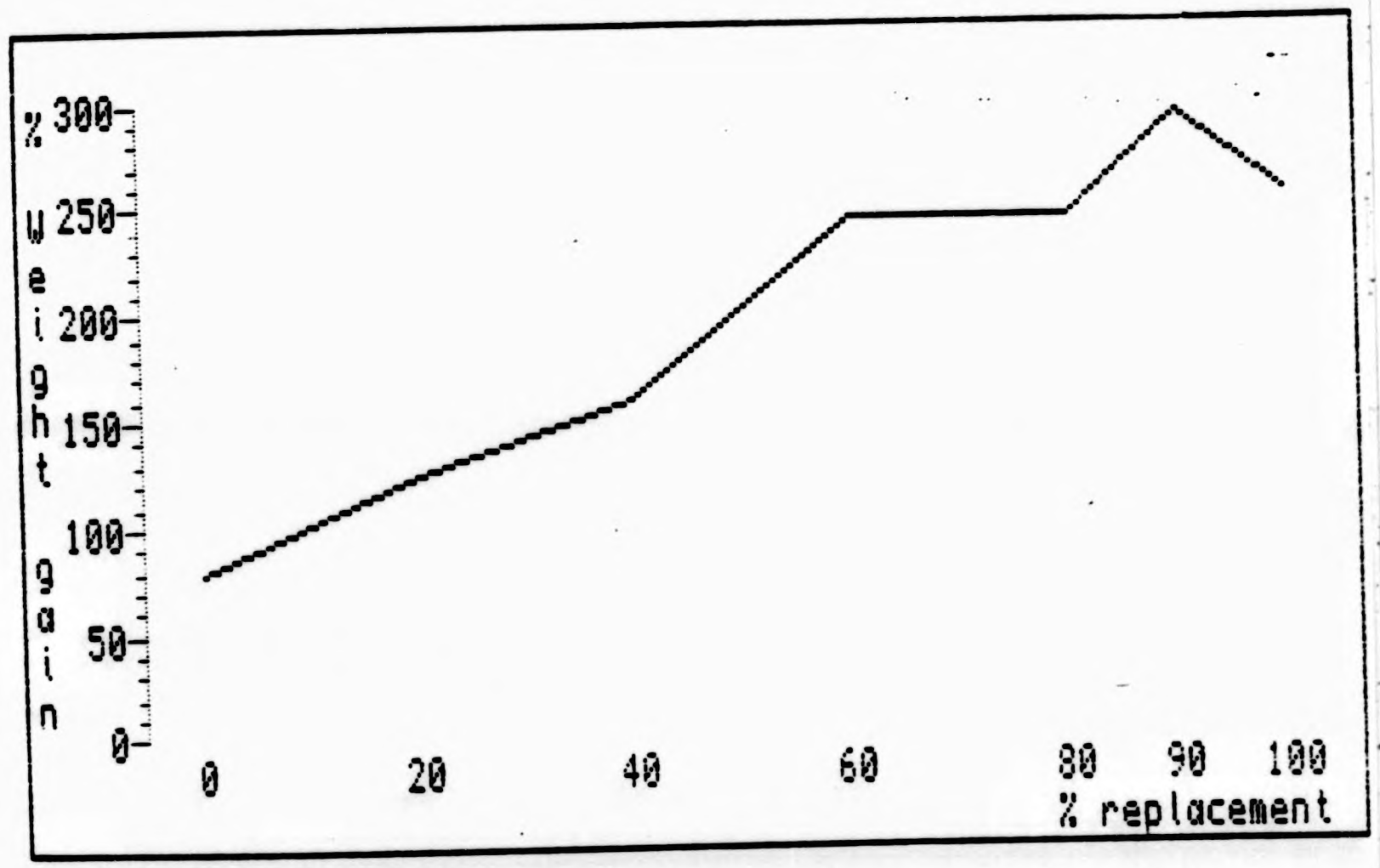

FIGURE 5.2 Overall mean weight gain (%) of fish fed diets containing increasing levels of Poultry by-product and hydrolysed feather meal as a replacement for Brown fish meal

TABLE 5.7 Weekly deaths per net cage and overall percentage mortality over the 18 week period

Treatments	1		2		3		4		5		6		7		No feeding
Replicates	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
0 week				3											1
2				1				1		1					1
4						1					1				3
6	1									1		1		1	
8	1	1						1		1			1	1	3
10			1									1			4
12							1					1		1	
14		1			1				1		1				1
16						1			1						
18															
TOTAL	2	2	1	4	1	2	1	2	2	3	2	3	1	3	13
%	10	10	5	20	5	10	5	10	10	15	10	15	5	15	65

fish, however, was 65%. Furthermore the mean weight of the survivors was reduced by almost half to 19.15g at the end of the trial (Appendix II; Fig. 5.1), thus demonstrating that any available natural food would have had a negligible effect on the overall growth performance of the fish fed the experimental rations.

The specific growth rate increased with increasing levels of PBHFm up to a maximum rate of 1.09% per day for fish fed the diet in which 90% brown fish meal protein was replaced by PBHFm, which was 2.5 times higher than that of fish fed the control (0.47% per day; Table 5.6; Fig. 5.3). Thus the fish fed the diet where 90% of the brown fish meal protein had been replaced by PBHFm displayed the best growth performance in terms of mean body weight, percentage weight gain, and specific growth rate.

The food conversion ratio decreased from 4.64 in the control diet to 1.29 to 100% protein replacement (Table 5.6; Fig. 5.4). The slight loss of appetite observed during weeks 6 to 10 did not affect the food conversion ratio.

A better assessment of the nutritional quality of the diets is calculated as the efficiency with which dietary protein is utilized by the fish. Protein efficiency ratio (PER) gives the weight of fish produced per unit of dietary protein. The PER of fish fed Diet 6 containing 90% of the protein as PBHFm was 2.6 times higher than that of the fish meal control (Diet 1; Table 5.6; Fig. 5.5). However, PER does not take into account variation in carcass composition, thus a more accurate assessment of protein utilization is

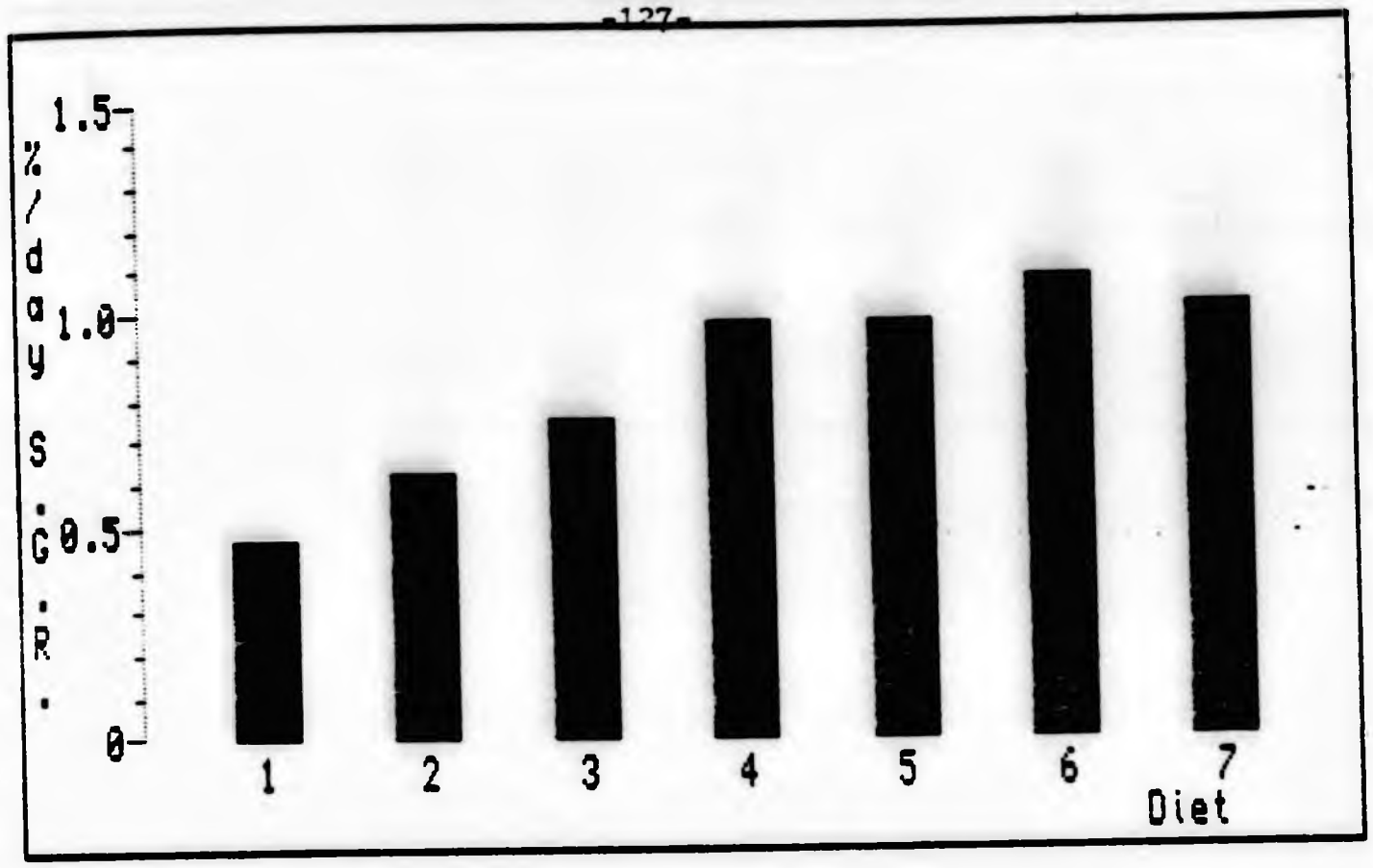

FIGURE 5.3 Specific growth rate (%/day) of fish fed the seven experimental diets

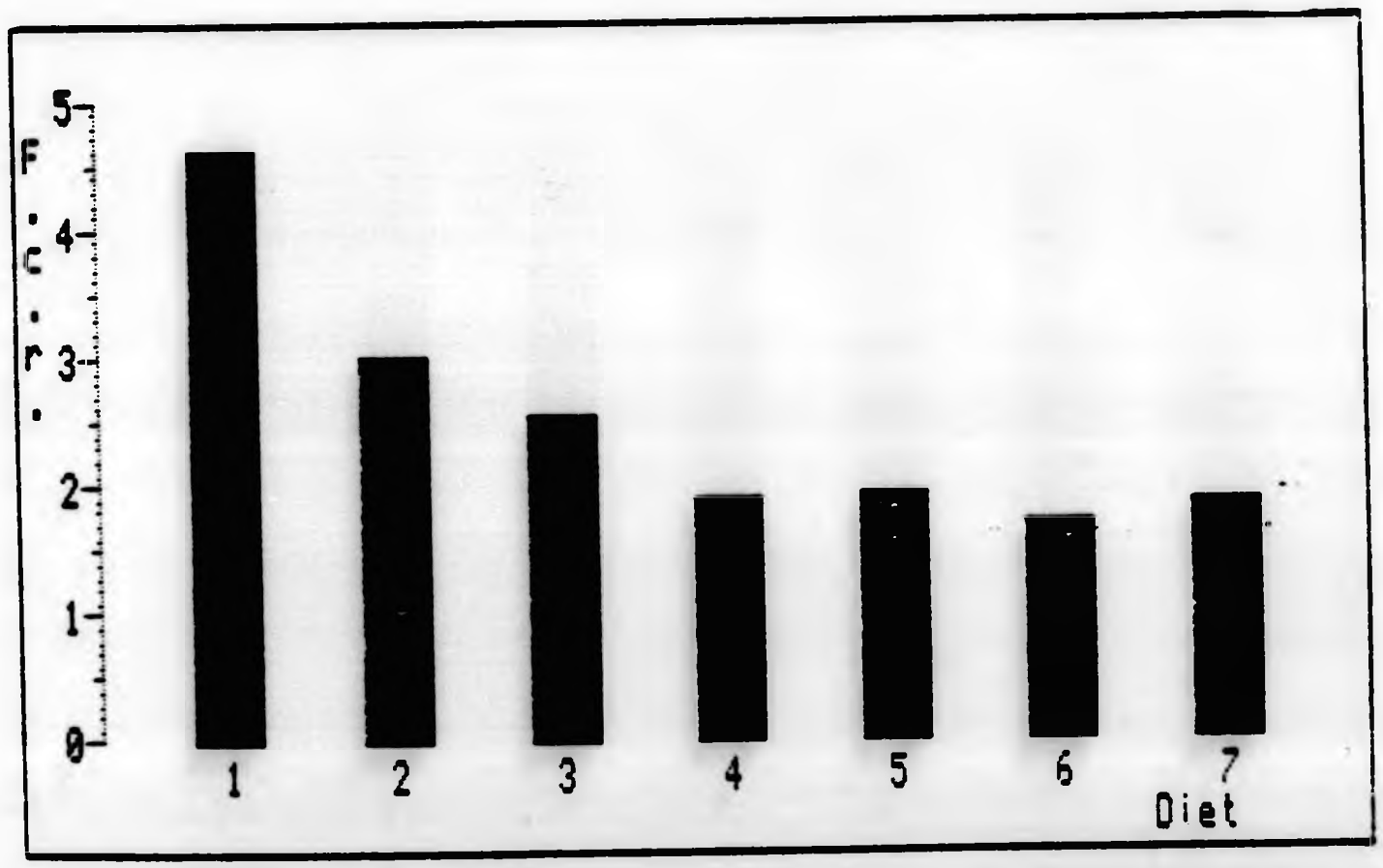

FIGURE 5.4 Food conversion ratio of fish fed the seven experimental diets

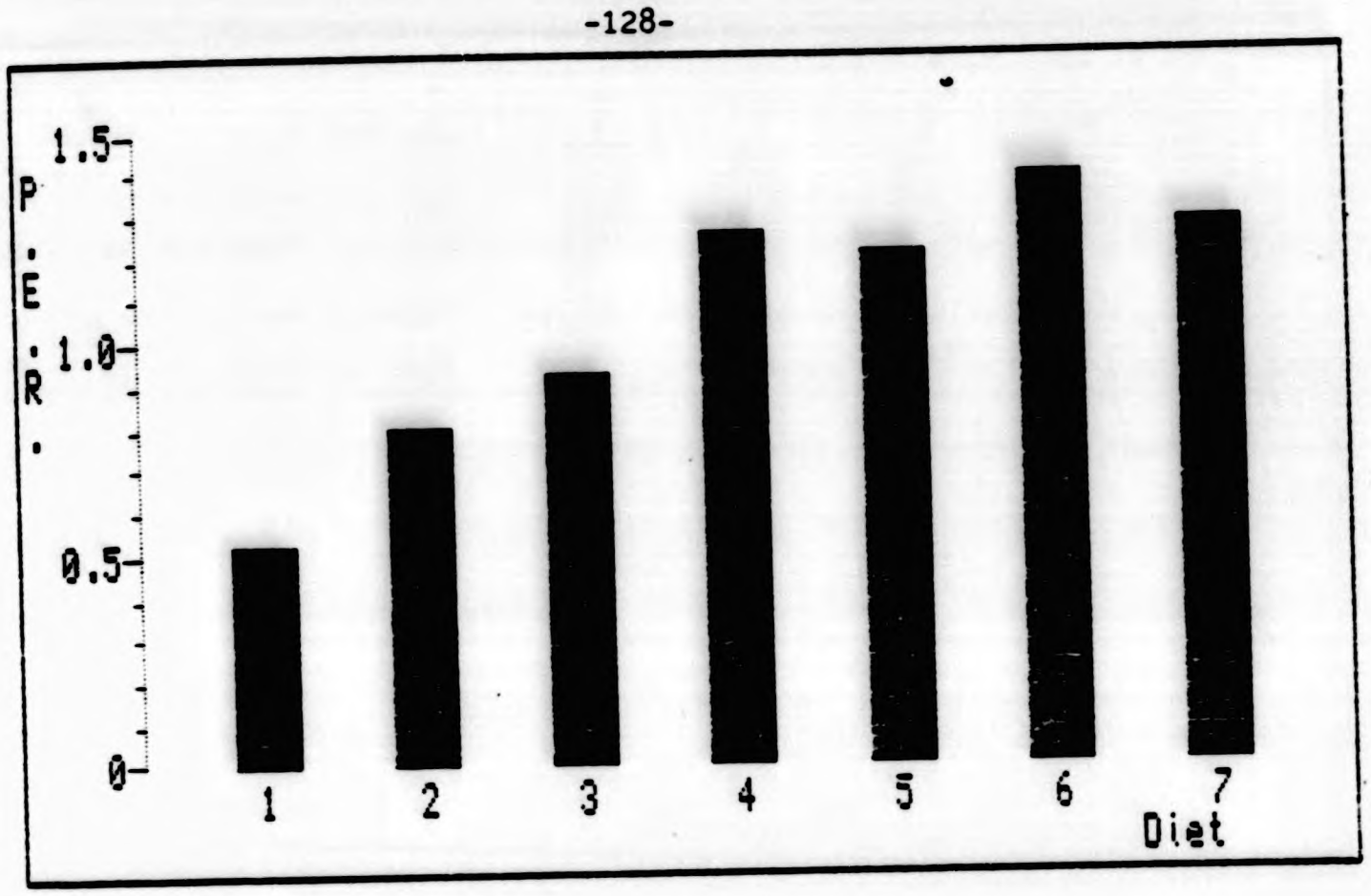

FIGURE 5.5 Protein efficiency ratio of fish fed the seven experimental diets

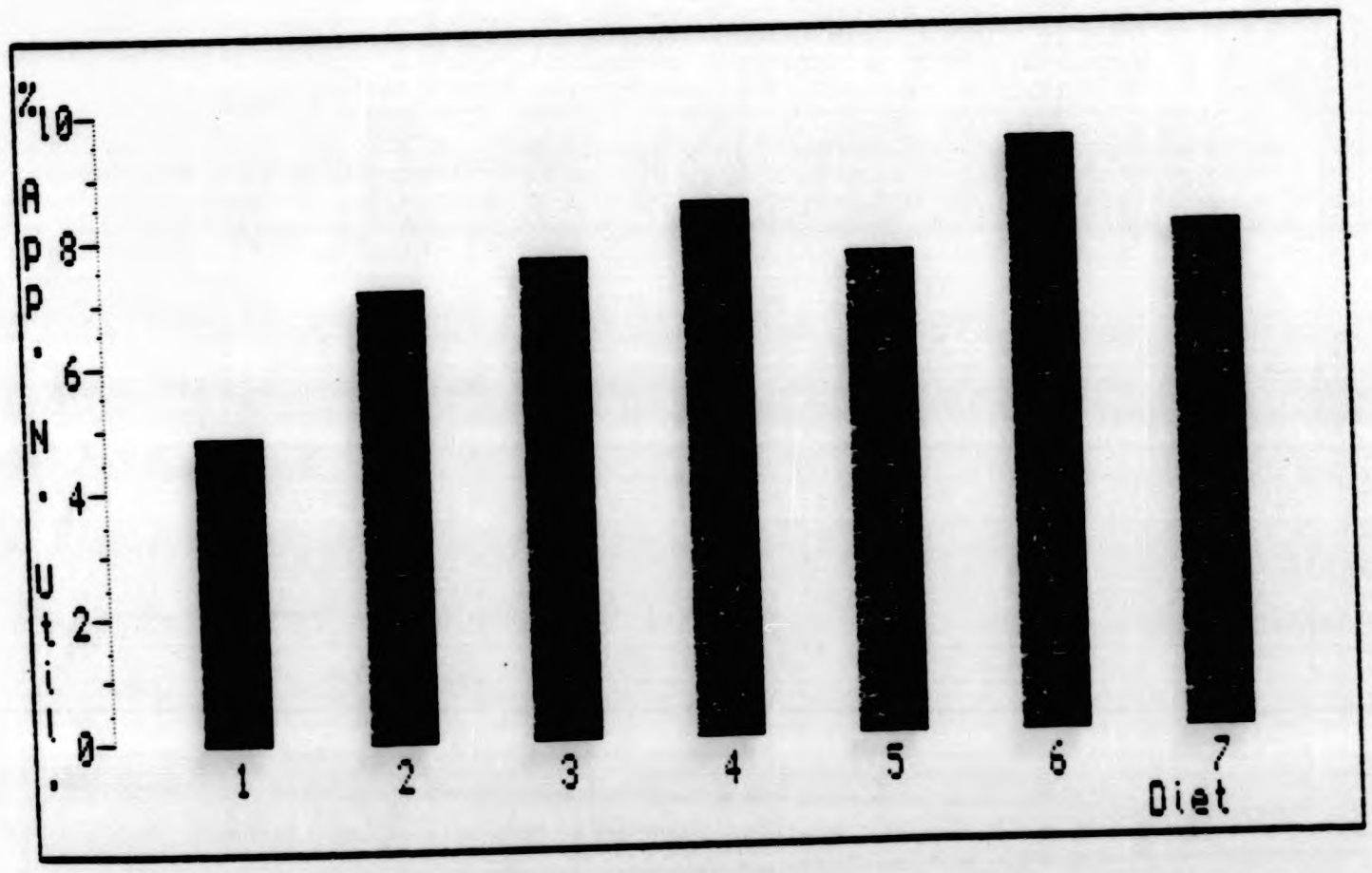

FIGURE 5.6 Apparent net protein utilization (%) of fish fed the seven experimental diets

given by apparent net protein utilization (apparent NPU). Apparent NPU increased two-fold from 4.90% for fish fed the fish meal control to 9.43% for fish fed Diet 6 (Table 5.6; Fig. 5.6). Nitrogen intake and nitrogen deposition followed the same trend as apparent NPU.

The growth performance of rainbow trout replicates fed the experimental diets is given in Appendix I.

5.3.3 Digestibility

The moisture content of faeces increased with increasing inclusion levels of PBHFm from 9.41% for fish fed Diet 2 to 14.41% for fish fed Diet 7 (Table 5.8; Fig. 5.7a). The moisture content of faeces from fish fed the fish meal control diet was 10.49% (Table 5.8; Fig. 5.7a).

As the dietary inclusion level of PBHFm increased there was a small decrease in the crude protein content of the faeces from 18.34% (Diet 2) to 16.18% (Diet 7). By contrast faeces from fish fed the control diet had a crude protein content of only 15.70% (Table 5.8; Fig. 5.7b). Differences in protein content were reflected in the apparent protein digestibility of the experimental diets. Thus, the apparent protein digestibility increased with increasing inclusion levels of PBHFm from 81.22% for 20% inclusion to 85.83% for 80% inclusion and then slightly decreased to 85.50% for 100% inclusion (Table 5.6). Fish fed the control diet had a coefficient similar to that of those fed Diet 3 (83.67% and 83.03%, respectively).

TABLE 5.8 Proximate composition and Cr_2O_3 content of faeces taken from rainbow trout after 18 weeks on the experimental diets (% dry weight)

Faeces composition (% dry wt.)	Dietary treatments 1	2	3	4	5	6	7
Moisture (%)	10.49(0.05)[1]	9.41(0.06)	9.91(0.07)	11.50(0.04)	13.19(0.11)	13.94(0.08)	14.41(0.07)
Crude protein (N x 6.25)	15.70(0.06)	18.34(0.22)	17.62(1.11)	19.15(1.83)	18.17(1.05)	16.66(0.89)	16.18(1.55)
Lipid (%)	10.55(0.41)	12.16(0.51)	12.01(0.31)	12.91(0.89)	12.82(1.01)	11.43(0.35)	12.35(0.53)
Ash (%)	42.62(0.33)	43.28(0.51)	38.19(0.23)	39.05(0.71)	34.92(0.47)	26.83(0.52)	18.61(0.81)
Cr_2O_3(%)	1.11(0.03)	1.17(0.01)	1.24(0.02)	1.52(0.04)	1.57(0.03)	1.46(0.03)	1.40(0.05)

[1] Standard deviation

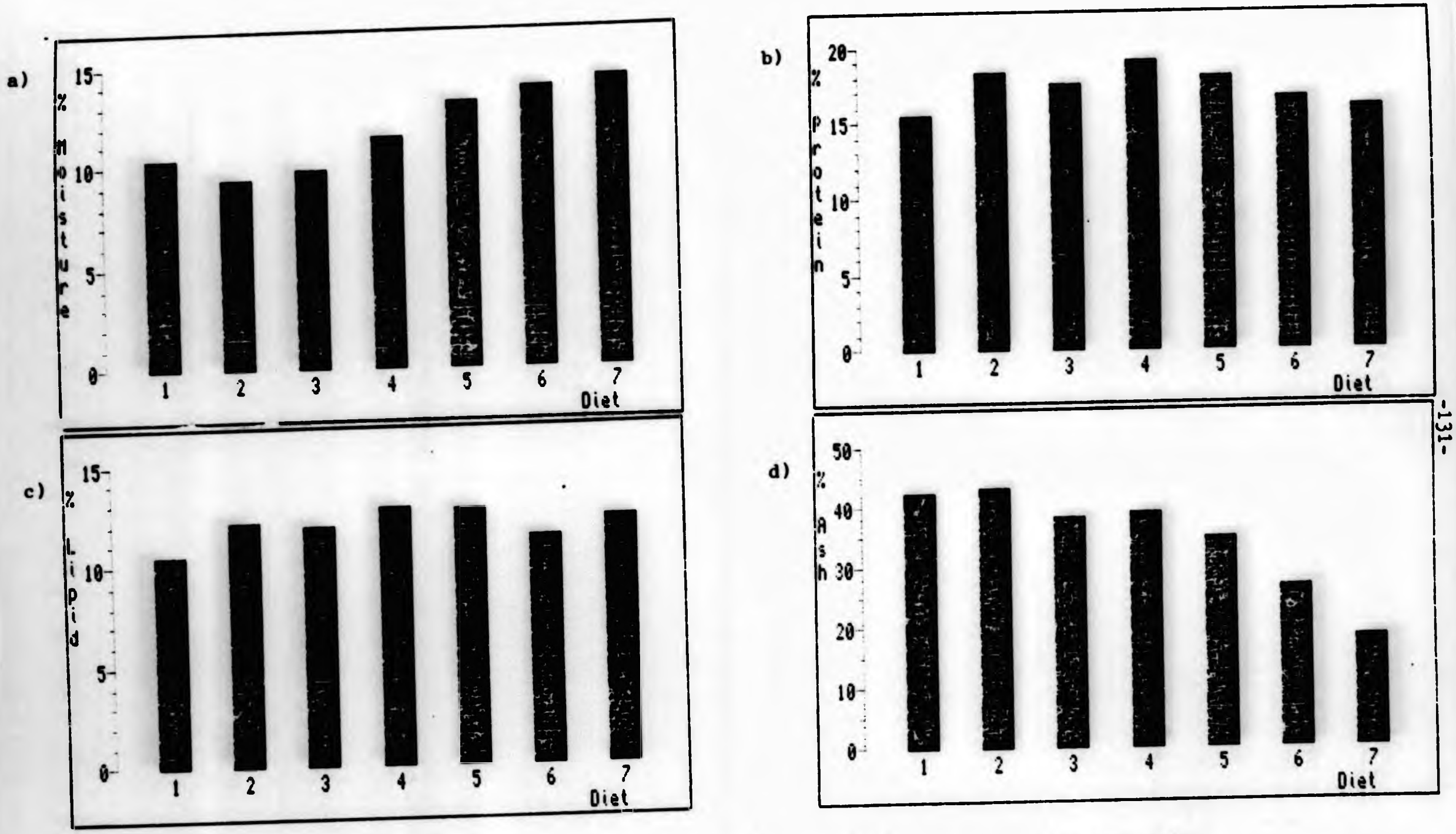

FIGURE 5.7 Proximate composition of the faeces from fish fed each of the seven experimental diets.
a) Moisture; b) Crude protein; c) Lipid; d) Ash

The lipid content of faeces from fish fed diets containing PBHFm showed only a slight variation between dietary treatments, varying from 11.43% (Diet 6) to 12.91% (Diet 4). Again faeces from fish fed the control diet had the lowest lipid content of only 10.55% (Table 5.8; Fig. 5.7c). The apparent lipid digestibility of the experimental diets varied significantly ($P < 0.05$) from 71.14% (Diet 2) to 79.96% (Diet 7). Hence digestibility again increased with an increase in the inclusion level of PBHFm. Fish fed the control diet had a coefficient similar to that of those fed Diet 3 (74.73% and 74.48%, respectively; Table 5.6).

The ash content of faeces decreased sharply from Diet 2 (43.28%) to Diet 7 (18.61%). Fish fed the control diet had an ash content of 42.62% which was only slightly lower than that of fish fed Diet 2 (Table 5.8; Fig. 5.7d). The apparent organic matter digestibility of the experimental diets varied significantly ($P < 0.05$) from 59.68% for Diet 2 to 67.62% for Diet 4. At PBHFm above 80% inclusion the digestibility decreased to 63.33% for 100% inclusion. However, with the exception of Diet 2 (20% protein replacement), the apparent organic matter digestibilities of all of the rations containing PBHFm were significantly ($P < 0.05$) higher than that of the fish meal control (57.76%; Table 5.6).

The proximate composition of faeces taken from rainbow trout replicates is given in Appendix III.

5.3.4 Liver Somatic Index and Blood Parameters

There were no significant ($P < 0.05$) differences in liver somatic index between dietary treatments (Table 5.6) with the exception of fish fed Diet 7 (100% protein replacement). The liver somatic index of fish fed Diet 7 was 1.23 compared with values of between 1.01 and 1.13 for fish fed the other six diets. This increase in liver somatic index indicates that the livers of fish fed the diet containing PBHFm as the sole protein source were slightly enlarged. Furthermore, it was noted that the livers of these fish were paler at the end of the trial than normal indicating a higher glycogen deposition.

Haematocrit values of fish fed rations containing PBHFm varied between 31.76% and 36.07% but these differences were not significant at the 95% level of significance. There were however, significant differences between these values and the value of 27.50% recorded from fish fed the control ration (Table 5.6). This difference indicates that these fish may have been slightly anaemic although this value is still within the normal range for healthy rainbow trout (Wedemeyer and Nelson, 1975; Miller *et al*., 1983; Railo *et al*., 1985).

The glucose in plasma followed a similar trend to haematocrit value (Table 5.6). Thus, fish fed the control diet (Diet 1) registered the lowest value of 75.33 mg per 100 cm^3, possibly because it was the diet with one of the lowest dietary carbohydrate level (Table 5.1). There was a significant ($P < 0.05$) difference between

this value and the values recorded in the other dietary treatments which varied, although not significantly, between 91.94 and 101.54mg per 100 cm^3 with the highest value recorded from fish fed Diet 4 (60% protein replacement). Diet 7 (100% protein replacement) which had the highest dietary carbohydrate level produced a lower value (97.44 mg per 100 cm^3) comparable to that of fish fed Diets 3 and 5 (40% and 80% protein replacement, respectively; Table 5.6). However plasma glucose levels of fish fed all six experimental diets based on PBHFm were within the normal range of values for healthy rainbow trout (Wedemeyer and Nelson, 1975; Miller et al., 1983). Fish fed the fish meal based control diet had a plasma glucose level of 75.33mg per 100 cm^3 which was slightly lower than the minimum value of 77mg per 100 cm^3 indicated by Wedemeyer and Nelson (1975).

The liver somatic index and blood parameters of rainbow trout replicates are given in Appendix I.

5.3.5 Carcass Composition

At the end of the 18 week growth trial the moisture content of fish fed all the experimental rations was significantly ($P < 0.05$) lower than the initial value of 75.84%. There was a significant trend of decreasing body moisture with increasing PBHFm inclusion level with a minimum value of 63.25% (100% protein replacement) and a maximum value of 68.80% (20% protein replacement) (Table 5.9; Fig. 5.8a).

The lipid content of fish fed all seven experimental rations increased significantly ($P < 0.05$) from 6.18% at the start of the

TABLE 5.9 Carcass composition of rainbow trout (280 fish, 35g) at the start and end of the experiment (18 weeks) based on 12 fish per treatment (% wet weight)

Carcass composition (% wet wt.)	Initial		Dietary treatments 1	2	3	4	5	6	7	± S.E.[1]
Moisture (%)	75.84^{h}	(1.22)[2]	69.53^{efg} (1.62)	68.80^{ef} (1.22)	67.54^{cde} (1.14)	66.28^{bcd} (1.67)	65.73^{bc} (1.55)	64.79^{ab} (1.47)	63.25^{a} (0.73)	0.779
Crude protein (N x 6.25)	16.07^{a}	(1.84)	18.41^{b} (1.33)	18.43^{b} (1.86)	18.22^{b} (1.23)	17.38^{ab} (0.74)	17.41^{ab} (0.93)	17.19^{ab} (1.37)	17.35^{ab} (0.75)	0.532
Lipid (%)	6.18^{a}	(0.66)	9.19^{b} (1.15)	9.98^{bc} (1.39)	12.07^{cde} (1.32)	14.40^{de} (1.72)	14.42^{de} (1.12)	16.04^{de} (1.06)	16.75^{e} (1.09)	0.892
Ash (%)	3.23^{abcd}	(0.84)	3.66^{d} (0.43)	3.63^{cd} (0.22)	3.41^{abcd} (0.22)	3.08^{ab} (0.33)	3.11^{abc} (0.30)	3.07^{a} (0.42)	3.19^{abcd} (0.09)	0.163
	101.32		100.79	100.84	101.24	101.14	100.66	101.09	100.59	

[1] Standard error; calculated from residual mean square in the analysis of variance

[2] Standard deviation

abcdefg Mean values for components with common superscripts are not significantly ($P < 0.05$) different

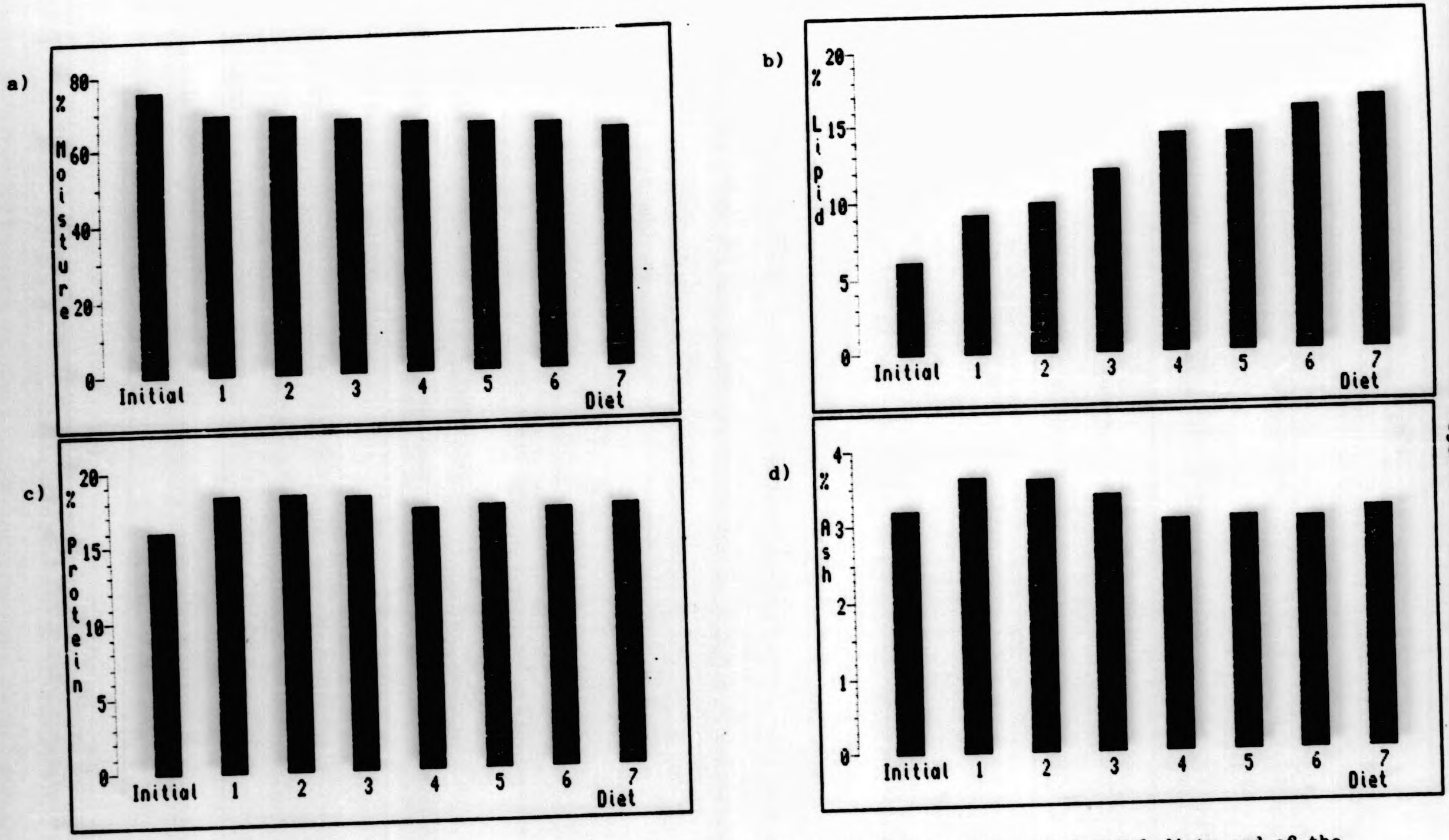

FIGURE 5.8 Proximate composition of the carcasses from fish fed each of the seven experimental diets and of the initial sample. a) Moisture; b) Lipid; c) Crude protein; d) Ash

trial to a maximum of 16.75% for fish Fed Diet 7. The lipid content of fish fed the control ration (Diet 1; 9.19%) was approximately 33% greater than the initial value. Furthermore, there was a trend of increasing lipid deposition with increasing inclusion level of PBHFm from 9.98% for fish fed Diet 2 to 16.75% for fish fed Diet 7. This increase in body lipid deposition was accompanied by a decrease in body moisture levels (Table 5.9; Fig. 5.8b).

There was an increase in the crude protein content of fish carcasses from 16.07% at the start of the trial to between 17.19% for fish fed Diet 6 and 18.43% for those fed Diet 2. However, the differences in protein content of fish fed Diets 4 to 7 were not significantly ($P < 0.05$) different from the initial value. The highest carcass protein levels were recorded from fish fed the fish meal control diet and the two diets containing the lowest inclusion levels of PBHFm (20% and 40% protein replacement; Table 5.9; Fig. 5.8c). From the analysis of carcass composition it is apparent that the greater weight gain of fish fed diets containing high inclusion levels of PBHFm was due, in part, to an increase in lipid deposition rather than of protein at the cost of both carcass crude protein and moisture content.

The ash content of fish carcasses varied from 3.07% (90% protein replacement) to 3.66% (fish meal control diet). However, no relationship between ash content and PBHFm inclusion level was indicated (Table 5.9; Fig. 5.8d).

The proximate composition of fish carcass replicates is given in Appendix IV.

5.3.6 Histological Examination

Histological examination of the liver, kidney, spleen, and gill tissues revealed no apparent differences between fish fed the seven experimental diets and those of healthy rainbow trout.

5.4 DISCUSSION

At all inclusion levels up to 100% fish meal replacement the performance of rainbow trout fed diets containing poultry by-product and hydrolysed feather meal (PBHFm) was better than that of those fed a fish meal control diet. Thus, fish fed diets containing increasing inclusion levels of PBHFm had a maximum mean final weight of 136.60g at 90% protein replacement (Diet 6) compared with a mean final weight of only 62.39g for those fed the fish meal control diet (Table 5.6). This trend of improved growth performance with increasing inclusion levels of poultry by-products has also been reported in salmonid fish by several authors. Wojno and Dabrowska (1984a) using rainbow trout in the same size range reported a maximum mean final weight of 107.10g for fish fed diets where 50% of the protein was supplied by poultry ofall meal (POm) compared with a mean final weight of only 80.4g for those fed a fish meal control diet. A similar trend for coho salmon (Oncorhynchus kisutch) fed diets containing varying levels of POm was also reported by Markert et al. (1977). Similarly, Higgs et al. (1979) using coho salmon found that growth performance improved with increasing dietary inclusion levels of a mixture of poultry by-product meal and hydrolysed feather meal up to a maximum of 75% protein replacement.

A good quality fish meal usually has an amino acid profile which comes close to satisfying the requirements of fish (Reinitz, unpublished; Tacon and Jackson, 1985). Other feedstuffs however, are almost always significantly deficient in at least one essential amino acid and thus it is extremely unusual for the total replacement

of fish meal by a single commodity to result in a better growth performance than that produced by a fish meal control ration.

The first limiting essential amino acids in all seven diets were phenylalanine and leucine. Levels of phenylalanine varied between 0.56% in Diet 1 and 0.54% in Diets 6 and 7 compared with a requirement level of 1.24%. Thus, in all rations only around 45% of the requirement level was present. Similarly, levels of leucine varied between 0.91% in Diet 1 and 0.84% in Diet 7 giving a minimum dietary concentration of 48% of the requirement level. Phenylalanine, however, can be spared by tyrosine (Mertz, 1972; Halver, 1975, 1976; Cowey, 1979; Ketola, 1982; Millikin, 1982; Walton, 1985; Wilton, 1985) and consequently it is only necessary for approximately 60% of the requirement level to be present provided the remaining 40% is supplied by tyrosine. This indeed appears to have been the case as the tyrosine content of the diets varied between 0.79g and 0.85g per 100g (Table 5.4). Thus leucine appears to have been the first limiting essential amino acid in all seven rations, and as a consequence only around half of the total protein would have been usable for growth (Halver, 1975, 1976).

The improvement in fish performance with increasing levels of PBHFm can not be correlated with leucine levels since the levels in all seven diets were similar, and in fact decreased slightly with increasing PBHFm content. The greatest variation in essential amino acid levels was in the lysine content. The fish meal contained only 1.68% lysine compared to 2.98% in the PBHFm. As a result the fish meal control diet (Diet 1) contained only 1.27% lysine compared

with a requirement of 2.12% and the lysine level increased from 1.42% in Diet 2 to 2.01% in Diet 7. Thus the control ration contained only 60% of the requirement level for lysine compared to 95% in Diet 7 based entirely on PBHFm. However, since only around half of the protein was available for growth due to the severe leucine deficiencies, this would have the effect of making these differences in lysine content irrelevant. Essential amino acid profiles do not give any indication of the availability of each amino acid and since lysine is the essential amino acid which is most susceptible to become chemically unavailable (Cowey et al., 1972; Cowey, 1979; Jauncey and Ross, 1982) it is possible that not all of the lysine was available. Unfortunately, levels of available lysine were not determined, but had a significant portion of the dietary lysine been rendered unavailable, and especially if this were more the case in the fish meal than the PBHFm, variation in the lysine content may help to explain the variation in performance between the treatments.

The fish meal control ration also contained the lowest level of valine, 1.12%, although this was only slightly lower than the requirement level of 1.24%. In addition, the levels of valine in the PBHFm based rations were only slightly higher varying between 1.14% and 1.18% (Table 5.4). All diets were also slightly deficient in arginine and furthermore the level decreased with increasing inclusion levels of PBHFm from 1.27% in the control to 1.10% in Diet 7 containing all of the dietary protein as PBHFm. Thus Diet 7 contained around 79% of the requirement level, although again

in view of the severe leucine deficiency, this variation would not have had a significant impact on differences in growth performance.

No determinations of dietary levels of the essential amino acid tryptophan were performed. Good quality fish meals usually contain around 1% of the crude protein as tryptophan (Windsor and Barlow, 1981). Thus, in a diet containing around 41% crude protein supplied by fish meal this would give a dietary level of 0.4g per 100g compared with a requirement of 0.2g per 100g (Ogino, 1980). Even if the tryptophan content of the fish meal used in this study was only half of that found in a good quality fish meal this should still have satisfied the requirements for this essential amino acid. However, tryptophan has been reported to be deficient in poultry by-products (Section 2.2) and therefore the diets with high inclusion levels of PBHFm may well have had tryptophan levels which were lower than the requirement level, particularly in Diet 7 where all of the fish meal was replaced by PBHFm.

This work was carried out at high water temperatures of around 22°C which is close to the upper limit for rainbow trout culture. It is therefore difficult to compare findings in this work with those of other authors since trials have generally been carried out at much lower water temperatures (Tiews et al., 1976; Gropp et al., 1979; Higgs et al., 1979; Wojno and Dabrowska, 1984a). The food intake of 2% body weight per day should have been adequate for good growth rates despite the higher energy requirements at these high water temperatures (Brocksen and Bugge, 1974; Elliot, 1979, 1982; Choubert et al., 1982). Growth rates in treatments

4 to 7 were reasonable and the food conversion ratios were quite good (Table 5.6). Growth of fish fed Diets 1 to 3 were very poor and higher growth rates should have been possible for all treatments if the diets had not had such significant essential amino acid deficiencies.

The improved growth performance of fish fed increasing levels of PBHFm was due in part to an increase in body lipid deposition. Fish fed the control ration, Diet 1, with a protein component based only on fish meal had a carcass lipid content of 9.19% which was significantly higher than the level of 6.18% at the start of the 18 week trial (Table 5.6). This increase in carcass lipid is likely to be a consequence of the high dietary lipid content of 17% (Table 5.3). The effect of high energy diets on carcass lipid deposition in fish has been examined by several authors. Lee and Putnam (1973) found that the carcass lipid component of rainbow trout fed diets containing 24% herring oil increased from the initial level of 6.3% to 13% at the end of an 18 week growth trial. In addition, Reinitz et al. (1978b), Austreng (1979), Buckley and Groves (1979), Cowey (1979, 1981), and Watanabe et al. (1979) reported that an excessive energy intake at moderate dietary protein levels leads to the accumulation of fat in fish with subsequent changes in carcass composition. In the current study body lipid deposition increased significantly with increasing inclusion levels of PBHFm up to a maximum of 16.75% carcass lipid at 100% fish meal replacement. Thus, although the final mean weight of fish fed Diet 7, where all of the fish meal was replaced by PBHFm, was 65g greater than that of those fed the control ration (Diet 1) based on fish meal alone, almost 40%

of this difference was attributable to fat. An increase in body lipid deposition with increasing inclusion levels of a poultry by-product was also reported by Wojno and Dabrowska (1984a) in trials using rainbow trout of a similar size. Final body lipid varied between 7.31% for the control fish fed a fish meal based ration to 9.94% in those fed a diet in which the protein was supplied entirely by poultry offal meal. These levels however, are significantly lower than the levels of up to 16.75% in the present study. This discrepancy is likely to be again related to dietary lipid levels since Wojno and Dabrowska (1984a) fed experimental rations containing only 10% lipid compared with 17% in this trial.

In conjunction with the increase in fish body lipid there was a small but significant decrease in body protein deposition from 18.41% (Diet 1) to 17.35% (Diet 7) and also in body moisture from 69.53% (Diet 1) to 63.25% (Diet 7; Table 5.9). This relationship between body lipid and body moisture and protein content has also been reported by Gulbrandsen and Utne (1977), Papoutsoglou and Papaparaskeva-Papoutsoglou (1978), Reinitz et al. (1978b), Buckley and Groves (1979), and Weatherley and Gill (1983). Thus the overall effect of increasing the inclusion level of PBHFm on fish carcasses was a significant increase in body lipid deposition at the expense of both body moisture and body protein which were significantly reduced.

Some of this variation in fish carcass composition could also have been attributable to differences in dietary carbohydrate levels. The level of nitrogen free extract (NFE) increased from 19.33% in the fish meal control ration (Diet 1) to 29.07% in Diet 7 where

all of the fish meal was replaced by PBHFm (Table 5.3). The NFE content of the fish meal control ration was much higher than the carbohydrate level of 6.8% added to the diet, but this may be explained by the high NFE content of 11.49% for the brown fish meal, which is significantly higher than the usual maximum level of around 8% for a good quality fish meal (Cullison, 1979; Göhl, 1981).

Refstie and Austreng (1981) reported that body moisture decreases with increasing carbohydrate levels and this indeed appears to have been the case for fish fed Diets 2 to 7 based on PBHFm. However, Refstie and Austreng (1981) found that carcass lipid levels also decreased whereas in this study there was a significant increase from 9.19% in fish fed Diet 1 to 16.75% for those fed Diet 7.

In addition, Reinitz (unpublished), Austreng et al. (1977), Refstie and Austreng (1981), and Hilton and Dixon (1982) have also reported that liver glycogen, liver weight, and consequently liver size, increased with increasing inclusion levels of dietary carbohydrate. There was some indication of a trend of increasing liver somatic index with increasing levels of PBHFm and as a consequence of increasing carbohydrate levels, however only the liver somatic index of fish fed Diet 7 (1.23) containing 100% of the protein as PBHFm was significantly different from the control value of 1.01 (Table 5.6). Furthermore, the livers of fish from this treatment were paler than those from the other six treatments which also indicated higher glycogen deposition (Phillips et al., 1966).

Although plasma glucose levels varied between 95.57 mg (Diet 2) and 101.54 mg per 100 cm^3 (Diet 4) for rations based on PBHFm, these differences were not significantly different. Therefore no correlation between carbohydrate level and plasma glucose was indicated. Wedemeyer and Nelson (1975) reported that the normal plasma glucose levels of healthy rainbow trout vary between 77 mg and 150 mg per 100 cm^3 and Miller *et al*. (1983) considered that the normal range was 72 mg to 218 mg per 100 cm^3. The plasma glucose levels recorded from fish fed the rations containing PBHFm were thus well within these ranges. However, the plasma glucose content of fish fed the fish meal control ration (Diet 1) was only 75.33 mg per 100 cm^3 which, in addition to being significantly lower than the levels found in fish fed the rations containing PBHFm, was also close to the minimum values reported above for healthy rainbow trout. This low level suggests that fish fed this ration were slightly hypoglycaemic although it is surprising that it is so different from the level recorded in fish fed Diet 2 which contained only 20% PBHFm and in fact had a lower NFE content.

There was a general trend for food digestibility values to increase significantly with increasing inclusion levels of PBHFm. The protein digestibility coefficient of fish fed the control diet (Diet 1) was 83.67% which is well within the range of reported values of between 60% and 95% for brown fish meals (Smith and Rumsey, 1976; Atack and Matty, 1979; Cho and Slinger, 1979; Lovell, 1981; Pfeffer, 1982; Watanabe *et al*., 1983). Protein digestibility of fish fed the diets based on PBHFm increased from 81.22% for fish fed the ration containing 20% of the protein as PBHFm to 85.83% for those

fed the ration where 80% of the protein was supplied by PBHFm (Table 5.6). However, only the protein digestibilities of fish fed Diets 5 to 7 were significantly higher than that of fish fed the control ration (Diet 1).

Unlike protein digestibility, the lipid digestibility value of 74.73% for fish fed the fish meal control diet, Diet 1, was significantly lower than the usual range of 89% to 97% for a good quality fish meal (Smith and Rumsey, 1976; Cho and Slinger, 1979). Lipid digestibility increased with increasing inclusion levels of PBHFm from 71.14% at 20% protein replacement (Diet 2) to a maximum coefficient of 79.96% at 100% protein replacement (Diet 7). These values, however, are significantly lower than those reported by Wojno and Dąbrawoska (1984a) for rainbow trout fed diets based on poultry offal meal. In addition, these authors reported a decrease in lipid digestibility from 93.95% for fish fed a diet containing 25% poultry offal meal to 90.20% for one where all of the protein was supplied by poultry offal meal. The range of lipid digestibility values in this study were also lower than the value of 83.60% reported by Cho and Slinger (1979) for a poultry by-product meal in a diet fed to rainbow trout where all of the protein was replaced by this product, although they were higher than the value of 71.5% reported by Smith and Rumsey (1976) for fish fed diets where the entire dietary protein was supplied by a poultry by-product meal. In addition to lower lipid digestibilities the apparent organic matter digestibilities were also low, varying between only 57.76% for fish fed the fish meal control diet to 67.62% for those fed Diet 4 where 60% of the protein was replaced by PBHFm.

There was no evidence from histopathological studies to indicate that the wide variation in performance between the dietary treatments resulted in any disorders. However, there was a significant variation in haematocrit. Fish fed the control diet (Diet 1) had a haematocrit value of only 27.50% compared to the significantly higher haematocrit values of between 34.82% (Diet 3) and 36.07% (Diet 5) for fish fed diets containing PBHFm. The lower haematocrit of the fish meal control fish suggests they were in poorer condition and may have been slightly anaemic. However, Wedemeyer and Nelson (1975) and Miller et al. (1983) proposed that the normal range of haematocrit values for healthy rainbow trout was 24% to 43% and 21% to 44%, respectively, and thus fish from all treatments fell into these ranges, while Railo et al. (1985) suggested a much narrower range of only 28.9% to 29.4% for rainbow trout. All haematocrit values were lower than the range of 45% to 53% proposed by Blaxhall and Daisley (1973) for healthy brown trout (Salmo trutta).

Since PBHFm is reported to be deficient in ω3 essential fatty acids (Higgs et al., 1979) it was necessary to supplement the experimental diets with fish oil to provide ω3 essential fatty acids required for good health and growth (Yu and Sinnhuber, 1979). This supplementation appears to have been adequate since there were no signs of essential fatty acid deficiencies such as increased body moisture, caudal fin erosion or liver pathology (Buckley and Groves, 1979; Cowey and Sargent, 1979; Cowey, 1981). A further problem associated with the use of PBHFm in rations at high inclusion levels is the necessity to formulate diets with a high lipid content.

The need to add some fish oil to supply essential fatty acids increases the lipid content still further.

In conclusion, fish fed the brown fish meal based diets had the poorest growth performance and feed utilization efficiency whereas the performance of fish fed the PBHFm based diets improved with increasing inclusion levels of PBHFm. The poor performance of fish fed the fish meal control ration was probably attributable to the poor quality of the fish meal. Compared with good quality fish meals it contained particularly low levels of the essential amino acids arginine, leucine, lysine and phenylalanine. The optimal inclusion level of PBHFm was between 80% and 90% of the protein equivalent to 34% to 36% of the entire diet. However, improvements in growth rate should be possible by improving the essential amino acid profile of these diets. This could be achieved by inclusion of other protein sources or supplementation with purified essential amino acids.

CHAPTER 6

THE NUTRITIONAL EVALUATION OF SIX BROWN FISH MEALS AS FEEDS FOR RAINBOW TROUT

6.1 INTRODUCTION

One of the major industries in Portugal is fish canning. Sardines (Sardina pilchardus) are caught and processed in the North of the country based from the fishing port of Matosinhos near Porto, whereas in the South tuna (Thunnus thynnus) is the main fish species caught and canned. By 1972 Portugal was reported by Coull (1972) to be the outstanding exporter of these canned fish products within Europe.

Fish meals in Portugal are produced mainly from the scraps of this canning industry although occasionally whole carcasses can be used should a surplus arise. Clearly this will lead to variability in quality. Furthermore, since the fish meal industry relies heavily on the scraps and surplus of this industry, a somewhat irregular production is often encountered.

In addition to sardines and tuna the other species most commonly used to produce fish meals in Portugal are mackerel (Scomber sp.), blue whiting (Micromesistius poutassou), snapper fish (Syngnathus sp.), trumpet fish (Macrarhamphosus sp.), and mackerel shad (Trachurus sp.).

In view of the relatively poor performance of the fish meal in the last trial, the nutritional quality of fish meals produced by the six main manufacturers in Portugal was evaluated. The total output by these fish meal producers and that of around a further 10 small production units is approximately 8,000 to 10,000 tons per year (fish meal plant managers' personal communications).

6.2 MATERIALS AND METHODS

6.2.1 Diets

25 Kilogrammes of each of six brown fish meals were obtained from six Portuguese fish meal plants located along the coast of Portugal (Fig. 9.1) as follows.

Brown fish meal A: Olfaixe-Produtos de Óleos e Farinhas de Peixe, Ltd., Portas Fronhas, Póvoa do Varzim

Brown fish meal B: Farinhas e Óleos de Peixe do Sul, Lda., Olhão, Algarve

Brown fish meal C: Óleos e Farinhas de Peixe, Ltd., Sociedade Produtora, Matosinhos

Brown fish meal D: Sociedade de Aproveitamentos de Detritos e Óleos de Peixe, Lda., Setúbal

Brown fish meal E: Sociedade Algarvia de Farinhas e Óleos, Lda., Olhão, Algarve

Brown fish meal F: Sociedade Industrial de Farinhas e Óleos de Peixe, Lda., Portimão, Algarve.

25 Kilogrammes of the bacterial SCP Methylophilus methylotrophus "Pruteen" was obtained from ICI Portuguesa, Lisboa. This bacterial SCP was used as the protein source in the control ration since it has a high protein content and an excellent amino acid profile which allows it to be included at very high inclusion levels in diets for rainbow trout without any significant loss of performance and feed utilization efficiency (Schulz and Oslage, 1976; Beck et al., 1979; Spinelli et al., 1979; Tiews et al., 1979; Kaushik and Luquet, 1980; Tacon, 1981; Tacon et al., 1983a).

On arrival at the laboratory the brown fish meals and "Pruteen" were immediately ground and the crude protein and lipid content were determined according to the methods given in Chapter 4.

Seven experimental diets were formulated on an isonitrogenous and isocaloric basis to contain 45% crude protein and 11% lipid (Table 6.6). Six of the diets each contained one of the six brown fish meals as the sole source of dietary protein and a seventh control diet was also formulated using "Pruteen" as the sole protein source.

The brown fish meals contained between 52.49% and 63.55% crude protein and between 7.06% and 12.36% lipid. The "Pruteen" contained 74.95% crude protein and 2.20% lipid (Table 6.3). The full dietary formulations are presented in Table 6.1. Seven kilogrammes of each diet were prepared as described in Chapter 4.

Diets contained sufficient fish oil to provide the necessary essential fatty acids.

The percentage of dietary carbohydrates in the diets varied from 0.4% to 12.9% and from 0.2% to 6.5% for corn starch and yellow dextrin respectively (Table 6.1). Thus the maximum carbohydrate content of 19% of the diet did not exceed the maximum recommended level of 20% (Phillips et al., 1948; Hilton and Dixon, 1982; Spannhof and Plantikow, 1983).

TABLE 6.1 Formulation of the experimental diets (% by weight)

Diet No.	1	2	3	4	5	6	7
"Pruteen"	66.10						
Fish meal A		75.00					
Fish meal B			93.60				
Fish meal C				80.00			
Fish meal D					83.00		
Fish meal E						89.00	
Fish meal F							80.00
Corn starch	12.90	9.50	0.40	8.80	5.10	3.70	8.10
Yellow dextrin	6.50	4.70	0.20	4.40	2.50	1.80	4.10
Cod liver oil[1]	9.71	6.01	1.01	2.01	4.61	0.71	3.01
Vitamin premix[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[3]	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Binder[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Potassium sorbate	0.20	0.20	0.20	0.20	0.20	0.20	0.20

1) Containing 150 mg/Kg diet of butylated hydroxy toluene (BDH Chemicals Ltd., Poole, Dorset, England)

2) According to Table 4.4

3) According to Table 4.5

4) Carboxymethylcellulose, dissodium salt, high viscosity (BDH Chemicals Ltd., Poole, Dorset, England)

The full chemical analysis carried out on both the foodstuffs and diets is summarised in Table 4.2 and the methods are described in Chapter 4.

6.2.2 Growth Trial

300 rainbow trout of mean weight 23g (23.05-23.82g; Appendix V) were obtained from Posto Aquícola de Manteigas, Serra da Estrela, and were allocated to 15 net cages as described in Chapter 4. Each experimental diet was allocated randomly to two net cages and a further net of 20 fish was unfed for the duration of the experimental period.

Fish were fed at a fixed rate of 2% body weight per day throughout the 18 week trial. The full experimental protocol of the trial is described in Chapter 4.

The mean water temperature during the experimental period was 15.8°C (9.0°-24°C ± 4.63) and the daily variation never exceeded 2°C (Table 6.2).

Statistical methods and the production of graphs were carried out as described in Chapter 4.

TABLE 6.2 Water temperature (°C) and standard deviation over the feeding period

Weeks	March	April	May	June	July	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0	10.0								
1	9.0					9.5	0.58		
2	9.0								
3	10.0								
4		11.0							
5		13.0				14.1	2.66		
6		15.5							
7		17.0							
8			17.0					15.8	4.63
9			16.5			15.9	1.03		
10			15.0						
11			15.0						
12				17.5					
13				16.0		18.4	2.56		
14				18.0					
15				22.0					
16				24.0					
17				22.0		22.7	1.15		
18				22.0					

6.3 RESULTS

6.3.1 Diets

The crude protein content of all six brown fish meals tested was relatively low varying from 52.49% for fish meal B to 63.55% for fish meal A (Table 6.3). In particular, fish meals B and E had crude protein levels well below 60%, which is considered to be the minimum protein content for a good quality fish meal (Windsor and Barlow, 1981). The crude protein content of the bacterial SCP was 74.95% (Table 6.3).

The amino acid profiles of the brown fish meals tested revealed that the sums of the amino acids analysed were not in agreement with the values of crude protein determined. This difference varied between 3.84% for brown fish meal A and 17.00% for brown fish meal E, but was also particularly high in fish meals C and D with differences of 14.42% and 14.29% respectively. All six brown fish meals were a good source of the nonessential amino acids glycine, proline, aspartic acid, and glutamic acid, and of the essential amino acids threonine, isoleucine, histidine, and methionine (Table 6.4). However they contained suboptimal levels of up to five of the essential amino acids and were particularly deficient in arginine, leucine, lysine, phenylalanine and valine. Leucine was the first limiting amino acid of brown fish meals C, D, E, and F, and phenylalanine of brown fish meals B and F. The bacterial SCP had an excellent amino acid profile although it was slightly deficient in arginine and leucine and deficient in both phenylalanine and valine. Phenylalanine was its first limiting amino acid (Table 6.4).

TABLE 6.3 Proximate composition, Cr_2O_3 and energy content of M. methylotrophus, "Pruteen" and of the six Brown fish meals (% dry weight)

Nutrient content (% dry weight)	"Pruteen"	Brown fish meal A	Brown fish meal B	Brown fish meal C	Brown fish meal D	Brown fish meal E	Brown fish meal F
Moisture (%)	9.14(0.03)[1]	5.60(0.02)	8.37(0.03)	6.44(0.01)	9.44(0.02)	6.21(0.01)	7.51(0.01)
Crude protein (N x 6.25)	74.95(0.13)	63.55(0.31)	52.49(0.25)	60.17(0.17)	59.87(0.31)	53.95(0.43)	60.87(0.51)
Lipid (%)	2.20(0.28)	7.06(0.31)	11.64(0.25)	12.07(0.43)	8.46(0.37)	12.36(0.21)	10.84(0.71)
Ash (%)	14.78(0.13)	23.43(0.17)	32.29(0.26)	23.64(0.14)	24.71(0.08)	31.54(1.31)	26.19(1.12)
Nitrogen free extract[2] (%)	-	-	-	-	-	-	-
Peroxide value (mEq/Kg oil)	0.00(-)	2.48(0.23)	1.58(0.21)	4.05(0.63)	2.04(0.12)	9.88(1.04)	0.49(0.01)
Acid insoluble ash (%)	0.28(0.08)	0.21(0.01)	0.39(0.06)	0.20(0.04)	0.20(0.08)	0.14(0.08)	1.47(0.12)
Crude fibre (%)	0.80(0.21)	0.99(0.15)	2.47(0.17)	0.54(0.15)	0.78(0.09)	0.56(0.07)	0.59(0.12)
Energy - ash fee (Kcal/g)	5.40(0.05)	6.27(0.18)	5.58(0.14)	6.28(0.01)	5.41(0.20)	5.46(0.17)	5.69(0.32)

1) Standard deviation

2) Nitrogen free extract = 100-(% moisture+% crude protein+% lipid+% ash+% crude fibre)

TABLE 6.4 Amino acid profile of M. methylotrophus "Pruteen" and of the six Brown fish meals and amino acid requirement of rainbow trout (g/100g dry weight)

Amino acid (g/100g dry wt)	"Pruteen"	Brown fish meal A	Brown fish meal B	Brown fish meal C	Brown fish meal D	Brown fish meal E	Brown fish meal F	Requirement of rainbow trout (% diet)*
Arginine	2.01	2.15	1.59	1.59	1.51	1.18	1.79	1.40, 40% (Ogino, 1980)
Histidine	3.01	2.93	2.67	2.50	2.40	1.85	2.46	0.64, 40% (Ogino, 1980)
Isoleucine	4.03	3.91	3.46	3.20	3.28	2.59	3.31	0.96, 40% (Ogino, 1980)
Leucine	2.40	2.33	0.96	0.94	0.98	0.97	1.16	1.76, 40% (Ogino, 1980)
Lysine	4.74	4.61	1.64	2.00	2.42	2.73	3.50	2.12, 40% (Ogino, 1980)
Methionine	2.51	2.44	1.34	1.53	1.26	1.24	1.65	0.55-0.75, 35% (Rumsey et al., 1983)
Phenylalanine	0.98	0.95	0.83	0.72	0.76	0.55	0.86	1.24, 40% (Ogino, 1980)
Threonine	3.42	3.32	2.45	2.43	2.20	2.22	2.47	1.36, 40% (Ogino, 1980)
Valine	1.13	1.10	1.35	1.34	1.22	1.07	1.40	1.24, 40% (Ogino, 1980)
Alanine	1.21	1.17	0.52	0.52	0.59	0.39	0.66	
Aspartic acid	5.34	5.18	4.52	4.99	4.14	3.37	5.23	
Cystine	1.08	1.05	0.72	0.60	0.56	0.49	0.62	0.30, 35% (Rumsey et al., 1983)
Glutamic acid	4.39	4.26	4.72	4.79	4.56	3.28	4.72	
Glycine	9.13	9.26	7.88	8.03	7.79	6.03	8.44	
Proline	6.17	6.00	7.59	6.30	7.51	5.66	8.16	
Serine	3.71	3.60	3.58	3.18	3.36	2.39	3.31	
Tyrosine	1.69	1.65	1.03	1.09	1.04	0.94	1.23	0.84, 40% (Ogino, 1980)
TOTAL	56.95	55.91	48.65	45.75	45.58	36.95	50.97	

* Percentage of crude protein in the diet

The lipid content of the brown fish meals varied between 7.06% for brown fish meal A and 12.36% for brown fish meal E. Thus only fish meals C and E (12.07% and 12.36% respectively) had lipid levels which were slightly outside the reported normal range of values of 8% to 12% lipid for a good quality brown fish meal (Windsor and Barlow, 1981). The lipid content of the bacterial SCP was 2.20% which is significantly lower than the reported values of 8% to 13% (Kaushik and Luquet, 1980; Tacon, 1981; Tacon et al., 1983a).

The ash contents of the brown fish meals were in all cases high, ranging from 23.43% for fish meal A to 32.29% for fish meal B (Table 6.3). These levels were all significantly higher than the reported maximum value of 21% for good quality brown fish meals (Windsor and Barlow, 1981). This high ash content indicates that the fish meals contained a high proportion of bone material which caused a concomitant fall in the crude protein content of the meals (Cho, 1980). The ash content of the bacterial SCP was 14.78% which is again higher than the normal range of 9% to 10% (Kaushik and Luquet, 1980; Tacon, 1981; Tacon et al., 1983a).

The peroxide values of the brown fish meals were low (<3 mEq/Kg oil) with the exception of fish meals C and E which had peroxide values of 4.05 and 9.88 mEq. per Kg oil respectively. These latter values are slightly higher than the recommended levels of 4 mEq/Kg oil (Billinski et al., 1978). The degree of oil peroxidation of the bacterial SCP used was negligible (Table 6.3).

The acid insoluble ash and crude fibre content of both the brown fish meals and the bacterial SCP were low (<3%; Table 6.3).

Both the brown fish meals and the bacterial SCP were good sources of all the minerals analysed, in particular of Na, Ca, and K for the former and of K, Ca, and Mg for the latter (Table 6.5). Nevertheless the brown fish meals tested had a slightly lower phosphorous content (1.05-1.35% dry weight) than the values reported by Windsor and Barlow (1981) of 1.50% to 2.98% dry weight.

The experimental diets had a crude protein content varying from 40.92% to 41.67% (Table 6.6), which is somewhat lower than the formulated value of 45%. All seven diets were deficient in leucine with levels ranging from 0.64% for Diet 4 to 1.52% for Diet 2 compared to the dietary requirement of 1.76% (Ogino, 1980) for rainbow trout. There was also a deficiency of the essential amino acid phenylalanine in all seven diets with levels ranging from 0.42% for Diet 6 to 0.66% for Diet 3, compared with a dietary requirement of 1.24% (Ogino, 1980). There was a significant lysine deficiency in Diets 3, 4 and 5 (1.30%, 1.36%, and 1.68% respectively) and a slight deficiency in Diet 6 (2.07%). With the exception of Diet 1 all diets were also slightly deficient in arginine and valine (Table 6.7).

The lipid content of the diets varied between 11.06% and 11.81% and was therefore close to the formulated value of 11% (Table 6.6). The energy content of the experimental diets varied between 5.12 and 5.94 Kcalories per gramme dry weight (Table 6.6).

TABLE 6.5 Concentration of mineral elements in M methylotrophus "Pruteen" and in six Brown fish meals and mineral requirement of rainbow trout

Elements	"Pruteen"	Brown fish meal A	Brown fish meal B	Brown fish meal C	Brown fish meal D	Brown fish meal E	Brown fish meal F	Requirement of rainbow trout (mg/100g dry wt)
Ca (g/100g)	3.15	10.75	7.66	6.71	7.57	8.85	8.35	650-750 (Ogino and Takeda, 1978)
Mg (g/100g)	0.32	0.24	0.27	0.22	0.21	0.24	0.23	50-70 (Ogino et al., 1978) (Knox et al., 1981)
K (g/100g)	1.01	1.26	2.30	1.29	1.43	1.45	2.12	160 (Frenzel and Pfeffer, 1982)
Na (g/100g)	0.13	2.16	6.10	2.16	3.01	4.85	1.99	220 (Frenzel and Pfeffer, 1982)
P (g/100g)	2.87	1.15	1.21	1.05	1.10	1.35	1.21	700-800 (Ogino and Takeda, 1978) 650 (Nose and Arai, 1979)
Zn (mg/100g)	5.29	18.40	31.27	18.65	22.13	17.38	18.18	1.5-3.0 (Ogino and Yang, 1978)

TABLE 6.6 Proximate composition, Cr_2O_3 and energy content of experimental diets (% dry weight)

Nutrient content (% dry weight)	1	2	3	4	5	6	7
Moisture (%)	3.25(0.03)[1]	2.68(0.10)	2.12(0.01)	2.47(0.25)	2.99(0.05)	2.85(0.05)	2.65(0.52)
Crude protein (N x 6.25)	41.45(0.17)	41.41(0.42)	41.67(0.15)	41.00(0.18)	41.47(0.33)	40.92(1.23)	41.24(0.48)
Lipid (%)	11.81(0.08)	11.06(0.08)	11.29(0.23)	11.29(0.09)	11.61(0.05)	11.48(0.02)	11.57(0.05)
Ash (%)	9.15(0.27)	18.63(1.23)	31.34(0.08)	22.11(1.21)	21.88(0.32)	28.89(0.03)	23.44(0.51)
Nitrogen free extract[2] (%)	33.72	26.11	13.18	20.51	20.58	14.97	20.70
Peroxide value (mEq/Kg oil)	0.00(-)	2.71(0.05)	3.09(0.02)	4.49(0.41)	1.09(0.32)	4.44(0.31)	2.16(0.25)
Acid insoluble ash (%)	0.12(0.00)	0.89(0.05)	0.66(0.32)	0.88(0.00)	0.73(0.03)	0.60(0.23)	0.61(0.08)
Crude fibre (%)	0.62(0.19)	0.11(0.22)	0.40(0.27)	2.62(0.31)	1.47(0.22)	0.89(0.15)	0.40(0.11)
Cr_2O_3 (%)	0.50(0.00)	0.49(0.00)	0.50(0.01)	0.50(0.00)	0.49(0.01)	0.47(0.02)	0.49(0.01)
Energy - ash free (Kcal/g)	5.36(0.33)	5.72(0.09)	5.12(0.03)	5.28(0.04)	5.40(0.41)	5.41(0.62)	5.94(0.19)

1) Standard deviation

2) Nitrogen free extract = 100-(% moisture+% crude protein+% lipid+% ash+% crude fibre)

TABLE 6.7 Amino acid profile of the experimental diets and amino acid requirement of rainbow trout (g/100 g dry weight)

Amino acid (g/100g dry wt)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Requirement of rainbow trout (% diet)
Arginine	1.11	1.40	1.26	1.08	1.04	0.89	1.21	1.40, 40%* (Ogino, 1980)
Histidine	1.66	1.91	2.12	1.70	1.66	1.40	1.67	0.64, 40% (Ogino, 1980)
Isoleucine	2.23	2.55	2.75	2.18	2.27	1.96	2.24	0.96, 40% (Ogino, 1980)
Leucine	1.33	1.52	0.86	0.64	0.68	0.73	0.78	1.76, 40% (Ogino, 1980)
Lysine	2.62	3.00	1.30	1.36	1.68	2.07	2.37	2.12, 40% (Ogino, 1980)
Methionine	1.39	1.59	1.06	2.34	0.87	0.94	1.12	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	0.54	0.62	0.66	0.49	0.53	0.42	0.58	1.24, 40% (Ogino, 1980)
Threonine	1.89	2.16	1.94	1.65	1.52	1.68	1.67	1.36, 40% (Ogino, 1980)
Valine	0.62	0.72	1.07	0.91	0.84	0.81	0.95	1.24, 40% (Ogino, 1980)
Alanine	0.67	0.76	0.41	0.27	0.41	0.29	0.45	
Aspartic acid	2.95	3.37	3.59	3.40	2.87	2.56	3.54	
Cystine	0.60	0.68	0.52	0.36	0.31	0.37	0.38	0.30, 35% (Rumsey et al. 1983)
Glutamic acid	2.43	2.77	3.75	3.26	3.16	2.49	3.20	
Glycine	5.05	6.03	6.25	5.47	5.39	4.57	5.72	
Proline	3.41	3.91	6.02	4.29	5.20	4.29	5.53	
Serine	2.05	2.34	2.84	2.17	2.33	1.81	2.24	
Tyrosine	0.93	1.07	0.82	0.74	0.72	0.71	0.83	0.84, 40% (Ogino, 1980)
TOTAL	30.37	39.40	37.22	32.31	31.48	27.99	34.48	

* Percentage of crude protein in the diet

6.2.2 Growth Performance and Feed Utilization Efficiency

Fish accepted the experimental diets readily and fed quite aggressively throughout the feeding period with the exception of fish fed the diet based on bacterial SCP (Diet 1). However, by the end of the first week these fish also consumed all of the diet although not so aggressively.

At the start of the trial there were no significant ($P < 0.05$) differences in mean weights of around 23g (23.21-23.56g) between treatments (Table 6.8). However, by the end of the 18 week growth trial fish fed the "Pruteen" based diet (Diet 1) had tripled their weight to 72.79g. By contrast, the best weight achieved by fish fed the fish meal based rations was only 69.07g (Diet 3) and the poorest was 51.89g (Diet 6). Thus the growth response of fish fed all six of the fish meal based rations was poorer than that of those fed the bacterial SCP based control diet (Table 6.8; Fig. 6.1). There were no significant ($P < 0.05$) differences in the final mean weights of fish fed diets containing fish meals C, D, and E (52.65g, 53.25g, and 51.89g respectively).

The mean overall mortality rate of fish fed the seven experimental diets was 10% although in treatments 7A, 3A, 6A, and 6B mortality rates of 15%, 20%, 20% and 35% respectively were recorded (Table 6.9). Thus, slightly higher mortalities were encountered within fish replicates fed Diet 6. The mortality rate of the unfed fish, however, was 95%. Furthermore the mean weight of the single remaining fish was reduced by almost one third to 17.97g at the end

TABLE 6.8 Growth performance, feed utilization efficiency, liver somatic index, and blood parameters of rainbow trout (280 fish, 23g) fed the experimental diets after 18 weeks

Mean values	1 "Pruteen"	2 Fish meal A	3 Fish meal B	4 Fish meal C	5 Fish meal D	6 Fish meal E	7 Fish meal F	± S.E.[1]
Mean initial weight (g)	23.56[a]	23.27[a]	23.50[a]	23.21[a]	23.53[a]	23.26[a]	23.21[a]	0.719
Mean final weight (g)	72.79[f]	64.54[e]	69.07[f]	52.65[ab]	53.25[abc]	51.89[a]	58.38[d]	1.430
Weight gain (%)	212.96	176.10	196.20	126.59	127.00	121.10	140.37	
Specific growth rate (%/day)	0.91	0.81	0.86	0.65	0.65	0.63	0.70	
Food intake (mg/day)	936	879	1039	772	779	804	863	
Protein intake (mg/day)	388	364	433	317	324	329	357	
Weight gain (mg/day)	398	328	366	233	237	223	259	
Food conversion ratio	2.35	2.68	2.84	3.31	3.29	3.60	3.33	
Protein efficiency ratio	1.02	0.90	0.84	0.73	0.73	0.68	0.72	
Nitrogen intake (mg/day)	62.08	58.24	69.28	50.72	51.84	52.64	57.12	
Nitrogen deposition (mg/day)	6.41	5.63	5.28	4.59	4.57	4.24	4.53	
Apparent net protein utilization (%)	10.32	9.67	7.62	9.05	8.81	8.05	7.93	
Apparent protein digestibility (%)	86.27[g]	76.71[bcd]	72.30[a]	76.57[bc]	78.03[e]	76.09[b]	78.09[ef]	0.404
Apparent lipid digestibility (%)	90.76[g]	74.33[b]	75.90[bc]	86.11[e]	78.25[cd]	86.77[ef]	59.84[a]	0.962
Apparent organic matter digestibility (%)	70.18[g]	54.34[bcd]	44.65[a]	55.41[bcde]	53.51[b]	53.80[bc]	58.25[f]	0.665
Liver somatic index	1.39[b]	1.42[b]	1.44[b]	1.17[ab]	1.06[a]	1.27[ab]	1.26[ab]	0.087
Haematocrit (%)	33.00[a]	38.80[g]	34.60[cd]	34.12[bc]	35.33[de]	37.00[f]	33.25[ab]	0.369
Haemoglobin (g/100 cm^3)	5.46[a]	5.71[a]	5.47[a]	5.15[a]	5.47[a]	4.79[a]	5.00[a]	0.300

1) Standard error; calculated from residual mean square in the analysis of variance

abcdefg Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

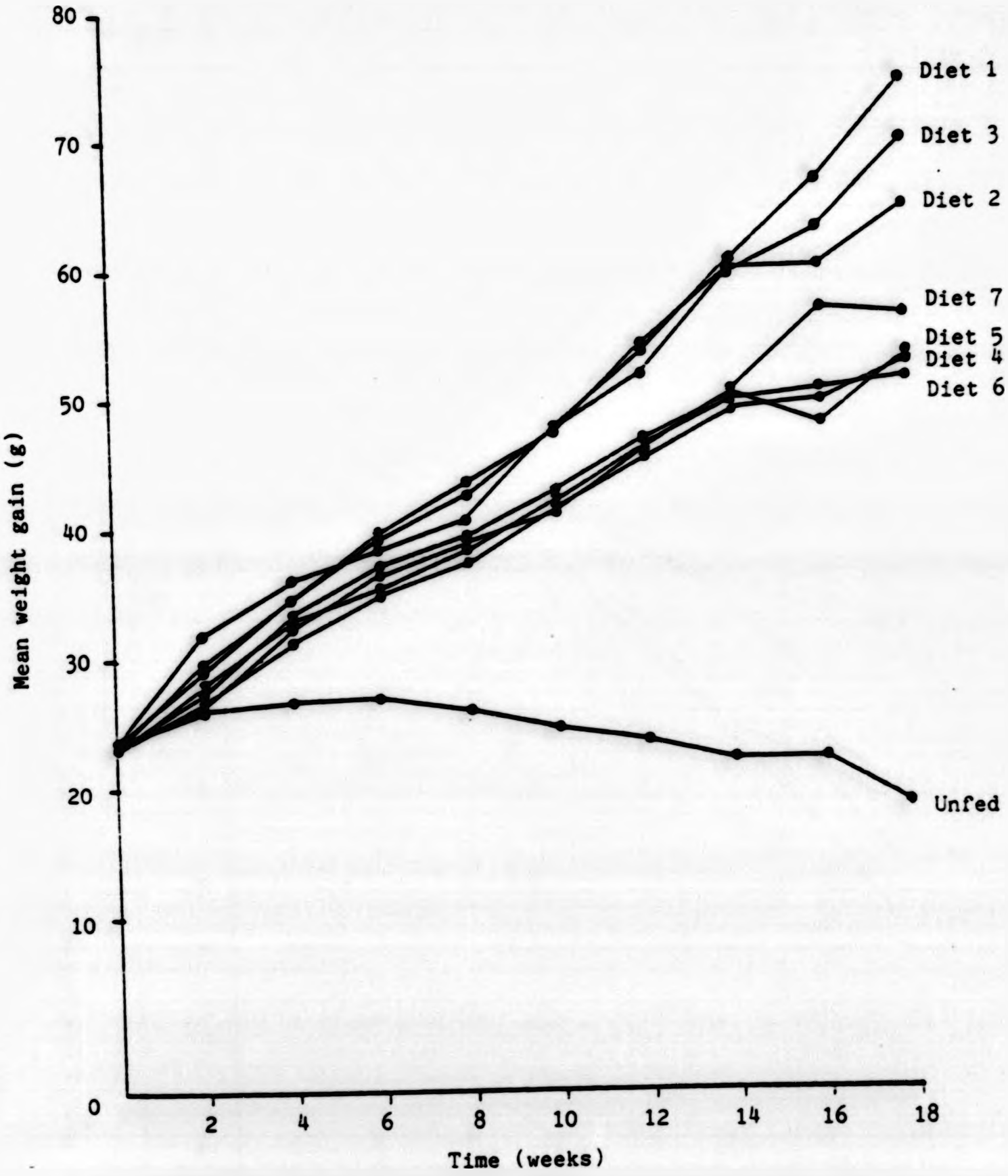

FIGURE 6.1 Overall mean weight gain (g) of rainbow trout at successive fortnightly intervals over the experimental test period

TABLE 6.9 Weekly deaths per net cage and overall percentage mortality over the 18 week feeding period

Treatments	1		2		3		4		5		6		7		No feeding
Replicates	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
0 week													1		
2													1		1
4															1
6	1														1
8	1	1			1		1				1		1		1
10		1	1	1	3	1					1	1	1	1	5
12									1		1	2			3
14												1			3
16											1	1			4
18											1	1			1
TOTAL	2	2	1	1	4	1	0	1	1	0	4	7	3	2	19
%	10	10	5	5	20	5	0	5	5	0	20	35	15	10	95

of the trial (Appendix VI; Fig. 6.1), thus demonstrating that any available natural food would have a negligible effect on the overall growth performance of the fish fed the experimental diets.

Fish fed the "Pruteen" control diet (Diet 1) had the highest specific growth rate of 0.91% per day whereas specific growth rates of fish fed the fish meal based rations were significantly ($P < 0.05$) lower ranging between 0.63% for Diet 6 and 0.86% per day for Diet 3. Fish fed Diets 4, 5 and 6 registered the poorest specific growth rates of 0.65%, 0.65% and 0.63% per day respectively (Table 6.8; Fig. 6.2).

The food conversion ratios of all six experimental diets based on the fish meals were high, ranging from 2.68 for fish fed Diet 2 to 3.60 for thosefed Diet 6. The best food conversion ratio however of 2.35 was again achieved by the fish fed the "Pruteen" based diet (Diet 1), although the food conversion ratio for Diet 2 of 2.68 was only slightly higher. Diets 4 to 7 registered very high food conversion ratios of between 3.29 for Diet 5 and 3.60 for Diet 6 (Table 6.8; Fig. 6.3).

The protein efficiency ratio (PER) follows the same trend as the food conversion ratio. Thus the best PER was achieved by fish fed the "Pruteen" control diet (1.02). The best PER by fish fed the fish meal based experimental diets was 0.90 (Diet 2) which was only 12% less than that of the control diet value (Table 6.8; Fig. 6.4). The apparent net protein utilization (apparent NPU), which gives a more accurate assessment of the protein utilization, again

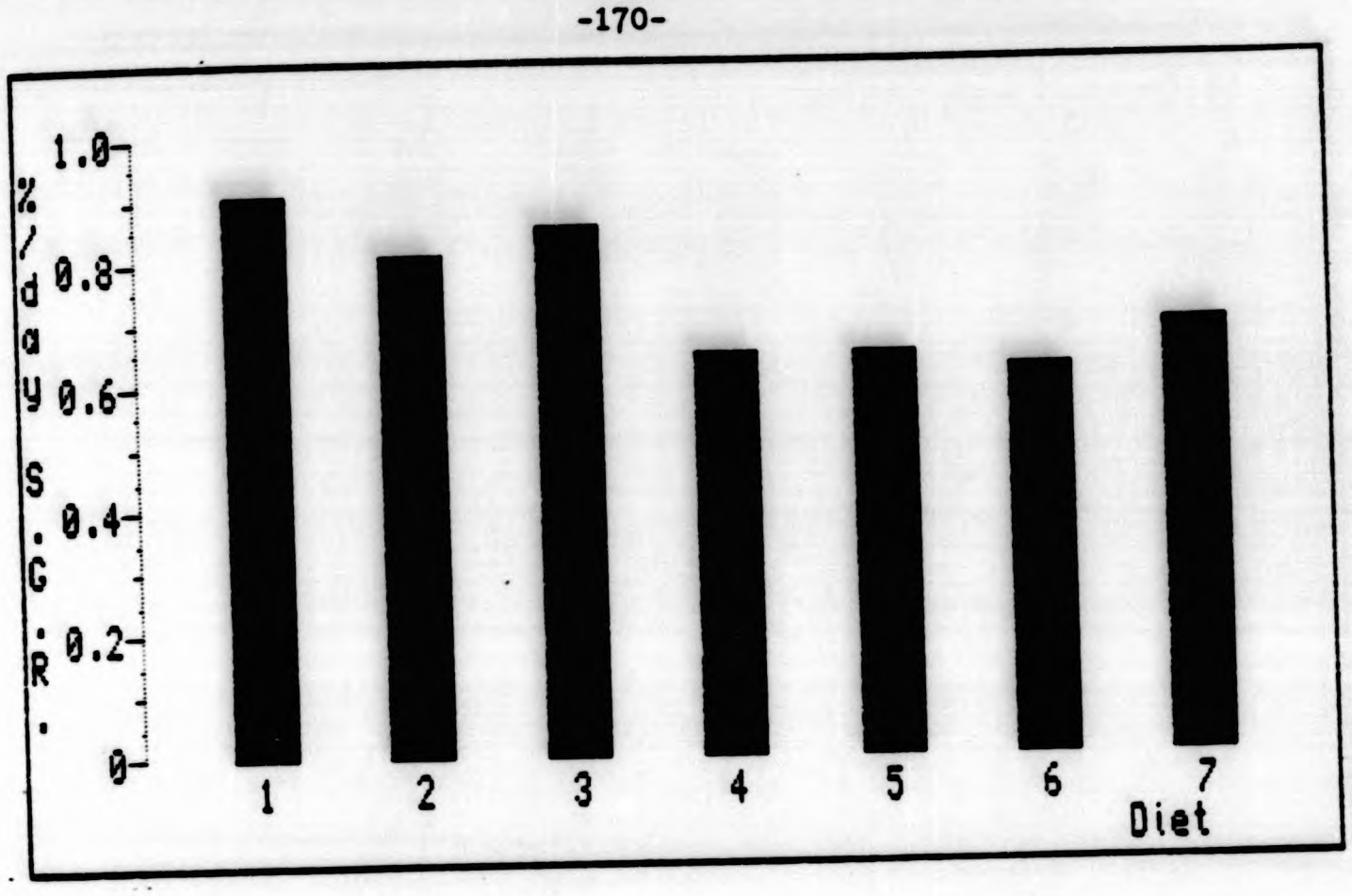

FIGURE 6.2 Specific growth rate (%/day) of fish fed the seven experimental diets

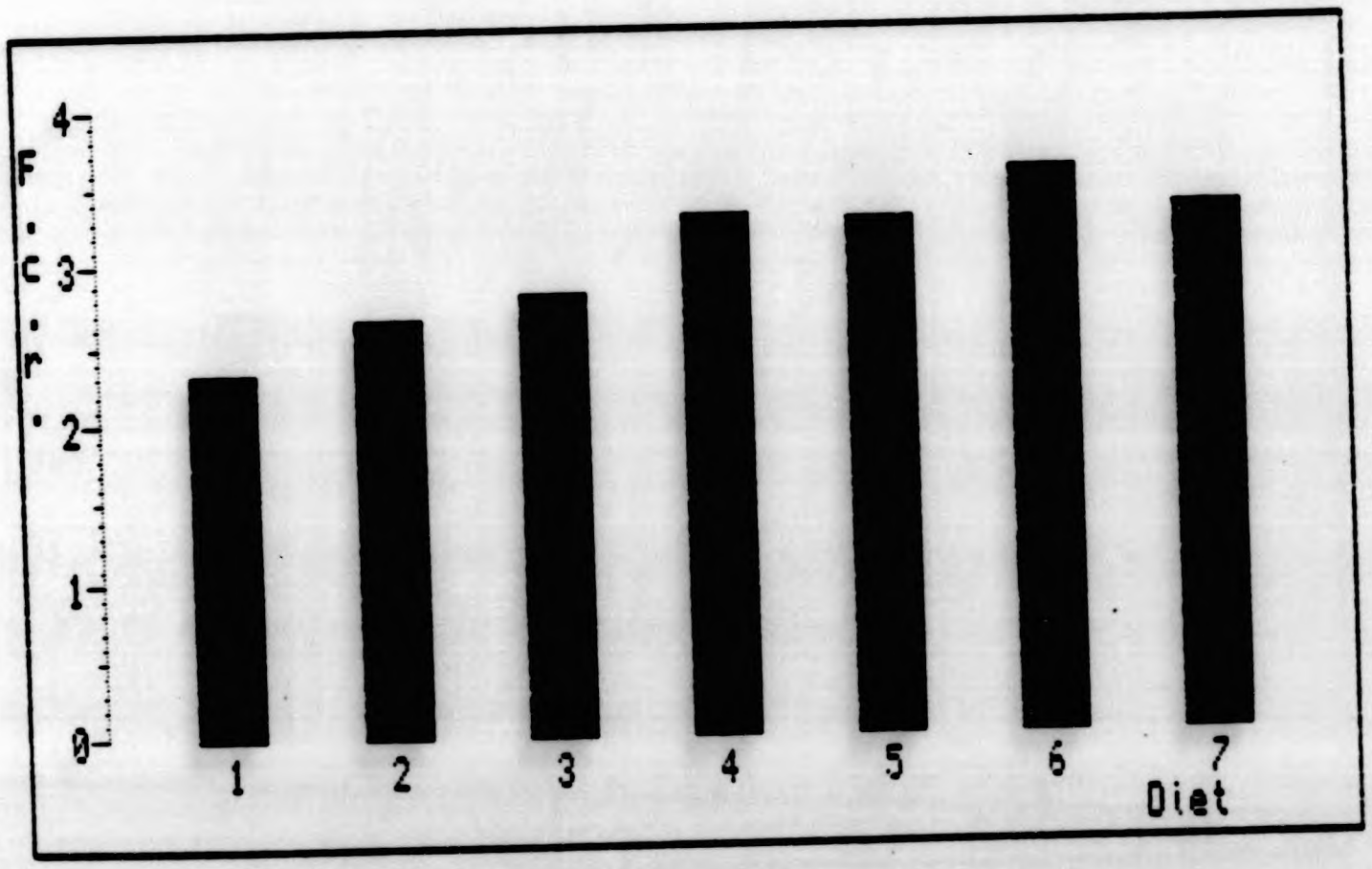

FIGURE 6.3 Food conversion ratio of fish fed the seven experimental diets

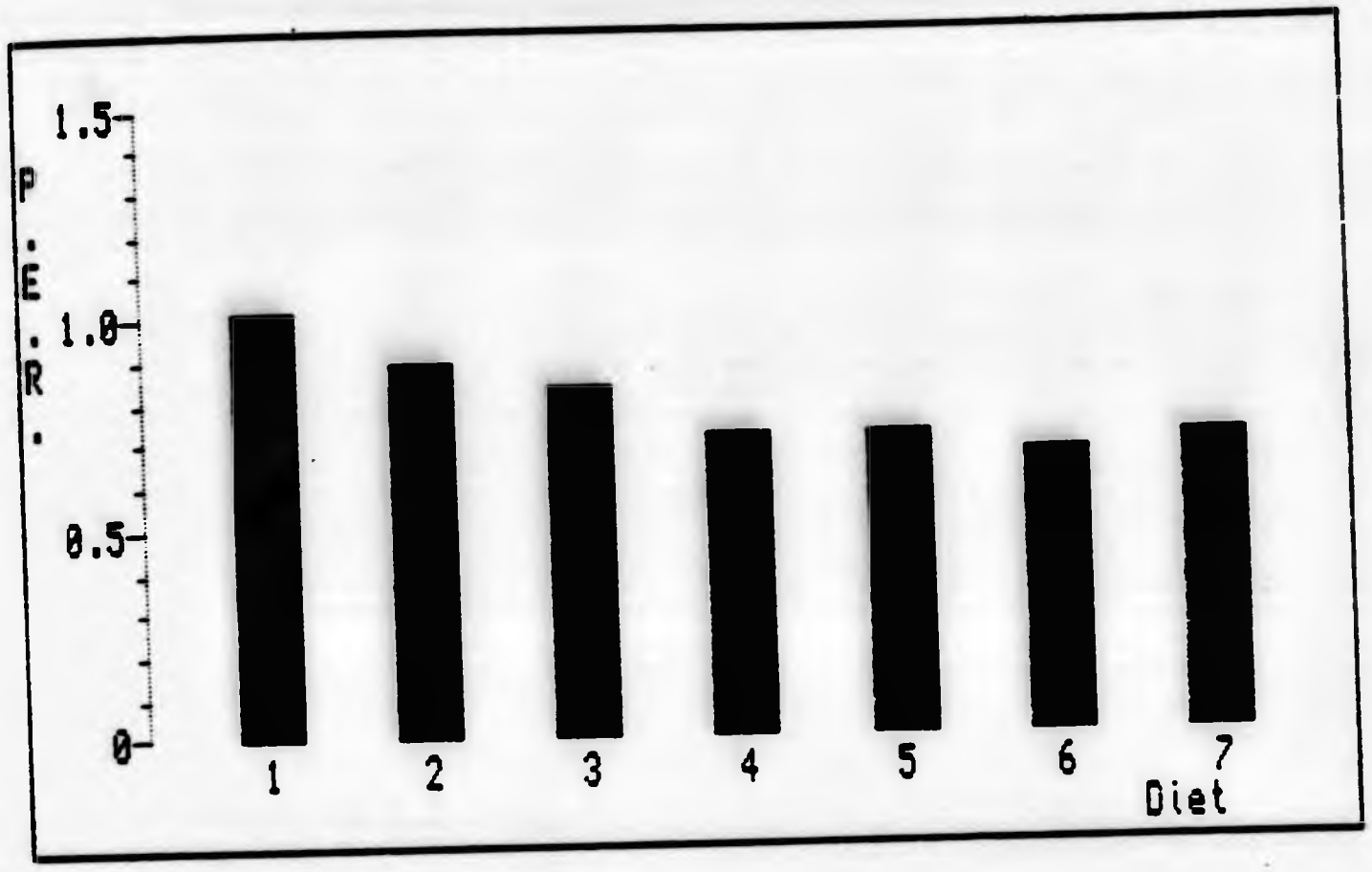

FIGURE 6.4 Protein efficiency ratio of fish fed the seven experimental diets

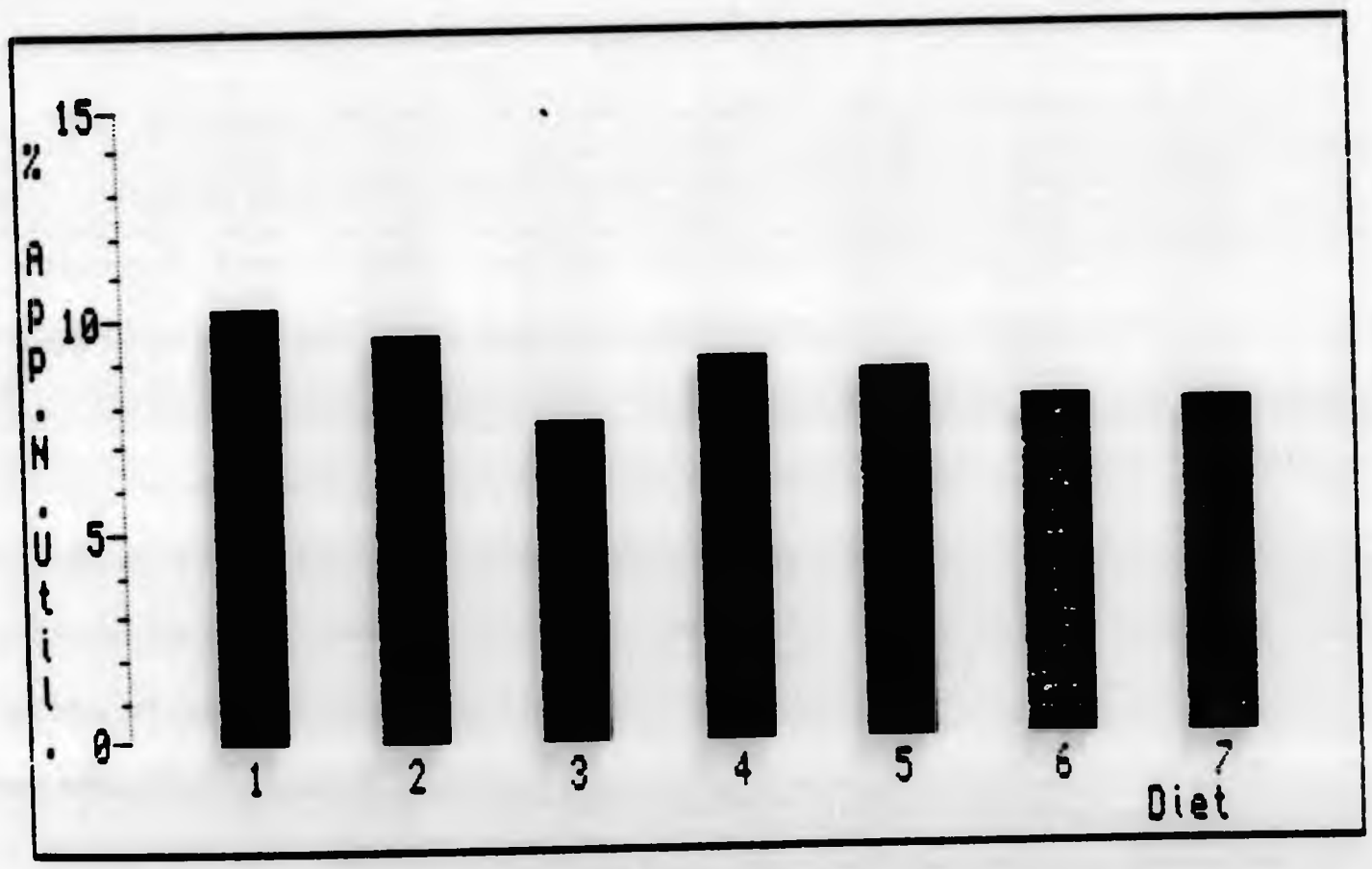

FIGURE 6.5 Apparent net protein utilization (%) of fish fed the seven experimental diets

shows that the best performance was registered by fish fed Diet 1 with an apparent NPU of 10.32%. Of the fish meal based diets the highest apparent NPU of 9.67% was achieved by fish fed Diet 2 and the lowest of 7.62% by those fed Diet 3 (Table 6.8; Fig. 6.5). It was noted that although fish fed Diet 6 had the worst PER they did not have the lowest apparent NPU. Thus in terms of maximum growth and feed utilization efficiency the best performance of fish fed the fish meal based rations was by fish fed Diet 3 containing fish meal B.

The growth performance of rainbow trout replicates is given in Appendix V.

6.3.3 Digestibility

The moisture content of faeces from fish fed the fish meal based rations varied between 3.97% (Diet 2) and 6.53% (Diet 3). By contrast faeces from fish fed the "Pruteen" based control diet (Diet 1) had a moisture content of 8.97% (Table 6.10; Fig. 6.6a).

The crude protein content of faeces varied between 17.90% (Diet 1) and 21.47% (Diet 7). The lowest value of 17.90% was for faeces produced by fish fed the control ration. Thus the crude protein content of faeces from fish fed all diets in which the dietary protein was supplied entirely by the brown fish meals were higher than those of fish fed the control diet (Table 6.10; Fig. 6.6b). Differences in levels of crude protein found in faeces from fish fed the experimental diets were reflected in the apparent protein digestibilities.

TABLE 6.10 Proximate composition and Cr_2O_3 content of faeces taken from rainbow trout after 18 weeks on the experimental diets (% dry weight)

Faeces composition (% dry weight)	Dietary treatments 1	2	3	4	5	6	7
Moisture (%)	8.97(1.61)[1]	3.97(0.95)	6.53(0.68)	4.80(0.57)	4.09(1.04)	4.60(2.09)	4.49(1.93)
Crude protein (N x 6.25)	17.90(0.48)	21.09(1.63)	20.48(0.65)	21.43(1.71)	19.42(0.21)	21.13(0.11)	21.47(0.38)
Lipid (%)	3.55(0.31)	17.69(0.60)	14.02(0.42)	15.91(0.57)	9.35(0.03)	6.47(1.41)	11.00(0.01)
Ash (%)	18.82(0.69)	43.28(3.11)	50.77(5.21)	42.58(0.35)	48.21(2.18)	51.14(1.61)	45.89(0.21)
Cr_2O_3 (%)	1.29(0.12)	1.07(0.06)	0.90(0.10)	1.11(0.02)	1.04(0.03)	1.00(0.00)	1.16(0.04)

1) Standard deviation

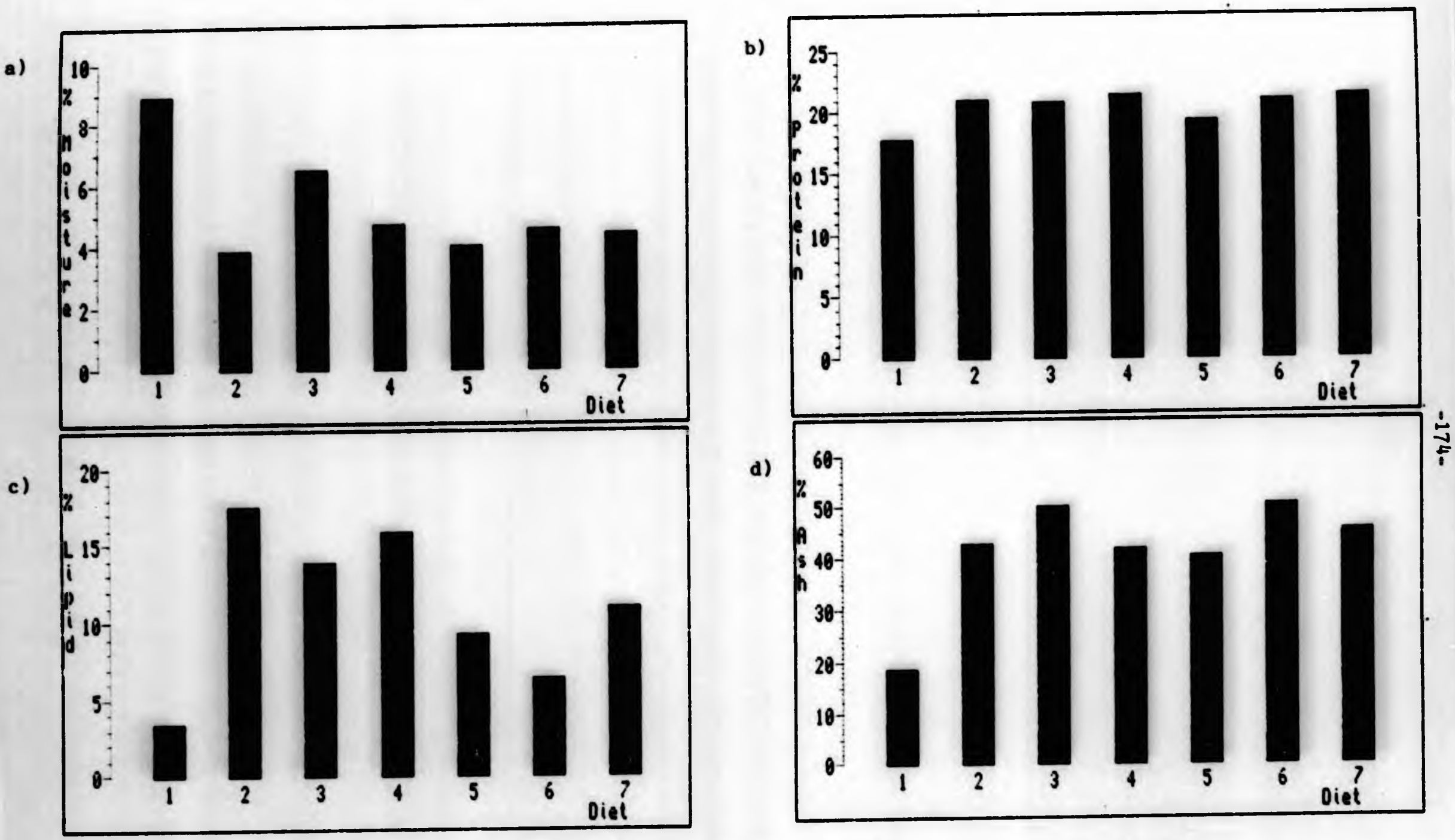

FIGURE 6.6 Proximate composition of the faeces from fish fed each of the seven experimental diets.
a) Moisture; b) Crude protein; c) Lipid; d) Ash

Apparent protein digestibility varied significantly ($P < 0.05$) between 72.30% for fish fed the diet based on fish meal B (Diet 3) and 86.27% for those fed the bacterial SCP control diet (Diet 1; Table 6.8). Although in terms of growth performance, Diet 3 gave the best specific growth rate, and also one of the best PER's and food conversion ratios (Table 6.8), the apparent protein digestibility was significantly poorer than for any other treatment.

Faeces from the control fish fed Diet 1 again contained the lowest level of lipid (3.55%). There was a wide variation in lipid content of faeces from fish fed the fish meal based diets from 6.47% for fish fed Diet 6 to 17.69% for fish fed Diet 2 (Table 6.10; Fig. 6.6c). Thus the lipid content of faeces from fish fed Diet 2 was five times higher than that of the control fish. The apparent lipid digestibility of the experimental diets varied significantly ($P<0.05$) from only 59.84% (Diet 7) to 90.76% (Diet 1), thus fish fed the control diet again had the best coefficient, while the best of the fish meal based diets was Diet 6 (86.77%; Table 6.8). Fish fed Diet 3 had one of the poorest apparent lipid digestibility coefficients of 75.90%.

The ash content of faeces from fish fed all six fish meal based experimental diets was high, ranging from 42.58% for fish fed Diet 4 to 51.14% for those fed Diet 6. By contrast, the ash content of faeces from the control fish fed the "Pruteen" based diet (Diet 1) was only 18.82% (Table 6.10; Fig. 6.6d). It therefore appears that the high dietary ash content was in part reflected in the ash content of fish faeces. Thus, faeces from fish fed Diet 3, which contained

the highest level of dietary ash (31.34%; Table 6.6), had one of the highest ash contents (50.77%) while those of fish fed Diet 1, which contained the lowest level of dietary ash (9.15%), had the lowest ash content (18.82%). One consequence of the high ash content of the fish meal based rations was low apparent organic matter digestibilities. The apparent organic matter digestibilities of fish fed the fish meal based experimental diets varied significantly ($P < 0.05$) between only 44.65% (Diet 3) and 58.25% (Diet 7). Again fish fed the control diet (Diet 1) had a significantly higher apparent organic matter digestibility of 70.18% (Table 6.8).

Thus although fish fed Diet 3 had the best absolute growth rate of fish fed the fish meal based rations, they achieved poor digestibility coefficients. The best overall digestibility coefficients by fish fed the fish meal based rations was by those fed Diets 4 and 5 containing fish meals C and D respectively.

The proximate composition of faeces taken from rainbow trout replicates is given in Appendix VII.

6.3.4 Liver Somatic Index and Blood Parameters

The liver somatic index of fish fed the seven experimental diets varied between 1.06 for fish fed Diet 5 and 1.44 for fish fed Diet 3. Fish fed Diet 5 had a liver somatic index significantly ($P < 0.05$) lower than that of fish fed the control ration (1.39; Table 6.8).

The haematocrit value varied significantly ($P < 0.05$) between 33.00% for fish fed Diet 1 and 38.80% for those fed Diet 2 (Table 6.8). Thus fish fed the bacterial SCP "Pruteen" diet had the lowest haematocrit value although this level is still within the normal range for healthy rainbow trout (Wedemeyer and Nelson, 1975; Miller et al., 1983; Railo et al., 1985).

Blood haemoglobin levels of the experimental fish varied between 4.79g per 100 cm^3 for fish fed Diet 6 and 5.71g per 100 cm^3 for those fed Diet 2. However, these differences were not significant at the 95% level (Table 6.8).

The liver somatic index and blood parameters of rainbow trout replicates are given in Appendix V.

6.3.5 Carcass Composition

At the end of the 18 week growth trial the carcass moisture content of fish fed all seven experimental diets had decreased significantly ($P < 0.05$) from 80.11% to between 68.88% for fish fed Diet 2 and 74.18% for those fed Diet 6 (Table 6.11; Fig. 6.7a). Fish fed the fish meal experimental diets had significantly higher moisture contents (70.77-74.18%) than those fed the control (68.88%).

In conjunction with the decrease in carcass moisture, there was a significant ($P < 0.05$) increase in carcass lipid over the 18 week feeding trial. At the start of the trial fish contained only 2.63% lipid, but this increased to a maximum of 11.29% in the

TABLE 6.11 Carcass composition of rainbow trout (280 fish, 23g) at the start and end of the experiment (18 weeks) based on 12 fish per treatment (% wet weight)

Carcass composition (% wet weight)	Initial	Dietary treatments 1	2	3	4	5	6	7	± S.E.[1]
Moisture (%)	e 80.11(0.14)[2]	a 68.88(0.93)	bc 71.08(1.09)	b 70.77(1.38)	d 73.70(0.31)	d 74.02(1.38)	d 74.18(0.55)	d 73.31(1.42)	0.628
Crude protein (N x 6.25) (%)	a 13.32(0.15)	bcde 16.91(0.58)	bcdefg 17.53(0.93)	h 18.88(0.41)	bcdef 17.16(0.67)	bc 16.68(0.64)	b 16.40(0.49)	bcd 16.72(0.04)	0.381
Lipid (%)	a 2.63(0.65)	h 11.29(0.87)	efg 7.60(0.80)	cdef 7.00(0.72)	bc 5.42(0.74)	bcd 5.49(0.19)	b 4.97(0.49)	bcde 6.22(0.39)	0.510
Ash (%)	a 3.85(0.19)	a 3.67(0.43)	a 3.64(0.20)	a 3.87(0.49)	a 3.63(0.28)	a 3.74(0.14)	a 4.06(0.14)	a 3.70(0.25)	0.143
	99.91	100.75	99.85	100.50	100.01	99.93	99.61	99.95	

1) Standard error; calculated from residual mean square in the analysis of variance

2) Standard deviation

abcdefgh Mean values for components with common superscripts are not significantly ($P < 0.05$) different

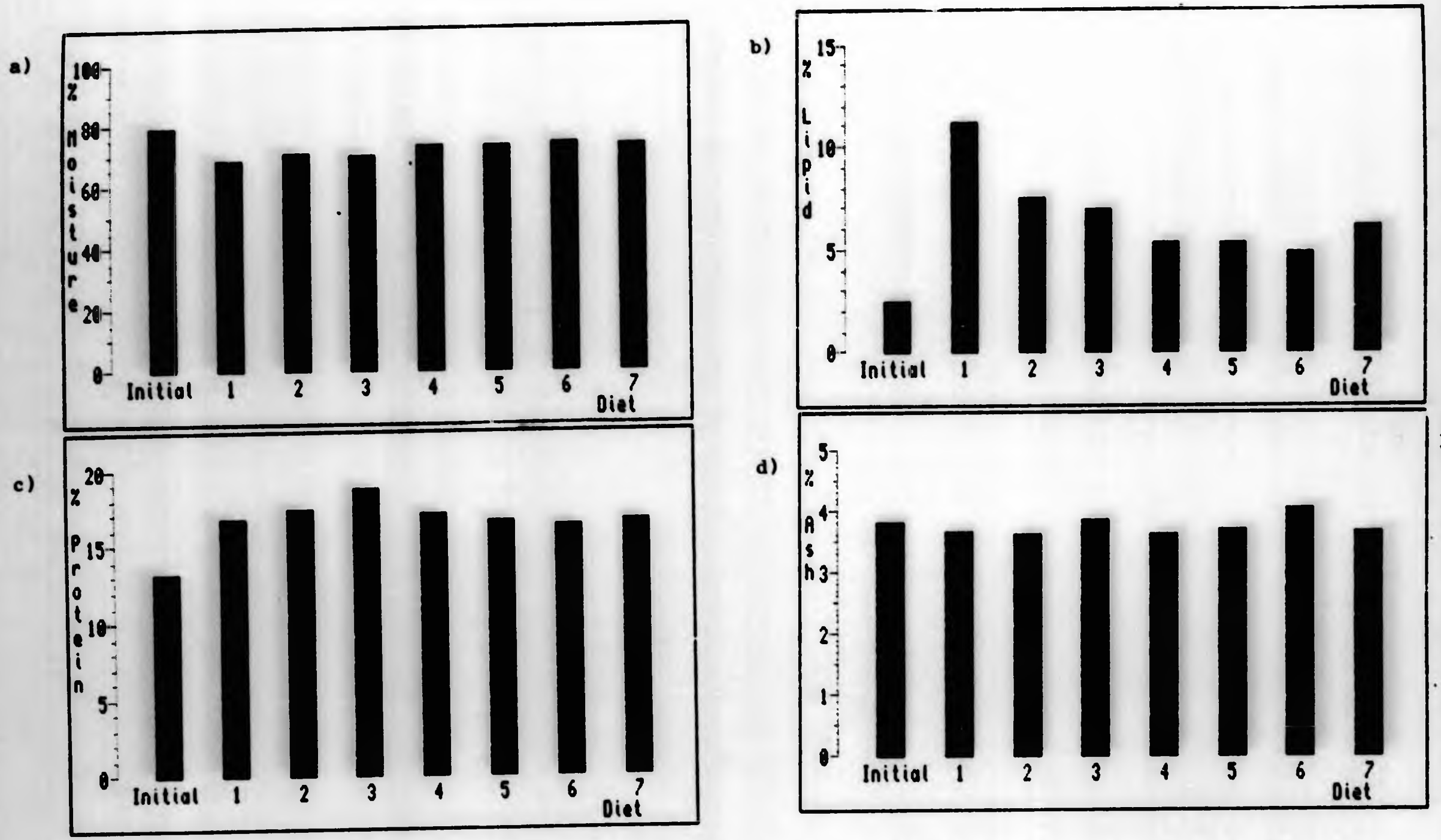

FIGURE 6.7 Proximate composition of the carcasses from fish fed each of the seven experimental diets and of the initial sample. a) Moisture; b) Lipid; c) Crude protein; d) Ash

"Pruteen" control fish (Table 6.11; Fig. 6.7b). Lipid levels of fish fed the fish meal based rations varied between 4.97% (Diet 6) and 7.60% (Diet 2).

There was a significant ($P<0.05$) increase in the carcass protein content of fish fed all seven experimental rations from 13.32% at the start of the trial to between 16.40% (Diet 6) and 18.88% (Diet 3) at the end. With a single exception there were no significant ($P < 0.05$) differences in protein content either between fish fed the fish meal based rations and those fed the "Pruteen" control, or between fish fed five of the fish meal based diets themselves. The protein content of fish fed Diet 3 (18.88%) however, was significantly higher than that of any treatment (Table 6.11; Fig. 6.7c).

The carcass ash content at the start of the trial was 3.85% and although the ash content of fish at the end varied between 3.64% and 4.06%, these differences were not signifcant ($P < 0.05$; Table 6.11; Fig. 6.7d).

The proximate composition of fish carcass replicates is given in Appendix VIII.

6.4 DISCUSSION

The growth performance and feed utilization efficiency of rainbow trout fed diets where five of the six fish meals tested were the sole source of dietary protein were significantly poorer than that of fish fed a "Pruteen" based control ration (Table 6.8). Only one of the fish meals, fish meal B, gave a growth response similar to the control ration. Generally the use of bacterial SCP as the sole source of dietary protein is not recommended since the maximum inclusion level without any loss of performance or feed utilization is 75% (Beck *et al*., 1979; Spinelli *et al*., 1979; Tiews *et al*., 1979; Kaushik and Luquet, 1980; Tacon *et al*., 1983a; Tacon and Jackson, 1985). It would thus be expected that fish fed the fish meal based diets should perform better than those fed a control ration based on "Pruteen", which was not the case. Indeed under the prevailing culture conditions, fish fed at a rate of 2% body weight per day giving food conversion ratios of around 2 would be expected to reach at least 100g by the end of an 18 week growth period (Ewos-feeding chart). However the best final mean weight was only 72g and the poorest 52g (Table 6.8). Another problem related to the use of "Pruteen" as the sole protein source is its acceptability by fish. Fish fed the bacterial SCP based control ration took longer to accept the new diet and thereafter they did not feed so aggressively as those fed the fish meal based diets, suggesting a slightly poorer palatability when compared with that of the fish meals.

From the analysis it is apparent that none of the fish meals tested was of a particularly high quality. The maximum crude protein

content was only 63.55% for fish meal A (Table 6.3) which is close to the lower limit of 60% for good quality fish meals (Windsor and Barlow, 1981). This fish meal produced one of the best growth responses (Table 6.8). Although the diet based on fish meal B produced the best growth rate of the fish meals, fish meal B had the lowest crude protein content of only 52.49%. Using fish meals with low protein content will necessarily lead to their use at high percentage by weight to obtain the required crude protein content thus reducing the available space for other ingredients.

Suboptimal levels of certain essential amino acids in the fish meals resulted in all the diets based on fish meal being deficient in between three and five of the nine essential amino acids analysed (Table 6.7). In comparison with good quality fish meals, all the fish meals tested contained low levels of leucine, particularly fish meals B to F which contained between 0.94% and 1.16% compared with levels of around 4% for good quality fish meals (Windsor and Barlow, 1981). This resulted in leucine being the first limiting essential amino acid in all the diets produced from these fish meals with the exception of Diet 6 containing fish meal F. In this case, although the leucine content was only 42% of the requirement level, only 34% of the required phenylalanine content was present in the diet. However, since tyrosine can spare phenylalanine (Mertz, 1972; Halver, 1975, 1976; Cowey, 1979; Ketola, 1982; Millikin, 1982; Walton, 1985; Wilton, 1985) the first limiting amino acid would have again been leucine. Thus the proportion of protein usable for growth in Diets 3 to 7 varied between only 36% in Diet 4 based on fish meal C which gave one of the poorest growth rates, and 49% in

Diet 3 based on fish meal B which gave the best growth rates. By contrast with Diets 3 to 7, Diet 2 based on fish meal A contained 86% of the leucine requirement level. Although the lowest chemical score in this diet was that of phenylalanine, the first limiting essential amino acid was probably valine since phenylalanine can be spared by tyrosine. Valine supplied 58% of the requirement level and therefore the level of usable protein using fish meal A was slightly better than that of fish meal B. Furthermore Diet 2 containing fish meal A was only deficient in three essential amino acids, leucine, phenylalanine, and valine compared with five in Diets 3 to 6 (arginine, leucine, lysine, phenylalanine, and valine) and four in Diet 7 (Table 6.7). Again tryptophan levels were not measured but it was unlikely to have been the first limiting amino acid since if only half the normal level of tryptophan was present in the fish meals it would still supply enough to satisfy the requirement of rainbow trout for this essential amino acid as indicated in Section 5.4.

The essential amino acid index (EAAI) gives a relatively good indication of the quality of a protein since the closer the EAAI is to 100, the closer the essential amino acid profile of the protein matches the requirement of the fish (Jauncey and Ross, 1982). The EAAIs were determined based on the nine essential amino acids analysed and calculated on each of the essential amino acid requirements indicated by Ogino (1980) and Rumsey et al. (1983). Diets 2, 3 and 7 (fish meals A, B and F) had the best EAAIs with values varying between around 82 and 85 and these diets produced the best growth performances. By contrast the EAAIs of Diets 4, 5 and 6 were around

only 76 and 78 and these rations produced the poorest growth responses (Table 6.8). The bacterial SCP, which had an EAAI of 83.15, was deficient in both the essential amino acids phenylalanine and valine (Table 6.4) with levels which were lower than the normal values indicated for this protein source (Table 2.31). This resulted in only 50% of the required valine level being present in the control ration, and thus only half of the protein was usable for growth which was close to the amount available in Diet 3 containing fish meal B.

The amount of unaccounted amino acids and of nonprotein nitrogen in the fish meals tested varied widely between around 4% and 17% with particularly high levels in fish meals C, D, and E (14.42%, 14.29%, and 17.00% respectively). Spinelli and Dassow (1979) and Jobling (1983) reported that fish normally have a nonprotein nitrogen content of around 0.5% and 2% respectively. By contrast, Niimi (1972) indicated that levels of nonprotein nitrogen vary between 2% and 38% dependent on fish species. Thus nonprotein nitrogen values of between 9% and 14% were indicated for flatfish, 14% and 18% for herrings and between 34% and 38% of the total nitrogen in elasmobranchs. By contrast a value of only 2% was indicated for sockeye salmon (Oncorhynchus nerka), a salmonid fish (Brett et al., 1969). It is possible that fish meals C, D, and E may have contained some of these fish species with high nonprotein nitrogen levels, but since neither true protein nor nonprotein nitrogen were measured in this trial it is not feasible to establish accurately the proportion of nonprotein nitrogen and of unaccounted amino acids.

The level of unaccounted amino acids and nonprotein nitrogen in the bacterial SCP was high (18.00%), but bacterial SCPs have been reported to contain between 8% and 16% by weight of nonprotein nitrogen (Kilhberg, 1972; Matty and Smith, 1978; Tacon and Jackson, 1985) and Braunde et al. (1977) reported a value of 14.7% for "Pruteen". It is therefore likely that only around 3% of the nitrogen content may be attributable to unaccounted amino acids.

The lipid content of all the fish meals tested was either within or only slightly higher than the normal range of reported values of 8% to 12% for good quality fish meals (Windsor and Barlow, 1981). The peroxide values of fish meals C and E were both higher than the recommended maximum level of 4 mEq/Kg oil (Billinski et al., 1978) which indicated a certain degree of lipid peroxidation. As a result the diets produced using these fish meals both had peroxide values of around 4.5 mEq/Kg oil. It is known that high peroxide values have the effect of decreasing the nutritive value of protein and lipid due to reactions between oxidized oil and protein and consequently lowering the energy content of the meal (Reinitz, unpublished; Cockerell et al., 1971; Lee and Sinnhuber, 1972; Opstvedt, 1974; Hilton et al., 1977; Castell, 1979; Eriksson, 1982; Watanabe et al., 1983; Bell and Cowey, 1985). Hung et al. (1980) however, reported that diets fed to rainbow trout containing oxidized fish oil with a peroxide value of 5 mEq/Kg oil had no significant effect on performance and mortality rate, when supplemented with an anti-oxidant.

The other main effect of oxidized oil is on protein and lipid digestibilities which are known to suffer a marked reduction, particularly the lipid digestibility (Nose and Toyama, 1966; Opstevdt, 1974; Watanabe et al., 1983). Undoubtedly this was not the case since lipid digestibility of Diets 4 and 6 containing oxidized oil had two of the best lipid digestibility coefficients. Furthermore protein digestibility did not appear to have been affected (Table 6.8). The increased peroxide values of fish meals C and E (Table 6.3) suggests that these fish meals may have been stored under adverse environmental conditions without adequate protection either before or after processing. High environmental temperatures are known to accelerate the process of oxidative rancidity (Cockerell et al., 1971; Windsor and Barlow, 1981; Stansby, 1983) and since these meals were manufactured during the spring when the environmental temperatures in Portugal normally begin to increase rapidly to around 25°-35°C it is possible that this accelerated the process of lipid peroxidation. In terms of final moisture content the processing of these fish meals seems to have been adequate since moisture levels (Table 6.3) were all well below the critical level of 13% - 14% above which spoilage is likely to occur (Cockerell et al., 1971; Windsor and Barlow, 1981).

The ash content of all of the fish meals tested was higher than the maximum recommended level of 21% for good quality fish meals (Windsor and Barlow, 1981). In particular fish meals B and E contained in excess of 30% ash (Table 6.3) and these high ash levels were largely responsible for the low crude protein content of the meals (Cho, 1980). Thus, where a high proportion of the

protein is to be supplied by these fish meals high inclusion levels are necessary to obtain the required dietary crude protein content and this therefore reduces the available space for the addition of other ingredients, such as carbohydrates. As a consequence there was a wide variation in the nitrogen free extract (NFE) content of the diets. The rations containing the six brown fish meals contained between 13.18% and 26.11% NFE whereas the high crude protein content of the bacterial SCP resulted in a very high dietary NFE value of 33.72% (Table 6.6).

Protein digestibilities of the fish meals tested in this study varied between 72.30% and 78.09% whereas very good quality brown fish meals have been reported to have protein digestibilities as high as 93% to 95% (McDonald et al., 1981; Watanabe et al., 1983; Wojno and Dabrowska, 1984a). Watanabe et al. (1983) reported protein digestibility coefficients of between 84% and 95% for three brown fish meals when they were included in diets for rainbow trout as the sole source of protein. Thus even the best coefficient of 78% obtained in Diet 7 containing fish meal F was well below the minimum coefficient indicated by these authors. These low protein digestibilities of the fish meal based diets was a reflection not only of the high dietary ash content but more importantly of the severe essential amino acid deficiencies. This is particularly clear in Diets 4, 5 and 6 where the total dietary protein usable for growth was reduced to only 36%, 39% and 42% respectively, and furthermore the poorest growth rates, food conversion ratios and PERs were indicated (Table 6.8).

The protein digestibility of the control ration (86.27%) was significantly higher than those of the fish meal based diets and furthermore it was better than the value of 82.40% reported by Kaushik and Luquet (1980) when this bacterial SCP entirely replaced herring meal protein in a 45% crude protein ration fed to rainbow trout. However, Atack and Matty (1979) indicated a true protein digestibility coefficient of 93.5% for "Pruteen" fed to rainbow trout as the sole dietary protein source.

Although there were significant differences in carcass composition between fish fed the six brown fish meals, particularly in the crude protein and lipid content, these variations were not very great and varied between 16.40% and 18.88% for the former and between 4.97% and 7.60% for the latter (Table 6.11). Fish fed the control ration based on "Pruteen" had a significantly different carcass composition from those fed the fish meals, particularly in terms of body lipid content. A significantly higher lipid content of 11.29% was indicated for fish fed the "Pruteen" based ration compared with lipid levels of only between 5% and 7% for those fed the fish meal based diets (Table 6.11). This is in accordance with the findings of Kaushik and Luquet (1980) who reported a slight increase in body lipid content of rainbow trout fed diets containing increasing inclusion levels of "Pruteen". By contrast, Atack and Matty (1979) found that rainbow trout fed a diet containing "Pruteen" as the sole source of dietary protein had a significantly lower lipid content than those fed a herring meal control ration.

The high food conversion ratios obtained with the fish meal based diets reflected the essential amino acid deficiencies of these meals. Thus food conversion ratios of around 3.3-3.6 were obtained in Diets 4, 5 and 6 where only around two-thirds of the total protein was usable for growth, while in Diets 2 and 3 half of the total protein was usable for growth and therefore better food conversion ratios of around 2.6-2.8 were indicated (Table 6.8).

The food conversion ratio of the control ration (2.35) was also quite high but was close to the value of 2.01 reported by Atack and Matty (1979) when "Pruteen" was the sole protein source, although Beck *et al*. (1979) obtained a much lower food conversion ratio of 1.3. This diet contained only 50% of the valine requirement which effectively reduced the amount of protein available for growth to half. It is possible that the relatively high food conversion ratio may also be attributable to the high NFE content (33.72%) of the "Pruteen" based ration since it has been reported that high dietary carbohydrate levels increase the food conversion ratio (Spannhof and Kühne, 1977; Refstie and Austreng, 1981).

Fish fed Diet 5 containing fish meal D had a significantly lower liver somatic index of 1.06 than those fed the control ration and Diets containing fish meals A and B. This is most likely to be attributable to the fish meal itself rather than to carbohydrate since only a carbohydrate level of 20% was indicated for this Diet and fish fed Diets 4 and 7 with similar carbohydrate levels had higher liver somatic indices although these were not significantly different at the 95% level (Table 6.8). The liver somatic index

of fish fed the "Pruteen" based ration was relatively high (1.39). This may have been due to the effect of this bacterial SCP on fish since it has been reported that rainbow trout fed diets containing "Pruteen" resulted in enlarged livers caused by extremely high glycogen levels stored inside the hepatic cells (Beck et al., 1979).

There were significant differences in the haematocrit values of fish fed the fish meal based diets (Table 6.8) but these differences were relatively small and values were all within the normal range of reported values for healthy rainbow trout (Wedemeyer and Nelson, 1975; Miller et al., 1983; Railo et al., 1985). Although there were no significant differences in haemoglobin content, fish fed Diets 4, 6 and 7 containing fish meals C, E and F had haemoglobin levels below the minimum value of 5.3g per 100 cm^3 reported by Wedemeyer and Nelson (1975) for healthy rainbow trout, while according to Lowe-Jinde and Niimi (1983) fish fed all dietary treatments had low haemoglobin contents since all values were below the range of between 7.8g and 9.8g per 100 cm^3 indicated by these authors for healthy rainbow trout.

In conclusion the fish meals tested had an overall chemical composition well below the normal standards for good quality fish meals with a low crude protein and a high ash content. Furthermore they had severe deficiencies in at least three essential amino acids and consequently their inclusion in experimental diets resulted in relatively poor growth performances and feed utilization efficiencies. Fish meals C, D and E gave the poorest growth responses and although the diet based on fish meal B produced the best weight

gain, the growth response was still somewhat poorer than would be expected for a good quality fish meal.

CHAPTER 7

THE NUTRITIONAL EVALUATION OF MEAT AND BONE MEAL AS A FEED FOR RAINBOW TROUT

7.1 INTRODUCTION

Meat and bone meal is one of the commonest by-products produced from slaughterhouse plants in Portugal with production costs 35% lower than that of Portuguese fish meals. The production of this commodity by one of the main processing plants amounts to 2800 tons per year (manager, personal communication). Although total Portuguese production is unknown, there are at least 80 similar plants scattered throughout the country.

A further important advantage associated with Meat and bone meal is its availability throughout the year. Thus supply is not subject to shortages and to seasonality which can be encountered with fish meals.

The use of Meat and bone meal and its nutritional quality in diets for rainbow trout was reviewed in Chapter 2. This animal by-product is commonly used in commercial rations although generally only at relatively low levels of up to 15% by weight. Nevertheless good results at higher inclusion levels in experimental diets for rainbow trout have been reported, as indicated in Chapter 2.

In view of its availability, relatively low cost and promising nutrient profile it was evaluated as a protein source in diets for rainbow trout.

7.2 MATERIALS AND METHODS

7.2.1 Diets

25 Kilogrammes of meat and bone meal were obtained from Manuel dos Santos Moura Lda, Porto (Fig. 9.1). The meal was produced from the scraps of the cattle slaughterhouse industry and from local butchers. 25 Kilogrammes of brown fish meal were supplied by Sociedade de Pescas do Oceano, Figueira da Foz, located at the centre of Portugal. This meal was mainly produced from whole fish carcasses and blended with an imported brown fish meal to improve its quality (fish meal plant manager, personal communication).

On arrival at the laboratory the meat and bone meal and the brown fish meal were immediately ground and their crude protein and and lipid content were determined according to the methods given in Chapter 4. During grinding some of the larger bone particles of the meat and bone meal were discarded, thus altering its proximate composition, which resulted in a meal with a better nutritional quality.

Six experimental diets were formulated using meat and bone meal and brown fish meal as the sole protein sources. The meat and bone meal contained 67.07% crude protein and 18.57% lipid. This crude protein content was higher than the usual level of around 50% and was a consequence of the removal of some of the bone material. The resulting meal was included in five experimental diets for rainbow trout. Thus a control ration in which brown fish meal (73.70% crude

protein and 7.22% lipid) supplied the dietary protein and five diets in which meat and bone meal replaced up to 100% of the brown fish meal protein were prepared.

Diet 1: control, 100% Brown fish meal protein

Diet 2: 20% Meat and bone meal protein + 80% Brown fish meal protein

Diet 3: 40% Meat and bone meal protein + 60% Brown fish meal protein

Diet 4: 60% Meat and bone meal protein + 40% Brown fish meal protein

Diet 5: 80% Meat and bone meal protein + 20% Brown fish meal protein

Diet 6: 100% Meat and bone meal protein

The full dietary formulations are presented in Table 7.1. All six experimental diets were formulated on an isonitrogenous and iso-caloric basis to contain 45% crude protein and 13% lipid (Table 7.3). Since the meat and bone meal contained a high level of lipid the diets were formulated to contain 13% lipid since this was the minimum level possible in a diet in which all the protein was supplied by meat and bone meal and which also contained sufficient fish oil to supply the necessary essential fatty acids.

Seven Kilogrammes of each diet were manufactured as described in Chapter 4.

The percentage of dietary carbohydrates varied from 14.1% to 15.4% for corn starch and from 7.1% to 7.7% for yellow dextrin (Table 7.1). Thus the overall carbohydrate content of between 21% and 23%

TABLE 7.1 Formulation of experimental diets (% by weight)

Diet No.	1	2	3	4	5	6
% inclusion MBm	0	20	40	60	80	100
Brown fish meal	65.40	52.40	39.30	26.20	13.10	
Meat and bone meal		14.30	28.60	42.90	57.20	71.50
Corn starch	14.10	14.30	14.60	14.90	15.20	15.40
Yellow dextrin	7.10	7.20	7.30	7.40	7.50	7.70
Cod liver oil[1]	8.61	7.01	5.41	3.81	2.21	0.61
Vitamin premix[2]	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[3]	1.09	1.09	1.09	1.09	1.09	1.09
Binder[4]	1.00	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50
Potassium sorbate	0.20	0.20	0.20	0.20	0.20	0.20

1) Containing 150 mg/Kg diet of Butylated hydroxytoluene (BDH Chemicals Ltd., Poole, Dorset, England)

2) According to Table 4.4

3) According to Table 4.5

4) Carboxymethylcellulose, dissodium salt, high viscosity (BDH Chemicals Ltd., Poole, Dorset, England)

of the diet was only slightly higher than the recommended maximum level of 20%.

The full chemical analysis carried out on both the ingredients and the diets is summarised in Table 4.2 and the methods are described in Chapter 4.

7.2.2 Growth Trial

260 rainbow trout of mean weight 36g (36.13g-36.82g; Appendix IX) were obtained from Truturão, Cernache, Coimbra and were allocated to 13 net cages as described in Chapter 4. Each experimental diet was allocated randomly to two net cages and a further cage of 20 fish was unfed during the experimental feeding period. Fish were fed at a fixed rate of 2% body weight per day throughout the 13 week trial. The full experimental protocol of the trial is described in Chapter 4.

The mean water temperature during the experimental test period was 9.3°C (5.0°-11.5°C ± 1.58) and the daily variation never exceeded 2°C (Table 7.2).

Statistical methods and the production of graphs were carried out as described in Chapter 4.

TABLE 7.2 Water temperature (°C) and standard deviation over the feeding period

Weeks	December	January	February	March	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0	10.0							
1	10.0				10.0	0.00		
2	10.0							
3		5.0						
4		7.5			7.5	1.78		
5		9.0						
6		8.5					9.3	1.58
7			9.5					
8			10.1		10.4	0.84		
9			11.5					
10			10.5					
11				9.0				
12				10.5	9.5	0.87		
13				9.0				

7.3 RESULTS

7.3.1 Diets

The meat and bone meal and the brown fish meal contained 67.07% and 73.70% crude protein content respectively (Table 7.3). The meat and bone meal tested was a good source of both nonessential amino acids glycine and proline and of the essential amino acids isoleucine, and histidine, but was deficient in leucine although slight deficiencies in the essential amino acids arginine, phenylalanine and valine were also noted. Leucine was the first limiting amino acid. The brown fish meal contained good levels of all the essential amino acids although arginine, leucine and phenylalanine were somewhat low. Leucine was the first limiting amino acid (Table 7.4).

The meat and bone meal contained 18.57% lipid which is high in comparison with the usual lipid range of between 2% and 12% for this animal by-product (Fowler and Banks, 1976; Skrede et al., 1980; McDonald et al., 1981; Tacon et al., 1984). The brown fish meal had a lipid content of 7.22% which is within the normal range of values for a good quality fish meal (Table 7.3; Windsor and Barlow, 1981).

The ash content of the meat and bone meal was 12.69% which is much lower than the reported normal range of values of 27% to 33% (Fowler and Banks, 1976; Skrede et al., 1980; McDonald et al., 1981; Tacon et al., 1984). This was a consequence of the partial removal of the bone fraction of the meal during processing at the laboratory.

TABLE 7.3 Proximate composition, Cr_2O_3 and energy content of Brown fish meal, Meat and bone meal, and experimental diets (% dry weight)

Nutrient composition (% dry weight)	Brown fish meal	Meat and bone meal	Dietary treatments 1	2	3	4	5	6
Moisture (%)	6.66(0.04)[1]	6.10(0.07)	2.54(0.29)	2.53(0.06)	2.49(0.07)	2.08(0.05)	1.66(0.09)	1.67(0.03)
Crude protein (N x 6.25)	73.70(0.21)	67.07(0.32)	46.56(0.50)	45.81(0.46)	45.57(0.58)	45.09(0.49)	44.71(0.42)	45.44(0.32)
Lipid (%)	7.22(0.27)	18.57(0.08)	13.01(0.52)	12.74(0.25)	12.91(0.25)	13.44(0.20)	13.24(0.23)	13.65(0.15)
Ash (%)	16.18(0.77)	12.69(0.20)	11.47(0.28)	11.90(0.81)	10.89(0.04)	10.72(0.06)	11.43(0.03)	10.65(0.22)
Nitrogen free extract[2] (%)	-	-	25.77	26.18	27.35	27.94	28.29	27.98
Peroxide value (mEq/Kg oil)	0.00(-)	0.00(-)	1.12(0.01)	1.57(0.06)	0.94(0.33)	0.17(0.01)	0.19(0.01)	0.17(0.01)
Acid insoluble ash (%)	0.23(0.03)	0.30(0.06)	0.93(0.07)	0.95(0.12)	0.99(0.13)	0.84(0.02)	0.83(0.03)	0.82(0.06)
Crude fibre (%)	0.13(0.02)	0.76(0.21)	0.65(0.07)	0.84(0.09)	0.79(0.05)	0.73(0.06)	0.67(0.07)	0.61(0.08)
Cr_2O_3 (%)	-	-	0.50(0.01)	0.53(0.07)	0.52(0.03)	0.51(0.01)	0.54(0.15)	0.54(0.01)
Energy -ash free (Kcal/g)	6.24(0.13)	6.36(0.09)	6.22(0.28)	6.12(0.04)	6.57(0.37)	6.24(0.06)	5.85(0.48)	6.34(0.39)

1) Standard deviation

2) Nitrogen free extract = 100 - (% moisture+% Crude protein+% Lipid+% Ash+% Crude fibre)

TABLE 7.4 Amino acid profile of Brown fish meal, Meat and bone meal, experimental diets and amino acid requirement of rainbow trout (g/100g dry weight)

Amino acid (g/100g dry wt)	Brown fish meal	Meat and bone meal	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Requirement of rainbow trout (% diet)
Arginine	2.13	2.03	1.34	1.34	1.34	1.33	1.34	1.37	1.40, 40% (Ogino, 1980)
Histidine	4.13	2.69	2.61	2.42	2.26	2.09	1.93	1.82	0.64, 40% (Ogino, 1980)
Isoleucine	3.82	3.69	2.41	2.40	2.42	2.42	2.33	2.50	0.96, 40% (Ogino, 1980)
Leucine	1.76	1.36	1.11	1.05	1.02	0.98	0.93	0.92	1.76, 40% (Ogino, 1980)
Lysine	3.49	4.09	2.20	2.29	2.40	2.50	2.60	2.77	2.12, 40% (Ogino, 1980)
Methionine	2.33	1.63	1.47	1.38	1.30	1.23	1.15	1.10	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	1.51	1.21	0.95	0.91	0.89	0.86	0.83	0.82	1.24, 40% (Ogino, 1980)
Threonine	3.64	2.91	2.30	2.21	2.14	2.06	1.99	1.97	1.36, 40% (Ogino, 1980)
Valine	2.27	1.39	1.43	1.32	1.22	1.11	1.01	0.94	1.24, 40% (Ogino, 1980)
Alanine	0.95	0.85	0.60	0.59	0.58	0.57	0.56	0.57	
Aspartic acid	6.32	4.88	3.99	3.81	3.66	3.52	3.37	3.31	
Cystine	0.98	0.79	0.62	0.60	0.57	0.56	0.54	0.53	0.30, 35% (Rumsey et al. 1983)
Glutamic acid	5.97	5.75	3.77	3.75	3.77	3.78	3.79	3.89	
Glycine	10.86	11.21	6.86	6.93	7.08	7.18	7.30	7.59	
Proline	8.13	10.66	5.14	5.50	5.92	6.29	6.67	7.22	
Serine	4.25	4.09	2.69	2.67	2.69	2.69	2.65	2.77	
Tyrosine	1.64	1.40	0.95	1.00	0.99	0.96	0.95	0.95	0.84, 40% (Ogino, 1980)
TOTAL	64.18	60.63	41.77	40.17	40.25	40.13	39.94	41.04	

* Percentage of crude protein in the diet

The ash content of the brown fish meal was 16.18% (Table 7.3) which again is higher than normal for a good quality fish meal (Windsor and Barlow, 1981).

The peroxide value, acid insoluble ash, and crude fibre levels of both meals were all very low with maximum values of 1.57 mEq/Kg oil, 0.99% and 0.84% respectively (Table 7.3).

Both the meat and bone meal and the brown fish meal were good sources of all the minerals analysed, in particular of K, Na, and Ca for the former and of K, Ca, and Zn for the latter (Table 7.5).

The moisture content of the experimental diets varied only between 1.66% and 2.54% (Table 7.3) and therefore a slight hardness of pellets is indicated.

The crude protein content of the experimental diets varied between 44.71% and 46.56% which is close to the formulated value of 45% (Table 7.3). All seven experimental diets were slightly deficient in arginine and deficient in leucine and this deficiency was exacerbated with increasing inclusion levels of meat and bone meal. All rations containing meat and bone meal were slightly deficient in phenylalanine, and again the level of this essential amino acid decreased with increasing dietary levels of meat and bone meal. Finally, Diets 3 to 6 containing 40% to 100% of the protein as meat and bone meal contained sub-optimal levels of valine (Table 7.4).

TABLE 7.5 Concentration of mineral elements in Brown fish meal and Meat and bone meal and mineral requirement of rainbow trout

Element	Brown fish meal	Meat and bone meal	Requirement of rainbow trout (mg/100g dry wt)	
Ca (g/100g)	4.78	3.53	650-750	(Ogino and TAkeda, 1978)
Mg (g/100g)	0.15	0.09	50-70	(Ogino *et al*., 1978) (Knox *et al*., 1981)
K (g/100g)	3.56	3.34	160	(Frenzel and Pfeffer, 1982)
Na (g/100g)	0.72	1.05	220	(Frenzel and Pfeffer, 1982)
P (g/100g)	1.20	0.98	700-800 650	(Ogino and Takeda, 1978) (Nose and Arai, 1979)
Zn (mg/100g)	13.71	10.58	1.5-3.0	(Ogino and Yang, 1978)

The lipid content of the experimental diets varied from 12.74% to 13.65% which is close to the formulated value of 13% (Table 7.3). The minimum dietary energy content was 5.85 Kcalories per gramme dry weight and the maximum was 6.57 Kcalories per gramme dry weight (Table 7.3).

7.3.2 Growth Response and Feed Utilization Efficiency

Both the fish meal based experimental diet (Diet 1) and the five meat and bone meal based diets (Diets 2-6) were immediately accepted by fish, even by those fed Diet 6 where all of the fish meal protein was replaced by meat and bone meal.

At the start of the trial there were no significant ($P < 0.05$) differences in mean fish weights of around 36g (36.33g-36.50g) between treatments (Table 7.6). The maximum weight gain was attained by fish fed the fish meal control diet (Diet 1). The final mean weight of 138.53g (Diet 1) was significantly ($P < 0.05$) higher than those of fish fed diets containing meat and bone meal. At the end of the 13 week trial there were no significant differences in the mean weights of fish fed the experimental diets containing between 20% and 80% of meat and bone meal protein (Table 7.6; Fig. 7.1). The final mean weight of fish fed Diet 6, however, where all the fish meal was replaced by meat and bone meal was only 99.55g (Table 7.6; Fig. 7.1) which is between 20% and 28% lower than all the other treatments. Despite not being significant differences there appears to have been a trend of decreasing growth with increasing inclusion levels of meat and bone meal (Table 7.6; Fig. 7.2). Differences

TABLE 7.6 Growth performance, feed utilization efficiency, liver somatic index, and blood parameters of rainbow trout (240 fish, 36g) fed the experimental diets after 13 weeks

Mean values	Dietary treatments 1	2	3	4	5	6	± S.E.[1]
Mean initial weight (g)	36.34[a]	36.50[a]	36.44[a]	36.38[a]	36.33[a]	36.44[a]	0.965
Mean final weight (g)	138.53[c]	135.42[b]	124.84[b]	131.38[b]	125.65[b]	99.55[a]	5.510
Weight gain (%)	280.89	270.99	242.64	261.22	232.20	173.31	
Specific growth rate (%/day)	1.47	1.44	1.36	1.41	1.32	1.10	
Food intake (mg/day)	1367	1354	1265	1316	1312	1171	
Protein intake (mg/day)	636	620	576	593	587	532	
Weight gain (mg/day)	1123	1087	971	1044	927	694	
Food conversion ratio	1.22	1.24	1.30	1.26	1.41	1.69	
Protein efficiency ratio	1.76	1.75	1.68	1.76	1.58	1.30	
Nitrogen intake (mg/day)	101.76	99.20	92.16	94.80	93.92	85.12	
Nitrogen deposition (mg/day)	11.03	10.96	10.54	11.00	9.87	8.15	
Apparent net protein utilization (%)	10.84	11.05	11.44	11.59	10.51	9.57	
Apparent protein digestibility (%)	80.70[f]	77.82[e]	74.88[cd]	74.09[c]	68.28[b]	65.85[d]	0.428
Apparent lipid digestibility (%)	86.55[e]	81.70[de]	77.40[cd]	71.56[bc]	65.33[ab]	59.27[a]	2.219
Apparent organic matter digestibility (%)	55.41[a]	50.96[a]	51.30[a]	52.38[a]	53.07[a]	54.76[a]	1.471
Liver somatic index	0.95[abc]	0.89[a]	0.93[ab]	1.01[cd]	1.06[d]	1.10[e]	0.025
Haematocrit (%)	44.08[ab]	46.25[c]	44.58[abc]	44.42[abc]	42.33[a]	45.75[abc]	0.961
Haemoglobin (g/100 cm^3)	8.89[a]	9.22[ab]	9.24[ab]	9.36[ab]	9.56[ab]	9.71[b]	0.226

1) Standard error; calculated from residual mean square in the analysis of variance

abcdef Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

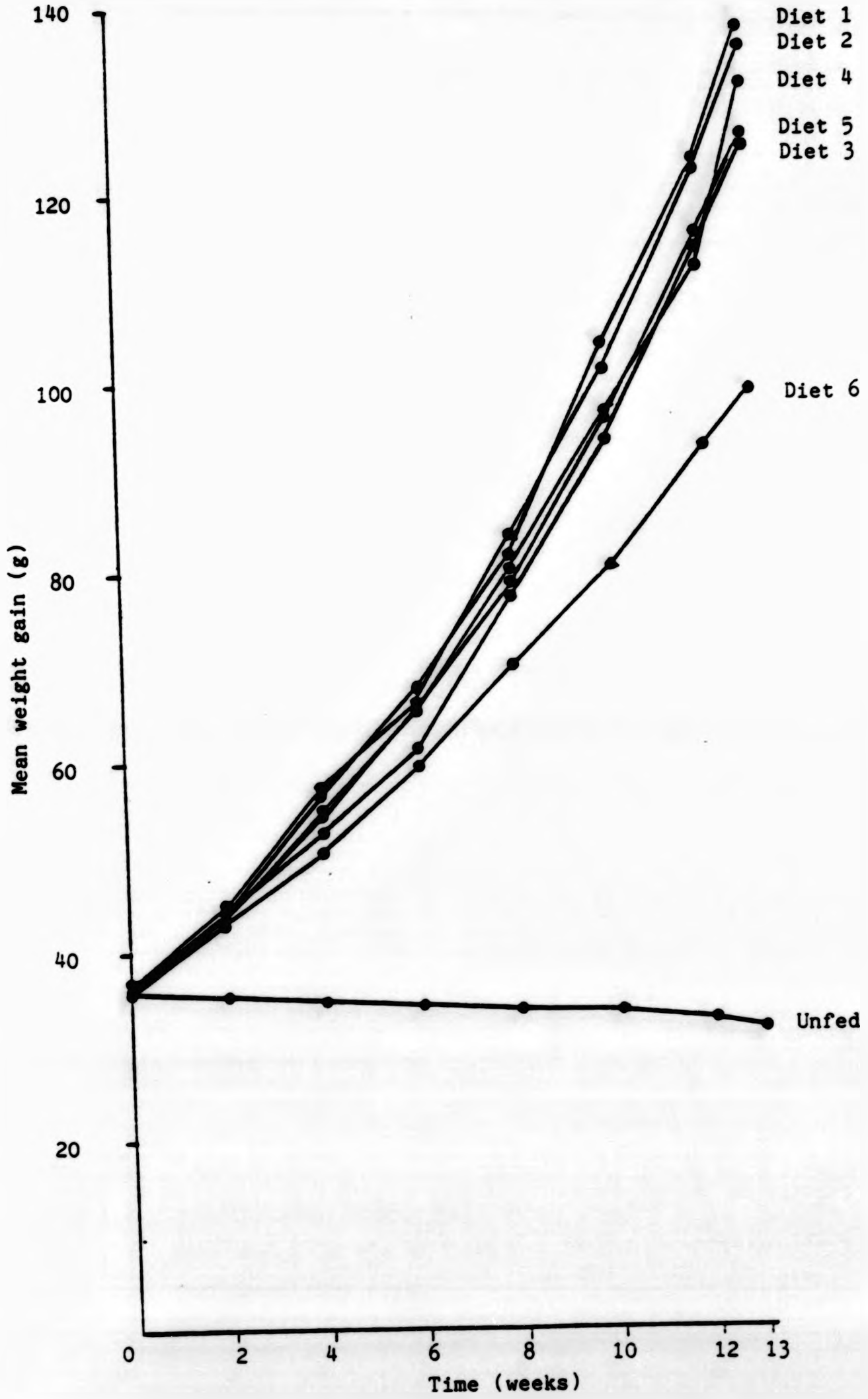

FIGURE 7.1 Overall mean weight gain (g) of rainbow trout at successive fortnightly intervals over the experimental test period

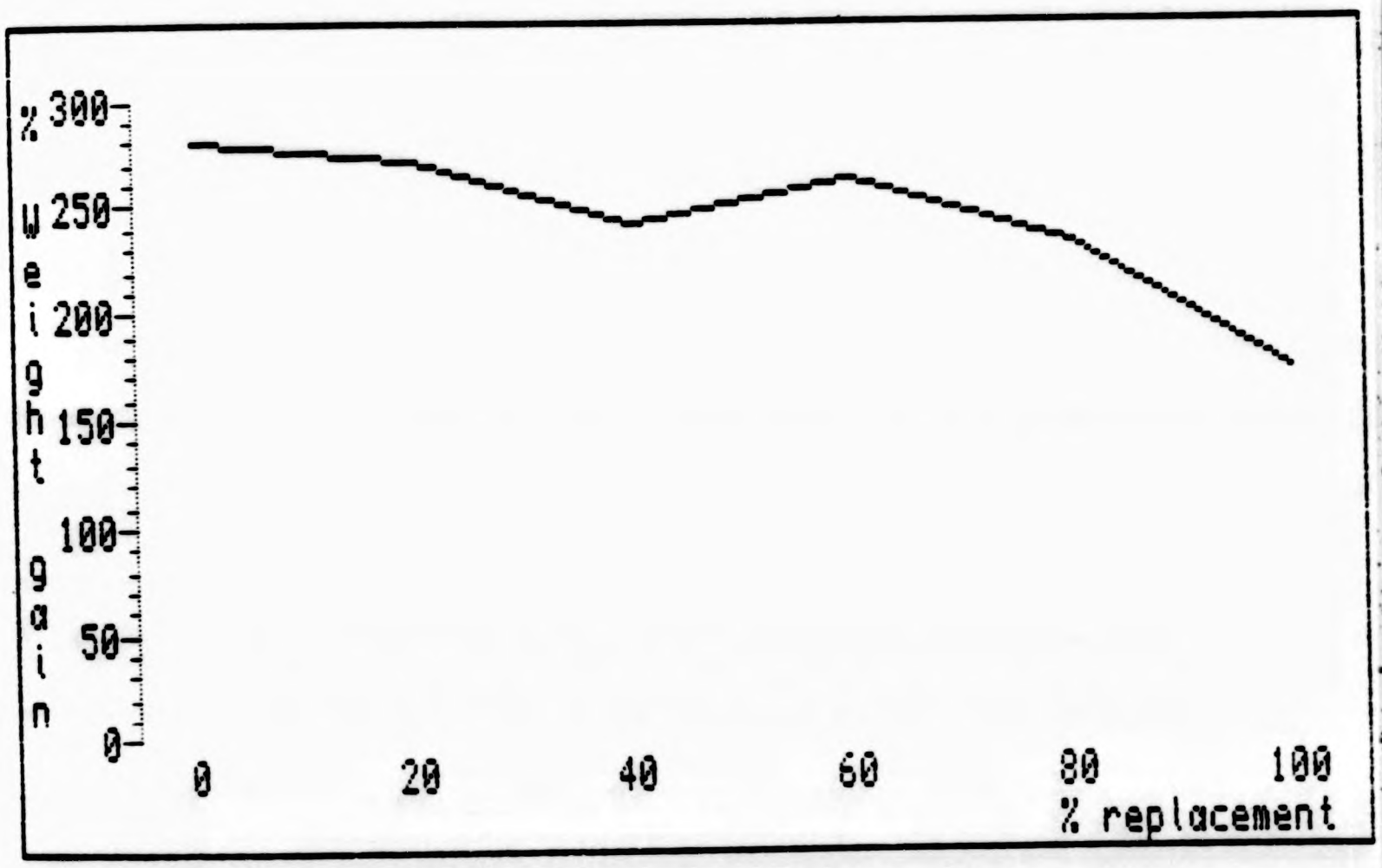

FIGURE 7.2 Overall mean weight gain (%) of fish fed diets containing increasing levels of Meat and bone meal as a replacement for Brown fish meal

would most certainly have been greater if the growth trial had lasted longer than 13 weeks.

Out of a total of 240 fish fed the six experimental rations there was only a single mortality in one replicate of fish fed Diet 1. In addition, no mortalities were recorded from the unfed cage of fish although the mean fish weight was reduced slightly from 36.02g at the start of the trial to 31.31g at the end of the 13 week trial (Appendix X; Fig. 7.1).

The specific growth rate decreased slightly with increasing levels of meat and bone meal from 1.47% per day for fish fed Diet 1 to 1.10% per day for those fed Diet 6 (Table 7.6; Fig. 7.3).

The food conversion ratio increased slightly from 1.22 for fish fed the control diet (Diet 1) to 1.69 at 100% protein replacement (Table 7.6; Fig. 7.4). Thus the food conversion ratios of Diets 5 and 6 in which 80% and 100% of the protein was meat and bone meal were only slightly poorer than that of those fed the fish meal control diet.

The protein efficiency ratio (PER) varied between 1.30 for fish fed Diet 6 (100% protein replacement) and 1.76 for those fed the fish meal control diet (Table 7.6; Fig. 7.5). There also appears to have been a trend of decreasing PER with increasing inclusion levels of meat and bone meal, although the PER of Diet 4 is equal to that of the control (Diet 1).

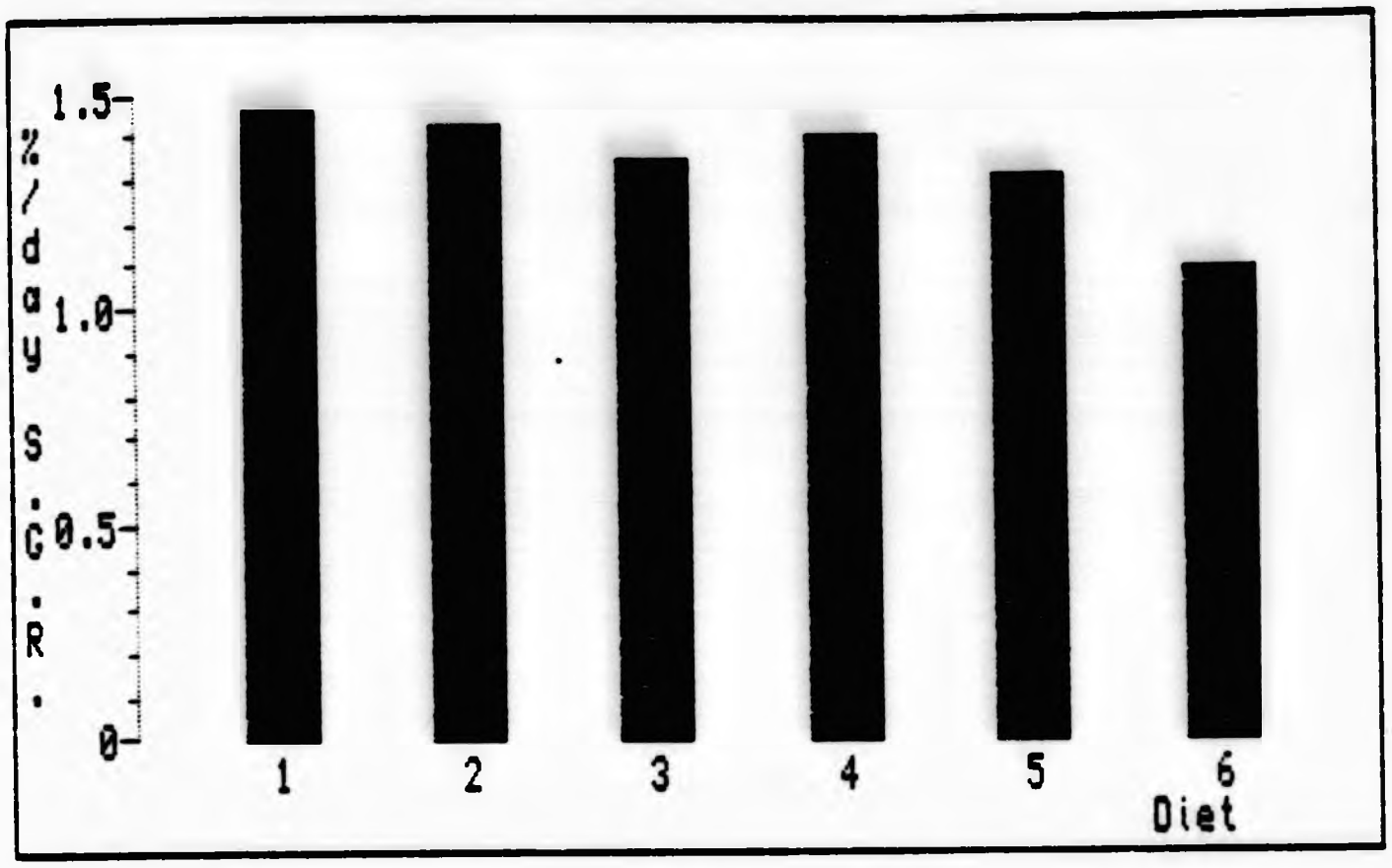

FIGURE 7.3 Specific growth rate (%/day) of fish fed the six experimental diets

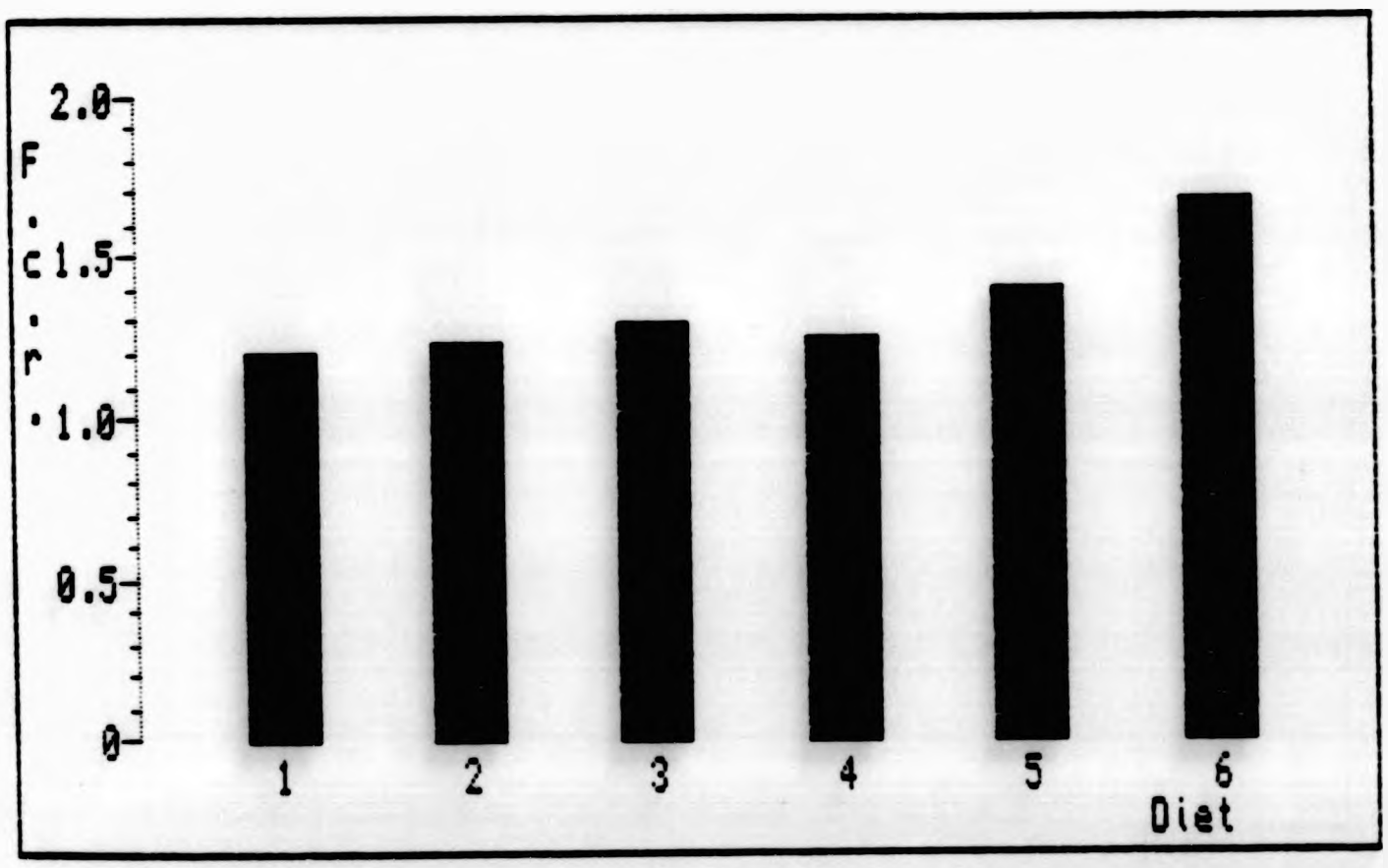

FIGURE 7.4 Food conversion ratio of fish fed the six experimental diets

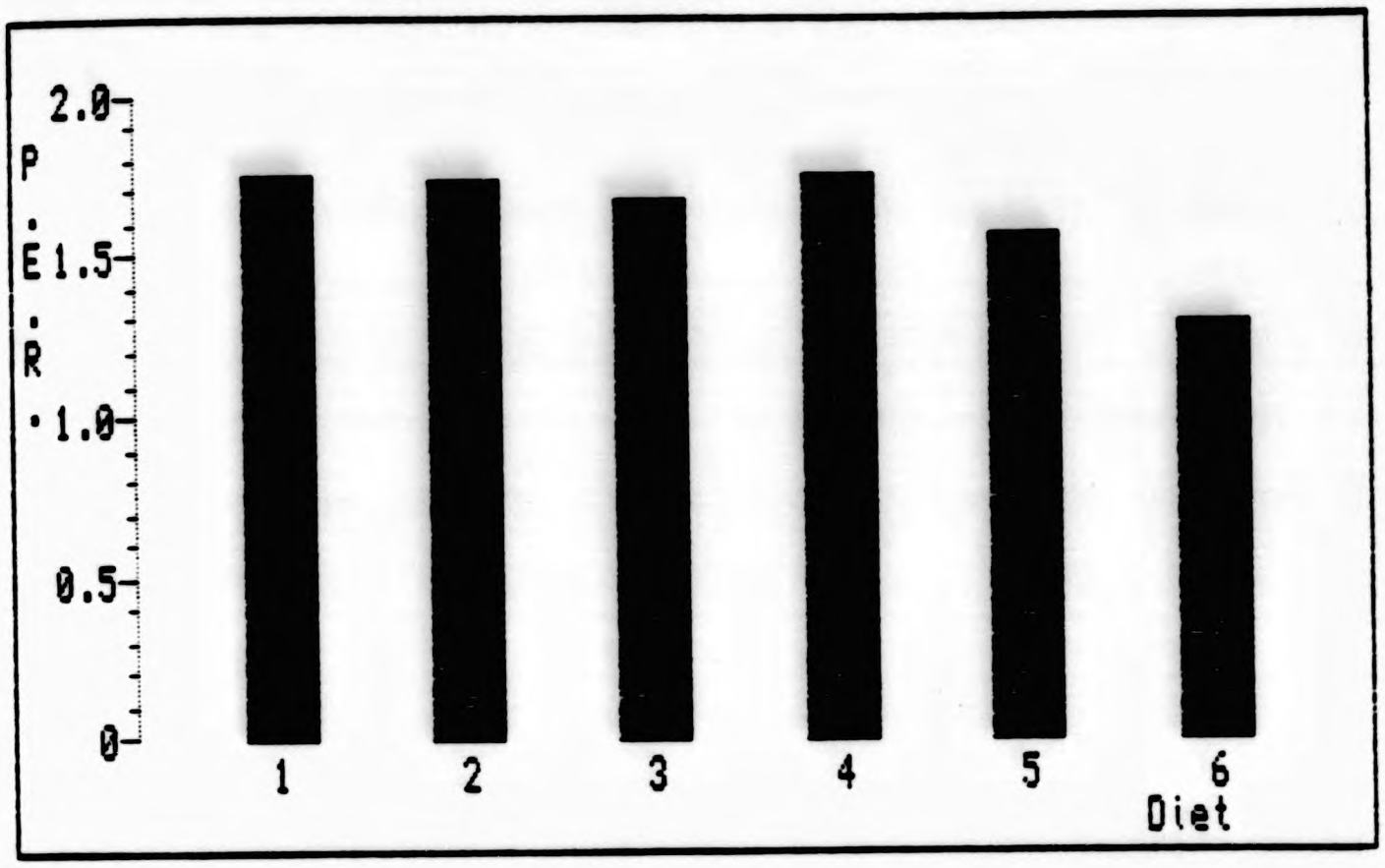

FIGURE 7.5 Protein efficiency ratio of fish fed the six experimental diets

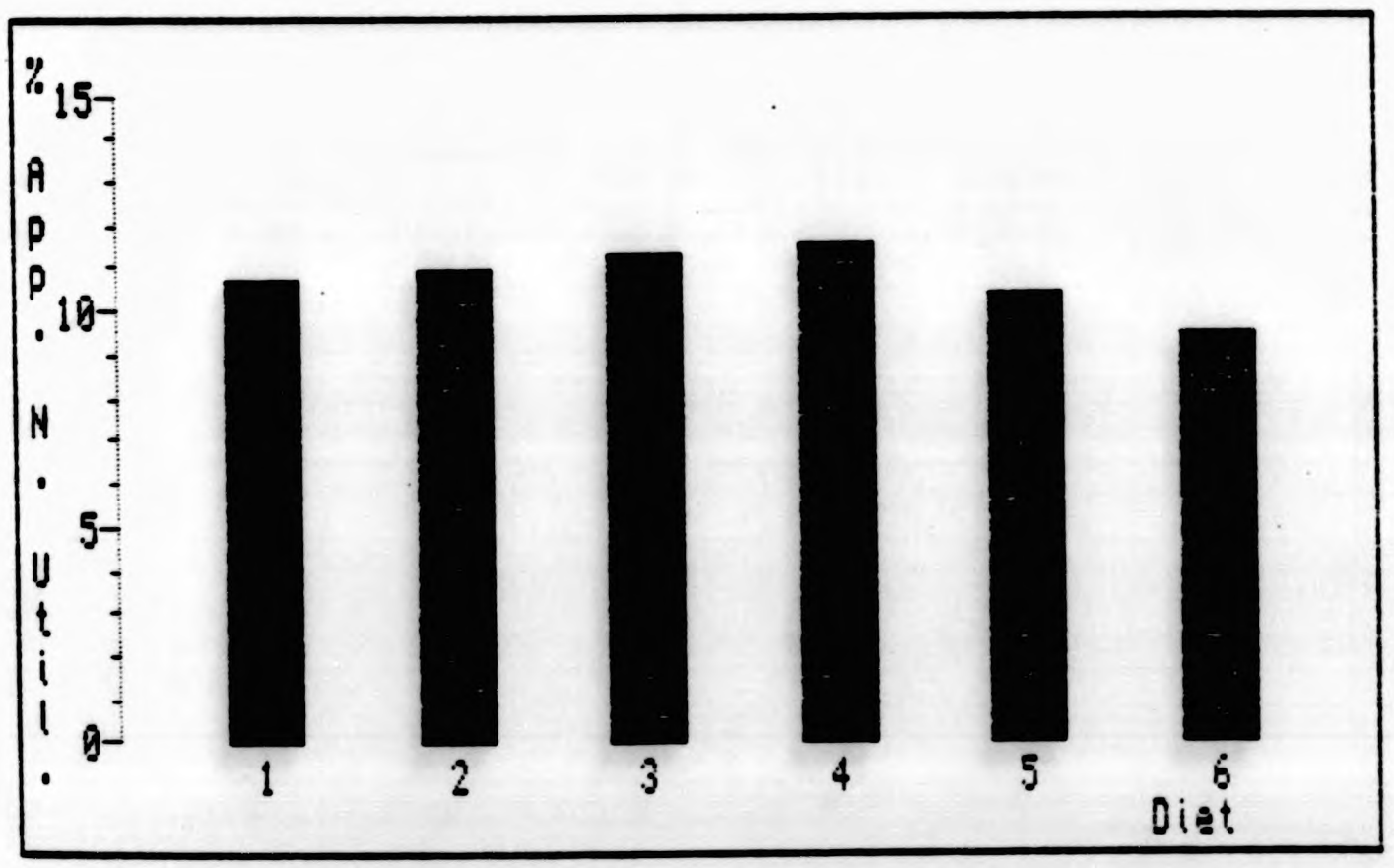

FIGURE 7.6 Apparent net protein utilization (%) of fish fed the six experimental diets

The apparent net protein utilization (apparent NPU) varied between a minimum value of 9.57% for fish fed Diet 6 and a maximum value of 11.59% for those fed Diet 4. The apparent NPU of fish fed the fish meal control diet was only 10.84% (Table 7.6; Fig. 7.6). No trend between the apparent NPU and meat and bone meal inclusion levels was indicated.

The growth performance of rainbow trout replicates fed the experimental diets is given in Appendix IX.

7.3.3 Digestibility

The moisture content of faeces from fish fed all dietary treatments was very low and varied between only 0.70% (Diet 2) and 0.95% (Diet 6; Table 7.7; Fig. 7.7a).

There was a trend of increasing faecal protein with increasing meat and bone meal inclusion levels from 20.60% for fish fed Diet 2 to 28.63% for those fed Diet 6. Faeces from fish fed the fish meal control diet (Diet 1) had a crude protein content of 20.13% which was close to the level recorded in faeces from fish fed Diet 2 (Table 7.7; Fig. 7.7b). Apparent protein digestibilities varied significantly ($P < 0.05$) between a maximum of 80.70% for fish fed the fish meal control diet (Diet 1) to 65.85% for those fed Diet 6 (100% protein replacement; Table 7.6). Thus the apparent protein digestibility of fish fed the meat and bone meal based diets decreased with increasing inclusion levels of meat and bone meal.

TABLE 7.7 Proximate composition and Cr_2O_3 of faeces taken from rainbow trout after 13 weeks on the experimental diets (% dry weight)

Faeces composition (% dry weight)	Dietary treatments 1	2	3	4	5	6
Moisture (%)	0.78(0.05)[1]	0.70(0.30)	0.75(0.30)	0.75(0.60)	0.95(0.20)	0.80(0.20)
Crude protein (N x 6.25)	20.13(2.19)	20.60(0.69)	23.08(1.00)	24.39(0.04)	27.21(0.07)	28.63(0.02)
Lipid (%)	3.90(2.31)	4.42(1.21)	5.83(2.00)	8.13(2.11)	8.81(2.81)	12.20(1.56)
Ash (%)	23.16(0.86)	23.38(1.02)	18.88(1.56)	19.34(0.43)	18.54(1.21)	16.82(0.06)
Cr_2O_3	1.12(0.01)	1.09(0.04)	1.06(0.04)	1.07(0.08)	1.05(0.05)	0.98(0.02)

1) Standard deviation

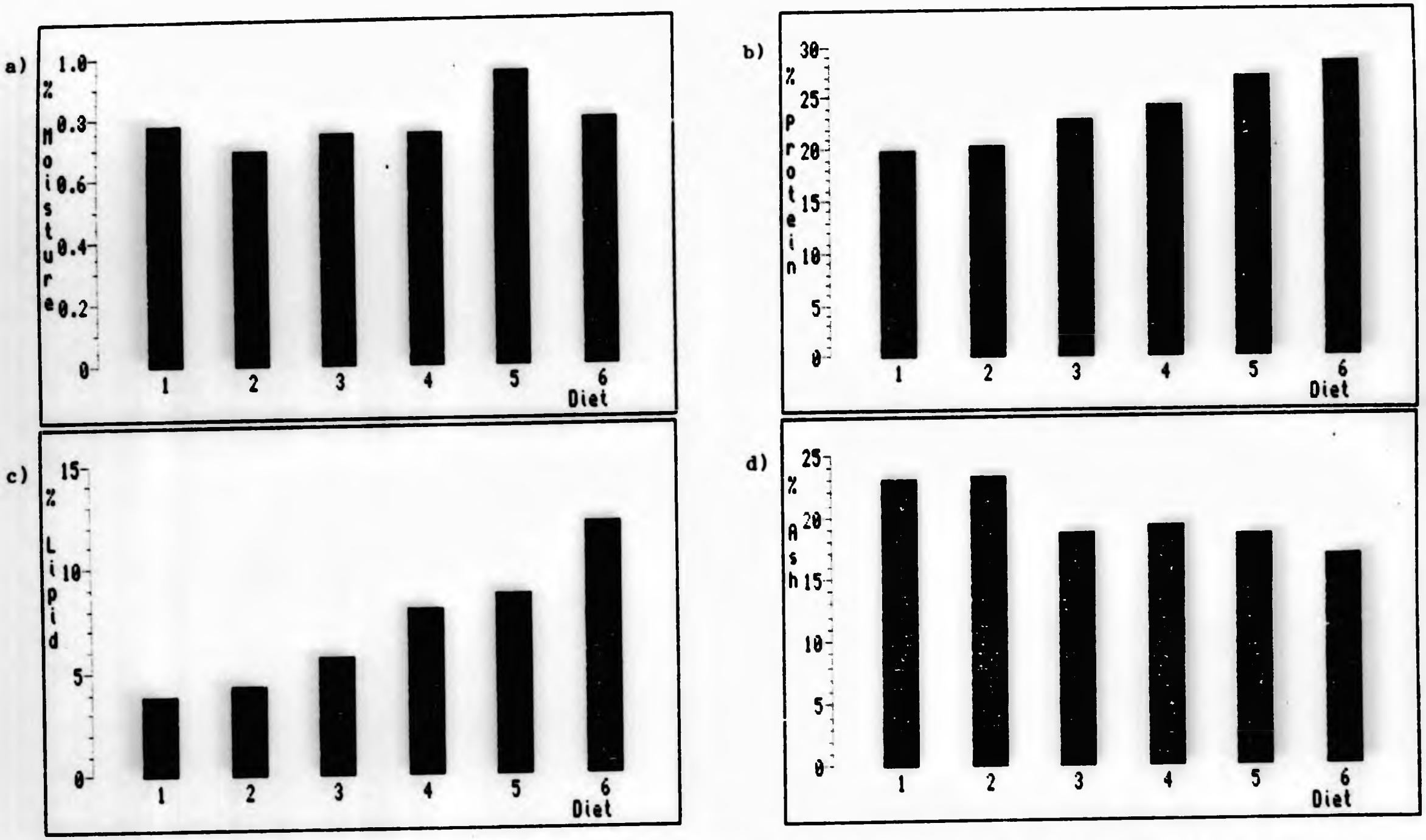

FIGURE 7.7 Proximate composition of the faeces from fish fed each of the six experimental diets. a) Moisture; b) Crude protein; c) Lipid; d) Ash

Faecal lipid also increased with increasing meat and bone meal inclusion levels from 4.42% (Diet 2) to 12.20% (Diet 6). The lipid content of faeces from fish fed Diet 6 (100% protein replacement) was three times higher than the level of 3.90% recorded in faeces from fish fed the fish meal control diet (Table 7.7; Fig. 7.7c). These differences were reflected in the apparent lipid digestibilities with a maximum of 86.55% for the control diet (Diet 1) and then a significant ($P < 0.05$) trend of decreasing digestibility with increasing inclusion levels of meat and bone meal from 81.70% for 20% protein inclusion level to 59.27% for 100% replacement (Table 7.6).

The ash content of faeces from fish fed Diets 1 and 2 were similar, 23.16% and 23.38% respectively. There was a general trend of decreasing ash content with increasing meat and bone meal inclusion levels to a minimum value of 16.82% in faeces from fish fed Diet 6 (Table 7.7; Fig. 7.7d). There were, however, no significant ($P < 0.05$) differences in apparent organic matter digestibilities and furthermore digestibility coefficients were low, varying between only 50% and 55% (Table 7.6).

The proximate composition of faeces taken from rainbow trout replicates is given in Appendix XI.

7.3.4 Liver Somatic Index and Blood Parameters

The liver somatic index increased with increasing inclusion levels of meat and bone meal from 0.89 (Diet 2) to 1.10 (Diet 6), varying significantly ($P < 0.05$) at inclusion levels higher than 40%

protein replacement (Table 7.6). The liver somatic index of fish fed the fish meal control diet (Diet 1) was similar to that of fish fed the diet in which 40% of the fish meal protein was replaced by meat and bone meal (Diet 3).

Although there were some significant ($P < 0.05$) differences in haematocrit values the range of values only varied between 44.08% and 46.25% (Table 7.6) which is within the normal range (Wedemeyer and Nelson, 1975; Blaxhall and Daisley, 1973; Miller et al., 1983; Railo et al., 1985). In addition no trend in haematocrit values with increasing inclusion levels of meat and bone meal was noted.

There was a trend of increasing blood haemoglobin with increasing meat and bone meal inclusion levels, from 9.22g per 100 cm^3 from fish fed Diet 2 (20% protein replacement) to 9.71g per 100 cm^3 from those fed Diet 6 (100% protein replacement). However only the blood haemoglobin levels of fish fed the fish meal control diet and those fed Diet 6 containing 100% of the protein as meat and bone meal were significantly ($P < 0.05$) different (Table 7.6).

The liver somatic index and blood parameters of rainbow trout replicates are given in Appendix IX.

7.3.5 Carcass Composition

By the end of the 13 week growth trial the moisture content of fish from all of the six dietary treatments had not changed significantly ($P < 0.05$) from the initial value of 71.55% (Table 7.8; Fig. 7.8a).

TABLE 7.8 Carcass composition of rainbow trout (240 fish, 36g) at the start and end of the experiment (13 weeks) based on 12 fish per treatment (% wet weight)

Carcass composition (% wet weight)	Initial	Dietary treatments 1	2	3	4	5	6	±S.E.[1]
Moisture (%)	a 71.55(0.41)[2]	a 70.97(0.67)	a 70.15(1.69)	a 72.68(0.40)	a 71.06(1.07)	a 71.89(0.68)	a 72.08(0.43)	1.052
Crude protein (N x 6.25)	ab 17.35(0.16)	ab 17.66(0.32)	b 18.35(1.38)	ab 17.28(0.16)	b 18.19(1.51)	ab 17.40(0.51)	a 16.91(0.76)	0.367
Lipid (%)	ab 7.71(0.06)	b 8.06(0.80)	ab 7.50(0.82)	a 6.29(0.45)	ab 7.29(1.02)	b 8.02(0.68)	b 8.73(0.51)	0.453
Ash (%)	abc 3.63(0.26)	ab 3.36(0.44)	c 4.13(0.43)	abc 3.56(0.25)	abc 3.64(0.16)	bc 4.07(0.41)	a 3.21(0.80)	0.219

1) Standard error; calculated from residual mean square in the analysis of variance

2) Standard deviation

abc Mean values for components with common superscripts are not significantly ($P < 0.05$) different

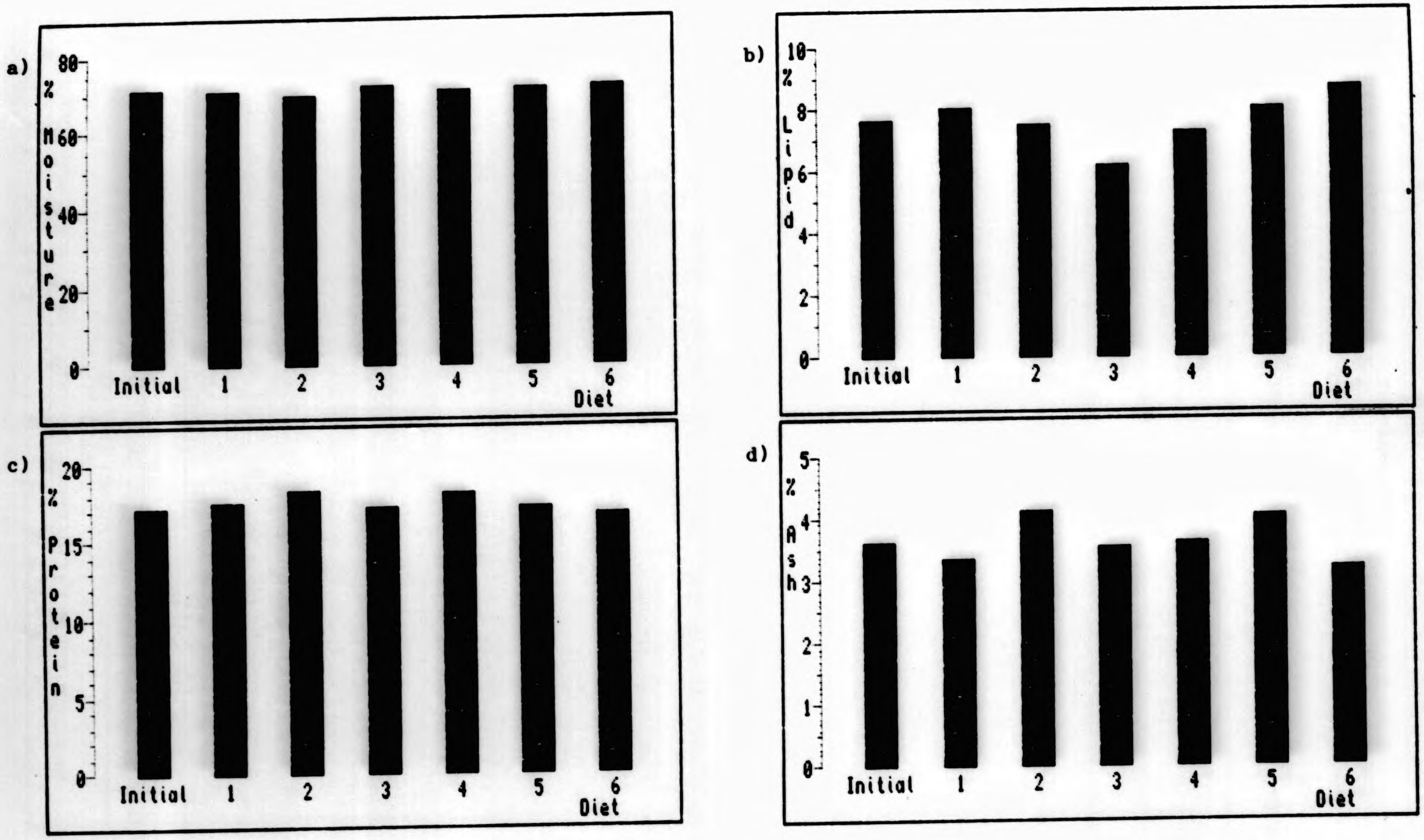

FIGURE 7.8 Proximate composition of the carcasses from fish fed each of the six experimental diets and of the initial sample. a) Moisture; b) Lipid; c) Crude protein; d) Ash

Although there were significant ($P < 0.05$) differences in carcass lipid levels between dietary treatments these differences were small varying between 6.29% for fish fed Diet 3 to 8.73% for those fed Diet 6 (100% protein replacement). No trend in lipid content of carcasses and meat and bone meal inclusion levels was noted. Furthermore lipid levels at the end of the trial were not significantly ($P < 0.05$) different from the level of 7.71% at the start of the trial (Table 7.8; Fig. 7.8b).

Differences in carcass protein levels between dietary treatments were also small varying between 16.91% for fish fed Diet 6 and 18.35% for those fed Diet 2. No relationship between the carcass protein content and meat and bone meal inclusion levels was indicated. The carcass protein content of fish fed the experimental diets were not significantly ($P < 0.05$) different from the initial value of 17.35% (Table 7.8; Fig. 7.8c).

Although there were significant ($P < 0.05$) differences in carcass ash levels at the end of the trial between dietary treatments these differences were small and varied between 3.21% (Diet 6) and 4.13% (Diet 2). No trend between the ash content of carcasses of fish fed the experimental diets and meat and bone meal inclusion levels was indicated. Furthermore the carcass ash levels at the end of the trial were not significantly ($P < 0.05$) different from the initial level of 3.63% (Table 7.8; Fig. 7.8d).

The proximate composition of fish carcass replicates is given in Appendix XII.

7.4 DISCUSSION

The growth performances of rainbow trout fed diets containing up to 80% of the protein as meat and bone meal replacing a good quality brown fish meal were only slightly poorer than that of the ration based on fish meal alone (Table 7.6). Nevertheless there was a trend of decreasing growth performance with increasing inclusion levels of meat and bone meal. Thus fish fed Diet 2 where only 20% of the fish meal protein was replaced by meat and bone meal had a mean final weight of 135.42g while those fed Diet 6 where all of the protein was supplied by meat and bone meal had a significantly lower mean final weight of only 99.55g (Table 7.6). This trend of decreasing growth performance with increasing inclusion levels of meat and bone meal has also been reported by Tiews et al. (1976) and Fowler and Banks (1976). Fowler and Banks (1976) reported that chinook salmon (Oncorhynchus tshawytscha) fed diets containing 77% and 88% meat and bone meal protein replacing herring meal had significantly reduced growth rates, although these authors used a meat and bone meal with a much lower crude protein content of only 50.4% compared with 67% for the meat and bone meal used in this trial. Tiews et al. (1976) also reported significantly lower growth performances for rainbow trout even when only 38% of the dietary protein was supplied by meat and bone meal. By contrast Tacon (1982b) reported a better growth performance by rainbow trout fed diets containing increasing inclusion levels of meat and bone meal up to a level of around 34% of the protein.

In conjunction with reduced growth performances, increasing inclusion levels of meat and bone meal also resulted in poorer feed

utilization efficiencies (Table 7.6). Thus fish fed the diet where all of the protein was supplied by brown fish meal (Diet 1) had a food conversion ratio of 1.22 while those fed the diet containing only meat and bone meal as the sole source of dietary protein (Diet 6) had a food conversion ratio of 1.69. This poorer feed utilization efficiency with increasing inclusion levels of meat and bone meal was also reported by Tiews et al. (1976). Thus a food conversion ratio of 1.36 was indicated for a diet where all of the protein was supplied by fish meal, while that where 36% of the protein was supplied by meat and bone meal was 1.64.

The high crude protein content and low ash content of the meat and bone meal tested were in part a consequence of discarding the large particles of bone during grinding. Thus the chemical composition of the meal was more characteristic of a meat meal than a meat and bone meal. The improved quality of the product is likely therefore to have contributed to the growth performance and feed utilization efficiency of fish fed the diets containing up to 80% of the protein as meat and bone meal being only slightly poorer than the fish meal control ration. Nevertheless the meat and bone meal tested was deficient in the essential amino acids arginine, leucine, phenylalanine, and valine (Table 7.4). Levels of leucine in the diets varied between 1.11% (Diet 1) and 0.92% (Diet 6) compared with a requirement of 1.76% (Ogino, 1980). Phenylalanine was also deficient in all rations varying between 0.95% (Diet 1) and 0.82% (Diet 6). However, at least 66% of the requirement level was present and furthermore phenylalanine can be spared by tyrosine (Mertz, 1972; Halver, 1975, 1976; Cowey, 1979; Ketola, 1982; Millikin, 1982;

Walton, 1985; Wilton, 1985). Arginine was only slightly deficient supplying at least 95% of the requirement, and valine was only deficient in Diets 3 to 6 but again at least 76% of the requirement for this essential amino acid was present. Thus leucine appears to have been the first limiting amino acid in all six rations supplying only between 77% (Diet 1) and 52% (Diet 6) of the requirement and as a consequence only half of the total protein would have been usable for growth in Diet 6 where all of the protein was supplied by meat and bone meal. Tryptophan was not analysed but this essential amino acid should not have been limiting in at least the first 3-4 experimental diets since the good quality fish meal used should have supplied enough tryptophan to satisfy the requirement of rainbow trout for this essential amino acid. Although meat and bone meals have been reported to be deficient in tryptophan (Chapter 2), it is not the first limiting amino acid (Skrede et al., 1980; McDonald et al., 1981; Menzies, 1982; Tacon et al., 1984; Tacon and Jackson, 1985) and therefore it is most unlikely that Diet 6 where all of the protein was supplied by meat and bone meal would have had tryptophan as its first limiting amino acid. Since tryptophan was not determined the essential amino acid index (EAAI) was calculated based on the nine remaining essential amino acids and on each of the essential amino acid requirements of rainbow trout indicated by Ogino (1980) and Rumsey et al. (1983). Thus the best EAAI of 92.82 was indicated for the fish meal control diet and thereafter it decreased with increasing inclusion levels of meat and bone meal from 91.54 in Diet 2 to 88.01 in Diet 6. As a consequence the mean final weight of 138.53g of fish fed the fish meal control ration decreased with increasing inclusion levels of meat and bone meal

from 135.42g in Diet 2 (20% protein replacement) to only 99.55g in Diet 6 (100% protein replacement) where only 52% of the total protein was available for growth.

The levels of unaccounted amino acids and nonprotein nitrogen for the meat and bone meal and brown fish meal was 6.44% and 9.52% respectively. As a consequence the percentage did not vary much in the dietary treatments and in all cases was less than 6% of the protein.

The meat and bone meal tested had a lipid content of 18.57% which is much higher than the reported values of below 12% (Fowler and Banks, 1976; Skrede et al., 1980; McDonald et al., 1981; Tacon, 1982b; Tacon et al., 1984). This is not the most suitable type of fat for fish although it has been reported that animal fat can replace a large proportion of fish oil with no adverse effects if diets are provided with the necessary essential fatty acids (Yu et al., 1977; Takeuchi et al., 1978c; Cowey et al., 1979; Reinitz and Yu, 1981; Yu and Sinnhuber, 1981). Nevertheless since animal fats are less prone to oxidation than fish oil (Castell et al., 1972; Watanabe et al., 1974a, b; Takeuchi and Watanabe, 1976, 1977; Yu and Sinnhuber, 1976; Castell, 1979; Castledine and Buckley, 1980; Reinitz and Yu, 1981; Henderson and Sargent, 1985), oxidative rancidity is much less likely to be a problem (Cockerell et al, 1971; Windsor and Barlow, 1981; Hung and Slinger, 1982). This seems to have been the case since no detectable lipid peroxidation was indicated for the meat and bone meal and in all rations peroxide values were very low and did not exceed 2 mEq/Kg oil. Lipid oxidation

of the experimental diets cannot therefore be used to explain the low protein and lipid digestibilities obtained with increasing inclusion levels of meat and bone meal. Protein digestibility decreased from 80% in the fish meal control ration to only 66% at 100% protein replacement and there was a concomitant fall in lipid digestibility from 86% to 59% (Table 7.6). Low protein and lipid digestibility coefficients of around 70% and 71% respectively, have also been reported by Smith and Rumsey (1976) for meat and bone meals included at 100% of the protein in diets for rainbow trout.

Fowler and Banks (1976) reported that high inclusion levels of meat and bone meal in diets for chinook salmon (Oncorhynchus tshawytscha) induced a mild nephrocalcinosis. No sign of nephrocalcinosis was indicated in this work although the meat and bone meal tested contained only 12% ash compared with 30% in the meal used by Fowler and Banks (1976). Thus the relatively low Ca content of 3.53% (Table 7.5) of the meal compared with normal levels of around 10% to 12% for meat and bone meals (Göhl, 1981; Menzies, 1982) is likely to have been insufficient to result in significant deposition in the kidney tubules (Roberts, 1978; Gillespie and Evans, 1979; Harrison and Richards, 1979). Due to the low ash content of both the fish meal and the meat and bone meal the dietary ash content were consequently low and varied only between 10.65% (Diet 6) and 11.90% (Diet 1; Table 7.3). Despite the dietary low ash content the apparent organic matter digestibilities were low although they increased slightly with increasing inclusion levels of meat and bone meal. However, differences in apparent organic matter digestibilities were not significant.

There were small differences in the nitrogen free extract (NFE) values indicated for the experimental diets which varied only between 25.77% and 28.29%. Furthermore no NFE was indicated for both the fish meal and the meat and bone meal. This absence of NFE in meat and bone meals was also reported by Göhl (1981) and McDonald et al. (1981).

The decrease in food digestibility, PER and food conversion ratio with increasing inclusion levels of meat and bone meal may be attributable not only to increased essential amino acid deficiencies with increasing levels of meat and bone meal, but also to the increase in dietary carbohydrate levels which have been reported to cause a reduction in food digestibility and feed utilization efficiency (Spannhof and Kühne, 1977; Refstie and Austreng, 1981). However, as indicated above, the increase in NFE with increasing levels of meat and bone meal was small with a maximum difference of less than 3% between Diets 1 and 6. Thus differences in carbohydrate levels are unlikely to have produced any of the variations recorded in nutritional parameters.

There were no significant changes in fish carcass composition produced by replacing fish meal with the meat and bone meal (Table 7.8). Thus no trend between moisture, lipid, crude protein or ash content and inclusion levels of meat and bone meal was indicated, although a slight reduction in the crude protein content in conjunction with a slight increase in body lipid in fish fed Diet 6, where all of the protein was supplied by meat and bone meal, was noted. A somewhat lower crude protein content with increasing inclusion

levels of meat and bone meal was also indicated by Fowler and Banks (1976) in chinook salmon (Oncorhynchus tshawytscha) although body lipid was also reported to decrease, while in this work a slightly higher lipid deposition was indicated (Section 7.3.5; Table 7.8).

There was a small but significant increase in liver somatic index with increasing inclusion levels of meat and bone meal from 0.95 (Diet 1) to 1.10 (Diet 6). Liver weight has been reported to increase with increasing dietary carbohydrate content (Reinitz, unpublished; Austreng et al., 1977; Refstie and Austreng, 1981; Hilton and Dixon, 1982). Although in this trial the maximum variation in dietary carbohydrate levels was of 3%.

A trend of increasing haemoglobin content with increasing inclusion levels of meat and bone meal was also indicated although haemoglobin levels varied only between 9.22g (Diet 1) and 9.71g per 100 cm^3 (Diet 6) and were all within the normal range of values for healthy rainbow trout (Wedemeyer and Nelson, 1975; Lowe-Jinde and Niimi, 1983). No relationship between haematocrit levels and meat and bone meal inclusion levels was indicated and furthermore fish fed all dietary treatments had haematocrit values (Table 7.6) within the normal range of values for healthy rainbow trout (Wedemeyer and Nelson, 1975; Miller et al., 1983; Railo et al., 1985).

In conclusion, the brown fish meal used as a control was of good quality with an amino acid profile only slightly deficient in arginine, leucine, and phenylalanine. The meat and bone meal, although having a high crude protein content and low ash content,

had severe deficiencies in both the essential amino acids leucine and phenylalanine. Despite these essential amino acid deficiencies the meat and bone meal tested successfully replaced up to 80% of the good quality fish meal protein without any significant loss of growth performance or effect on fish carcass composition.

CHAPTER 8

THE NUTRITIONAL EVALUATION OF POULTRY BY-PRODUCT AND HYDROLYSED FEATHER MEAL AND MEAT AND BONE MEAL IN A COMPOUND DIET FOR RAINBOW TROUT

8.1 INTRODUCTION

The results of growth trials carried out to evaluate a PBHFm and a meat and bone meal readily available in Portugal, indicated that they could replace up to 90% and 80% respectively of the fish meal component in rations for rainbow trout without any significant loss of performance. In the case of the PBHFm however, the fish meal used as a control was not of a particularly high quality and it is therefore likely that the optimum inclusion level is somewhat lower than the 90% indicated.

In order to develop further a practical diet for rainbow trout in Portugal, a feeding trial was designed to evaluate rations containing different inclusion levels of both of these animal by-products in conjunction with a good quality fish meal. In this trial the maximum inclusion levels for the PBHFm and the meat and bone meal were set at 50% and 60% of the protein component respectively, while the maximum fish meal replacement level for any combination of these products was 70%.

8.2 MATERIALS AND METHODS

8.2.1 Diets

Further batches of 25 Kilogrammes of PBHFm and 25 Kilogrammes of meat and bone meal were obtained from Soaves, Pomarelho, Guimarães and from Manuel dos Santos Moura Lda., respectively. 25 Kilogrammes of a good quality brown fish meal was supplied by Sociedade de Pescas do Oceano, Figueira da Foz.

On arrival at the laboratory the three protein sources were immediately ground and their crude protein and lipid content were determined according to the methods given in Chapter 4.

Six experimental diets were formulated using PBHFm, meat and bone meal and brown fish meal as the principle protein sources. The PBHFm had a crude protein content of 56.18% and a lipid content of 26.56% and the brown fish meal had a crude protein and lipid content of 68.36% and 6.12% respectively. For both the PBHFm and the brown fish meal these values were close to the levels in the previous batches. The meat and bone meal, however, contained only 43.58% crude protein compared to 67.07% crude protein in the previous batch. According to the Fertilizers and Feeding Stuff Regulations (HMSO, 1973) the minimum crude protein content of a meat and bone meal should be 45%. The lipid content of 14.14% was similar to the level in the previous batch (Table 8.3).

The selection of inclusion levels of each product was based on fish performances in the previous experiments. Thus the protein

component of the diets consisted of:

Diet 1: control, 100% Brown fish meal protein

Diet 2: 50% PBHFm protein + 50% Brown fish meal protein

Diet 3: 60% Meat and bone meal protein + 40% Brown fish meal protein

Diet 4: 30% PBHFm protein + 40% Meat and bone meal protein + 30% Brown fish meal protein

Diet 5: 20% PBHFm protein + 50% Meat and bone meal protein + 30% Brown fish meal protein

Diet 6: 30% PBHFm protein + 30% Meat and bone meal protein + 40% Brown fish meal protein

Thus the PBHFm was used to replace up to 50% of the brown fish meal and meat and bone meal replaced a maximum of 60% brown fish meal. The full dietary formulations are presented in Table 8.1.

All six diets were formulated on an isonitrogenous and isocaloric basis and contained 41% crude protein in order to match the crude protein content of a commercial ration which was used to provide a further control. Due to the high lipid content of the PBHFm (26.56%) the diets were formulated to contain 13% lipid. However all diets contained sufficient fish oil to supply the necessary essential fatty acids. Seven kilogrammes of each diet were manufactured as described in Chapter 4.

The commercial control ration consisted of a pelleted trout diet manufactured in Portugal by Truturão, Cernache, Coimbra, a

TABLE 8.1 Formulation of the experimental diets (% by weight)

Diet No	1	2	3	4	5	6	7
% inclusion PBHFm	0	50	0	30	20	30	Commercial
% inclusion MBm	0	0	60	40	50	30	diet
Brown fish meal	62.60	31.30	25.00	18.80	18.80	25.00	
PBHFm		39.60		23.70	15.90	23.80	
MBm			61.30	40.80	51.00	30.60	
Corn starch	15.50	15.20	3.90	7.60	5.70	9.50	
Yellow dextrin	7.70	7.60	1.90	3.80	2.80	4.80	
Cod liver oil[1]	9.41	1.51	3.11	0.51	1.01	1.51	1.50
Vitamin premix[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[3]	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Binder[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Potassium sorbate	0.20	0.20	0.20	0.20	0.20	0.20	0.20

1) Containing 150 mg/kg diet of Butylated hydroxitoluene (BDH Chemicals Ltd., Poole, Dorset, England)

2) According to Table 4.4

3) According to Table 4.5

4) Carboxymethylcellulose, dissodium salt, high viscosity (BDH Chemicals Ltd., Poole, Dorset, England)

trout farmer who produces trout rations for his own consumption. The diet contained 40.90% crude protein and 11.5% lipid. Seven kilogrammes of this ration were ground and mixed with 2% vitamin premix, 1% mineral premix, 1% binder, 0.5% Cr_2O_3, and 0.2% potassium sorbate. Finally 1.5% cod liver oil was added to increase the lipid content to the formulated level of 13% and the diet was re-pelletised as described in Chapter 4.

The percentage of dietary carbohydrates varied from 3.9% to 15.5% for corn starch and from 1.9% to 7.7% for yellow dextrin (Table 8.1). Thus the overall carbohydrate content of Diets 1 and 2 (23.2% and 22.8% respectively) was slightly higher than the recommended maximum level of 20%.

The full chemical analysis carried out on both the foodstuffs and the diets is summarised in Table 4.2 and the methods were described in Chapter 4.

8.2.2 Growth Trial

300 rainbow trout of mean weight 19g (18.74-19.89g; Appendix XIII) were obtained from Inha, S. João da Madeira located near Porto and were allocated to 15 net cages as described in Chapter 4. Each experimental diet was allocated randomly to two net cages and a further cage of 20 fish was unfed during the experimental test period. Fish were fed at a fixed rate of 2% body weight per day throughout the 12 week trial. The full experimental protocol of the trial is described in Chapter 4.

The mean water temperature during the experimental period was 20.9°C (19-23°C ± 0.95) and the daily variation never exceeded 2°C (Table 8.2).

Statistical methods and the production of graphs were carried out as described in Chapter 4.

TABLE 8.2 Water temperature (°C) and standard deviation over the feeding period

Weeks	July	August	September	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0	19.0						
1	21.0						
2	21.0			21.0	1.41		
3	23.0						
4	21.0						
5		21.0				20.9	0.95
6		21.0		21.2	0.50		
7		21.0					
8		22.0					
9			21.0				
10			21.0	20.5	0.58		
11			20.0				
12			20.0				

8.3 RESULTS

8.3.1 Diets

The PBHFm had a crude protein and a true protein content of 56.18% and 52.93% respectively. Thus the nonprotein nitrogen component calculated by difference was 3.25%, which is slightly lower than the value of 4.31% determined directly by the trichloroacetic acid method (Table 8.3). Again the Kjeldahl factor of 6.25 may not have been the most appropriate value to employ (see Section 5.3.1). The meat and bone meal contained 43.58% crude protein and had a true protein content of 42.04%, while the brown fish meal had a crude protein content of 68.36% and a true protein content of 67.17%. Thus the calculated nonprotein nitrogen value of the meat and bone meal was 1.50% and that for the brown fish meal was 1.19%. These compared with nonprotein nitrogen values determined by the trichloroacetic acid method of 1.09% and 1.05% for meat and bone meal and brown fish meal respectively (Table 8.3).

The PBHFm tested was a good source of both the nonessential amino acids proline and glycine and of both the essential amino acids isoleucine and methionine, but a poor source of leucine, phenylalanine and valine. Valine was its first limiting amino acid. The meat and bone meal was a good source of both the nonessential amino acids proline and glycine but a poor source of leucine, methionine, phenylalanine, threonine, and valine. Leucine was its first limiting amino acid. Finally the brown fish meal was a good source of the nonessential amino acids glycine, glutamic acid, and proline, and of the essential amino acids lysine, histidine and isoleucine, but

TABLE 8.3 Proximate composition, Cr_2O_3 and energy content of Brown fish meal, Poultry by-product and hydrolysed feather meal (PBHFm), Meat and bone meal (MBm) and experimental diets (% dry weight)

Nutrient composition (% dry weight)	Brown fish meal	PBHFm	MBm	Dietary treatments 1	2	3	4	5	6	7
Moisture (%)	4.04(0.03)[1]	7.82(0.02)	2.67(0.04)	1.14(0.08)	1.34(0.04)	1.85(0.01)	1.57(0.06)	1.80(0.03)	1.98(0.03)	1.51(0.04)
Crude protein (N x 6.25)	68.36(0.53)	56.18(0.21)	43.58(0.09)	42.35(0.26)	42.50(0.19)	42.50(0.26)	42.76(0.16)	42.87(0.45)	42.36(0.33)	40.90(0.36)
True protein (%)	67.17(0.71)	52.93(0.53)	42.04(0.79)	41.33(0.58)	40.35(1.05)	38.99(0.98)	40.57(0.75)	40.94(0.57)	39.97(0.81)	38.95(0.38)
Nonprotein nitrogen (%)	1.05(0.41)	4.31(0.89)	1.09(0.47)	0.71(0.31)	1.98(0.88)	1.01(0.73)	1.43(0.58)	1.52(0.41)	1.75(0.54)	0.59(0.21)
Lipid (%)	6.12(0.26)	26.56(0.10)	14.14(0.38)	12.21(0.14)	13.73(0.13)	12.65(0.03)	13.02(0.57)	13.50(0.45)	13.81(0.37)	13.83(0.44)
Ash (%)	14.86(0.27)	5.63(0.26)	39.30(0.40)	11.32(0.45)	9.83(0.51)	29.12(0.10)	22.43(0.87)	25.28(0.17)	19.13(0.91)	15.14(0.55)
Nitrogen free extract[2] (%)	6.16	1.39	-	33.05	32.21	15.81	20.17	16.26	22.73	29.10
Peroxide value (mEq/Kg oil)	0.04(0.01)	0.00(-)	0.00(-)	0.58(0.01)	0.00(-)	0.00(-)	0.00(-)	0.00(-)	0.00(-)	0.58(0.09)
Acid insoluble ash (%)	0.61(0.03)	0.23(0.06)	0.23(0.09)	1.89(0.01)	1.76(0.03)	0.59(0.05)	0.80(0.08)	0.65(0.01)	0.94(0.09)	0.89(0.07)
Crude fibre (%)	0.60(0.03)	1.36(0.25)	0.95(0.13)	0.24(0.33)	0.56(0.16)	0.57(0.04)	0.81(0.14)	0.70(0.18)	0.63(0.13)	0.88(0.16)
Cr_2O_3 (%)	-	-	-	0.49(0.01)	0.50(0.01)	0.49(0.02)	0.50(0.01)	0.49(0.03)	0.48(0.01)	0.50(0.02)
Energy - ash free Kcal/g	5.64(0.57)	6.21(0.18)	6.80(0.26)	5.14(0.03)	5.49(0.13)	5.81(0.15)	5.45(0.17)	5.88(0.51)	5.68(0.52)	5.58(0.04)

1) Standard deviation

2) Nitrogen free extract = 100 - (% moisture+% True protein+% Nonprotein nitrogen+% Lipid+% Ash+% Crude fibre)

a poor source of arginine, leucine and phenylalanine. Leucine was the first limiting amino acid (Table 8.4).

The lipid content of the PBHFm and of the brown fish meal were 26.56% and 6.12% respectively, which are both within the normal range reported for these animal by-products (Jackson and Fulton, 1971; Burgos et al., 1974; Bhargava and O'Neil, 1975; Göhl, 1981; Windsor and Barlow, 1981; Bielorai et al., 1983). The lipid content of the meat and bone meal however was 14.14% (Table 8.3) which is higher than the normal range of 2% to 12% (Fowler and Banks, 1976; Skrede et al., 1980; McDonald et al., 1981; Tacon et al., 1984).

The ash content of the PBHFm, meat and bone meal and brown fish meal were 5.63%, 39.30% and 14.86% respectively (Table 8.3). The ash content of the meat and bone meal was significantly higher than the level of the last batch (12.69%) and also higher than the normal range of reported values (27-33%).

The peroxide value, acid insoluble ash, and crude fibre values of all three animal by-products were very low, in each case less than 2 mEq/Kg oil and 2% respectively (Table 8.3).

The PBHFm, meat and bone meal, and brown fish meal were all good sources of all the minerals analysed in particular of K, Zn, and Na for the former, Ca, K, and Na for the second, and K, Ca, and Zn for the latter (Table 8.5).

TABLE 8.4 Amino acid profile of Brown fish meal, Poultry by-product and hydrolysed feather meal (PBHFm), Meat and bone meal (MBm), experimental diets and amino acid requirement of rainbow trout (g/100g dry weight)

Amino acid (g/100g dry wt)	Brown fish meal	PBHFm	MBm	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Requirement of rainbow trout (% of diet)
Arginine	2.06	1.89	1.45	1.28	1.53	1.36	1.39	1.39	1.36	1.44	1.40, 40% (Ogino, 1980)
Histidine	4.55	1.55	1.39	2.82	2.00	1.94	1.74	1.78	1.88	2.08	0.64, 40% (Ogino, 1980)
Isoleucine	4.80	3.97	1.63	2.97	2.99	2.14	2.45	2.30	2.56	2.77	0.96, 40% (Ogino, 1980)
Leucine	1.59	1.21	0.46	0.98	0.95	0.66	0.76	0.71	0.79	0.97	1.76, 40% (Ogino, 1980)
Lysine	6.36	3.49	3.01	3.94	3.30	3.34	3.17	3.21	3.25	3.08	2.12, 40% (Ogino, 1980)
Methionine	2.45	1.85	0.70	1.52	1.46	1.02	1.16	1.08	1.23	1.17	0.55-0.75, 35% (Rumsey et al. 1983)
Phenylalanine	1.41	0.48	0.41	0.87	0.62	0.59	0.53	0.50	0.58	0.97	1.24, 40% (Ogino, 1980)
Threonine	3.54	2.49	1.10	2.19	2.04	1.52	1.66	1.59	1.76	2.02	1.36, 40% (Ogino, 1980)
Valine	2.07	0.28	0.38	1.28	0.75	0.73	0.60	0.62	0.68	1.12	1.24, 40% (Ogino, 1980)
Alanine	1.13	2.44	0.49	0.70	1.27	0.57	0.96	0.82	0.97	0.79	
Aspartic acid	4.87	3.65	1.82	3.02	2.89	2.27	2.45	2.37	2.56	3.53	
Cystine	0.99	0.88	0.36	0.61	0.64	0.46	0.52	0.50	0.55	0.61	2.30, 35% (Rumsey et al. 1983)
Glutamic acid	7.18	4.76	3.27	4.45	4.03	3.69	3.72	3.69	3.81	3.65	
Glycine	10.12	7.40	6.68	6.27	5.94	6.43	6.21	6.31	6.13	5.84	
Proline	6.99	8.19	10.82	4.33	5.27	8.07	7.43	7.88	6.73	4.41	
Serine	4.64	5.75	2.15	2.87	3.61	2.41	3.02	2.81	3.08	2.99	
Tyrosine	1.52	1.41	0.54	0.94	1.00	0.69	0.81	0.75	1.25	0.82	0.84, 40% (Ogino, 1980)
TOTAL	66.27	51.69	36.66	41.04	40.29	37.89	38.58	38.31	39.17	39.26	

* Percentage of crude protein in the diet

TABLE 8.5 Concentration of mineral elements in Brown fish meal, Poultry by-product and hydrolysed feather meal (PBHFm), Meat and bone meal, commercial diet and mineral requirement of rainbow trout

Element	Brown fish meal	PBHFm	Meat and bone meal	Commercial Diet	Requirement of rainbow trout (mg/100g dry wt)	
Ca (g/100g)	3.93	1.42	13.72	3.14	650-750	(Ogino and Takeda, 1978)
Mg (g/100g)	0.16	0.07	0.17	0.16	60-70	(Ogino et al, 1978) (Knox et al., 1978)
K (g/100g)	3.93	1.79	1.89	3.82	160	(Frenzel and Pfeffer, 1982)
Na (g/100g)	0.67	0.43	1.34	2.14	220	(Frenzel and Pfeffer, 1982)
P (g/100g)	0.97	0.79	0.51	0.63	700-800 650	(Ogino and Takeda, 1978) (Nose and Arai, 1979)
Zn (mg/100g)	13.62	7.86	5.25	9.48	1.5-3.0	(Ogino and Yang, 1978)

The crude protein content of the commercial based ration (Diet 7) was 40.90% which is close to the formulated level of 41%. The crude protein levels of the remaining six rations were somewhat higher, varying between 42.35% and 42.87% (Table 8.3). The true protein content varied between 38.95% for Diet 7 and 41.33% for Diet 1.

All rations were deficient in leucine and phenylalanine. The leucine level in Diet 3 was particularly low, 0.66%, compared with a dietary requirement of 1.76% (Ogino, 1980) and with a maximum dietary level of only 0.98% in Diet 1. Phenylalanine levels varied between 0.50% in Diet 5 and 0.97% in Diet 7 (commercial ration) compared with a dietary requirement of 1.24% (Ogino, 1980). All diets except Diets 2 and 7 were slightly deficient in arginine with a minimum level of 1.28% in Diet 1 compared with a dietary requirement of 1.40% (Ogino, 1980). Finally, with the exception of Diet 1, all rations were deficient in valine with levels ranging from 0.60% in Diet 4 to 1.12% in Diet 7 (Table 8.4).

The lipid content of the experimental diets varied from 12.21% to 13.83% which is close to the formulated value of 13% (Table 8.3). The energy content of the diets varied between 5.14 and 5.88 Kcalories per gramme dry weight (Table 8.3).

8.3.2 Growth Performance and Feed Utilization Efficiency

Fish accepted the experimental diets readily within a few days and thereafter they were consumed quite aggressively even at water temperatures as high as 23°C.

At the start of the trial there were no significant ($P < 0.05$) differences in mean fish weights of around 19g (18.95-19.59g) between treatments (Table 8.6). However by the end of the 12 week growth trial the mean final weights varied between 48.05g for fish fed Diet 5 and 87.61g for those fed Diet 2 (Table 8.6; Fig. 8.1). There were no significant ($P < 0.05$) differences between the final weights of fish fed the fish meal based control diet and those fed Diet 2 where 50% of the fish meal protein was replaced by PBHFm. Both of these experimental diets performed better than the commercial ration (Diet 7) which produced fish with a mean final weight of only 72.22g. The poorest growth performance resulted from feeding the diets containing high inclusion levels of meat and bone meal (Diets 3 and 5), which as a consequence also contained the lowest levels of brown fish meal (Table 8.6).

The mortality rate of fish fed the experimental diets varied between 5% and 30% and no correlation between mortality rate and dietary treatments was indicated (Table 8.7). The mortality rate of the unfed fish however was 95%. Furthermore the weight of the remaining fish was only 15.00g (Appendix XIV; Fig. 8.1), thus demonstrating that any available natural food would have had a negligible effect on the overall growth performance of fish fed the experimental diets.

The specific growth rate of fish fed Diet 2 containing 50% of the protein as PBHFm was 1.81% per day which was slightly higher than that of those fed the control ration. Again the poorest specific growth rates were produced by diets containing high proportions

TABLE 8.6 Growth performance, feed utilization efficiency, liver somatic index, and blood parameters of rainbow trout (280 fish, 19g) fed the experimental diets after 12 weeks

Mean values	Dietary treatments 1	2	3	4	5	6	7	±S.E.[1]
Mean initial weight (g)	19.20^{a}	19.12^{a}	19.45^{a}	19.59^{a}	18.95^{a}	19.26^{a}	19.25^{a}	0.383
Mean final weight (g)	85.89^{f}	87.61^{f}	52.49^{ab}	60.79^{bc}	48.05^{a}	67.74^{cd}	72.22^{de}	2.969
Weight gain (%)	347.34	358.21	169.87	210.31	153.56	251.71	275.17	
Specific growth rate (%/day)	1.78	1.81	1.18	1.36	1.11	1.49	1.57	
Food intake (mg/day)	1098	1100	846	876	782	913	1005	
Protein intake (mg/day)	454	456	351	366	327	378	411	
Weight gain (mg/day)	794	815	393	490	346	577	630	
Food conversion ratio	1.38	1.35	2.15	1.79	2.26	1.58	1.59	
Protein efficiency ratio	1.75	1.79	1.12	1.34	1.06	1.53	1.53	
Nitrogen intake (mg/day)	72.64	72.96	56.16	58.56	52.32	60.48	65.76	
Nitrogen deposition (mg/day)	10.93	11.17	7.00	8.37	6.61	9.54	9.58	
Apparent net protein utilization (%)	15.05	15.31	12.46	14.29	12.63	15.77	14.57	
Apparent protein digestibility (%)	84.06^{e}	83.36^{e}	69.20^{a}	74.97^{b}	76.83^{c}	80.61^{d}	84.29^{e}	0.509
Apparent lipid digestibility (%)	81.92^{c}	85.79^{de}	69.27^{a}	76.63^{b}	82.14^{cd}	86.63^{ef}	89.94^{g}	0.939
Apparent organic matter digestibility (%)	61.81^{de}	65.93^{f}	44.14^{a}	50.65^{b}	55.08^{c}	61.26^{d}	64.33^{ef}	0.833
Liver somatic index	1.37^{abcd}	1.50^{cd}	1.58^{d}	1.26^{abc}	1.23^{ab}	1.43^{abcd}	1.22^{a}	0.079
Haematocrit (%)	41.00^{bc}	37.38^{ab}	35.88^{a}	38.50^{abc}	40.50^{bc}	40.50^{bc}	42.00^{c}	1.343
Haemoglobin (g/100 cm^3)	8.75^{ab}	8.12^{ab}	7.91^{a}	8.22^{ab}	8.64^{ab}	8.71^{ab}	8.96^{b}	0.272

[1] Standard error; calculated from residual mean square in the analysis of variance

abcdefg Mean values for components with the same superscript are not significantly ($P < 0.05$) different

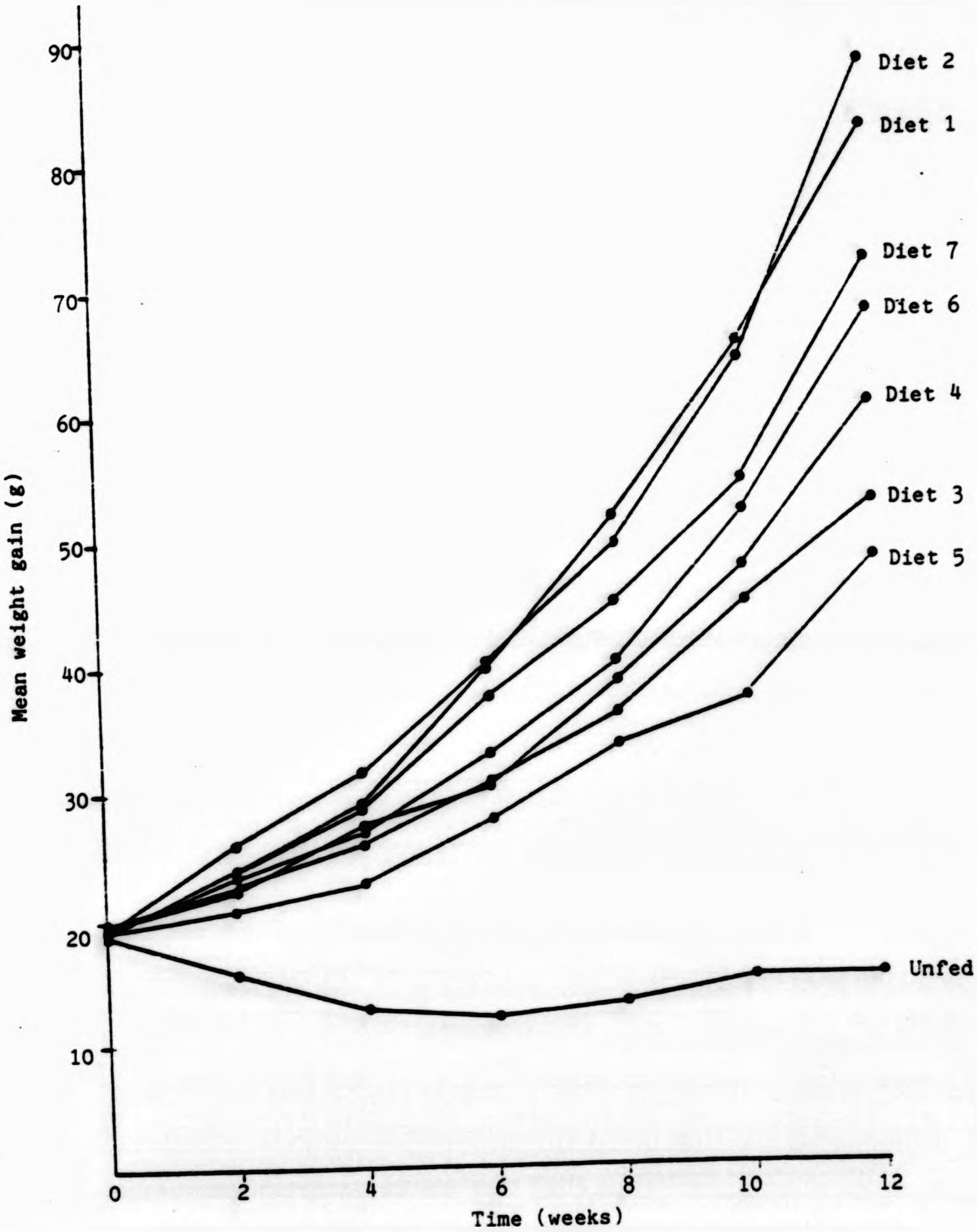

FIGURE 8.1 Overall mean weight gain (g) of rainbow trout at successive fortnightly intervals over the experimental test period

TABLE 8.7 Weekly deaths per net cage and overall percentage mortality over the 12 week trial

Treatments	1		2		3		4		5		6		7		No feeding
Replicates	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
0															
2			1	2		1	1			1	1		2		
4		1			1							1			1
6		3								1		1		1	9
8		1			2						1			1	2
10			2		1					1	1				5
12	2		1		1	1				2	3	1			2
TOTAL	2	5	4	2	5	2	1	0	0	5	6	3	2	2	19
%	10	25	20	10	25	10	5	0	0	25	30	15	10	10	95

of the protein component as meat and bone meal, thus Diets 3 and 5 (60 and 50% meat and bone meal protein replacement respectively) had specific growth rates of only 1.18% and 1.11% per day respectively. The specific growth rate of fish fed Diet 6 where 60% of the dietary protein was supplied by a combination of PBHFm and meat and bone meal was slightly lower than that of fish fed the commercial control diet (Table 8.6; Fig. 8.2).

The food conversion ratio of Diet 2 (1.40) was only slightly higher than that of the control ration (1.38), although this was still lower than the value of 1.59 for the commercial ration (Diet 7). Again food conversion ratios of Diets 3 and 5 were the poorest (2.15 and 2.26 respectively). The food conversion ratio of Diet 6 where 60% of the protein was supplied by equal proportions of PBHFm and meat and bone meal was close to that obtained for the commercial ration (Table 8.6; Fig. 8.3).

The protein efficiency ratio (PER) followed the same trend as both specific growth rates and food conversion ratios. Thus the PER of fish fed Diet 2 (1.79) was slightly better than that of those fed the control diet (Diet 1; 1.75) and both of these diets performed better than Diet 7, the commercial ration (1.53). The poorest performance in terms of PER was again attained by fish fed Diets 3 and 5 (1.12 and 1.06 respectively). Finally the PER of fish fed Diet 6 was again close to that of those fed the commercial ration (Table 8.6; Fig. 8.4). Apparent net protein utilization (apparent NPU) however, did not follow exactly the same trend as the PER. The highest apparent NPUs were for fish fed Diets 2 and 6,

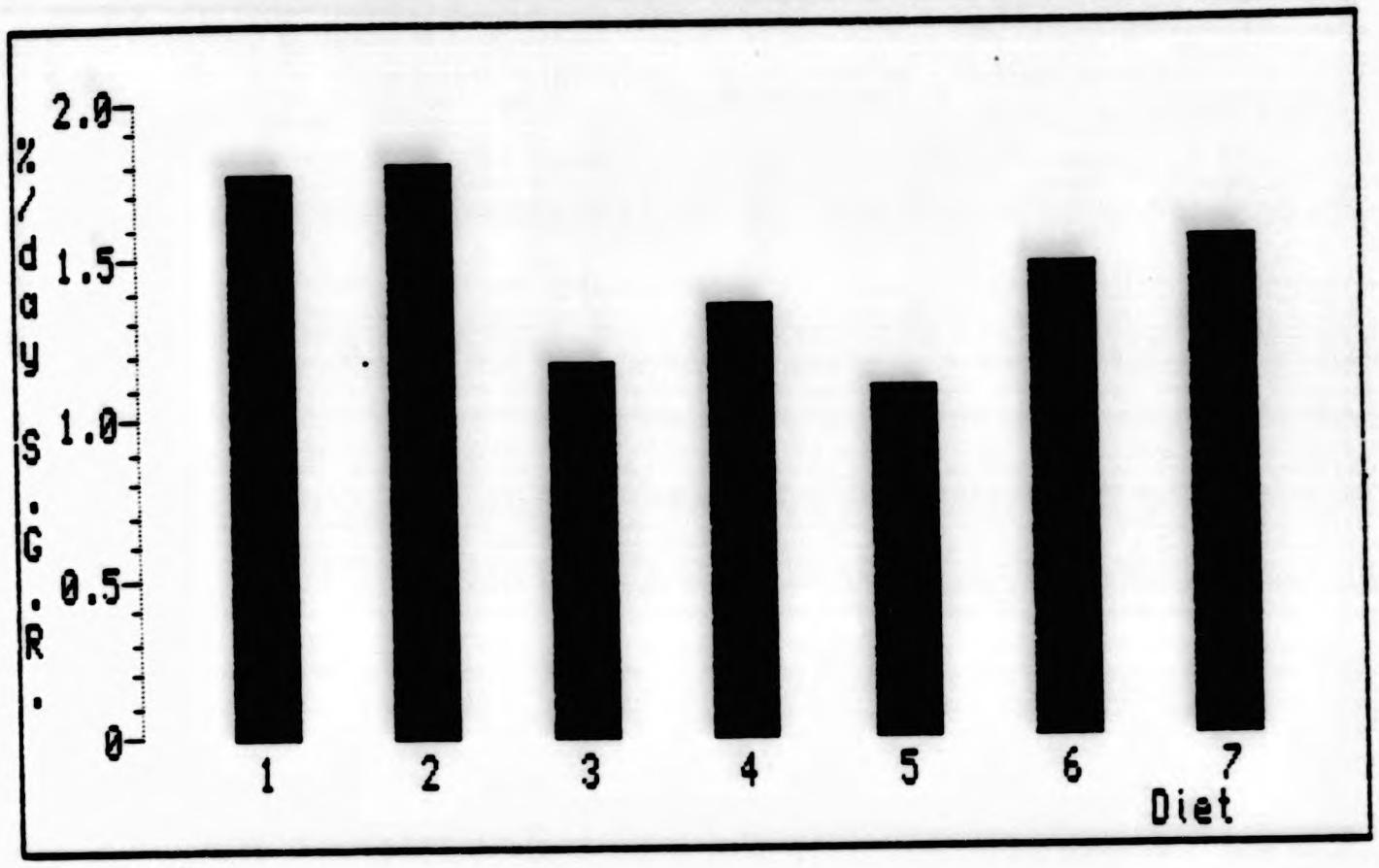

FIGURE 8.2 Specific growth rate (%/day) of fish fed the six experimental diets and the commercial ration

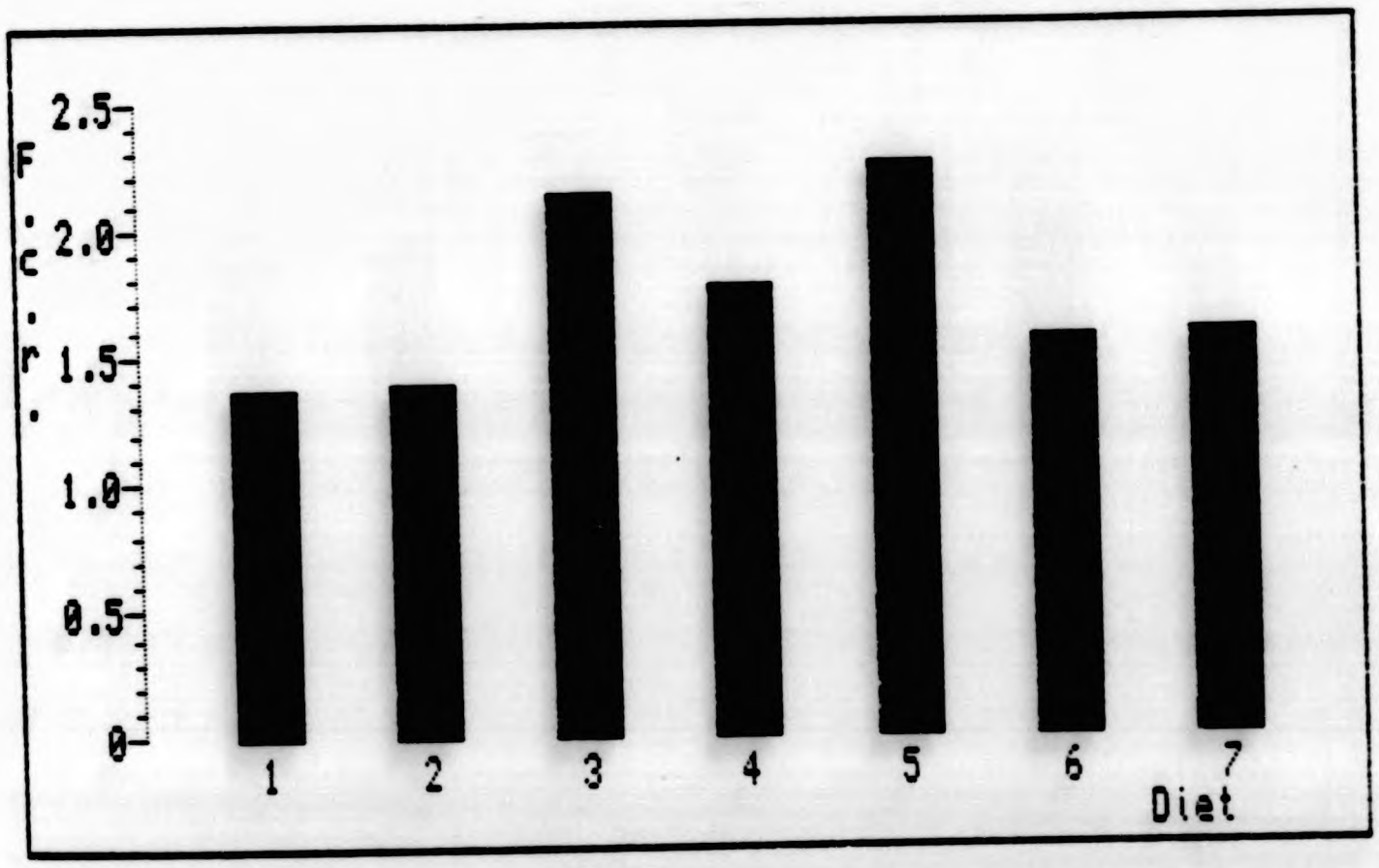

FIGURE 8.3 Food conversion ratio of fish fed the six experimental diets and the commercial ration

FIGURE 8.4 Protein efficiency ratio of fish fed the six experimental diets and the commercial ration

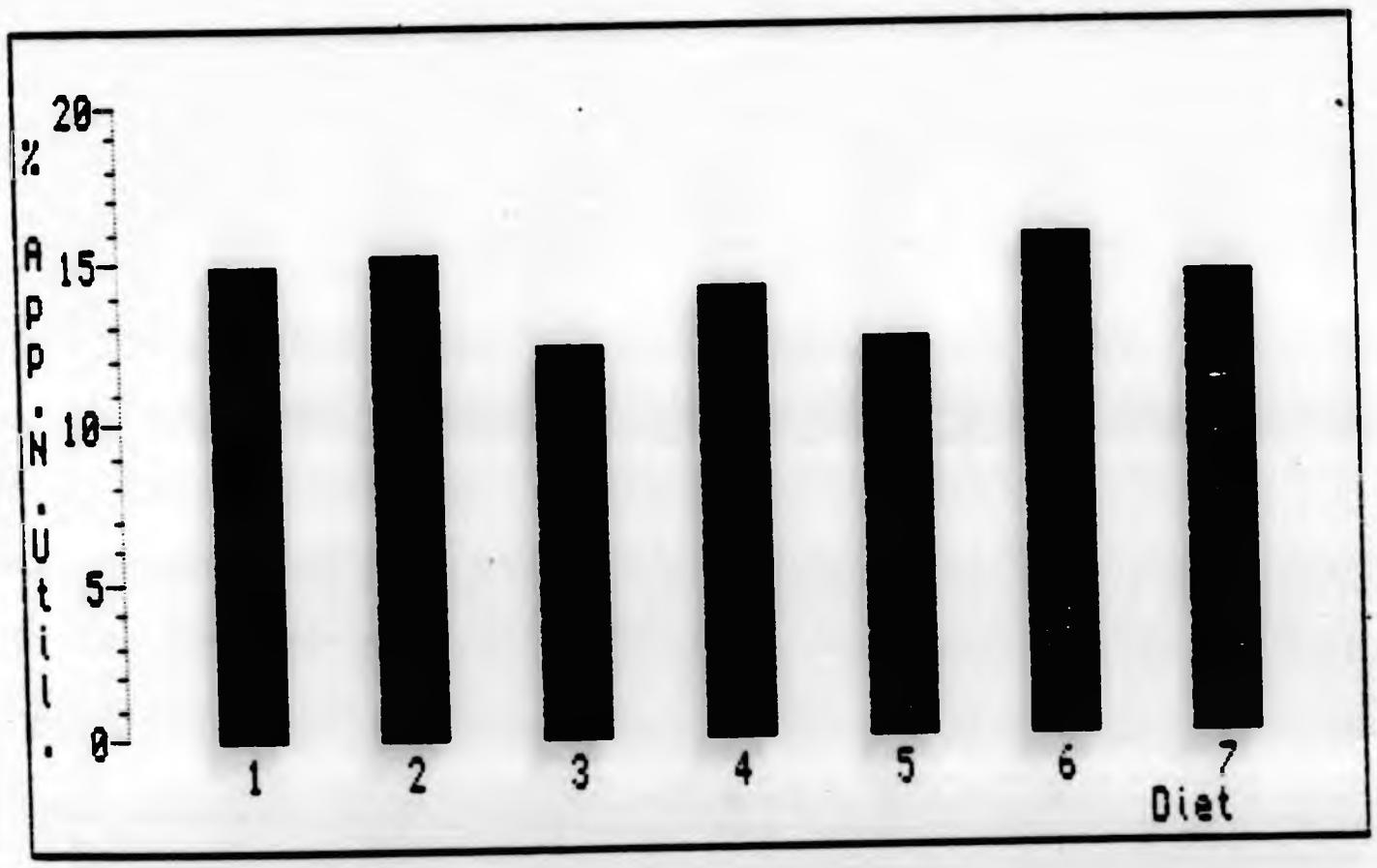

FIGURE 8.5 Apparent net protein utilization (%) of fish fed the six experimental diets and the commercial ration

although these values were only slightly higher than the value of 15.05% for the control ration (Diet 1). Again the poorest apparent NPUs were registered by fish fed Diets 3 and 5 with values of 12.46% and 12.63% respectively (Table 8.6; Fig. 8.5).

The growth performance of rainbow trout replicates fed the experimental diets is given in Appendix XIII.

8.3.3 Digestibility

The moisture content of faeces varied from 2.43% for fish fed Diet 2 (50% meat and bone meal protein replacement) to 5.29% for those fed Diet 3 (20% PBHFm + 50% meat and bone meal protein replacement). The moisture contents of faeces from fish fed the fish meal control diet and from those fed the commercial ration were 2.61% and 3.59% respectively (Table 8.8; Fig. 8.6a).

The lowest crude protein levels in faeces were from fish fed the fish meal control diet and from those fed the commercial ration (17.20% and 17.88% respectively). The crude protein content of faeces from fish fed diets containing PBHFm and meat and bone meal varied between 20.07% and 22.79% respectively (Table 8.8; Fig. 8.6b) and the crude protein content increased with increasing inclusion levels of meat and bone meal.

The best apparent protein digestibilities were by fish fed the fish meal control diet (Diet 1; 84.06%) and the commercial diet (Diet 7; 84.29%), although fish fed Diet 2 containing 50% PBHFm had a coefficient of 83.36% which was not significantly different

TABLE 8.8 Proximate composition of faeces taken from rainbow trout after 12 weeks on the experimental diets and commercial ration (% dry weight)

Faeces composition (% dry weight)	Dietary treatments 1	2	3	4	5	6	7
Moisture (%)	2.61(0.50)[1]	2.43(0.37)	2.63(0.24)	3.21(0.28)	5.29(4.04)	2.91(0.57)	3.59(0.29)
Crude protein (N x 6.25)	17.20(0.26)	20.07(0.84)	22.79(0.38)	20.99(1.29)	21.47(0.39)	20.58(0.90)	17.88(0.65)
Lipid (%)	5.88(1.30)	5.67(1.65)	6.94(1.27)	6.14(1.51)	5.33(0.17)	4.75(1.03)	3.97(1.70)
Ash (%)	25.38(0.12)	21.39(1.25)	53.77(0.25)	51.10(3.21)	56.89(2.45)	43.75(0.03)	37.77(2.65)
Cr_2O_3 (%)	1.28(0.09)	1.45(0.07)	0.87(0.07)	1.01(0.10)	1.09(0.05)	1.23(0.08)	1.40(0.12)

1) Standard deviation

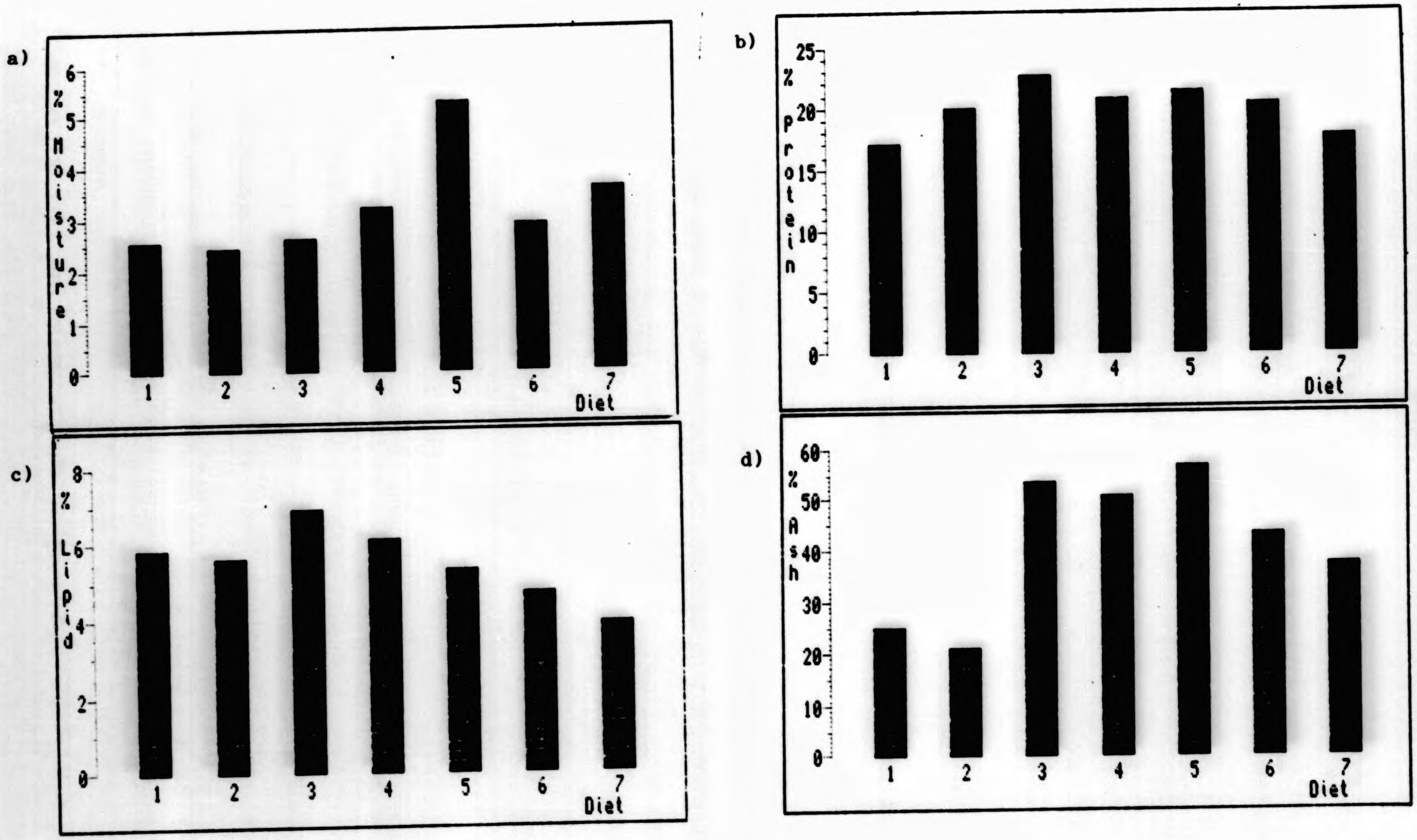

FIGURE 8.6 Proximate composition of the faeces from fish fed each of the six experimental diets and the commercial ration. a) Moisture; b) Crude protein; c) Lipid; d) Ash

from these. The poorest apparent protein digestibility was 69.20% by fish fed Diet 3 containing the highest level of meat and bone meal (Table 8.6). Protein digestibility decreased with increasing inclusion levels of meat and bone meal from 80.61% for fish fed Diet 6 containing 30% meat and bone meal to only 69.20% for fish fed Diet 3 containing 60% of the protein as meat and bone meal.

The lipid content of faeces varied from 3.97% (Diet 7) to 6.94% (Diet 3). The lowest lipid content of 3.97% was in faeces from fish fed the commercial ration (Diet 7) while the faecal lipid content of fish fed the control diet was 5.88% (Table 8.8; Fig. 8.6c). As had been the case with protein levels lipid levels increased with increasing inclusion levels of meat and bone meal. The apparent lipid digestibility of the commercial ration was 89.94% which was significantly ($P < 0.05$) higher than for any other treatments (Table 8.6). The poorest apparent lipid digestibility of only 69.27% was by fish fed Diet 3, thus digestibilities again decreased with increasing inclusion levels of meat and bone meal.

There was a wide variation in the ash content of faeces between dietary treatments, from 21.39% for fish fed Diet 2 to 56.89% for those fed Diet 5 (Table 8.8; Fig. 8.6d). The best apparent organic matter digestibility coefficient was by fish fed Diet 2 although this did not differ significantly from the coefficient for the commercial ration (Diet 7). There was no significant difference between the apparent organic matter digestibility of the control ration, Diet 1 (61.81%) and Diet 6 (61.26%) containing a combination of PBHFm and meat and bone meal. Again the poorest apparent organic

matter digestibility of only 44.14% was by fish fed Diet 3 where 60% of the protein was supplied by meat and bone meal (Table 8.6).

Thus the overall trend appears to have been for digestibilities to decrease with increasing inclusion levels of meat and bone meal, although in almost all cases this was also accompanied by a decrease in the levels of the brown fish meal.

The proximate composition of faeces taken from rainbow trout replicates is given in Appendix XV.

8.3.4 Liver Somatix Index and Blood Parameters

The liver somatic index of fish fed the experimental diets varied between 1.22 for fish fed the commercial diet (Diet 7) and 1.58 for those fed Diet 3. Although there were significant ($P<0.05$) differences, particularly between the values for fish fed Diets 3 and 7, the liver somatic indeces did not differ significantly from the control value of 1.37 (Table 8.6).

The haematocrit value varied significantly ($P < 0.05$) between 35.88% for fish fed Diet 3 and 42.00% for those fed the commercial diet (Diet 7). The only value which differed significantly from the control was that of Diet 3 with a haematocrit value of 35.88%. However all values are still within the normal range for healthy rainbow trout (Wedemeyer and Nelson, 1975; Miller et al., 1983; Railo et al., 1985).

Blood haemoglobin levels varied between 7.91g per 100 cm^3 for fish fed Diet 3 and 8.96g per 100 cm^3 for those fed Diet 7 (commercial ration). However these differences were not significant ($P < 0.05$) and furthermore these values are all within the normal range reported for healthy rainbow trout (Lowe-Jinde and Niimi, 1973; Wedemeyer and Nelson, 1975).

The liver somatic index and blood parameters of rainbow trout replicates are given in Appendix XIII.

8.3.5 Carcass Composition

At the end of the 12 week growth trial the moisture content of fish fed all experimental diets was significantly ($P < 0.05$) lower than the initial value of 71.56% (Table 8.9; Fig. 8.7a). The moisture content of fish fed the experimental rations varied between 68.49% (Diet 1) and 69.97% (Diet 5) although the differences were not significant at the 95 percent level.

The lipid content of fish fed all the experimental diets varied between 8.75% for fish fed Diet 6 and 9.78% for those fed Diet 4. These levels were all significantly ($P < 0.05$) higher than the initial value of 6.27%. Again there were no significant differences between dietary treatments with the exception of fish fed Diet 4 which had a significantly higher carcass lipid content of 9.78% (Table 8.9; Fig. 8.7b).

TABLE 8.9 Carcass composition of rainbow trout (280 fish, 19g) at the start and end of the experiment (12 weeks) based on 12 fish per treatment (% wet weight)

Carcass composition (% wet weight)	Initial	Dietary treatments 1	2	3	4	5	6	7	± S.E.[1]
Moisture (%)	h 71.56(0.05)[2]	a 68.49(0.63)	abcd 69.52(0.55)	abcde 69.73(0.46)	ab 69.03(1.04)	abcdefg 69.97(0.33)	abc 69.17(1.17)	abcdef 69.83(1.11)	0.437
Crude protein (N x 6.25)	a 18.60(0.50)	a 17.84(0.38)	a 17.79(0.46)	a 17.26(0.42)	a 17.31(0.61)	a 17.23(0.33)	a 17.70(0.74)	a 17.50(0.75)	0.933
Lipid (%)	a 6.27(0.39)	bc 9.62(0.67)	bc 8.99(0.37)	bc 8.93(0.32)	c 9.78(0.49)	bc 8.96(1.04)	bc 8.75(0.73)	bc 9.20(0.63)	0.286
Ash (%)	ab 3.83(0.13)	bc 4.31(0.43)	abc 3.97(0.51)	bc 4.29(0.55)	bc 4.27(0.53)	abc 4.08(0.41)	c 4.52(0.53)	a 3.70(0.11)	0.167
	100.26	100.26	100.27	100.21	100.39	100.24	100.14	100.23	

1) Standard error; calculated from residual mean square in the analysis of variance

2) Standard deviation

abc..fgh Mean values of components with common superscripts are not significantly ($P < 0.05$) different

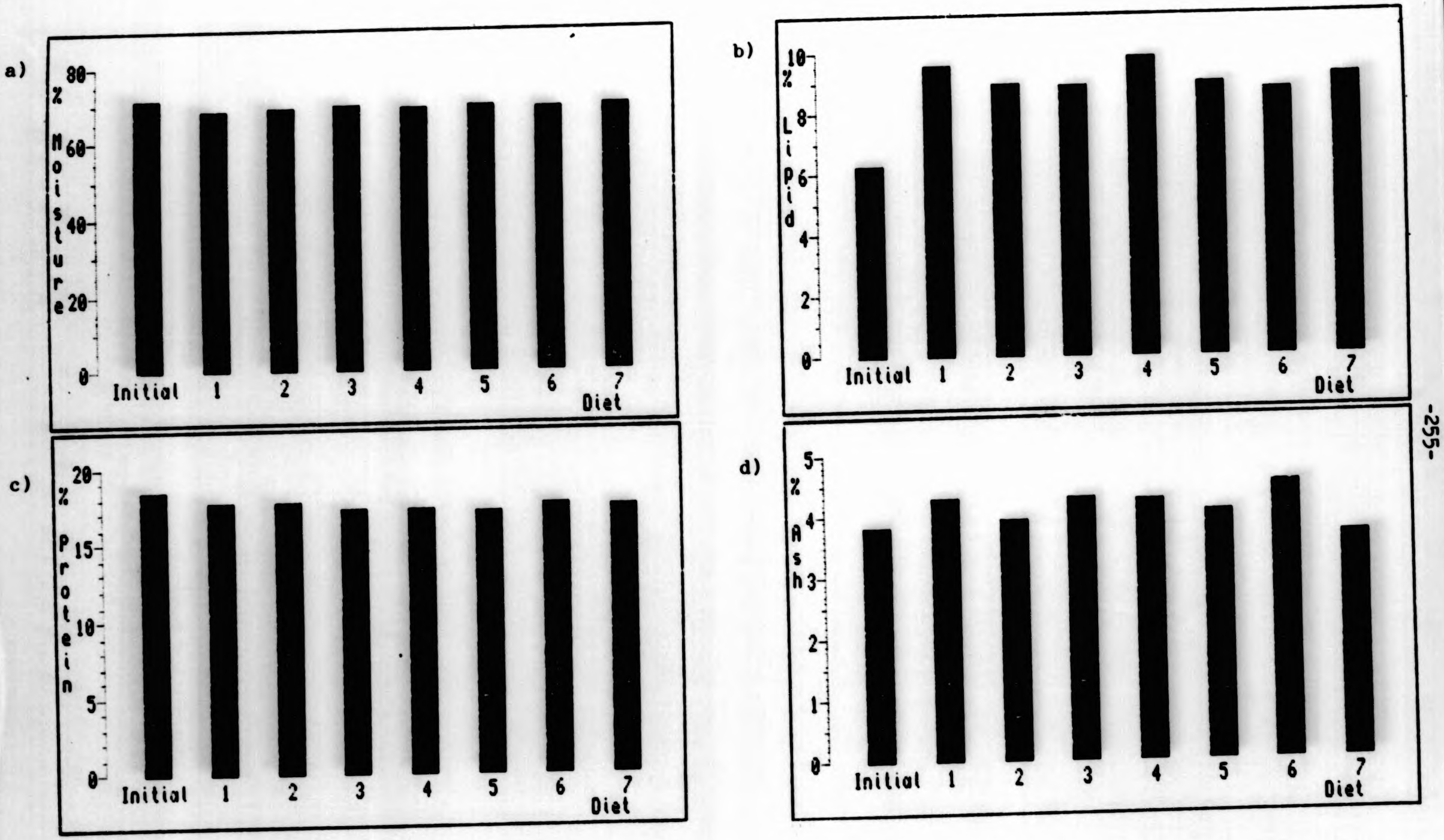

FIGURE 8.7 Proximate composition of the carcasses from fish fed the six experimental diets and the commercial ration and of the initial sample. a) Moisture; b) Lipid; c) Crude protein; d) Ash

None of the dietary treatments produced a significant ($P < 0.05$) change from the initial carcass protein content of 18.60%. The protein content of fish fed the seven experimental rations varied, although not significantly between 17.23% and 17.84% (Table 8.9; Fig. 8.7c).

With the exception of fish fed Diet 6 the carcass ash content of fish fed the experimental diets increased from 3.83% at the start of the trial to between 3.97% and 4.52% at the end. There was a significant ($P < 0.05$) decrease in carcass ash of fish fed the commercially based ration, Diet 7, from 3.83% at the start of the trial to 3.70% after 12 weeks (Table 8.9; Fig. 8.7d).

The proximate composition of fish carcass replicates is given in Appendix XVI.

8.4 DISCUSSION

The best growth performance was by fish fed Diet 2 where only half of the fish meal component was replaced by PBHFm. The final mean weight of these fish, 87.61g, was slightly higher, although not significantly, than that of the fish fed the fish meal control ration, Diet 1 (Table 8.6). Both growth responses were better than that of fish fed a commercial trout ration (Diet 7) which had a final mean weight of only 72.22g. This may be attributable, at least in part, to the somewhat lower true protein content of only 38.95% in the commercial ration compared with levels of around 39% to 42% in the six other diets (Table 8.3). The poorest growth response was by fish fed Diet 5 containing both one of the highest inclusion levels of meat and bone meal (50%) and also one of the lowest levels of fish meal (30%). Fish fed this ration increased their biomass by less than 2.5 times compared with around 4.5 times by fish fed Diets 1 and 2. An apparent trend of decreasing growth performances with increasing inclusion levels of meat and bone meal was also indicated. Thus, fish fed diets in which meat and bone meal supplied 30%, 40%, 50%, and 60% of the dietary protein had decreasing growth responses with final mean weights of around 68g, 61g, 48g, and 52g respectively (Table 8.6). The relatively higher final mean weight of 52.49g of fish fed Diet 3 containing the highest meat and bone meal component was most likely due to the higher fish meal inclusion level of 40% compared with only 30% in Diets 4 and 5. The growth response of fish fed diets containing combinations of the three by-products was best when the fish meal and the PBHFm were included at high inclusion levels and conversely was worse

when the fish meal component was largely replaced by meat and bone meal. Thus, fish fed Diet 6 containing 70% of the protein as fish meal and PBHFm (1.3:1 ratio) had a final mean weight of around 68g while those fed Diet 5 where only 50% of the protein was supplied by these two products (1.5 fish meal:1 PBHFm ratio) had a final mean weight of only around 48g. Fish fed Diets 3 and 5 which both contained 40% of the protein as fish meal, had significantly different growth responses. Thus fish fed Diet 3 containing the remaining 60% of the protein as only meat and bone meal had a final mean weight of only around 52g while those fed Diet 6 where the remaining 60% of the protein was supplied by both PBHFm and meat and bone meal (1:1 ratio) had a significantly better final mean weight of around 68g. Overall the results indicate that combinations of protein sources give the best growth responses, and that where meat and bone meals similar to the one tested in this work are included in diets for rainbow trout, its inclusion should be restricted to a relatively low level of below 30%.

The significant differences in growth response among fish fed the seven experimental diets were related to differences in amino acid profiles of the diets resulting from varying deficiencies in dietary protein sources (Section 8.3.1). Thus Diet 2 containing 50% of the protein as PBHFm was deficient in leucine, phenylalanine and valine which supplied 54%, 50%, and 60% of the requirement levels respectively (Table 8.4). Since phenylalanine can be spared by tyrosine (Mertz, 1972; Halver, 1975, 1976; Cowey, 1979; Ketola, 1982; Millikin, 1982; Walton, 1985; Wilton, 1985) the first limiting amino acid was leucine, and therefore only 54% of the protein was

usable for growth. Similarly the control ration was deficient in both the essential amino acids leucine and phenylalanine which supplied 55% and 70% of the requirement levels and therefore the first limiting amino acid was also leucine. Thus, although a better essential amino acid index (EAAI) of 95.73 was indicated for the control ration compared with an EAAI of only 84.94 for Diet 2, both experimental diets had around 55% of the protein usuable for growth and consequently similar growth performances by fish fed these diets were attained (Table 8.6). As had been the case with Diet 2, the commercial trout ration (Diet 7) had an essential amino acid profile deficient in leucine, phenylalanine, and valine. Leucine was again the first limiting amino acid since it supplied only around 55% of the requirement level while both phenylalanine and valine supplied around 78% and 90% of the requirement levels respectively. The EAAI indicated for this ration was high, 91.52, and better than that indicated for Diet 2 (84.94). However, despite similarities in the essential amino acid profiles of fish fed Diets 1, 2 and 7, the growth response of the fish fed the commercial control ration was significantly poorer than that of the fish fed either Diets 1 or 2. The formulation of the commercial ration was not known and hence it is not possible to explain these differences in performances with any certainty. The main differences in chemical composition between these three diets were the slightly lower protein content of the commercial ration and also a slightly higher ash content of 15% compared with only around 10% and 11% in Diets 1 and 2. In any case, both the significant essential amino acid deficiencies and the relatively poor growth performance produced by the commercial control diet indicate that the quality of this commercial trout ration was not of a particularly high standard.

Diets containing increasing inclusion levels of meat and bone meal were severely deficient in the essential amino acids leucine, phenylalanine and valine. Thus Diet 3 containing the highest meat and bone meal inclusion level (60% protein replacement) reflected the severe essential amino acid deficiencies of this by-product and therefore only 37%, 47% and 59% of the leucine, phenylalanine and valine requirement levels respectively, were present. Leucine was therefore the first limiting amino acid and consequently only around one third of the total protein would have been available for growth, and this probably explains why one of the poorest growth performances resulted from feeding this ration. In conjunction with the poor essential amino acid profile the diet also contained the highest dietary ash content of around 29%.

The first limiting amino acid in Diets 6, 4, and 5 containing 30% to 50% of the protein as meat and bone meal was leucine with levels of around 44%, 43%, and 40% of the requirement level for rainbow trout respectively. Thus, although there was a trend of decreasing leucine content with increasing inclusion levels of meat and bone meal, the small differences are unlikely to have been the only reason for the significant differences in fish performance. Indeed, it is probable that the high ash content of the meat and bone meal of around 39% would also have contributed for the significant differences in fish performance with increasing inclusion levels of this product. Thus fish fed Diets 6, 4 and 5 containing 30% to 50% of the protein as meat and bone meal had increasing dietary ash contents of 19%, 22%, and 25% respectively.

The best food conversion ratios of between 1.4 and 1.7 and the best PER's of around 1.5 - 1.8 were achieved by fish fed the control ration (Diet 1), the diet containing 50% of the protein as PBHFm (Diet 2), and the commercial trout ration (Diet 7; Table 8.6). Food utilization efficiency was also depressed with increasing inclusion levels of meat and bone meal and therefore a food conversion ratio of 1.58 and a PER of 1.53 were indicated for Diet 6 containing only 30% of the protein as meat and bone meal, while one of the poorest food conversion ratios of 2.15 and one of the lowest PERs of 1.12 were indicated for Diet 3 where 60% of the protein was supplied by this product. However the poorest growth performance and food conversion ratio of 2.26 and the lowest PER of 1.06 were indicated for Diet 5 containing only 50% of the protein as meat and bone meal, but this was almost certainly related to the lower fish meal inclusion level of 30% of the protein compared to that of 40% in Diet 3. These findings are in agreement with those of Fowler and Banks (1976) and Tiews et al. (1976) who found that chinook salmon (Oncorhynchus tshawytscha) and rainbow trout fed diets containing increasing inclusion levels of meat and bone meal had reduced growth responses and feed utilization efficiencies as indicated in Section 7.4. Furthermore Tiews et al. (1976) reported that even at an inclusion level of 38% of the protein meat and bone meal is already a poor protein source for rainbow trout. The best growth performance and feed utilization efficiency among fish fed diets containing different proportions of the three by-products was achieved by those fed Diet 6 containing fish meal, PBHFm and meat and bone meal in the ratio 1.3:1:1 respectively.

The digestibilities of the experimental diets followed the same trend as the growth performance and feed utilization efficiency (Table 8.6; Section 8.3.3). Thus the highest apparent protein digestibilities were indicated for fish fed the fish meal control ration (Diet 1), the diet containing 50% of the protein as PBHFm (Diet 2), and the commercial trout ration (Diet 7) with coefficients of around 84%. The protein digestibility of fish fed the control ration containing only fish meal as the protein source was within the normal range of values of between 60% and 95% indicated for brown fish meals (Smith and Rumsey, 1976; Atack and Matty, 1979; Cho and Slinger, 1979; Lovell, 1981; Pfeffer, 1982; Watanabe et al., 1983). Furthermore the coefficient of 83.36% obtained by fish fed Diet 2 was better than that reported by Wojno and Dabrowska (1984a) when rainbow trout were fed a diet containing 50% of the protein as poultry offal meal. The apparent protein digestibility also decreased with increasing inclusion levels of meat and bone meal from around 80% for fish fed Diet 6 where 30% of the protein was supplied by meat and bone meal, to only 69% for fish fed Diet 3 where 60% of the protein was supplied by this product. The best apparent protein digestibility coefficient among fish fed diets containing different proportions of these three by-products was again Diet 6.

Similarly, the highest apparent lipid digestibilities were attained by fish fed Diets 1, 2 and 7 although significantly different coefficients of around 82%, 86%, and 90% respectively were indicated (Table 8.6). Unlike the apparent protein digestibility of fish fed the control ration, the apparent lipid digestibility coefficient

of around 82% was somewhat lower than the normal range of between 89% to 97% indicated for a good quality fish meal (Smith and Rumsey, 1976; Cho and Slinger, 1979). The apparent lipid digestibility coefficient of 86% indicated in this work for fish fed Diet 2 was also lower than that of 93.58% reported by Wojno and Dabrowska (1984a) when rainbow trout were fed a diet where 50% of the fish meal protein was replaced by a poultry offal meal. As was the case with the apparent protein digestibility, the apparent lipid digestibility also decreased with increasing inclusion levels of meat and bone meal and therefore a coefficient of around 86% was indicated for fish fed Diet 6 containing 30% of the protein as meat and bone meal, while a much lower coefficient of only 69% was indicated for those fed Diet 3 where 60% of the protein was supplied by this product.

Overall apparent organic matter digestibility of fish fed the experimental diets was low and followed the same trend as the apparent protein and lipid digestibilities. A particularly low coefficient of only around 44% was indicated for fish fed Diet 3 containing the highest inclusion level of meat and bone meal. This low apparent organic matter digestibility was a consequence of the high dietary ash content of 29% indicated for this ration (Table 8.3) which resulted from the high ash content of 39.20% in the meat and bone meal. This level of ash is higher than the maximum value of 33% indicated for a meat and bone meal (Fowler and Banks, 1976; Skrede et al., 1980; McDonald et al., 1981; Tacon et al., 1984) and as a consequence the meal also had a somewhat lower crude protein content of 44% compared with the usual minimum value of 45% for this product.

The carcass composition of fish fed the experimental diets where the dietary protein was supplied by one, two or three of the by-products and the commercial ration were similar at the 95% level of significance. Only fish fed the commercial trout ration had a significantly lower ash content although it was not significantly different from the level in the initial fish sample.

The liver somatic indices of fish fed the experimental diets were similar with the exceptions of the liver somatic index of 1.58 indicated for fish fed Diet 3 containing the highest meat and bone meal inclusion level and that of those fed the commercial trout ration, Diet 7, with a significantly lower index of 1.22. No correlation between liver weight and carbohydrate levels was indicated since fish fed Diet 3 containing the highest liver somatic index had a nitrogen free extract (NFE) content of only around 16%, while those fed Diet 7 containing one of the highest NFE values of around 29% had a liver somatic index of only 1.22 (Tables 8.3 and 8.6). Thus the relatively high liver somatic index of Diet 3 may be attributable to the meat and bone meal itself although no apparent trend of increasing liver somatic index with increasing inclusion levels of this product was indicated. Tacon (1982b) reported a slight decrease in liver weights of fish fed diets containing increasing inclusion levels of meat and bone meal at levels of up to 36% of the protein while thereafter a slight increasing in the liver somatic index was indicated for those fed the diets containing the highest inclusion level (40%) of this product.

Both the haematocrit and haemoglobin contents of fish fed the experimental diets were all normal for healthy rainbow trout (Wedemeyer and Nelson, 1975; Lowe-Jinde and Niimi, 1983; Miller et al., 1983; Railo et al., 1985).

In conclusion, fish fed a diet in which half of the protein was supplied by PBHFm had a growth performance and feed utilization efficiency similar to that attained by fish fed a good quality fish meal based ration. Although both of these rations were deficient in leucine, they resulted in better growth performance than that produced by a commercial trout ration manufactured in Portugal. The commercial ration had a similar leucine deficiency which therefore indicates serious shortfalls in the formulation of the only commercial ration produced in Portugal. The growth performance and feed utilization efficiency of fish fed diets containing increasing inclusion levels of meat and bone meal decreased due to the severe essential amino acid deficiencies of this by-product which thus caused substantial reductions in the level of protein available for growth. The best growth response and feed utilization efficiency among fish fed diets containing different proportions of all three by-products was with Diet 6 containing 30% PBHFm, 30% meat and bone meal and 40% of the protein as brown fish meal. The growth performance of fish fed this diet was only slightly poorer, although not significantly, than that achieved using the commercial trout ration, and it appears that the reduced performance when compared with that of Diet 2 was a result either of a decrease in the fish meal component or an excessive level of meat and bone meal.

CHAPTER 9

FEEDSTUFFS SURVEY

9.1 INTRODUCTION

The evaluation of a potential ingredient for inclusion in fish rations should not be based on the results of the chemical analysis of a single sample (Tacon and Ferns, 1978/1979). A number of samples should be taken, ideally over a period of a year, to determine the degree of variability between batches of a product. This is particularly important when evaluating by-products since it is often the case that less attention is focused on the quality of by-products than on that of the primary product (Ørskov, 1977).

The composition of by-products may vary from one locality to another and there is frequently seasonal variation, particularly in fish products where the type and quality of fish caught may fluctuate widely (Ørskov, 1977). Therefore in order to make a full assessment of the animal by-products evaluated in the preceding growth trials, monthly samples of each of the six brown fish meals, the poultry by-product and hydrolysed feather meal (PBHFm) and the meat and bone meal were collected over a period of a year and analysed to investigate the degree of variation in chemical composition.

9.2 MATERIALS AND METHODS

1 Kilogramme of each of the following feedstuffs was collected at monthly intervals for a period of one year between March 1984 and February 1985 for the six brown fish meals, between May 1983 and April 1984 for the PBHFm, and between December 1984 and November 1985 for the meat and bone meal.

Brown fish meal A:	Olfaixe-Produtos de Óleos e Farinhas de Peixe, Ltd., Portas Fronhas, Póvoa do Varzim
Brown fish meal B:	Farinhas e Óleos de Peixe do Sul, Lda., Olhão, Algarve
Brown fish meal C:	Óleos e Farinhas de Peixe, Ltd., Sociedade Produtora, Matosinhos
Brown fish meal D:	Sociedade de Aproveitamentos de Detritos e Óleos de Peixe, Ltd., Setúbal
Brown fish meal E:	Sociedade Algarvia de Farinhas e Óleos, Lda., Olhão, Algarve
Brown fish meal F:	Sociedade Industrial de Farinhas e Óleos de Peixe, Lda., Portimão, Algarve
PBHFm:	Soaves, Pomarelho, Guimarães
Meat and bone meal:	Manuel dos Santos Moura, Lda., Porto.

The location of each of the processing plants is indicated in Fig. 9.1.

On arrival at the laboratory the samples were stored in sealed polythene bags in a deep freeze at -20°C until required for subsequent analysis. The analysis carried out is summarised in Table 4.2 and the methods are described in Chapter 4. Levels of amino acids and

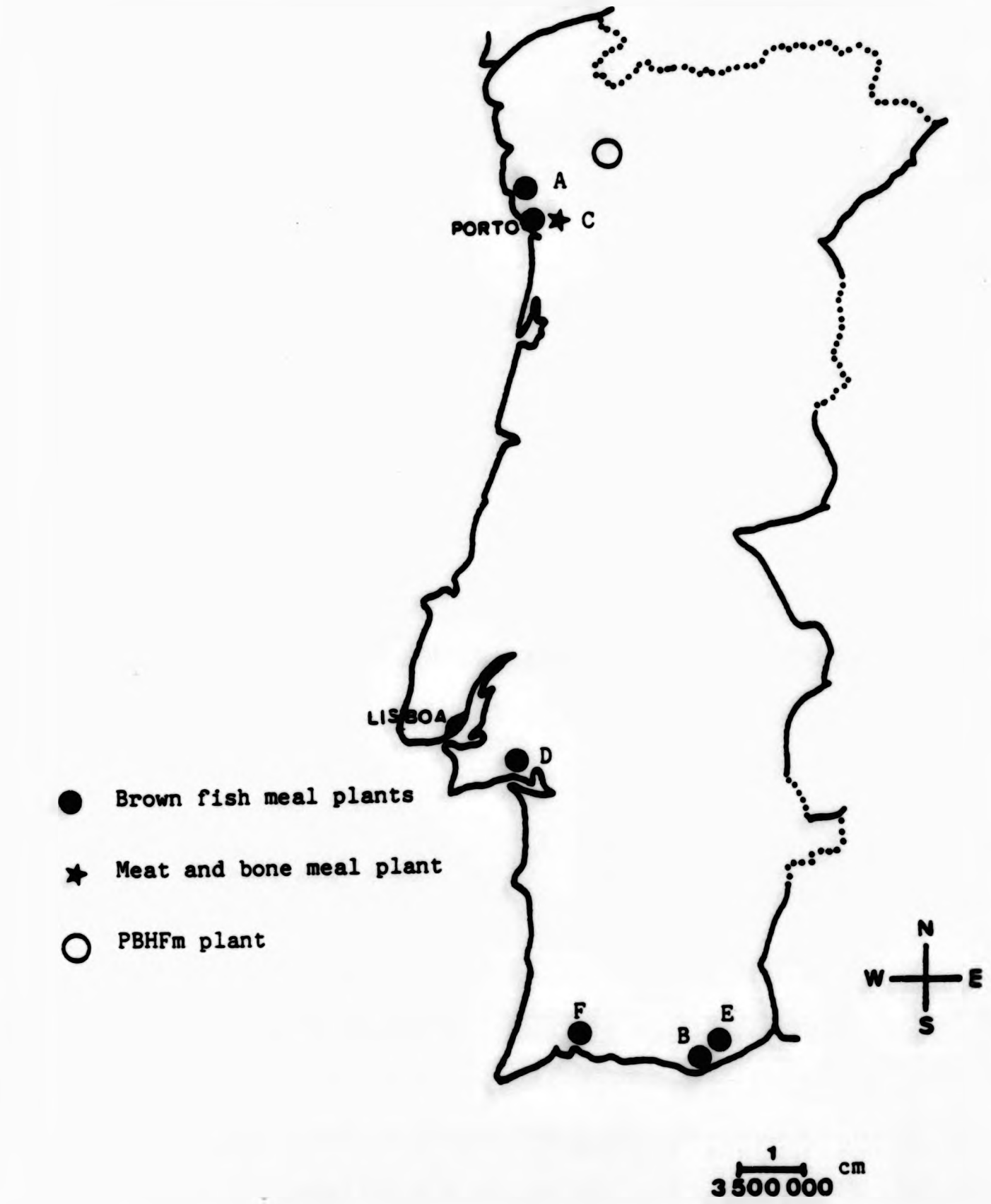

FIGURE 9.1 Location of the feedstuff processing plants in Portugal

minerals were only determined a total of four times for each by-product, on samples collected in April, July, November and January, which thus covered the four seasons of the year. In addition particle size analysis was also carried out on all samples. 100g of each sample was passed through a series of 5 Retsch sieves (4 mm, 3 mm, 2 mm, 1 mm, and 0.5 mm). The material retained in each sieve was weighed on a Mettler PC 4400 delta range balance to the nearest 0.01g and expressed as a percentage of the total sample weight.

9.3 RESULTS

9.3.1 Brown Fish Meals

No samples of brown fish meals A and F were collected in August since the fish meal processing plants closed down at this time for staff vacations.

There was a wide variation in the particle size distribution between fish meals, and in some cases from one month to another. Overall, fish meals A, D, and F had the most consistent particle distribution and in addition contained the highest proportion of particles smaller than 1 mm. Fish meals B, C, and E contained the highest proportion of particles bigger than 3 mm although the maximum level of particles which were greater than 4 mm never exceeded 3%. The particle size analysis of the six brown fish meals is given in Appendices XVII to XXII and in Figure 9.2.

There was a wide variation in the moisture content of the six brown fish meals (Tables 9.1 to 9.6; Fig. 9.3). The moisture content of fish meals D and F was particularly consistent and varied only between 7% and 10%. The remaining four fish meals exhibited a much wider variation with a minimum moisture content of around 5% to 6% while the maximum level was 14.11% in the August sample of fish meal C. This variation in moisture appears to have been random

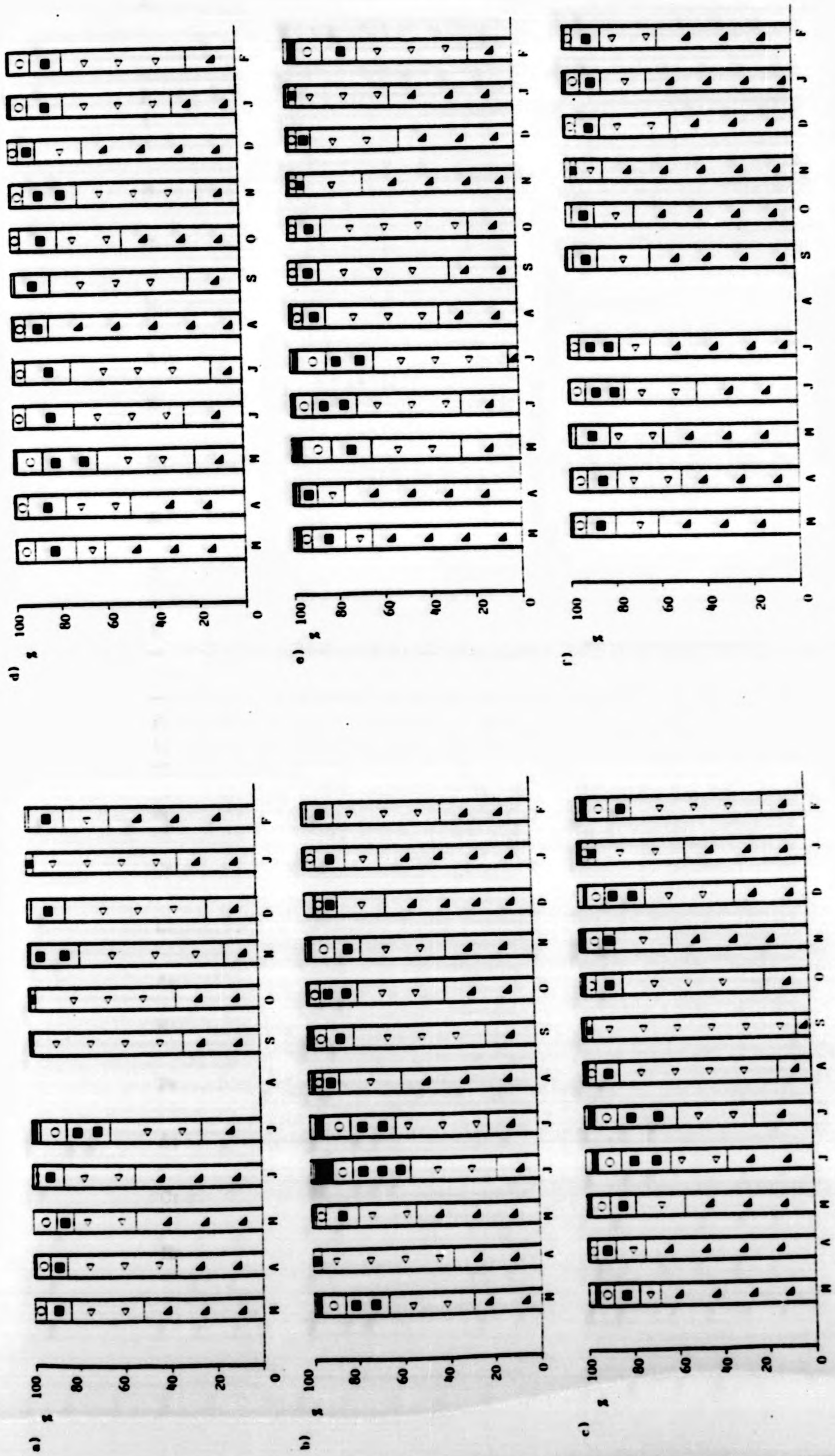

FIGURE 9.2 Monthly particle size distributions of Broom fish meals A to F between March 1984 and February 1985

> 4mm ▨ ; < 4>3mm ▨ ; < 3>2mm ▨ ; < 2>1mm ▨ ; < 1>0.5mm ■ ; <0.5mm □

TABLE 9.1 Monthly proximate composition and energy content of samples of Brown fish meal A between March 1984 and February 1984 (% dry weight)

Proximate composition (% dry weight)	MARCH	APRIL	MAY	JUNE	JULY	AUGUST
Moisture (%)	a 5.60(0.02)[2]	f 7.62(0.03)	f 7.64(0.03)	e 7.01(0.04)	g 9.86(0.12)	
Crude protein (N x 6.25)	h 63.55(0.31)	e 59.24(0.03)	efg 60.24(0.51)	a 53.36(1.61)	abc 54.06(0.43)	
Lipid (%)	bcd 7.06(0.31)	b 6.70(0.21)	f 8.66(0.15)	a 3.70(0.46)	fg 9.02(0.08)	
Ash (%)	h 23.43(0.17)	bcd 16.85(0.03)	g 21.25(0.21)	j 28.76(0.21)	k 30.85(0.28)	
Nitrogen free extract[3](%)	-	8.55	1.51	6.28	-	
Peroxide value (mEq/Kg oil)	abcde 2.48(0.23)	abcde 1.55(0.03)	abcde 0.90(0.03)	abcde 3.10(0.14)	abcd 0.60(0.21)	
Acid insoluble ash (%)	a 0.21(0.01)	cdef 0.90(0.03)	c 0.64(0.05)	g 1.36(0.16)	fg 1.14(0.03)	
Crude fibre (%)	hi 0.99(0.15)	ij 1.04(0.06)	de 0.70(0.03)	f 0.89(0.05)	k 1.15(0.06)	
Energy - ash free (Kcal/g)	a 6.27(0.18)	a 6.20(0.18)	a 6.83(0.85	a 5.94(0.43)	a 6.03(0.28)	

Proximate composition (% dry weight)	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	± S.E.[1]
Moisture (%)	c 6.63(0.01)	d 6.30(0.06)	gh 9.88(0.03)	cd 6.66(0.02)	i 10.96(0.09)	j 12.51(0.23)	0.037
Crude protein (N x 6.25)	hi 66.29(1.42)	ij 65.34(0.54)	d 55.87(0.52)	j 67.29(0.48)	ef 59.91(0.43)	ab 53.96(0.17)	0.534
Lipid (%)	j 2.00(0.04)	j 12.05(0.51)	fgh 9.33(0.82)	e 7.77(0.37)	bc 6.92(0.33)	i 9.80(0.21)	0.183
Ash (%)	b 6.59(0.11)	e 18.32(0.47)	i 25.72(0.59)	ef 18.90(1.03)	bc 16.83(0.92)	a 12.51(0.21)	0.395
Nitrogen fee extract[3](%)	-	-	-	-	4.69	10.63	
Peroxide value (mEq/Kg oil)	e 4.30(0.42)	ab 0.80(0.30)	a 0.20(0.08)	abcd 0.60(0.08)	abc 0.37(0.17)	abcde 1.51(0.08)	1.010
Acid insoluble ash (%)	cde 0.85(0.04)	b 0.59(0.03)	ab 0.36(0.02)	fg 1.15(0.03)	cde 0.85(0.03)	cd 0.75(0.36)	0.080
Crude fibre (%)	gh 0.95(0.03)	a 0.38(0.03)	bc 0.60(0.04)	fg 0.90(0.01)	d 0.69(0.03)	b 0.59(0.22)	0.015
Energy - ash free (Kcal/g)	a 6.98(0.70)	a 6.83(0.55)	a 6.25(0.28)	a 6.12(0.29)	a 6.15(0.20)	a 6.81(0.40)	0.449

1) Standard error; calculated from residual mean square in the analysis of variance
2) Standard deviation
3) Nitrogen free extract = 100-(% moisture+% crude protein+% lipid+% ash+% crude fibre)

abc...ijk Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

TABLE 9.1 Monthly proximate composition and energy content of samples of Brown fish meal A between March 1984 and February 1984 (% dry weight)

Proximate composition (% dry weight)	MARCH	APRIL	MAY	JUNE	JULY	AUGUST
Moisture (%)	a 5.60(0.02)[2]	f 7.62(0.03)	f 7.64(0.03)	e 7.01(0.04)	g 9.86(0.12)	
Crude protein (N x 6.25)	h 63.55(0.31)	e 59.24(0.03)	efg 60.24(0.51)	a 53.36(1.61)	abc 54.06(0.43)	
Lipid (%)	bcd 7.06(0.31)	b 6.70(0.21)	f 8.66(0.15)	a 3.70(0.46)	fg 9.02(0.08)	
Ash (%)	h 23.43(0.17)	bcd 16.85(0.03)	g 21.25(0.21)	j 28.76(0.21)	k 30.85(0.28)	
Nitrogen free extract[3](%)	-	8.55	1.51	6.28	-	
Peroxide value (mEq/Kg oil)	abcde 2.48(0.23)	abcde 1.55(0.03)	abcde 0.90(0.03)	abcde 3.10(0.14)	abcd 0.60(0.21)	
Acid insoluble ash (%)	a 0.21(0.01)	cdef 0.90(0.03)	c 0.64(0.05)	g 1.36(0.16)	fg 1.14(0.03)	
Crude fibre (%)	hi 0.99(0.15)	ij 1.04(0.06)	de 0.70(0.03)	f 0.89(0.05)	k 1.15(0.06)	
Energy - ash free (Kcal/g)	a 6.27(0.18)	a 6.20(0.18)	a 6.83(0.85	a 5.94(0.43)	a 6.03(0.28)	

Proximate composition (% dry weight)	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	± S.E.[1]
Moisture (%)	c 6.63(0.01)	d 6.30(0.06)	gh 9.88(0.03)	cd 6.66(0.02)	i 10.96(0.09)	j 12.51(0.23)	0.037
Crude protein (N x 6.25)	hi 66.29(1.42)	ij 65.34(0.54)	d 55.87(0.52)	j 67.29(0.48)	ef 59.91(0.43)	ab 53.96(0.17)	0.534
Lipid (%)	j 2.00(0.04)	j 12.05(0.51)	fgh 9.33(0.82)	e 7.77(0.37)	bc 6.92(0.93)	i 9.80(0.21)	0.183
Ash (%)	b 6.59(0.11)	e 18.32(0.47)	i 25.72(0.59)	ef 18.90(1.03)	bc 16.83(0.92)	a 12.51(0.21)	0.395
Nitrogen fee extract[3](%)	-	-	-	-	4.69	10.63	
Peroxide value (mEq/Kg oil)	e 4.30(0.42)	ab 0.80(0.30)	a 0.20(0.08)	abcd 0.60(0.08)	abc 0.37(0.17)	abcde 1.51(0.08)	1.010
Acid insoluble ash (%)	cde 0.85(0.04)	b 0.59(0.03)	ab 0.36(0.02)	fg 1.15(0.03)	cde 0.85(0.03)	cd 0.75(0.36)	0.080
Crude fibre (%)	gh 0.95(0.03)	a 0.38(0.03)	bc 0.60(0.04)	fg 0.90(0.01)	d 0.69(0.03)	b 0.59(0.22)	0.015
Energy - ash free (Kcal/g)	a 6.98(0.70)	a 6.83(0.55)	a 6.25(0.28)	a 6.12(0.29)	a 6.15(0.20)	a 6.81(0.40)	0.449

1) Standard error; calculated from residual mean square in the analysis of variance
2) Standard deviation
3) Nitrogen free extract = 100-(% moisture+% crude protein+% lipid+% ash+% crude fibre)
abc...ijk Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

TABLE 9.2 Monthly proximate composition and energy content of samples of Brown fish meal B between March 1984 and February 1985 (% dry weight)

Proximate composition (% dry weight)	MARCH	APRIL	MAY	JUNE	JULY	AUGUST
Moisture (%)	c 6.61(0.05)[2]	cd 6.63(0.17)	g 8.37(0.03)	i 9.50(0.26)	k 11.38(0.16)	a 4.60(0.09)
Crude protein (N x 6.25)	cd 52.07(0.82)	cdef 53.68(0.81)	cde 52.49(0.25)	b 49.71(0.25)	c 52.06(0.51)	j 59.84(0.41)
Lipid (%)	a 3.65(0.43)	hijk 12.69(0.18)	fghi 11.64(0.25)	c 7.82(0.97)	fgh 11.52(0.34)	b 4.93(0.25)
Ash (%)	efg 25.69(1.47)	a 19.05(0.83)	hi 32.29(0.26)	j 35.84(0.87)	de 24.70(0.26)	a 15.05(0.91)
Nitrogen free extract[3](%)	11.68	7.38	-	-	-	14.41
Peroxide value (mEq/Kg oil)	abcde 1.03(0.19)	abcdefgh 2.04(0.42)	abcdef 1.58(0.21)	a 0.45(0.06)	abcd 1.00(0.14)	abc 0.90(0.14)
Acid insoluble ash (%)	ab 0.43(0.15)	abc 0.45(0.21)	a 0.39(0.06)	k 3.34(0.03)	fgh 1.16(0.03)	abcdef 0.66(0.12)
Crude fibre (%)	ab 0.30(0.08)	bc 0.40(0.06)	j 2.47(0.17)	gh 0.88(0.05)	cde 0.47(0.05)	i 1.17(0.07)
Energy - ash free (Kcal/g)	abcd 5.35(0.26)	abcde 5.68(0.17)	abcde 5.58(0.14)	ab 5.21(0.52)	cde 5.91(0.37)	ab 5.21(0.25)

Proximate composition (% dry weight)	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	± S.E.[1]
Moisture (%)	e 6.82(0.11)	f 7.86(0.08)	h 9.22(0.17)	b 6.47(0.06)	g 8.37(0.21)	j 9.72(0.04)	0.041
Crude protein (N x 6.25)	gh 55.68(1.87)	ij 58.59(0.27)	fg 55.26(0.73)	gh 56.69(0.91)	i 58.39(0.81)	a 40.09(1.18)	0.569
Lipid (%)	k 13.88(0.17)	de 9.50(0.32)	d 9.36(0.05)	ghij 11.85(0.12)	def 10.50(0.30)	fg 11.09(0.17)	0.351
Ash (%)	bc 19.40(0.15)	h 28.83(0.59)	ef 25.16(0.75)	fgh 27.50(1.71)	d 22.30(0.07)	k 37.60(2.17)	0.797
Nitrogen free extract[3](%)	3.77	-	0.18	-	-	0.98	
Peroxide value (mEq/Kg oil)	l 49.60(3.39)	k 13.16(3.05)	abcdefg 1.78(0.35)	abcdefghi 2.86(0.98)	abcdefghij 5.40(1.51)	ab 0.48(0.09)	1.926
Acid insoluble ash (%)	abcd 0.46(0.09)	abcdef 0.57(0.07)	bcdefg 0.92(0.11)	fghi 1.17(0.31)	j 2.18(0.03)	abcde 0.57(0.06)	0.167
Crude fibre (%)	cd 0.45(0.04)	a 0.26(0.02)	g 0.82(0.05)	i 1.17(0.21)	j 2.47(0.09)	cdef 0.52(0.19)	0.041
Energy - ash free (Kcal/g)	e 6.23(0.37)	de 6.01(0.41)	de 6.03(0.09)	de 6.02(0.21)	abcde 5.63(0.28)	abc 5.25(0.11)	0.198

1) Standard error; calculated from residual mean square in the analysis of variance
2) Standard deviation
3) Nitrogen free extract = 100-(% moisture+% crude protein+% lipid+% ash+% crude fibre)

abc...jkl Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

TABLE 9.4 Monthly proximate composition and energy content of samples of Brown fish meal D between March 1984 and February 1985 (% dry weight)

Proximate composition (% dry weight)	MARCH	APRIL	MAY	JUNE	JULY	AUGUST
Moisture (%)	defg 9.44(0.02)[2]	cdefg 8.86(0.41)	defg 9.67(0.47)	bcdef 8.45(0.35)	bcd 8.29(0.16)	abc 7.43(0.19)
Crude protein (N x 6.25)	cdefg 59.87(0.31)	a 51.34(1.41)	bcde 58.36(1.31)	bc 56.26(0.51)	cdefg 59.83(0.99)	cdefghi 60.22(0.87)
Lipid (%)	e 8.46(0.37)	a 4.75(0.81)	abcd 5.87(0.47)	e 8.43(0.61)	ab 5.21(0.37)	e 8.27(0.41)
Ash (%)	def 24.71(0.08)	bcde 23.90(0.41)	ef 26.94(0.88)	ef 25.22(0.93)	a 19.00(0.75)	ab 20.42(0.91)
Nitrogen free extract[3](%)	-	10.19	-	0.89	6.70	2.51
Peroxide value (mEq/Kg oil)	fgh 2.04(0.12)	cdef 1.60(0.21)	a 0.60(0.07)	fghi 2.15(0.17)	k 4.16(0.19)	fg 2.02(0.15)
Acid insoluble ash (%)	a 0.20(0.08)	gh 1.63(0.11)	efg 1.40(0.02)	def 1.13(0.05)	i 2.15(0.15)	i 2.17(0.10)
Crude fibre (%)	bc 0.78(0.09)	gh 0.96(0.04)	j 1.06(0.01)	ab 0.75(0.01)	ghi 0.97(0.02)	l 1.15(0.01)
Energy - ash free (Kcal/g)	a 5.41(0.20)	a 5.16(0.11)	a 5.38(0.09)	a 5.39(0.08)	a 5.21(0.10)	a 5.43(0.11)

Proximate composition (% dry weight)	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	± S.E.[1]
Moisture (%)	a 6.58(0.09)	defg 8.89(0.03)	g 10.27(0.35)	g 9.91(0.06)	bcde 8.44(0.21)	ab 7.31(0.32)	0.418
Crude protein (N x 6.25)	k 66.28(0.86)	cdefghij 60.63(0.81)	bcdef 59.63(1.41)	k 66.86(1.32)	bcd 57.29(0.90)	ab 55.92(0.81)	1.382
Lipid (%)	abc 5.33(0.79)	e 9.22(0.43)	e 9.48(0.61)	de 7.74(0.59)	e 8.23(0.76)	e 8.98(0.34)	0.568
Ash (%)	abcd 20.91(0.47)	ef 25.80(1.05)	ef 26.58(0.53)	abc 20.45(0.44)	def 24.86(1.17)	f 28.29(1.14)	1.151
Nitrogen free extract[3](%)	0.16	-	-	-	0.30	-	
Peroxide value (mEq/Kg oil)	cdef 1.60(0.09)	ab 0.88(0.08)	abcd 1.20(0.09)	bcde 1.33(0.31)	abc 1.16(0.15)	j 3.17(0.09)	0.195
Acid insoluble ash (%)	hi 1.92(0.07)	hi 1.86(0.08)	d 0.92(0.04)	abc 0.35(0.09)	de 1.06(0.07)	ab 0.33(0.09)	0.106
Crude fibre (%)	a 0.74(0.02)	k 1.10(0.02)	g 0.94(0.01)	d 0.84(0.02)	e 0.88(0.01)	ef 0.90(0.03)	0.012
Energy - ash free (Kcal/g)	a 5.38(0.08)	a 5.63(0.03)	a 5.69(0.09)	a 5.58(0.08)	a 5.28(0.15)	a 5.39(0.10)	0.139

1) Standard error; calculated from residual mean square in the analysis of variance
2) Standard deviation
3) Nitrogen free extract = 100-(%moisture+% crude protein+% lipid+% ash+% crude fibre)

abc...jkl Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

TABLE 9.6 Monthly proximate composition and energy content of samples of Brown fish meal F between March 1984 and February 1985 (% dry weight)

Proximate composition (% dry weight)	MARCH	APRIL	MAY	JUNE	JULY	AUGUST
Moisture (%)	b 7.51(0.01)[2]	d 8.92(0.03)	e 9.26(0.06)	a 7.05(0.11)	j 10.29(0.04)	
Crude protein (N x 6.25)	efg 60.87(0.51)	efgh 61.26(0.91)	abcd 55.92(1.07)	j 68.00(0.84)	abcde 57.77(1.04)	
Lipid (%)	j 10.84(0.71)	efgh 9.44(0.09)	efhgi 9.72(0.15)	a 1.87(0.12)	bc 3.51(0.03)	
Ash (%)	gh 26.19(1.12)	b 20.44(0.41)	defg 25.04(0.47)	a 18.05(0.29)	i 28.92(0.42)	
Nitrogen free extract[3](%)	-	-	-	4.19	-	
Peroxide value (mEq/kg oil)	ab 0.49(0.01)	abcdef 1.16(0.21)	cdefghi 2.11(0.45)	ij 3.50(0.18)	k 12.80(1.71)	
Acid insoluble ash (%)	j 1.47(0.12)	gh 1.16(0.06)	k 2.15(0.09)	abcdef 0.97(0.10)	abc 0.91(0.02)	
Crude fibre (%)	a 0.59(0.12)	bcdefg 0.92(0.07)	bcde 0.86(0.11)	bcd 0.84(0.05)	ab 0.73(0.03)	
Energy - ash free (Kcal/g)	a 5.69(0.32)	a 5.70(0.15)	a 5.48(0.19)	a 5.12(0.35)	a 5.02(0.21)	

Proximate composition (% dry weight)	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	± S.E.[1]
Moisture (%)	g 9.77(0.06)	gh 9.82(0.05)	c 7.94(0.21)	ef 9.32(0.07)	ghi 9.92(0.05)	ghij 10.04(0.09)	0.088
Crude protein (N x 6.25)	ab 55.29(0.81)	a 54.92(0.51)	ghi 62.45(0.79)	abcdef 57.92(0.89)	abcdef 57.92(1.10)	abc 55.84(0.71)	1.214
Lipid (%)	bcd 3.52(0.11)	b 3.10(0.07)	efg 9.33(0.09)	e 8.95(0.10)	ef 9.22(0.31)	fghij 10.11(0.17)	0.298
Ash (%)	i 28.77(0.35)	ghi 27.04(0.47)	cdef 23.32(0.81)	cde 23.17(0.75)	bc 22.11(0.75)	cde 23.17(0.19)	0.604
Nitrogen free extract[3](%)	1.69	3.96	-	-	-	0.01	
Peroxide value (mEq/Kg oil)	abcdefgh 1.19(0.03)	abcde 1.01(0.19)	abc 0.63(0.06)	abcdefg 1.18(0.51)	abc 0.61(0.31)	a 0.47(0.27)	0.436
Acid insoluble ash (%)	fg 1.13(0.01)	ab 0.87(0.04)	ghi 1.17(0.05)	abcd 0.92(0.05)	abcde 0.93(0.06)	a 0.85(0.07)	0.052
Crude fibre (%)	cdefgh 0.96(0.04)	i 1.16(0.08)	i 1.20(0.05)	bcdef 0.88(0.07)	cdefghi 1.02(0.07)	bc 0.83(0.03)	0.056
Energy - ash free (Kcal/g)	a 5.08(0.10)	a 5.03(0.09)	a 5.65(0.10)	a 5.41(0.31)	a 5.57(0.31)	a 5.58(0.27)	0.199

1) Standard error; calculated from residual mean square in the analysis of variance
2) Standard deviation
3) Nitrogen free extract = 100-(% moisture+% crude protein+% lipid+% ash+% crude fibre)

abc...ijk Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

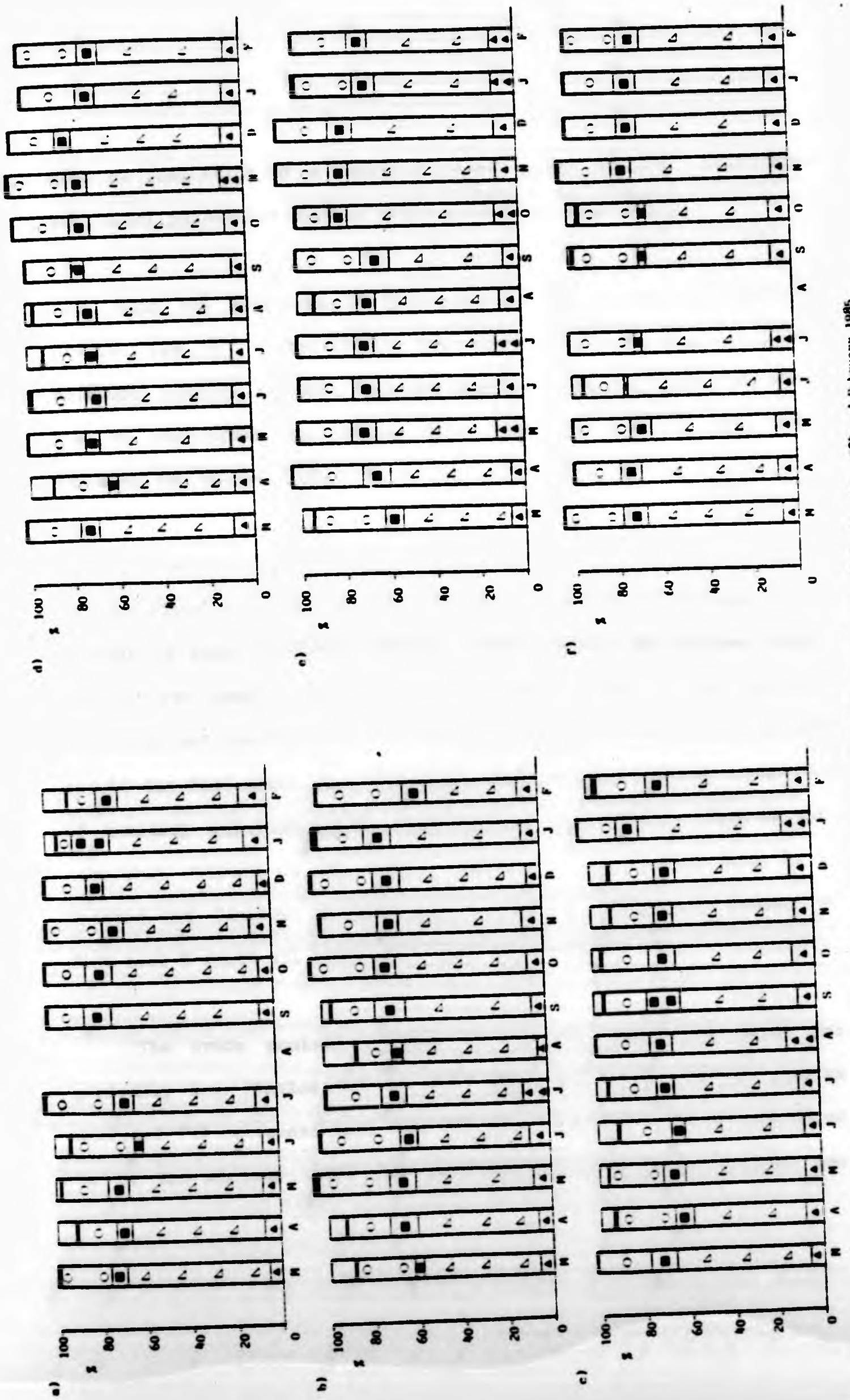

FIGURE 9.3 Monthly proximate composition of Brown Fish meals between March 1984 and February 1985
% moisture ☐ ; % crude protein ▨ ; % lipid ▩ ; % ash ▨ ; % crude fibre ■ ;

with no indication of a seasonal trend and no apparent correlation with other parameters such as lipid content was indicated.

With the exception of fish meals C and E where four and six of the samples, respectively, had moisture levels above 10%, the moisture content of most samples varied between 5% and 10% which can be considered the normal range for good quality fish meals (Windsor and Barlow, 1981).

Of the 70 samples of fish meals analysed only six had a moisture content close to or higher than 14% where the risk of spoilage during storage is high (Cockerell et al., 1971; Windsor and Barlow, 1981). Some of the samples however contained high levels of both moisture and lipid and consequently the risk of spoilage would be even greater should the fish meals be stored under adverse environmental conditions of humidity and temperature (Cockerell et al., 1971). This is particularly evident in the samples taken in July of fish meal B, in October and January of fish meal C, and in March and January of fish meal E which all contained lipid levels above 10%.

The crude protein content of the six brown fish meals was generally low (Tables 9.1 to 9.6; Fig. 9.3). Of the 70 samples analysed 74% contained less than 60% crude protein which is considered to be the minimum level for good quality fish meals (Windsor and

Barlow, 1981). This was particularly the case for fish meal B which had a crude protein content varying between only 40.09% and 59.84%. The highest crude protein content recorded was 68.00% which is still well below the crude protein content of 79% for a very good quality fish meal (Göhl, 1981). With the exception of two samples of both meals, the crude protein content of fish meals B and C were consistently low, and although levels in the other four meals were generally higher, the variation from month to month tended to be greater. No seasonal trend in crude protein was apparent.

Amino acids were calculated as a percentage of the protein to remove variation caused by differing levels of crude protein (Table 9.7). With few exceptions the samples of all six brown fish meals were to a greater or lesser extent deficient in arginine, leucine, lysine, phenylalanine, and valine. Good quality fish meals usually contain around 6% arginine, 7% leucine, 7% lysine, 4% phenylalanine, and 5% valine (Windsor and Barlow, 1981). The fish meals were particularly deficient in leucine and phenylalanine. Thus leucine supplied less than half of the requirement for rainbow trout in 44% of the samples and phenylalanine supplied less than half of the requirement in 56% of the samples, although phenylalanine can be spared by tyrosine.

Based on the four samples analysed, no seasonal trend in essential amino acid content was apparent although wide variations in the levels of essential amino acids between samples frequently occurred despite variations in protein content having been taken into account. For example, the level of isoleucine in the July

TABLE 9.7 Amino acid profile of feedstuffs in April (A), July (Ju), November (N) and January (Ja)

Amino acid (% of protein)	Brown fish meal A				Brown fish meal B				Brown fish meal C				Brown fish meal D				Requirement of rainbow trout (% protein)
	A	Ju	N	Ja	A	Ju	N	Ja	A	Ju	N	Ja	A	Ju	N	Ja	
Arginine	3.63	2.52	2.88	3.37	2.96	2.92	3.31	3.12	2.55	2.48	3.43	2.90	2.94	2.82	3.40	3.18	3.50, 40% (Ogino, 1980)
Histidine	4.94	4.97	4.96	4.61	4.97	4.38	5.85	5.39	6.25	4.92	5.91	8.58	5.76	5.13	5.42	5.04	1.60, 40% (Ogino, 1980)
Isoleucine	6.60	5.62	9.86	6.24	6.44	6.65	6.68	7.19	6.32	7.67	7.68	6.45	6.39	6.74	7.06	7.33	2.40, 40% (Ogino, 1980)
Leucine	3.93	4.24	3.56	2.40	1.79	1.84	4.20	2.67	1.86	3.72	3.11	2.71	1.91	2.26	3.27	2.48	4.40, 40% (Ogino, 1980)
Lysine	7.78	5.42	4.62	3.37	3.05	4.13	9.86	5.62	3.95	4.92	4.61	6.45	4.71	1.19	4.24	5.38	5.30, 40% (Ogino, 1980)
Methionine	4.12	2.81	3.22	4.41	2.50	3.78	3.58	3.49	3.02	2.80	3.06	3.98	2.45	4.51	3.76	3.25	1.60-2.10, 35% (Rumsey et al. 1983)
Phenylalanine	1.60	1.00	2.24	1.99	1.55	1.36	2.32	1.69	1.42	1.36	2.19	1.72	1.48	2.19	2.20	1.82	3.10, 40% (Ogino, 1980)
Threonine	5.60	3.59	6.35	4.69	4.56	4.72	5.30	5.03	5.90	5.77	5.95	5.56	4.84	4.28	5.75	5.67	3.40, 40% (Ogino, 1980)
Valine	1.86	2.00	3.10	2.97	2.51	2.84	3.58	2.88	2.65	2.03	3.46	3.18	2.38	2.66	3.56	2.90	3.10, 40% (Ogino, 1980)
Alanine	1.97	0.72	1.34	0.93	0.97	0.79	1.28	1.64	1.03	0.66	0.92	1.35	1.15	1.67	0.96	1.57	
Aspartic acid	8.74	8.45	8.38	8.39	8.42	8.18	8.03	8.77	9.85	7.54	8.99	7.75	8.06	7.00	8.35	9.18	
Cystine	1.77	1.16	1.27	1.28	1.34	1.38	1.43	1.44	1.18	1.26	1.53	1.63	1.09	1.67	1.34	1.13	0.86, 35% (Rumsey et al. 1983)
Glutamic acid	7.19	9.54	8.50	9.73	8.79	9.57	8.07	9.85	9.46	7.42	9.05	9.05	8.88	9.19	8.64	10.87	
Glycine	7.19	12.17	12.17	14.29	14.68	14.23	11.53	15.29	15.86	14.60	15.63	15.99	15.17	14.21	15.34	15.78	
Proline	10.13	11.17	9.77	14.54	14.14	12.01	9.79	12.24	12.44	10.60	9.97	11.42	14.62	11.16	9.79	13.27	
Serine	6.08	7.01	5.69	6.94	6.67	5.15	6.80	5.93	4.30	6.34	6.95	6.03	6.54	5.58	6.79	6.82	
Tyrosine	2.78	1.87	2.51	2.10	1.92	2.15	2.88	2.29	2.15	1.74	3.08	2.08	2.03	2.24	2.85	3.05	2.10, 40% (Ogino, 1980)
TOTAL	85.91	84.26	90.42	92.25	87.26	86.08	94.49	94.53	90.19	85.82	95.52	96.83	90.40	84.50	92.72	98.72	
% of meal	50.89	45.55	50.52	55.27	46.84	44.81	52.22	55.20	45.67	47.78	51.84	61.82	46.41	50.55	55.29	56.56	

* Percentage of crude protein in the diet

TABLE 9.7 (cont'd)

Amino acid (% of protein)	Brown fish meal E				Brown fish meal F				Poultry by-product and hydrolysed feather meal				Meat and bone meal				Requirement of rainbow trout (% protein)*
	A	Ju	N	Ja	A	Ju	N	Ja	A	Ju	N	Ja	A	Ju	N	Ja	
Arginine	2.63	3.19	6.25	2.72	2.92	2.94	3.47	3.35	2.92	2.68	3.12	2.75	3.25	4.06	4.14	4.19	3.50, 40% (Ogino, 1980)
Histidine	4.11	4.94	5.09	5.20	4.02	4.85	5.16	5.63	4.17	1.95	3.13	3.93	4.30	4.58	3.35	4.44	1.60, 40% (Ogino, 1980)
Isoleucine	5.76	0.67	7.22	6.16	5.40	6.06	6.63	7.72	7.07	5.35	6.58	6.67	5.92	6.30	6.55	6.10	2.40, 40% (Ogino, 1980)
Leucine	2.15	2.64	2.87	1.12	1.35	2.25	2.64	2.02	2.23	1.78	1.74	2.11	2.18	2.35	1.42	2.24	4.40, 40% (Ogino, 1980)
Lysine	6.08	4.59	4.51	2.30	5.71	5.63	4.16	6.94	5.34	10.01	5.72	5.03	6.57	6.01	7.45	6.75	5.30, 40% (Ogino, 1980)
Methionine	2.76	3.54	3.85	2.25	2.69	3.13	1.04	4.68	3.04	3.73	4.20	2.87	2.61	2.75	2.17	2.69	1.60-2.10, 35% (Rumsey et al. 1983)
Phenylalanine	1.22	1.61	2.52	1.45	1.40	1.40	5.94	1.67	1.43	1.37	1.57	1.35	1.94	1.48	0.94	1.99	3.10, 40% (Ogino, 1980)
Threonine	4.93	5.43	3.32	2.07	2.28	3.01	3.04	3.80	3.13	1.13	1.24	2.94	2.22	1.75	1.07	2.29	3.40, 40% (Ogino, 1980)
Valine	2.37	2.55	1.30	0.82	1.08	1.23	1.18	1.12	2.09	4.04	3.43	1.97	1.37	0.83	1.80	1.40	3.10, 40% (Ogino, 1980)
Alanine	0.87	1.62	7.37	7.90	8.54	9.14	6.74	9.56	7.60	7.17	6.14	7.15	7.82	4.06	7.06	8.07	
Aspartic acid	7.49	8.24	1.51	1.30	1.01	1.38	1.39	1.73	0.72	1.84	1.47	0.67	1.43	1.48	0.24	0.38	
Cystine	1.09	1.40	7.89	7.71	7.70	8.54	7.24	10.20	9.08	7.51	7.17	8.57	9.21	8.65	9.44	9.50	0.86, 35% (Rumsey et al. 1983)
Glutamic acid	7.30	8.59	13.61	14.27	13.78	16.53	12.47	13.78	14.26	11.98	10.90	13.45	17.96	18.53	18.54	17.68	
Glycine	13.42	14.07	10.46	11.92	13.32	13.35	9.58	11.10	12.94	11.18	10.93	12.20	17.07	19.34	23.40	16.77	
Proline	12.59	11.19	7.02	7.90	5.40	5.33	6.42	6.92	6.63	12.55	10.20	6.24	6.57	7.74	5.88	6.75	
Serine	5.30	5.41	5.89	4.06	4.03	4.67	5.40	5.63	5.18	5.68	5.18	4.88	4.65	4.85	3.73	4.81	
Tyrosine	2.09	0.22	2.84	1.99	2.01	2.54	2.61	2.85	2.23	1.84	2.73	2.11	2.24	2.49	1.72	2.31	2.10, 40% (Ogino, 1980)
TOTAL	82.16	79.90	93.52	81.14	82.64	91.98	85.11	98.70	90.06	91.79	85.45	84.89	97.31	97.25	98.90	98.36	
% of meal	44.33	43.83	61.96	42.40	50.63	53.14	53.15	57.17	50.96	56.84	46.35	50.31	48.16	50.48	46.08	46.47	

* Percentage of crude protein in the diet

sample of fish meal E was only 0.67%, compared with levels of between 5.76% and 7.22% for the remaining three samples. Methionine levels generally varied between 2.5% and 4.5% of the protein and were thus adequate to supply the requirement of rainbow trout for this essential amino acid. However, the methionine content of the November sample of fish meal F was only 1.04%, and as a consequence the use of this fish meal as the sole source of dietary protein would have resulted in a diet containing only around 65% of the dietary requirement for rainbow trout.

Wide variations were also found in levels of most of the non-essential amino acids although again no seasonal trend was apparent (Table 9.7). This was particularly the case in the alanine content of fish meal E which varied between 0.87% and 7.90%. In comparison with the other five fish meals, fish meal F contained consistently high levels of alanine and cystine, but much lower levels of aspartic acid and proline.

In each of the samples of the six brown fish meals analysed the sum of the amino acids was between 0.73% and 11.03% less than the crude protein content. Thus, allowing a maximum level of 2% for nonprotein nitrogen (Jobling, 1983) up to 9% of the amino acids were not accounted for.

The lipid content of 88% of the samples was within the normal range of reported values of between 8% and 12% (Windsor and Barlow, 1981). Two samples taken in September of fish meals B and C had particularly high lipid levels of 13.88% and 15.95% respectively,

and levels as low as 1.87% were also recorded (Tables 9.1 to 9.6; Fig. 9.3). Fish meals D and E exhibited the narrowest range of variation in lipid levels, whereas the greatest variation was in fish meals A and B. No seasonal trend in lipid content was indicated and no correlation with other parameters such as moisture content was apparent.

12 of the fish meal samples had peroxide values above the maximum recommended level of 4 mEq/Kg oil (Billinski et al., 1978). Furthermore, in conjunction with these high peroxide values, half of these samples contained more than 10% lipid. The September samples of fish meals B and E contained around 13% lipid and had particularly high peroxide values of 49.60 and 31.00 mEq/Kg oil respectively. The July sample of fish meal F also had a high peroxide value of 12.80 mEq/Kg oil, but in this case it only contained 3.51% lipid.

The ash content of the fish meals was generally high with more than 70% of samples having an ash content higher than the usual maximum level of 21% for good quality fish meals (Windsor and Barlow, 1981). The greatest variation was in the ash content of fish meals A and B with levels varying between 6.59% and 30.85% in the former and 15.05% and 37.60% in the latter (Tables 9.1 and 9.2; Fig. 9.3a,b). Fish meal C had the most consistent ash content with levels varying between only 18.56% and 25.71%. Although no seasonal trend in ash content was apparent, a negative correlation between ash content and crude protein content was indicated. This relationship was particularly evident in the sample of fish meal B taken in February which contained the highest ash content (37.60%) but also had the

lowest level of crude protein (40.09%), while the October sample of fish meal E only contained 14.81% ash and as a consequence had one of the highest crude protein contents of 66.29%. There was a general trend for the samples which had the highest ash content to have a high proportion of large particles.

Almost half of the samples contained some NFE, and in six samples the calculated values exceeded the usual maximum level of around 8% (Cullison, 1979; Göhl, 1981), particularly in the August sample of fish meal B with a NFE of 14.41% (Tables 9.1 to 9.6; Fig. 9.3). Nine of the samples of fish meal C contained NFE with levels ranging from 3% to 9%, whereas NFE was only present in three of the samples of fish meal A.

With very few exceptions all six fish meals contained adequate levels of the six minerals analysed to satisfy the dietary requirements of rainbow trout. Again, no definite seasonal trend in mineral levels was indicated although the April samples of all six meals generally contained the highest levels of Ca, K, and Zn (Table 9.8). There were however wide variations between samples in the levels of certain minerals. Furthermore, although the overall high ash content of the fish meals did result in high levels of certain minerals, in particular Ca, the relationship between ash content and levels of minerals was not always consistent. For example, the January sample of fish meal A contained only 5% Ca, compared with 10.75% in the April sample despite both samples containing around 16% ash. Ca levels were between one and three times higher than the usual range of values of between 1.95% and 3.95% for good

TABLE 9.8 Concentration of mineral elements of feedstuffs in April (A), July (Ju), November (N) and January (Ja)

Elements	Brown fish meal A				Brown fish meal B				Brown fish meal C				Brown fish meal D			
	A	Ju	N	Ja	A	Ju	N	Ja	A	Ju	N	Ja	A	Ju	N	Ja
Ca (g/100g)	10.75	8.49	6.35	5.18	7.66	5.06	4.48	5.89	6.71	5.53	5.70	5.18	7.57	5.18	8.07	9.17
Mg (g/100g)	0.24	0.25	0.05	0.15	0.27	0.15	0.18	0.19	0.22	0.15	0.13	0.13	0.21	0.13	0.18	0.23
K (g/100g)	1.26	0.43	0.52	0.57	2.30	0.32	0.22	0.27	1.29	1.83	1.37	1.97	1.43	0.20	0.24	0.43
Na (g/100g)	2.16	2.57	1.23	1.39	6.10	5.11	10.56	7.16	2.16	2.28	2.00	1.77	3.01	1.77	2.20	2.23
P (g/100g)	1.13	1.17	1.21	1.19	1.21	1.25	1.18	1.23	1.05	1.10	1.15	1.20	1.10	1.15	1.19	1.17
Zn (mg/100g)	18.40	18.04	20.46	6.11	31.27	10.57	11.50	10.46	18.65	16.49	14.89	12.08	22.13	12.08	12.98	19.13

Elements	Brown fish meal E				Brown fish meal F				Poultry by-product and bydrolysed feather meal				Meat and bone meal			
	A	Ju	N	Ja	A	Ju	N	Ja	A	Ju	N	Ja	A	Ju	N	Ja
Ca (g/100g)	8.86	7.54	7.14	6.82	8.35	9.81	7.11	7.16	1.66	1.28	3.05	5.89	13.46	13.42	13.20	3.53
Mg (g/100g)	0.24	0.23	0.21	0.21	0.23	0.25	0.22	0.22	0.06	0.05	0.09	0.15	0.16	0.17	0.16	0.09
K (g/100g)	1.45	0.17	0.22	0.20	2.12	0.17	0.19	0.19	0.29	0.19	0.38	3.47	1.12	1.84	1.50	3.34
Na (g/100g)	4.85	3.05	2.47	2.87	1.99	2.07	2.39	2.24	0.70	0.49	1.09	1.28	1.31	1.31	1.43	1.05
P (g/100g)	1.35	1.21	1.33	1.24	1.21	1.18	1.17	1.24	1.41	1.27	1.33	1.39	0.97	1.05	1.10	0.98
Zn (mg/100g)	17.38	17.63	18.42	16.96	18.18	17.69	15.13	14.12	13.07	13.13	19.93	19.44	5.40	5.11	5.99	10.58

quality fish meals (Windsor and Barlow, 1981). Levels of Na were also much higher than the reported values of 0.42% and 0.87% for Peruvian anchovy and herring meals respectively, with fish meal B containing particularly high levels of 5% to 11% Na. Fish meals A, B, and D exhibited particularly wide fluctuations in levels of Zn, although levels were in general higher than the usual range of 11.1 mg to 12.0 mg per 100g for good quality fish meals (Windsor and Barlow, 1981). Levels of Mg showed the least variation between samples and in almost all cases values were in the normal range for fish meals. With the exception of the April sample in five of the fish meals which were consistently high, there was little variation in levels of K between samples. In almost all cases however, values were below the levels of between 0.65% and 1.20% recorded in good quality fish meals (Windsor and Barlow, 1981), with the exception of fish meal C with higher K levels which varied between 1.29% and 1.97%.

Levels of P showed no wide variations and were fairly consistent between fish meals varying between 1.05% and 1.35%. In all cases P levels were below the usual range of 1.50% and 2.60% indicated for good quality fish meals (Windsor and Barlow, 1981).

Acid insoluble ash and crude fibre were in all six fish meals analysed very low and only in four and two samples respectively, were they slightly higher than 2% (Tables 9.1 to 9.6). With very few exceptions there were no great variations in the energy content of individual fish meals from one month to another (Tables 9.1 to 9.6).

9.3.2 Poultry By-product and Hydrolysed Feather Meal (PBHFm)

No sample was collected in August since the processing plant closed down at this time for staff vacations.

At least 80% of the particles in all samples were less than 2 mm, with most particles falling into the size range 0.5 mm to 2 mm. All samples contained some particles which were greater than 3 mm, although the proportion which did so never exceeded 4% (Appendix XXIII; Fig. 9.4a).

The moisture content of the samples varied significantly ($P < 0.05$) between 8.24% and 17.12% (Table 9.9; Fig. 9.4b). The sample taken in July had an exceptionally high moisture content of 17.12% and was therefore most likely to undergo spoilage during storage (Cockerell et al., 1971; Windsor and Barlow, 1981). Samples taken in May, November, December, and February also had moisture levels which were higher than the normal range of reported values of 3% to 10% for poultry by-products (Jackson and Fulton, 1971; Burgos et al., 1974; Bhargava and O'Neil, 1975; Göhl, 1981; Bielorai et al., 1983).

In general the variation in the crude protein content from month to month was small (Table 9.9; Fig. 9.4b). However, the crude protein content of the sample taken in June (66.61%) was exceptionally high while the November sample contained only 54.24% crude protein. Nevertheless, with one exception, all values were within the normal range of reported values for poultry by-products of between 56%

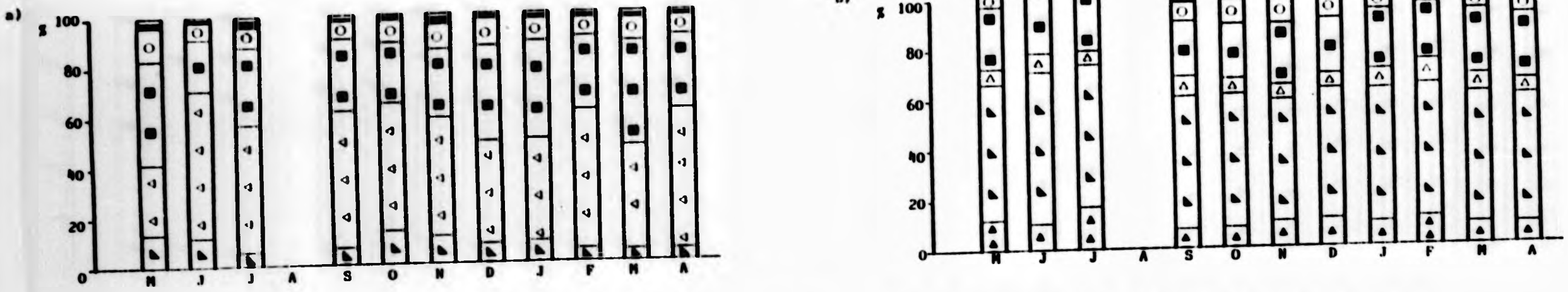

FIGURE 9.4 Monthly particle size distribution (a) and proximate composition (b) of Poultry by-product and hydrolysed feather meal between March 1983 and April 1984

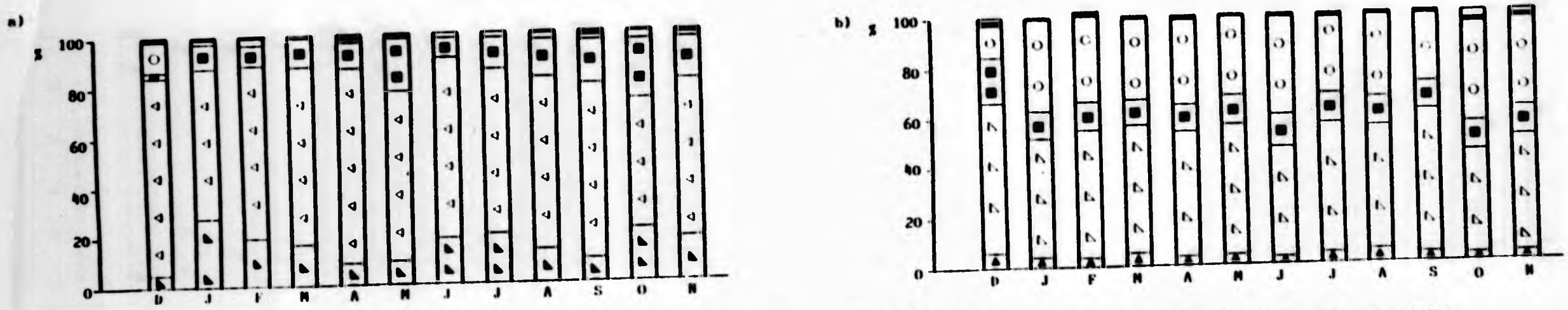

FIGURE 9.5 Monthly particle size distribution (a) and proximate composition (b) of Meat and bone meal between December 1984 and November 1985

> 4mm ; < 4> 3mm ; < 3> 2mm ; < 2 > 1mm ; <1> 0.5mm ; < 0.5mm

% moisture ; % crude protein ; % true protein ; % NPN ; % lipid ; % ash ; % crude fibre ; % NFE

TABLE 9.9 Monthly proximate composition and energy content of samples of Poultry by-product and hydrolysed feather meal between May 1983 and April 1984 (% dry weight)

Proximate composition (% dry weight)	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER
Moisture (%)	h 12.31(0.05)[2]	cf 10.65(0.10)	j 17.12(0.11)		a 7.82(0.08)	b 8.24(0.10)
Crude protein (N x 6.25)	defg 59.24(0.50)	k 66.61(0.67)	j 61.92(0.71)		def 58.33(0.08)	cde 58.24(0.15)
True protein (%) (Lowry method)	defghi 53.75(0.50)	k 60.78(0.47)	j 56.79(0.42)		cdefg 53.12(0.75)	cdef 53.05(0.22)
Nonprotein nitrogen (%) (TCA method)	cde 6.59(0.22)	fg 7.18(0.13)	ab 5.76(0.51)		fgh 7.54(0.29)	cd 6.55(0.32)
Lipid (%)	gh 25.18(0.35)	d 23.59(0.30)	efg 24.61(0.37)		abc 22.61(0.07)	a 21.91(0.21)
Ash (%)	abc 5.66(0.21)	abcd 6.07(0.27)	a 5.21(0.19)		ef 7.49(0.18)	hij 10.12(0.47)
Nitrogen free extract[3](%)	-	-	-		0.54	-
Peroxide value (mEq/Kg oil)	k 2.30(0.05)	cde 0.80(0.10)	a 0.10(0.01)		ab 0.25(0.07)	bc 0.52(0.02)
Acid insoluble ash (%)	j 4.07(0.04)	bc 0.26(0.02)	d 0.42(0.03)		b 0.23(0.01)	i 1.02(0.07)
Crude fibre (%)	hi 1.22(0.05)	k 1.46(0.08)	hij 1.25(0.01)		cd 0.88(0.02)	h 1.16(0.03)
Energy - ash free (Kcal/g)	a 5.99(0.10)	a 6.08(0.08)	a 6.15(0.09)		a 6.21(0.15)	a 6.01(0.49)

Proximate composition (% dry weight)	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	APRIL	± S.E.[1]
Moisture (%)	ef 10.16(0.11)	h 11.22(0.19)	e 9.24(0.06)	g 11.16(0.05)	cd 8.68(0.07)	bc 8.40(0.03)	0.090
Crude protein (N x 6.25)	a 54.24(0.25)	b 56.26(0.71)	defgh 59.26(0.35)	ghi 60.16(0.21)	bcd 57.64(0.41)	defgh 56.59(0.21)	0.486
True protein (%) (Lowry method)	a 48.79(0.42)	bcd 52.05(0.77)	cdefgh 53.50(0.75)	cde 52.90(0.32)	bc 51.81(0.46)	b 50.89(0.33)	0.547
Nonprotein nitrogen (%) (TCA method)	ab 5.76(0.51)	a 5.64(0.59)	fg 7.53(0.32)	i 9.73(0.35)	def 7.30(0.35)	abc 6.28(0.03)	0.258
Lipid (%)	g 24.83(0.43)	ab 22.17(0.17)	def 23.91(0.21)	de 23.80(0.15)	ghi 26.12(0.18)	j 26.71(0.12)	0.238
Ash (%)	hi 9.82(0.25)	h 9.36(0.34)	efg 7.56(0.25)	k 11.26(0.15)	ab 5.62(0.35)	de 6.96(0.45)	0.307
Nitrogen free extract[3](%)	-	-	-	-	-	-	
Peroxide value (mEq/Kg oil)	cdefg 0.85(0.07)	efgh 1.11(0.31)	cdef 0.82(0.06)	j 1.87(0.21)	bcd 0.61(0.08)	hi 1.30(0.04)	0.122
Acid insoluble ash (%)	gh 0.92(0.03)	a 0.16(0.01)	bc 0.26(0.01)	def 0.45(0.01)	g 0.87(0.05)	de 0.44(0.05)	0.021
Crude fibre (%)	ab 0.76(0.01)	abc 0.83(0.03)	cde 0.92(0.01)	a 0.71(0.51)	efg 1.03(0.03)	ef 1.01(0.04)	0.034
Energy - ash free (Kcal/g)	a 5.94(0.17)	a 5.83(0.13)	a 6.01(0.11)	a 6.03(0.33)	a 6.02(0.12)	a 5.98(0.07)	0.198

1) Standard error; calculated from residual mean square in the analysis of variance
2) Standard deviation
3) Nitrogen free extract = 100-(% moisture+% true protein+% nonprotein nitrogen+% lipid+% ash+% crude fibre)

abc...ijk Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

and 76% (see Chapter 2). The variation in crude protein content appears to have been random with no indication of seasonal trend.

The true protein content derived from the Lowry method varied between 48.79% and 60.78% (Table 9.9; Fig. 9.4b). Two slightly different ranges of values were obtained for the nonprotein nitrogen component. Values calculated by difference using the Lowry method varied between 4.21% and 7.26% whereas the direct approach of precipitating nonprotein nitrogen using trichloroacetic acid gave values of between 5.64% and 9.73% (Table 9.9; Fig. 9.4b). This discrepancy in results was possibly due to the use of 6.25 as the Kjeldahl factor which, as indicated in Chapter 5, may not have been appropriate as it has been reported that the nitrogen content of protein from different sources is not the same (Jobling, 1983) and may vary between 12% and 19% (Tacon, 1979a).

The amino acid profile shows that the PBHFm samples were good sources of the nonessential amino acids glycine and proline and of the essential amino acids histidine and methionine. All samples were significantly deficient in leucine, phenylalanine, and threonine (Table 9.7) compared with requirements for rainbow trout of 4.4%, 3.1%, and 3.4% of the protein respectively (Ogino, 1980). Valine was also deficient in the April and January samples.

Although variation in amino acids caused by varying levels of crude protein were taken into account, there was still a certain degree of variability in the levels of many amino acids. This was particularly evident in the essential amino acids histidine,

and lysine which varied between 1.95% and 4.17% and between 5.03% and 10.01% respectively (Table 9.7).

With the exception of the April sample the sum of the amino acids analysed was between 2.44% and 5.08% less than the true protein levels which represents the percentage attributable to unaccounted amino acids such as tryptophan.

Lipid levels were within the normal range for poultry by-products (Jackson and Fulton, 1971; Burgos et al., 1974; Bhargava and O'Neil, 1975; Göhl, 1981; Bielorai et al., 1983) and although there were significant ($P < 0.05$) differences, the variation from month to month was not great with levels varying between 21.91% and 26.71% (Table 9.9; Fig. 9.4b). Peroxide values of the samples were all low with none exceeding 2.3 mEq/Kg oil (Table 9.9).

Although the ash content varied significantly ($P < 0.05$) between 5.21% and 11.26% (Table 9.9; Fig. 94b) these values are in the lower range of reported values of 6.84% to 20.6% for poultry by-products (Jackson and Fulton, 1971; Burgos et al., 1974; Bhargava and O'Neil, 1975; Göhl, 1981; Bielorai et al., 1983).

None of the samples contained NFE with the exception of the September sample which had a calculated level of only 0.54% (Table 9.9; Fig. 9.4b).

With the exception of Mg, in almost all samples levels of the remaining five minerals analysed were adequate to satisfy

the requirements of rainbow trout at high inclusion levels of PBHFm. Levels of minerals, and in particular of Ca and K (Table 9.8), tended to be highest in the January samples, although this did not coincide with a particularly high ash content.

Acid insoluble ash and crude fibre were low, not exceeding 2% (Table 9.9). The energy content of the samples varied, although not significantly ($P < 0.05$) between 5.94 and 6.21 Kcalories per gramme dry weight (Table 9.9).

9.3.3 Meat and Bone Meal

With the exception of the sample taken in December at least 96% of the particles were smaller than 2 mm, and at least 83% were consistently less than 1 mm. The percentage of particles bigger than 3 mm never exceeded 1.5% (Appendix XXIV; Fig. 9.5a).

Although there were statistically significant ($P < 0.05$) differences between samples, the moisture content was consistently low with a maximum level of only 6.10% (Table 9.10; Fig. 9.5b).

With the exception of June when the crude protein content was of only 43.58%, there was very little variation in protein levels of around 50% between January and August (Table 9.10). Thereafter the crude protein content fluctuated widely, with levels of 41.09% and 60.05% (Table 9.10; Fig. 9.5b) which are both outside the range of values of 45% to 55% stipulated by the Fertilizers and Feeding Stuff Regulations (HMSO, 1973) for a meat and bone meal. Compared

TABLE 9.10 Monthly proximate composition and energy content of samples of Meat and bone meal between December 1984 and November 1985 (% dry weight)

Proximate composition (% dry weight)	DECEMBER	JANUARY	FEBRUARY	MARCH	APRIL	MAY
Moisture (%)	k 6.10(0.07)[2]	defgh 4.17(0.21)	defg 3.96(0.10)	i 5.15(0.35)	cde 3.84(0.27)	def 3.94(0.04)
Crude protein (N x 6.25)	l 60.05(0.32)	cd 47.24(0.45)	defg 50.31(0.16)	efgh 51.11(0.87)	cde 49.49(0.59)	efghij 52.24(0.88)
Lipid (%)	l 18.57(0.08)	abcde 11.31(0.15)	abcdefg 11.75(0.47)	a 10.46(0.61)	abc 11.11(0.19)	abcdef 11.73(0.81)
Ash (%)	a 19.71(0.20)	hij 36.41(0.31)	gh 34.63(0.23)	cde 32.31(0.63)	fg 34.39(0.45)	cd 31.49(0.83)
Nitrogen free extract[3](%)	-	-	-	-	0.30	-
Peroxide value (mEq/Kg oil)	a 0.09(0.03)	a 0.29(0.02)	fgh 1.26(0.10)	i 2.09(0.07)	fg 1.24(0.13)	abc 0.66(0.05)
Acid insoluble ash (%)	abcd 0.30(0.09)	abc 0.29(0.11)	f 0.77(0.08)	ef 0.68(0.21)	abcde 0.34(0.09)	abcdef 0.46(0.10)
Crude fibre (%)	a 0.76(0.24)	ab 0.89(0.02)	b 1.16(0.11)	ab 1.00(0.06)	ab 0.87(0.07)	ab 0.82(0.07)
Energy - ash free (Kcal/g)	abcdefgh 6.36(0.09)	abcdefg 6.34(0.07)	a 6.01(0.09)	i 7.25(0.11)	bcdefgh 6.75(0.09)	ij 7.48(0.10)

Proximate composition (% dry weight)	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	± S.E.[1]
Moisture (%)	a 2.67(0.33)	defgh 4.17(0.09)	j 5.22(0.10)	cd 3.74(0.15)	ab 2.94(0.25)	abc 3.13(0.25)	0.216
Crude protein (N x 6.25)	ab 43.58(0.03)	efghi 51.91(0.71)	cdef 49.52(0.91)	k 57.37(1.25)	a 41.09(0.28)	bc 46.59(0.28)	1.016
Lipid (%)	hijk 13.39(0.03)	cdefghij 12.06(0.25)	abcdefgh 11.94(0.63)	ab 10.47(0.51)	abcd 11.27(0.67)	bcdefghi 12.03(0.43)	0.453
Ash (%)	jk 39.30(0.03)	c 30.71(0.51)	def 32.56(0.61)	b 27.67(0.51)	l 40.41(0.25)	ghi 35.30(0.44)	0.533
Nitrogen free extract[3](%)	0.30	0.02	-	-	3.11	1.94	
Peroxide value (mEq/Kg oil)	0.00(0.00)	ab 0.65(0.11)	bcd 0.75(0.12)	bcde 0.82(0.11)	i 1.74(0.18)	bcdef 0.97(0.09)	0.111
Acid insoluble ash (%)	a 0.23(0.02)	f 0.76(0.05)	abcdef 0.55(0.08)	abcdef 0.45(0.09)	ab 0.24(0.07)	cdef 0.66(0.21)	0.108
Crude fibre (%)	a 0.76(0.08)	b 1.13(0.09)	ab 1.11(0.15)	ab 0.91(0.41)	b 1.18(0.11)	ab 1.01(0.03)	0.106
Energy - ash free (Kcal/g)	abcdefgh 6.36(0.07)	abcde 6.27(0.09)	abc 6.20(0.12)	abcdef 6.29(0.21)	ab 6.18(0.41)	abcd 6.25(0.04)	0.189

1) Standard error; calculated from residual mean square in the analysis of variance
2) Standard deviation
3) Nitrogen free extract = 100-(% moisture+% crude protein+% lipid+% ash+% crude fibre)

abc...jkl Mean values for components with the same superscripts are not significantly ($P < 0.05$) different

with the levels for other meat and bone meals all samples contained very low levels of the essential amino acids leucine, phenylalanine, threonine, and valine (Table 9.7). No seasonal trend was indicated and with very few exceptions the amino acids of the four samples analysed were reasonably consistent throughout the year.

The sums of the amino acids analysed were close to the levels of crude protein and therefore the percentage of unaccounted amino acids and of nonprotein nitrogen varied only between 0.52% and 1.43%.

With the exception of the sample taken in December, the lipid content was very consistent throughout the year varying only between 10.46% and 13.39% (Table 9.10; Fig. 9.5b). However, four of the samples had lipid levels higher than the normal maximum of 12% for meat and bone meals (Fowler and Banks, 1976; Skrede et al., 1980; McDonald et al., 1981; Tacon et al., 1984) and the December sample had an exceptionally high lipid content of 18.57%.

The ash content of all but one sample was consistently high and only four of the samples fell within the normal range of 27% to 33% for a meat and bone meal. The October sample contained the highest ash (40.41%) and the lowest crude protein (41.09%) levels while the December sample contained only 19.71% ash and had a crude protein content of 60% (Table 9.10; Fig. 9.5). Thus the composition of the latter sample was more characteristic of a meat meal than a meat and bone meal.

Only five samples contained NFE and in each of these levels did not exceed 4% (Table 9.10; Fig. 9.5b).

Samples supplied enough of all six minerals to satisfy the requirements of rainbow trout fed diets containing high inclusion levels of meat and bone meal. Mineral levels in the April, July, and November samples were very consistent and they also contained very similar ash levels of around 30% to 35%. The December sample contained only 19.71% ash and also had significantly different mineral levels. Even taking ash differences into account, the Ca content was very low whereas K and Zn levels were very much higher (Table 9.8).

Acid insoluble ash and crude fibre were all very low, not exceeding 1% and 2% respectively (Table 9.10). There were no significant ($P < 0.05$) differences in energy content of around 6 to 6.30 Kcalories per gramme dry weight (Table 9.10) for 10 of the samples.

9.4 DISCUSSION

The survey revealed that the crude protein levels of the six brown fish meals tested were very variable both from one processing plant to another and within the same plant from one sample to another (Tables 9.1 to 9.6). In terms of a consistent crude protein content and good amino acid profile the best fish meals were fish meals B and C and although the crude protein content of certain samples of fish meal A were higher, this was not consistently the case throughout the year. In the feeding trial comparing these six fish meals brown fish meals A and B produced the best growth performances and feed utilization efficiencies (see Chapter 6). The poorest fish meals in terms of crude protein content and amino acid profile were fish meals B, E and F and indeed the inclusion of fish meals E and F as the sole source of dietary protein produced the poorest growth performance in the feeding trial, although severe essential amino acid deficiencies were indicated for all samples of the fish meals tested (Table 9.7).

Fish meals D and F had the most consistent moisture content whereas levels in the other four fish meals were much more variable. Nevertheless the moisture content of the fish meals were in general lower than 10% although 9% of the samples overall had moisture contents close to or higher than the level of 14% where the risk of spoilage is high (Windsor and Barlow, 1981). Levels of moisture in excess of 14% normally allow the proliferation of moulds and furthermore Cockerell et al. (1971) reported that even lower levels of between 8% and 12% are sufficient to allow insect infestation

if products are inadequately stored in terms of environmental humidity and temperature.

The lipid content of the fish meals were in general within the normal range of reported values for good quality fish meals, although 20% of the samples had lipid levels close to the maximum value of 12% and a further 8% had lipid levels greater than 12%. The best fish meals in terms of low and more consistent lipid content were fish meals D and E with lipid levels varying only between 5% and 9% and between 8% and 12% respectively (Tables 9.4 and 9.5).

Fish oils are highly unsaturated and are thus very prone to oxidation (Reinitz, unpublished; Cockerell et al., 1971; Castell, 1979; Reinitz and Yu, 1981; Windsor and Barlow, 1981; Chan et al., 1982; Hung and Slinger, 1982; Hardy et al., 1983; Stansby, 1983; Watanabe et al., 1983). Assuming that the percentage of unsaturated fatty acids is constant in the oil fraction of fish meals, then oxidative rancidity is more likely to occur in fish meals with high lipid levels (Reinitz, unpublished). Indeed this appears to have been the case since extremely high peroxide values were associated with high lipid levels in at least seven samples. This was particularly the case in one sample of each of fish meals B and E which had peroxide values of 49.60 and 31.00 mEq/Kg oil respectively, in conjunction with high lipid levels of 13.88% for the former and 12.43% for the latter (Tables 9.2 and 9.5). Under inadequate conditions of storage, fish meals with high lipid levels would therefore suffer oxidative lipid peroxidation. This process leads to the production of hydroperoxides and is accompanied by secondary reactions

that result in off flavours and the destruction of essential fatty acids. The products resulting from these reactions can then react with free protein amino groups such as the E amino group of lysine thus reducing lysine availability and consequently the nutritive value of the protein (Reinitz, unpublished; Cockerell et al., 1971; Lee and Sinnhuber, 1972; Opstvedt, 1974; Hilton et al., 1977; Castell, 1979; Eriksson, 1982; Watanabe et al., 1983; Bell and Cowey, 1985). Furthermore oxidation of unsaturated fatty acids also destroys the fat soluble vitamins A, D, and E which are highly susceptible to oxidation (Cockerell et al., 1971; Castell, 1979; Hung et al., 1980, 1981; Chan et al., 1982).

To prevent lipids becoming rancid the addition of antioxidants to fish meals is general practice to stabilize the fat, particularly if their lipid content is higher than 10% (Reinitz, unpublished; Cockerell et al., 1971; Windsor and Barlow, 1981; Min et al., 1982; Logani et al., 1983). In view of the relatively high number of samples with peroxide values which exceeded the indicated level of 4 mEq/Kg oil for good quality fish meals (Billinski et al., 1978), it appears either that the addition of antioxidants is not a common practice among Portuguese fish meal producers or that the levels of antioxidants added were not sufficient to satisfactorily inhibit oxidation. Alternatively oxidative rancidity may have begun during storage of the raw materials prior to processing particularly if high environmental temperatures had occurred at that time. This may well have been the case since the two samples of fish meals B and E with peroxide values of 49.60 and 31.00 mEq/Kg oil (Tables 9.2 and 9.5) came from Algarve and both meals were processed in

September where environmental temperatures as high as 37°C may be experienced and this will indeed lead to faster rates of oxidative lipid rancidity (Cockerell et al., 1971; Windsor and Barlow, 1981; Stansby, 1983).

In conjunction with high lipid levels, high moisture levels were also recorded in some fish meal samples (Tables 9.2 to 9.6) and therefore the risk of spoilage is even greater. In addition to the risk of insect infestation and mould proliferation, ketonic lipid rancidity may also occur due to the proliferation of the micro-organisms Aspergillus and Pennicillium groups. These organisms can produce methylalkyl-ketones in oils particularly if the lipid fraction is rich in oleic acid once this causes the development of a most objectionable taste and odour. Ketones themselves can also react with free protein amino groups which reduces even further the protein quality of the meal (Cockerell et al., 1971).

Of all the parameters analysed, ash content was the most variable with levels ranging from 6.59% to 37.60% (Tables 9.1 and 9.2 respectively. This wide variation in ash content not only between processing plants but also within the same plant from one month to another, is a sign of inadequate quality control. Lack of adequate quality control is also evident in the wide variation in particle size (Appendices XVII to XXII).

High ash levels were most probably a consequence of the use of large proportions of fish scraps which contain high levels of bone material. In Portugal these scraps are mainly available from

the fish canning industry and when used in the production of fish meals they increase the ash content and therefore reduce the crude protein content of the meal (Cho, 1980). Although in all fish meals the overall ash content was high, fish meals C, D, and E had a relatively consistent ash content throughout the year (Tables 9.3, 9.4, and 9.5). Based on the particle size analysis, grinding as part of a pretreatment process prior to diet production is recommended.

The high degree of variation in proximate composition of the fish meals in Portugal may in part be attributable to seasonality of catches and also to the availability of certain fish species after the fish canning industry has been supplied. Thus, when a surplus arises fish meals are produced mainly with whole carcasses and then a better quality fish meal is obtained. By contrast, when the canning fish industry absorbs almost all, or indeed all of the fish available on the market, fish meals are then produced mainly with the scraps of this industry and consequently quality is reduced and a fish meal with higher ash content and lower crude protein is produced (Cho, 1980).

Variations in fish meal quality may not only be due to seasonal differences in catches but also to different fisheries. In the South of Portugal, in Algarve, where three of the fish meal processing plants are located (Fig. 9.1), catches are more heavily directed to mackerel and blue whiting. The use of these two oily fish species to supply fish meal processing plants in conjunction with much higher environmental temperatures experienced in this region compared to

the North, will lead to a faster spoilage of the raw materials prior to processing if proper storage is not implemented (Reinitz, unpublished; Cockerell et al., 1971; Windsor and Barlow, 1981; Stansby, 1983). The significant differences in the amino acid profile of fish meal F produced in Algarve, also suggests that a different fish species was used in the manufacture of this meal.

Overall the six brown fish meals analysed were good sources of Ca, Na, Mg, and Zn (Table 9.8), although K and P levels were in almost all samples below the normal range for good quality fish meals (Windsor and Barlow, 1981). Particularly high concentrations of both Ca and Na were recorded in all fish meals. Sodium levels of between 4.48% and 10.75% were recorded compared with levels of less than 1% for good quality fish meals (Windsor and Barlow, 1981). Assuming that most of the Na was in the form of NaCl, it is possible that this could have reduced growth rates and feed utilization efficiency as reported by Zaugg and McLain (1969) for coho salmon and Tacon and Silva (1983) in rainbow trout fed diets containing up to 12% salt. Nevertheless MacLeod (1978) in contrast to Zaugg and McLain (1969) found no decrease in either growth or feed utilization efficiency in rainbow trout fed diets containing up to 8.5% salt.

It is generally accepted that for most fish species there is an optimal level in the diet for both Ca and P, and hence an optimal ratio Ca:P. This ratio varied widely between 3.80 and 9.50 compared with a normal Ca:P ratio varying between 1.3 to 1.8 (Windsor and Barlow, 1981), although it has also been reported that Ca levels

have no effect on the requirement of rainbow trout for P (Lall, 1979; Nose and Arai, 1979; Millikin, 1982). It has also been reported that consumption of excess levels of Ca or P increases the dietary Mg requirement since these minerals can decrease Mg absorption (Lall, 1979; Millikin, 1982). Nevertheless, since almost all samples of the six brown fish meals were good sources of Mg, this antagonistic effect would not necessarily have resulted in Mg deficiency should these fish meals have been fed as the sole source of protein in diets for rainbow trout without mineral supplements.

The growth trial carried out to assess the six brown fish meals (Chapter 6) indicated that they were generally of a poorer quality than the fish meals usually used in good quality trout feeds. This survey has confirmed this, and also shown that quality can be very variable. The biggest problems were their high ash content resulting in lower crude protein levels and also some problems with oil rancidity. The use of any of these meals in feeds would therefore depend on very stringent quality control measures in the feed plant. Based on both the growth trial and the survey the best quality brown fish meal was B.

The PBHFm was a much more consistent product than the brown fish meals. The crude protein content was much higher and the variation random but small (Table 9.9). In comparison to the production of fish meals, seasonal trends in the manufacture of poultry by-products from chickens produced under intensive culture conditions are less likely to occur since carcass composition is controlled within fairly strict limits and therefore any variation is likely

to be indicative of different proportions of chickens used during processing (Burgos et al., 1974). Although the PBHFm was deficient in certain essential amino acids these deficiencies were consistent throughout the year. Consequently a feed formulator could be confident that these shortfalls could be remedied either by using this by-product in combination with other feedstuffs rich in these essential amino acids or by the addition of adequate amino acid supplements.

The overall lipid content of the PBHFm was high, but again it was generally constant (Table 9.9). The high lipid content of this animal by-product is one of the main constraints in diets formulation. Furthermore, poultry by-products have been reported to be deficient in ω3 essential fatty acids (Higgs et al., 1979) and therefore this meal can not be considered an ideal lipid source for rainbow trout (Castell et al., 1972; Watanabe et al., 1974a, b; Takeuchi and Watanabe, 1976, 1977; Yu And Sinnhuber, 1976; Castell, 1979; Castledine and Buckley, 1980; Reinitz and Yu, 1981; Henderson and Sargent, 1985). On the other hand the lipid is less saturated than fish oil and therefore less prone to oxidation during storage. This indeed seems to have been the case since very little oxidation was indicated despite the high lipid levels and on no occasion was a peroxide value higher than 4 mEq/Kg oil recorded (Table 9.9).

The PBHFm had a low but consistent ash content and indeed the particle size analysis (Appendix XXIII) reveals a consistent product with a high proportion of small particles, although grinding prior to diet manufacture would still be advisable. PBHFm is a consistently good quality product and therefore the success at high inclusion

levels indicated in the growth trials (Chapters 5 and 8) could be expected on a regular basis.

The meat and bone meal also had a much more consistent composition than any of the six brown fish meals tested. The crude protein content although lower than that of the fish meals and the PBHFm was fairly constant throughout the year (Table 9.10). Although the meat and bone meal had several essential amino acids deficiencies, the level of these essential amino acids were again fairly constant in the four samples analysed (Table 9.7). Nevertheless as demonstrated in the feeding trials in Chapters 7 and 8 these essential amino acid deficiencies will restrict the use of this foodstuff at high inclusion levels and its use in combination with other suitable protein sources is therefore recommended.

The lipid content was relatively high but little variation from one month to another was indicated (Table 9.10). In comparison with PBHFm and in particular with fish meals, lipid from slaughterhouse animals such as sheep and cattle, is highly saturated and therefore oxidative lipid peroxidation is less likely to occur. However it is not ideal for fish due to the lack of $\omega 3$ essential fatty acids although it has been reported that animal fat can replace a large proportion of fish oil with no adverse effects in a nutritionally balanced diet (Yu et al., 1977; Takeuchi et al., 1978c; Cowey et al., 1979; Reinitz and Yu, 1981; Yu and Sinnhuber, 1981). The meat and bone meal tested had fairly low lipid levels when compared with the fish meals and in particular with the PBHFm and therefore the risk of spoilage during storage is also less likely to occur.

Furthermore the moisture content was consistently low and therefore this risk is even further reduced. In fact very low peroxide values were recorded in all 12 samples tested (Table 9.10).

Although the overall ash content was high it was consistent throughout the year. Due to the overall high ash content samples had high levels of Ca although relatively low levels of P and Mg (Table 9.8) compared with the requirements of rainbow trout, particularly if this foodstuff is to be included at high inclusion levels without mineral supplements. A ratio of around 12 between Ca and P is therefore indicated compared with a Ca:P ratio of around 2 reported by Gðhl (1981) and Menzies (1982). Furthermore, in conjunction with high Ca levels relatively low Mg levels were also indicated and this may reduce Mg absorption and therefore a Mg deficiency may develop should this foodstuff be used as the sole protein source in a diet with no mineral supplement.

The high ash content in conjunction with deficiencies in the essential amino acids profile appear to be the two main constraints associated with the use of this by-product at high inclusion levels in diets for rainbow trout. Results from the growth trial (Chapter 8) indeed indicated that this by-product should not be used at levels above 30% of the dietary protein compared with good quality fish meal.

In conclusion, the six brown fish meals had a variable and in general poor quality in comparison with good quality fish meals,

while the PBHFm and the meat and bone meal had a much more consistent quality throughout the year. Furthermore these two protein sources are more reliable in terms of availability throughout the year than fish meals.

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSIONS

10. GENERAL DISCUSSION AND CONCLUSIONS

The Poultry by-product and hydrolysed feather meal (PBHFm) evaluated in diets for rainbow trout where the dietary fish meal protein was gradually replaced by this by-product indicated that it is a valuable source of protein and although deficient in both the essential acids leucine and phenylalanine can successfully replace fish meal at levels of up to 80% to 90% of the protein without any adverse effect on growth performance and feed utilization efficiency (Sections 5.3.2 and 5.4). These findings are broadly in agreement with those of Tiews et al. (1976, 1979) and Gropp et al. (1979) and with those of Higgs et al. (1979) who fed diets containing different proportions of Poultry by-product meal (PBm) and Hydrolysed feather meal (HFm) to rainbow trout and coho salmon. These two by-products successfully replaced up to 75% of the fish meal protein in a practical ration and even 100% protein replacement was successful when the diets were supplemented with deficient essential amino acids (Tiews et al., 1976; Gropp et al., 1979; Higgs et al., 1979).

Despite a decrease in performance by fish fed a ration containing 100% of the protein as Poultry by-product and hydrolysed feather meal (PBHFm), fish fed all rations containing this by-product performed better than those fed a control ration based only on fish meal. This therefore indicated that the fish meal used in the diets was of a poor quality, and this indeed appears to have been the case since the meal was deficient in the essential amino acids arginine, leucine, lysine and phenylalanine, and furthermore only 52% of the leucine requirement of rainbow trout was present in

the control ration where all the dietary protein was supplied by this fish meal.

Increasing inclusion levels of Poultry by-product and hydrolysed feather meal (PBHFm) caused a significant alteration in carcass composition and in particular in lipid deposition. Body lipid levels in fish fed the ration based on this poultry by-product alone were almost three times greater than those of fish fed the fish meal control ration (Section 5.4). This effect of increasing body lipid with increasing inclusion levels of poultry by-products was also reported by Wojno and Dabrowska (1984a) when rainbow trout were fed diets containing a Poultry offal meal (POm; Section 5.4) although the increase in body lipid content was not so great as that indicated in this work. This can probably be attributed to the lower dietary lipid content of only 10% used by these authors compared with a much higher level of 17% used in this study. Indeed in the later trial where fish were fed a diet containing 50% of the protein as Poultry by-product and hydrolysed feather meal but with a dietary lipid content of only 13%, there was no significant difference in carcass composition between these fish and those fed a fish meal based ration (Section 8.4). It was also noted that fish fed diets containing increasing inclusion levels of Poultry by-product and hydrolysed feather meal (PBHFm) had higher liver somatic indeces although this increase was only significantly different at the 100% protein replacement level. Furthermore the fish fed this dietary treatment had pale livers which, according to Phillips et al. (1966), indicates very high glycogen deposition in the liver. However, it is possible that this may have been attributable to the trend

of increasing dietary carbohydrate levels which accompanied the increase in inclusion levels of this by-product.

The survey demonstrated that the Poultry by-product and hydrolysed feather meal (PBHFm) is of a reliable quality throughout the year. The two main constraints associated with the use of this product at high inclusion levels in rations for rainbow trout are the deficiencies in the essential amino acids leucine, phenylalanine and threonine and the high lipid content. The amino acid deficiencies are fairly constant throughout the year, and could be corrected either by the addition of other by-products rich in these deficient amino acids or alternatively by adequate amino acid supplements (Sections 9.3.2 and 9.4). The very high lipid content of this meal may restrict its use at high inclusion levels. Furthermore, lipid from poultry by-products are deficient in ω3 essential fatty acids and consequently diets containing high inclusion levels of this meal need to be supplemented with a secondary lipid source to provide these essential fatty acids, thus increasing even further the dietary lipid level of a diet.

The first trial highlighted the poor quality of the Brown fish meal used as a control and this indeed was confirmed in this trial comparing six Brown fish meals from six processing plants in Portugal. With the exception of only one of the meals, growth performances and feed utilization efficiencies of fish fed diets based on these meals were significantly poorer than that of those fed a control ration based on "Pruteen" (Sections 6.3.2 and 6.4). The poor fish performances were attributable mainly to the low crude protein and

high ash levels which were both outside the levels recommended by Windsor and Barlow (1981) for good quality fish meals.

The experimental rations were particularly deficient in the essential amino acids arginine, leucine, lysine, phenylalanine and valine and therefore the protein available for growth was reduced to between 36% and 86% (Section 6.4). Furthermore the year long survey demonstrated that the six brown fish meals were not of a consistent quality. There were wide fluctuations in chemical composition, although the crude protein content was generally low and the ash content high compared with the normal levels for good quality fish meals (Sections 9.3.1 and 9.4). This variation in chemical composition was also accompanied by a variable particle size composition indicating inadequate quality control during manufacture.

The relatively high lipid content of the fish meals makes them highly susceptible to oxidative lipid peroxidation, especially in 22% of the 70 samples analysed in the survey which contained lipid levels higher than the maximum level recommended by Windsor and Barlow (1981) for good quality fish meals. Furthermore, associated with this high lipid content some fish meal samples also contained high moisture levels and therefore the risk of spoilage is even greater, since ketonic lipid rancidity may occur, particularly if these meals are stored under unfavourable conditions of humidity and temperature (Cockerell et al., 1971; Windsor and Barlow, 1981; Stansby, 1983). Portugal has a temperature climate in the North and a Mediterranean type climate in the South, Algarve, and therefore very high environmental temperatures are normally experienced in the spring and summer, particularly in Algarve. Very high peroxide

values were indeed recorded for some of the fish meal samples which therefore indicated either prolonged storage of the raw materials under unfavourable conditions prior to processing or inadequate protection by antioxidants (Section 9.4).

Thus, based on both the growth trial and the survey, it appears that obtaining a good quality fish meal of consistent quality is difficult in Portugal. However, as a result of further investigations, a manufacturer was finally located who produced a good quality fish meal, although the improved quality was obtained by blending a locally produced fish meal with an imported high quality meal. This fish meal was used as the control protein source in the last two feeding trials.

The growth performance and feed utilization efficiency of fish fed diets containing up to 80% of the protein as Meat and bone meal was not significantly different from those fed a control ration containing a good quality fish meal (Sections 7.3.2 and 7.4). However the pretreatment of the Meat and bone meal removed a significant part of the ash content and as a consequence increased the crude protein content and thus, in effect, a product with the characteristics of a meat meal was assessed (Section 7.4). The untreated Meat and bone meal was incorporated in rations in the final trial and from the growth performances it was apparent that the maximum inclusion level was below 30% of the protein (Section 8.4). Thus the partial removal of the bone fraction had a beneficial effect on the nutritive value of the product. However, the removal of the bone fraction is unlikely to be feasible, particularly in terms

of cost. It would be more advisable to use a meat meal either alone or in conjunction with a meat and bone meal although this would again increase ration production costs since Portuguese meat meals are much more expensive and are also less available than meat and bone meals.

The relatively unbalanced essential amino acid profile of the Meat and bone meal precludes its inclusion at high levels since even at a level of 30% of the protein growth performance is already significantly reduced, principally as a result of deficiencies in arginine, leucine, phenylalanine and valine (Section 8.4). One method to offset essential amino acid deficiencies is to use combinations of different protein sources and this indeed was one of the aims of the final trial where different proportions of the three by-products were assessed.

Based on growth performance and feed utilization efficiency the best combination of the three animal by-products were 40% Fish meal, 30% Poultry by-product and hydrolysed feather meal and 30% Meat and bone meal. However fish fed this diet had a significantly poorer growth performance and feed utilization efficiency compared with those fed either a fish meal control ration or a commercial trout ration (Section 8.4). In the final trial the best performance was attained by the ration completely devoid of Meat and bone meal but where half of the protein was provided by Poultry by-product and hydrolysed feather meal and half by Fish meal.

Throughout this study the three Animal by-products have all been deficient in as many as three to five essential amino acids, and in almost all cases the first limiting dietary essential amino acid has been leucine. As a consequence certain diets have only supplied as little as 36% of the leucine requirement for rainbow trout with the best value only 86%. This was even the case in the only commercial trout ration produced in Portugal where only 55% of the leucine requirement was present. Thus, in order to improve the nutritional balance of rations produced from locally available ingredients in Portugal one or more of the following measures must be taken. Improvements in processing of the by-products with higher quality control measures, particularly in the manufacture of fish meals, are necessary although the implementation of this measure would not be easy. Alternatively, rations could be formulated to include further protein sources selected to offset essential amino acid deficiencies. For example, blood meal and hydrolysed feather meal are rich sources of leucine and can be included in diets for rainbow trout at levels of up to 12% and 20% of the diet respectively (Higgs et al., 1979; Koops et al., 1979; Tacon, 1983; Hardy et al., 1984). Finally, there is the option of adding synthetic amino acids to make up deficiencies, but this would undoubtedly produce a substantial increase in production costs.

Like any other commercial industry aquaculture must be profitable. Feeds are one of the main variable costs in intensive aquaculture (Shang, 1981) and according to Tacon and Jackson (1985) can amount to between 40% and 60% of total operating costs. This is particularly the case in farming rainbow trout where Animal by-

products represent a large proportion of feed costs which increase the final costs of rations (Chapter 1). The main factor causing variation in the cost of diet production is fluctuation in the price of ingredients. As a consequence of these fluctuations the cost of producing a ration based on a fixed formulation is likely to be very variable. Least cost formulation packages designed to produce nutritionally balanced rations for the least cost are routinely used in the more advanced animal production industries such as swine and poultry. Their use in fish diet production is increasing, particularly as more information on dietary requirements becomes available. Ultimately however, only after further information becomes available on the nutritional quality of ingredients for fish when used in varying proportions will least cost formulation gain widespread acceptance in the fish feed industry (Reinitz, unpublished).

Feed costs can be reduced either by an improvement in the food conversion ratio or by lowering the unit price of feeds or by a combination of these two factors, and this can be achieved by utilizing locally available ingredients instead of imported ones (Shang, 1981). Although the cost of ingredients is an important factor determining the final price of a feed and therefore its economic feasibility, the cost per unit of fish production is a much more accurate method of assessing the real economic impact of a particular ingredient on fish feed costs since a much more reliable cost effectiveness analysis is obtained when the price of an ingredient and food conversion ratio are related (Crampton, 1985). As far as protein sources are concerned, the cost per

Kilogramme of a particular ingredient may be lower than that of, for example, fish meal. However, it is the cost per gramme of protein which is important and particularly the cost of available protein.

In order to evaluate the cost effectiveness of using Poultry by-product and hydrolysed feather meal and Meat and bone meal to replace Fish meal, and also to compare the relative costs of the rations based on the six Brown fish meals the production costs of the experimental rations were calculated (Table 10.2 and Appendices XXV to XXVIII). This took into account not only the price of the ingredients, but also the cost of electricity and water used in diet manufacture (Table 10.1). It should be noted that in comparison with practical commercial diets, the costs of the experimental rations were very high. The high prices indicated for the experimental rations were caused by the inclusion of products like chromic oxide, potassium sorbate, and a very good quality binder and vitamin premix and also to the use of dextrin and corn starch added both as dietary carbohydrate sources and bulking agents.

In practical rations cheaper ingredients than dextrin and corn starch are added as carbohydrate sources and bulking agents since purified ingredients are not economically feasible in a commercial scale fish feed production. Although dietary carbohydrates were present at relatively high inclusion levels in some of the experimental diets, levels did not exceed the recommended 25% inclusion level (Edwards et al., 1977; Bergot, 1979; Hilton et al., 1982).

TABLE 10.1 Price of the ingredients, electricity and water costs (in Escudos ($)/Kg and Sterling pounds (£)/Kg diet). (Prices refer to December 1985).

Ingredients	Escudos/Kg ($)	Sterling/Kg pounds (£)
Brown fish meal	62.00	0.26956
Poultry by-product and hydrolysed feather meal	50.00	0.21739
Meat and bone meal	40.00	0.17391
"Pruteen"	151.80	0.66000
Corn starch	240.00	1.04348
Yellow dextrin	145.00	0.63043
Cod liver oil	250.00	1.08696
Vitamin premix	2,300.00	10.00000
Mineral premix	2,515.03	10.93491
Carboxymethylcellulose high viscosity	5,985.00	26.02174
Potassium sorbate	120,000.00	521.73913
Butylated hydroxytoluene	10,000.00	43.47826
Cr_2O_3	7,800.00	33.91304
Electricity (Kwt)	3.50	0.01522
Water (m^3)	18.00	0.07826

1 Sterling pound (£) = Escudos 230.00

TABLE 10.2 Cost comparison of experimental diets per Kilogramme of feed and per Kilogramme of production (1 Sterling pound = Escudos 230.00)

		DIET 1		DIET 2		DIET 3		DIET 4		DIET 5		DIET 6		DIET 7	
		Escudo ($)	Sterling pound (£)	Escudo ($)	Sterling pound (£)	Escudo ($)	Sterling pound (£)	Escudo ($)	Sterling pound (£)	Escudo ($)	Sterling pound (£)	Escudo ($)	Sterling pound (£)	Escudo ($)	Sterling pound (£)
PBHFm trial	Price/Kg feed	611.076	2.6568	608.958	2.6476	606.581	2.6373	605.975	2.6347	604.879	2.6230	605.521	2.6327	606.163	2.6355
	Price/Kg production	2835.393	12.328	1845.143	8.0224	1571.045	6.8306	1169.532	5.0849	1158.563	5.1546	1053.606	4.5810	1151.710	5.0074
Brown fish meal trial	Price/Kg feed	681.537	2.9632	609.194	2.6487	584.231	2.5401	600.559	2.6111	598.994	2.6043	589.254	2.5620	601.324	2.6144
	Price/Kg production	1601.605	6.9635	1632.640	7.0984	1659.216	7.2140	1987.850	8.6428	1970.690	8.5682	2121.314	9.2231	2002.409	8.7061
Meat and bone meal trial	Price/Kg feed	626.272	2.7229	620.462	2.6977	614.735	2.6728	609.008	2.6479	603.281	2.6229	597.649	2.5985		
	Price/Kg production	764.052	3.3220	769.372	3.3451	799.155	3.4746	767.350	3.3363	850.626	3.6984	1010.027	4.3914		
Compound trial	Price/Kg feed	630.006	2.7391	609.975	2.6521	584.724	2.5423	586.680	2.5508	584.230	2.5401	607.080	2.6395		
	Price/Kg production	869.408	3.7800	853.965	3.7129	1257.156	5.4659	1050.157	4.5659	1320.360	5.7407	959.186	4.1704		

Current prices of Poultry by-product and hydrolysed feather meal and Meat and bone meal available in Portugal are 19% and 35% respectively cheaper than that of Portuguese Fish meals (Table 10.1). Consequently experimental rations based on these two animal by-products were less expensive than those based on fish meal alone (Table 10.2; Appendices XXV to XXVII). However, in the Poultry by-product and hydrolysed feather meal trial the variation in costs was not so great as might have been expected, varying only between 2.66 and 2.63 Sterling pounds (Escudos 611.00 and 606.00 respectively) per Kilogramme of diet. This was due mainly to the increasing inclusion levels of both dextrin and corn starch with increasing inclusion levels of Poultry by-product and hydrolysed feather meal, and as a result the high costs of these purified ingredients offset the saving of including Poultry by-product and hydrolysed feather meal instead of Fish meal. From Appendix XXV it can be seen that the cost of supplying the protein component of the fish meal control ration was 21 pence (Escudos 49.00) per Kilogramme compared with only 15 pence (Escudos 35.00) where all the fish meal was replaced by Poultry by-product and hydrolysed feather meal. In the Meat and bone meal trial carbohydrate costs were relatively constant, the main variation in ingredient costs other than protein sources was produced by the variation in the level of cod liver oil.

In the Poultry by-product and hydrolysed feather meal trial the differences in costs per Kilogramme of fish production were much more dramatic. The cost of producing a Kilogramme of fish using the fish meal based control ration was 12.33 Sterling pounds (Escudos 2835.00) compared with only 4.58 Sterling pounds (Escudos

1053.00) when 90% of the fish meal was replaced by Poultry by-product and hydrolysed feather meal (Table 10.2). This again highlighted the poor quality of the fish meal used since dietary costs of the fish meal control ration both in this trial and in the final trial where a high quality fish meal was utilised were very similar (2.66 and 2.74 Sterling pounds respectively; Table 10.2). However, the cost per Kilogramme fish production in the first trial was 12.33 Sterling pounds compared with only 3.78 Sterling pounds in the final experiment (Table 10.2).

Although in the fish meals trial (Chapter 6) the "Pruteen" based diet was the most expensive, fish production costs were the lowest despite the "Pruteen" component being around twice the price of other fish meals (Table 10.2; Appendix XXVI). However, fish production costs of the "Pruteen" ration were almost twice those of the fish meal control rations used in the last two trials where a good quality fish meal was used. Based on fish production costs, the best fish meal in Experiment 2 was Fish meal A although it was also the most expensive of the six Fish meal based rations evaluated in this trial. Again however, the fish production costs using this ration were almost two times higher than that incurred for the high quality Fish meal rations used in the last two trials (Table 10.2; Appendix XXVI).

In the Meat and bone meal trial the cost per unit production at the 20% Meat and bone meal inclusion level was similar to that of the control based on good quality fish meal. Thereafter there was a very significant trend of increasing cost per unit of production

with increasing inclusion levels of this product, so that at 100% inclusion level the cost per Kilogramme production was 32% greater than that of the fish meal based ration (Table 10.2; Appendix XXVII).

Although all of the experimental rations with different combinations of the animal protein sources were cheaper than the ration based on Fish meal alone, in fish production terms only the ration containing 50% of the protein as Poultry by-product and hydrolysed feather meal was more cost effective. Of the rations containing different combinations of all three Animal by-products, the cheapest in terms of fish production costs was the one containing Brown fish meal, Poultry by-product and hydrolysed feather meal and Meat and bone meal in the ratio 1.3:1:1 (Diet 6; Table 10.2; Appendix XXVIII).

Based on the information accumulated in the thesis certain recommendations can be made concerning the feasibility of developing a fish feed manufacturing industry to produce nutritionally balanced diets for rainbow trout in Portugal. Although there are adequate supplies of locally produced fish meals in Portugal, it appears that they are generally of a relatively poor quality and are of variable chemical composition. Furthermore the only high quality meal located contained a significant proportion of an imported meal. It is therefore important either to locate a reliable source of a good quality meal, or to try to encourage manufacturers to produce a more reliable product with a higher crude protein and lower ash content. Alternatively the dependence of feed manufacture on fish meal could be reduced by providing the protein component from alternative sources. Adequate quantities of both Poultry by-product

and hydrolysed feather meal and Meat and bone meal are available in Portugal to support a substantial fish feed manufacturing industry. It has also been demonstrated in this work that they are both of a consistent quality. The results obtained with Poultry by-product and hydrolysed feather meal indicate that this material can be successfully incorporated in rations at relatively high inclusion levels. The maximum inclusion level for the Meat and bone meal however, is significantly lower. Unfortunately both of these by-products and the fish meals had significant essential amino acid deficiencies and formulating rations based on combinations of these three protein sources did not produce a ration with an amino acid profile balanced for rainbow trout. Thus, in order to improve the nutritional quality it would be necessary to evaluate further protein sources which would offset the deficiencies, particularly those in leucine levels. The option of incorporating synthetic essential amino acids is unlikely to be cost effective. There are also adequate levels of plant proteins available in Portugal including rapeseed meal, sunflower meal and sweet lupin meal which could be included in practical rations to provide both energy and bulk. Ultimately however, the success of developing a fish feed industry in Portugal would depend on further more detailed evaluation of the cost of producing rations from locally available ingredients and on the availability and suitability of further protein sources which could be used to improve the nutritional balance of the rations developed in this study.

In the short term the data accumulated in this thesis could be used to improve the existing small scale diet manufacture industry in

Portugal and to act as a basis for further developments in Portuguese feed manufacture.

REFERENCES

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, WATER POLLUTION CONTROL FEDERATION (1985). Metals by atomic absorption spectrometry. In: APHA, AWWA, WPCF (Editors) Standard Methods For The Examination Of Water And Waste Water, 16th edition: pp143-261

ARTHUR, D. (1971). Selenium content of some feed ingredients available in Canada. Can. J. Ani. Sci., 51: pp71-74

ÅSGÅRD, T. & AUSTRENG, E. (1981). Fish silage for salmonids: a cheap way of utilizing wastes as feed. Feedstuffs, 53: pp22-24

ASGARD, T. & AUSTRENG, E. (1985). Casein silage as food for salmonids. Aquaculture, 48: pp233-252

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1980). Official Methods of Analysis. Association of Official Analytical Chemists, Washington D.C.

ATACK, T. & MATTY, A. J. (1979). The evaluation of some single-cell proteins in the diet of rainbow trout: II. The determination of net protein utilization, biological values and true digestibility. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Heeneman, Berlin: pp261-273

AUSTRENG, E. (1978a). Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture, 13: pp265-272

AUSTRENG, E. (1978b). Fat-containing bleaching earth as a feed constituent for rainbow trout. Aquaculture, 15: pp333-343

AUSTRENG, E. (1979). Fat levels and fat sources in dry diets for salmonid fish. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology. Vol. 2, H. Heeneman, Berlin: pp313-326

AUSTRENG, E. & REFFSTIE, T. (1979). Effect of varying dietary protein level in different families of rainbow trout. Aquaculture 18: pp145-156

AUSTRENG, E., RISA, S., EDWARDS, D. J., & HVIDSTEN, H. (1977). Carbohydrate in rainbow trout diets. II. Influence of carbohydrate levels on chemical composition and feed utilization of fish from different families. Aquaculture 11: pp39-50

BEAMISH, F. W. H. & THOMAS, E. (1984). Effects of dietary protein and lipid on nitrogen losses in rainbow trout, Salmo gairdneri. Aquaculture 41: pp359-371

BECK, H., GROPP, J., KOOPS, H. & TIEWS, K. (1979). Single cell protein in trout diets. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology. Vol. 2, H. Heeneman, Berling: pp269-281

BELL, J. G. & COWEY, C. B. (1985). Roles of vitamin E and selenium in the prevention of pathologies related to fatty acid oxidation in salmonids. In: C. B. Cowey, A. M. Mackie, and J. G. Bell (Editors), Nutrition and Feeding in Fish, Academic Press, London: pp333-347

BERGOT, F. (1979). Carbohydrate in rainbow trout: effects of the level and source of carbohydrate and the number of meals on growth and body composition. Aquaculture 18: pp157-167

BHARGAVA, K. K. & O'NEIL, J. B. (1975). Composition and utilization of poultry by-product and hydrolysed feather meal in broiler diets. Poultry Sci., 54: pp1511-1518

BIELORAI, R., IOSIF, B. & HARDUF, Z. (1983). The nutritive value of poultry by-product meal for chicks. Nutr. Rep. Intern.,27, pp891-898

BIELORAI, R., IOSIF, B., NEUMARK, H. & ALUMOT, E. (1982). Low nutritional value of feather-meal protein for chicks. J. Nutr.,112:. pp249-254

BILLINSKI, E., JONAS, R. E. E. & LAU, Y. C. (1978). Chill stowage and development of rancidity in frozen pacific herring. J. Fish. Res. Board Can.,35: pp473-477

BLAXHALL, P. C. & DAISLEY, K. W. (1973). Routine haematological methods for use with fish blood. J. Fish. Biol.,5: pp771-781

BLIGH, E. G. & DYER, W. J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol.,37: pp911-917

BLOSS, R. E., LUECKE, R. W., HOEFER, J. A., THORP, Jr., F. & MCMILLEN, W. N. (1953). Supplementation of a corn-meat and bone scrap ration for weanling pigs. J. Anim. Sci.,12: pp102-106

BONDARI, K. & SHEPPARD, D. C. (1981). Soldier fly larvae as feed in commercial fish production. Aquaculture, 24: pp103-109

BRAATEN,B.R.(1979). Bioenergetics - a review on methodology. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp461-504

BRAUNDE, R., HOSKING, Z. D., MITCHELL, K. G., PLONKA, S. & SAMBROOK, I. E. (1977). Pruteen, a new source of protein for growing pig. I. Metabolic experiment: utilization of nitrogen. Livestock Production Science, 4: pp79-89

BRETT, J. R., SHELBOURN, J. E. & SHOOP, C. T. (1969). Growth rate and body composition of fingerling sockeye salmon, Oncorhynchus nerka, in relation to temperature and ration size. J. Fish. Res. Board Can., 26: pp2363-2394

BROCKSEN, R. W. & BUGGE, J. P. (1974). Preliminary investigations on the influence of temperature on food assimilation by rainbow trout, Salmo gairdneri Richardson. J. Fish. Biol., 6: pp93-97

BROMLEY, P. J. & SMART, G. (1981). The effects of the major food categories on growth, composition and food conversion in rainbow trout (Salmo gairdneri Richardson). Aquaculture, 23: pp325-336

BROWN, E. C. (1977). Portugal. In: World fish farming cultivation and economics. AVI Publishing Company Inc., West Point, Connecticut, 1977: pp209-210

BUCKLEY, J. T. & GROVES, T. D. D. (1979). Influence of feed on the body composition of finfish. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin : pp335-343

BURGOS, A., FLOYD, J. I. & STEPHENSON, E. L. (1974). The aminoacid content and availability of different samples of poultry by-product meal, and feather meal. Poultry Sci., 53: pp198-203

BURROWS, I. (1984). The potential of earthworm composts for growing plants. Presented at the International Conference on Earthworms in Waste and Environmental Management, Cambridge, UK, July 1984: pp23-27

CALVERT, C. C., MARTIN, R. D. & MORGAN, N. O. (1969). Housefly pupae as food for poultry. J. Econ. Entomol., 62: pp938-939

CASTELL, J. D. (1979). Review of lipid requirements of finfish. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Heeneman, Berlin: pp59-83

CASTELL, J. D., LEE, D. J. & SINNHUBER, R. A. (1972). Essential fatty acids in the diet of rainbow trout (Salmo gairdneri): lipid metabolism and fatty acid composition. J. Nutr., 102: pp93-100

CASTELL, J. D. & TIEWS, K. (1980). Report of the EIFAC, IUNS and ICES working group on standardization of methodology in fish nutrition research. EIFAC Technical Paper, 36: pp1-21

CASTLEDINE, A. J. & BUCKLEY, J. T. (1980). Distribution and mobility of ω3 fatty acids in rainbow trout fed varying levels and types of dietary lipid. J. Nutr.,110: pp675-685

CHAN, H. W-S, COXON, D. T., PEERS, K. E. & PRICE, K. R. (1982). Oxidative reactions of unsaturated lipids. Food Chemistry, 9: pp21-34

CHO, C. Y. (1980). Recent advances in the diet formulation and the nutrition of salmonid fishes - type of fat and its quality. Proceedings of the Sixteenth Annual Nutrition Conference for Feed Manufacturers, Guelph: pp23-27

CHO, C. Y. & SLINGER, S. J. (1979). Apparent digestibility measurement in feedstuffs for rainbow trout. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp239-247

CHOUBERT, G., FAUCONNEAU, B. & LUQUET, P. (1982). Influence d'une élévation de la temperature de l'eau sur la digestibilité de la matière sèche, de l'azote et de l'énergie de l'aliment distribué à la truite arc-en-ciel (Salmo gairdneri Rich.). Reprod. Nutr. Develop., 22: pp941-949

CHOUBERT, Jr., G. & LUQUET, P. (1983). Utilization of shrimp meal for rainbow trout (Salmo gairdneri Rich.) pigmentation. Influence of fat content of the diet. Aquaculture, 32: pp19-26

CHUBB, L. G. (1982) Anti-nutritive factors in animal feedstuffs. In: W. Haresigh (Editor), Recent Advances in Animal Nutrition, pp21-37

CHVAPIL, M., ULREICH, J. B. & HUNTER, B. (1980). Complementation of predigested proteins: a nutritionally and economically advantageous method of by-products utilization. J. Nutr., 110: pp1319-1326

COCKERELL, I., FRANCIS, B. & HALLIDAY, D. (1971). Changes in nutritive value of concentrate feedingstuffs during storage. Tropical Products Institute, London, United Kingdom

COULL, J. R. (1972). The Fisheries of Europe and Economic Geography. Bell's Advanced Economic Geographies. R. O. Buchanan (Editor), 1972: pp218

COWEY, C. B. (1975). Aspects of protein utilization by fish. Proc. Nutr. Soc., 34: pp57-63

COWEY, C. B. (1976). Use of synthetic diets and biochemical criteria in the assessment of nutrient requirement of fish. J. Fish. Res. Board Can., 33: pp1040-1045

COWEY, C. B. (1979). Protein and aminoacid requirements of finfish. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Heeneman, Berlin: pp3-16

COWEY, C. B. (1981). The food and feeding of captive fish. In: A. D. Hawkins (Editor), Aquarium Systems. Academic Press, London: pp223-246

COWEY, C. B., ADRON, J. W., HARDY, R., SMITH, J. G. M. & WALTON, M. J. (1979). Utilization by rainbow trout of diets containing partially rendered hide fleshings. Aquaculture, 16: pp199-209

COWEY, C. B., POPE, J. A., ADRON, J. W. & BLAIR A. (1972). Studies on the nutrition of marine flatfish. The protein requirement of plaice (Pleuronectes platessa). Br. J. Nutr., 28: pp447-456

COWEY, C. B. & SARGENT, J. R. (1979). Nutrition. In: Fish Physiology, Vol. VIII. Academic Press Inc: pp1-69.

COWEY, C. B. & TACON, A. G. J. (1983). Fish nutrition - relevance to marine invertebrates. In: G. D. Pruder, C. J. Longdon & D. E. Conklin (Editors), Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approach to Shellfish Nutrition, Baton Rouge Louisiana State University: pp13-30

CRAMPTON, V. O. (1985). The application of nutritional findings to the formulation of practical diets. In: C. B. Cowey, A. M. Mackie, & J. G. Bell (Editors), Nutrition and Feeding in Fish, Academic Press, London: pp447-464

CRAMPTON, E. W. & HARRIS, L. E. (1969). Applied Animal Nutrition, second edition, W. H. Freeman & Co., San Francisco: pp753,

CULLISON, A. E. (1979). Feeds and Feeding, second edition. Reston Publishing Co. Inc., Reston, Virginia: pp595

DABROWSKA, H. (1984). Effect of dietary proteins on the free amino acid content in rainbow trout (Salmo gairdneri Rich.) muscles. Comp. Biochem. Physiol., 77: pp553-556

DABROWSKA, H. & WOJNO, T. (1977). Studies on the utilization by rainbow trout (Salmo gairdneri Rich.) of feed mixtures containing soya bean meal and an addition of amino acids. Aquaculture, 10: pp297-310

DABROWSKI, K., HASSARD, S., QUINN, J. & PITCHER, T. J. (1980). The effect of Geotrichum candidum protein substitution in pelleted fish feed on the growth of rainbow trout (Salmo gairdneri Rich.) and on utilization of the diet. Aquaculture, 21: pp213-232

DAVIS, E. M., RUMSEY, G. L. & NICKUM, J. G. (1976). Egg-processing wastes as a replacement protein source in salmonid diets. Prog. Fish Cult., 38: pp20-22

DUNCAN, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11: pp1-42

EASTOE, J. E. & LONG, J. E. (1960). The aminoacid composition of processed bones and meat. J. Sci. Fd. Agric., 11: pp87-92

EDWARDS, D. J., AUSTRENG, E., RISA, S. & GJEDREM, T. (1977). Carbohydrate in rainbow trout. I. Growth of fish of different families fed diets containing different proportions of carbohydrate. Aquaculture, 11: pp31-38

EISENREICH, S. J., BANNERMAN, R. T. & ARMSTRONG, D. E. (1975). A simplified phosphorous analysis technique. Environ. Lett., 9: pp45-53

ELLIOT, J. M. (1979). Energetics of freshwater teleosts. Symp. Zool. Soc. London, 44: pp39-61

ELLIOT, J. M. (1982). The effects of temperature and ration size on the growth and energetics of salmonids in captivity. Comp. Biochem. Physiol., 73B: pp81-89

ELLIS, J. N. (1979). The use of natural and synthetic carotenoids in the diet to color the flesh of salmonids. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp353-364

ERIKSSON, C. E. (1982). Lipid oxidation catalysts and inhibitors in raw materials and processed foods. Food Chemistry, 9: pp3-19

FAUCONNEAU, B., CHOUBERT, G., BLANC, D., BREQUE, J. & LUQUET, P. (1983). Influence of environmental temperature on flow rate of foodstuffs through the gastrointestinal tract of rainbow trout. Aquaculture, 34: pp27-39

FAUCONNEAU, B. & LUQUET, P. (1984). Influence de la fréquence de distribution des protéines sur la croissance et l'efficacité alimentaire chez la truite arc-en-ciel (Salmo gairdneri R.). Ann. Zootech., 33: pp245-254

FOWLER, L. G. & BANKS, J. L. (1976). Animal and vegetable substitutes for fish meal in the Abernathy diet, 1973. Prog. Fish. Cult., 38: pp123-126

FRENZEL, E. & PFEFFER, E. (1982). Investigations on the mineral need of rainbow trout (Salmo gairdneri R.). Arch. Tierernahrung, 32: pp1-8

FURUKAWA, A. & TSUKAHARA, H. (1966). On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jpn. Soc. Sci. Fish., 32: pp502-506

GILLESPIE, D. C. & EVANS, R. E. (1979). Composition of granules from kidneys of rainbow trout (Salmo gairdneri) with nephrocalcinosis. J. Fish. Res. Board Can., 36: pp683-685

GÖHL, B. (1981). Tropical Feeds. FAO Animal Production and Health Series, 12, Rome: 529pp

GOLDBLATT, M. J., CONKLIN, D. E. & BROWN, W. D. (1979). Nutrient leaching from pelleted rations. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp117-130

GRAYTON, B. D. & BEAMISH, F. W. H. (1977). Effects of feeding frequency on food intake, growth and body composition of rainbow trout (Salmo gairdneri). Aquaculture, 11: pp159-172

GROPP, J., KOOPS, H., TIEWS, K. & BECK, H. (1979). Replacement of fish meal in trout feeds by other feedstuffs. In: T. V. R. Pillay and W. A. Dill (Editors), Advances in Aquaculture. Fishing News Books Ltd., England: pp596-601

GULBRANDSEN, K. E. & UTNE, F. (1977). The protein requirement on energy basis for rainbow trout (Salmo gairdneri). Fisk. Dir. Skr. Ernaring, 1: pp75-85

HALVER, J. E. (1975). Nutritional Research with Salmonids. Proceedings of the First International Conference on Aquaculture Nutrition, 1975, Lewes, Rehobath, Delaware: pp25-37

HALVER, J. E. (1976). Formulating practical diets for fish. J. Fish. Res. Board Can., 33: pp1032-1039

HARDY, R. W., MUGRDITCHIAN, D. S. & IWAOKA, W. T. (1983). Storage stability of lipids in a dry salmonid diet. Aquaculture, 34: pp239-246

HARDY, R. W., SHEARER, K. D. & SPINELLI, J. (1984). The nutritional properties of co-dried fish silage in rainbow trout (Salmo gairdneri) dry diets. Aquaculture, 38: pp35-44

HARRISON, J. G. & RICHARDS, R. H. (1979). The pathology and histopathology of nephrocalcinosis in rainbow trout, Salmo gairdneri Richardson, in fresh water. J. Fish Diseases, 2: pp1-12

HENDERSON, R. J. & SARGENT, J. R. (1985). Fatty acid metabolism in fish. In: C. B. Cowey, A. M. Mackie & J. G. Bell (Editors) Nutrition and Feeding in Fish, Academic Press, London: pp349-364

HENSON, J. N., BEESON, W. M. & PERRY, T. W. (1954). Vitamin, amino acid and antibiotic supplementation of corn-meat by-product rations for swine. J. Anim. Sci., 13: pp885-898

HICKLING, C. F. (1962). Fish Culture. Faber and Faber, London: 259pp

HIGGS, D. A., MARKERT, J. R., MACQUARRIE, D. W., MCBRIDE, J. R. DOSANJH, B. S., NICHOLS, C. & HOSKINS, G. (1979). Development of practical dry diets for Coho salmon, Oncorhynchus kisutch, using poultry by-product meal, feather meal, soybean meal, and rapeseed meal as major protein sources. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp191-218

HILTON, J. W. (1983). Potential of freeze-dried worm meal as a replacement for fish meal in trout diet formulations. Aquaculture, 32: pp277-283

HILTON, J. W., ATKINSON, J. L. & SLINGER, S. J. (1982). Maximum tolerable level, digestion, and metabolism of D-glucose (cerelose) in rainbow trout (Salmo gairdneri) reared on a practical trout diet. Can. J. Fish. Aquat. Sci., 39: pp1229-1234

HILTON, J. W., CHO, C. Y. & SLINGER, S. J. (1977). Factors affecting the stability of supplemental ascorbic acid in practical trout diets. J. Fish. Res. Board Can., 34: pp683-687

HILTON, J. W., CHO, C. Y. & SLINGER, S. J. (1981). Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (Salmo gairdneri R.). Aquaculture, 25: pp185-194

HILTON, J. W. & DIXON, D. G. (1982). Effect of increased liver glycogen and liver weight on liver function in rainbow trout, Salmo gairdneri Richardson: recovery from anaesthesia and plasma ^{35}S-sulphobromophthalein clearance. J. Fish Diseases, 5: pp185-195

HMSO (1973). The Fertilizers and Feeding Stuffs Regulations. HMSO 1521, London

HOKANSON, K. E. F., KLEINER, C. F. & THORSLUND, T. W. (1977). Effect of constant temperatures and diel temperature fluctuations on specific growth and mortality rates and yield of juvenile rainbow trout, Salmo gairdneri. J. Fish. Res. Board Can., 34: pp639-648

HUNG, S. S. O., CHO, C. Y. & SLINGER, S. J. (1980). Measurement of oxidation in fish oil and its effect on vitamin E nutrition of rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci., 37: pp1248-1253

HUNG, S. S. O., CHO, C. Y. & SLINGER, S. J. (1981). Effect of oxidized fish oil, DL-α-tocopherol acetate and ethoxyquin supplementation on the vitamin E nutrition of rainbow trout (Salmo gairdneri) fed practical diets. J. Nutr., 111: pp648-657

HUNG, S. S. O. & SLINGER, S. J. (1980). Effect of oxidized fish oil on the ascorbic acid nutrition of rainbow trout (Salmo gairdneri). Int. J. Vitamin Nutr. Res., 50: pp393-400

HUNG, S. S. O. & SLINGER, S. J. (1982). Effect of dietary vitamin E on rainbow trout (Salmo gairdneri). Int. J. Vitamin Nutr. Res., 52: pp120-125

HYVARINEN, A. & NIKKILA, E. (1962). Specific determination of blood glucose with o-toluidine. Clin. Chim. Acta, 7: pp140-143

ICHHPONANI, J. S. & LODHI, G. N. (1976). Re-cycling animal waste as feed: a review. Indian J. Anim. Sci., 46: pp237-243

JACKSON, A. J., CAPPER, B. S. & MATTY, A. J. (1982). Evaluation of some plants protein in complete diets for the tilapia Sarotherodon mossambicus. Aquaculture, 27: pp97-109

JACKSON, N. & FULTON, R. B. (1971). Composition of feather and offal meal and its value as a protein supplement in the diet of broilers. J. Sci. Fd. Agric., 22: pp38-42

JACKSON, A. J., KERR, A. K., BULLOCK, A. M. (1984a). Fish silage as a dietary ingredient for salmon. II. Preliminary growth findings and nutritional pathology. Aquaculture, 40: pp283-291

JACKSON, A. J., KERR, A. K. & COWEY, C. B. (1984b). Fish silage as a dietary ingredient for salmon. I. Nutritional and storage characteristics. Aquaculture, 38: pp211-220

JAUNCEY, K. & ROSS, B. (1982). A guide to Tilapia feeds and feeding. Institute of Aquaculture, University of Stirling, 111pp

JOBLING, M. (1983). A short review and critique of methodology used in fish growth and nutrition studies. J. Fish. Biol., 23: pp685-703

JOHNSEN, F. & SKREDE, A. (1981). Evaluation of fish viscera silage as a feed resource. Acta Agric. Scand., 31: pp21-28

KAUSHIK, S. & LUQUET, P. (1976). Etude de la digestibilité des acides aminés de régimes à base de zéine chez la truite arc-en-ciel. Ann. Hydrobiol., 7: pp11-19

KAUSHIK, S. J. & LUQUET, P. (1980). Influence of bacterial protein incorporation and of sulphur amino acid supplementation to such diets on growth of rainbow trout, Salmo gairdneri Richardson. Aquaculture, 19: pp163-175

KETOLA, H. G. (1975). Mineral supplementation of diets containing sybean meal as a source of protein for rainbow trout. Prog. Fish Cult., 37: pp73-75

KETOLA, H. G. (1982). Aminoacid nutrition of fish: requirements and supplementation of diets. Comp. Biochem. Physiol., 73B: pp17-24

KIHLBERG, R. (1972). The microbe as a source of food. Ann. Rev. Microbiol., 26: p427-466

KING, R. H. & CAMPBELL, R. G. (1978). Blood meal as a source of protein for grower/finisher pigs. Anim. Feed. Sci. Technol., 3: pp191-200

KNOX, D., COWEY, C. B. & ADRON, J. W. (1981). Studies on the nutrition of salmonid fish. The magnesium requirement of rainbow trout, Salmo gairdneri. Br. J. Nutr., 45: pp137-148

KOOPS, H., TIEWS, K., GROOP, J. & BECK, H. (1979). Krill in trout diets. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp281-292

KORN, S. & MACEDO, D. (1973). Determination of fat content in fish with a non-toxic, non-inflammable solvent. J. Fish. Res. Board Can., 30: pp1880-1881

LALL, S. P. (1979). Minerals in finfish nutrition. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Heeneman, Berlin: pp85-89

LALL, S. P. & BISHOP, F. J. (1977). Studies on mineral and protein utilization by atlantic salmon (Salmo salar) growth in sea water. Fish. Mar. Serv. Tech. Rep. 688: pp1-17

LARSEN, H. N. & SNIESZKO, S. F. (1961). Comparison of various methods of determination of haemoglobin in trout blood. Prog. Fish. Cult., 23: pp8-17

LEE, D. J. & PUTNAM, C. B. (1973). The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr., 103: pp916-922

LEE, D. J. & SINNHUBER, R. O. (1972). Lipid requirements. In: J. E. Halver (Editor), Fish Nutrition. Academic Press, New York, London: pp145-180

LEE, D. J. & WALES, J. H. (1973). Observed liver changes in rainbow trout (Salmo gairdneri) fed varying levels of casein-gelatin mixture and herring oil in experimental diets. J. Fish. Res. Board Can., 30: pp1017-1020

LILLIE, R. J., SIZEMORE, J. R. & DENTON, C. A. (1956). Feather meal in chick nutrition. Poultry Sci., 35: pp316-318

LOGANI, M. K., SHAH, B. & DAVIES, R. E. (1983). Effect of anti-oxidants on the photooxidation of fatty acids. Lipids, 18: pp259-263

LOVELL, T. (1979). Phosphorus in fish feeds. Commercial Fish Farmer and Aquac. News, Vo. 5: 5pp

LOVELL, T. (1981). How important is fishmeal in fish feeds? Aquaculture Magazine, 7: pp36-37

LOWE-JINDE, L. & NIIMI, A. J. (1983). Influence of sampling on the interpretation of haematological measurements of rainbow trout, Salmo gairdneri. Can. J. Zool., 61: pp396-402

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: pp265-275

LOYACANO, Jr., H. A. (1974). Pupae of face fly as food for channel catfish. Proc. Ann. Conf. Southeast Assoc. Game Fish Comm., 28: pp228-231

LUCE, W. G., PEO, E. R. & HUDMAN, D. B. (1970). Effect of amino acid supplementation of rations containing meat and bone scraps on rate of gain, feed conversion and digestibility of certain ration components for growing finishing swine. J. Anim. Sci., 31: pp521-527

LUKOWICZ, M. (1979). Experiences with Krill (Euphausia superba Dana) in the diet for young carp (Cyprinus carpio L.). In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp293-302

LYUBIMOVA, T. G., NAUMOV, A. G. & LAGUNOV, L. L. (1973). Prospects of the utilization of Krill and other non-conventional resources of the world ocean. FI:FMD/73/S-46, (FAO report)

MACKIE, I. M. (1982). General review of fish protein hydrolysates. Anim. Feed. Sci. Technol., 7: pp113-124

MACLEOD, M. (1978). Relationship between dietary sodium chloride, food intake and food conversion in the rainbow trout. J. Fish. Biol., 13: pp73-78

MARKERT, J. R., HIGGS, D. A., DYE, H. M. & MACQUARRIE, D. W. (1977). Influence of bovine growth hormone on growth rate, appetite, and food conversion of yearling coho salmon (Oncorhynchus kisutch) fed two diets of different composition. Can. J. Zool., 55: pp74-83

MATHIAS, J. A., MARTIN, J., YURKOWSKI, M., LARK, J. G. I., PARST, M. & TABACHECK, J. L. (1982). Harvest and nutritional quality of Gammarus lacustris for trout culture. Trans. Am. Fish. Soc., 111: pp83-89

MATTY, A. J. & SMITH, P. (1978). Evaluation of a yeast, a bacterium and an alga as a protein source for rainbow trout. I. Effect of protein level on growth, gross conversion efficiency and protein conversion efficiency. Aquaculture, 14: pp235-246

MATTY, A. J. & SMITH, P. (1979). Evaluation of a yeast, bacterium and alga as a protein source for rainbow trout. I. Effect of protein level on growth, gross conversion efficiency and protein conversion efficiency. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Heeneman, Berlin: pp261-273

MAYNARD, L. A. & LOOSLI, J. K. (1969). Animal Nutrition. McGraw Hill, New York, 6th edition: 613pp

MCDONALD, P., EDWARDS, R. A. & GREENHALGH, J. F. D. (1981). Animal Nutrition, 3rd edition. Longman Inc., New York: 479pp

MEADE, R. J. & TETER, W. S. (1957). The influence of calcium pentothenate, tryptophan and methionine supplementation and source of protein upon performance of growing swine fed corn-meat and bone scraps rations. J. Anim. Sci., 16: pp892-900

MENZIES, J. (1982). Report presented to Forrest Feeding Quality Meat and Bone Meal, Newarthill, Motherwell, Scotland

MERTZ, E. D. (1972). The protein and amino acid needs. In: J. E. Halver (Editor), Fish Nutrition. Academic Press, London: pp105-143

MEZINCESCU, M. D. & SZABO, F. (1936). Method for the determination of the non-protein nitrogen. J. Biol. Chem., 115: pp131-138

MEYERS, S. P. & RUTLEDGE, J. E. (1971). Economic utilization of crustacean meals. Feedstuffs, 43: pp46

MILLER, E. R. (1977). Formulating swine, poultry rations using flash-dried blood meal. Feedstuffs, 49: pp22-23

MILLER, W. R., HENDRICKS, A. C. & CAIRNS, Nr., J. (1983). Normal ranges for diagnostically important haematological and blood chemistry characteristics of rainbow trout (Salmo gairdneri). Can. J. Fish. Aquat. Sci., 40: pp420-425

MILLIKIN, M. R. (1982). Qualitative and quantitative nutrient requirements of fishes: a review. Fishery Bulletin, 80: pp655-686

MILNE, P. H. (1976). Engineering and the economics of aquaculture. J. Fish. Res. Board Can., 33: pp888-898

MIN, D. B., TICKNOR, D. & SCHWEIZER, D. (1982). Analysis of anti-oxidants in oil. JAOCS, 59: pp378-380

MORAN, E. T. & SUMMERS, J. D. (1968). Keratins as sources of protein for the growing chick. 5. Practical application of feather and hog hair meals in broiler diets: effects on growth, feed utilization and carcass quality. Poultry Sci., 47: pp940-945

MORRIS, W. C. & BALLOUN, S. L. (1973a). Effect of processing methods on utilization of feather meal by broiler chicks. Poultry Sci., 52: pp858-866

MORRIS, W. C. & BALLOUN, S. L. (1973b). Evaluation of five differently processed feather meals by nitrogen retention, net protein values, xanthine dehydrogenase activity and chemical analysis. Poultry Sci., 52: pp1075-1084

MUNRO, N. & FLECK, A. (1969). Analysis for nitrogenous constituents. In: H. N. Munro (Editor), Mammalian Protein Meals, Vol. 8. Academic Press Inc., London: pp435-437

NABER, E. C. & MORGAN, C. L. (1956). Feather meal and poultry meat scrap in chick starting rations. Poultry Sci., 35: pp888-895

NABER, E. C., TOUCHURN, S. P., BARNETT, B. D. & MORGAN, C. L. (1961). Effect of processing methods and amino acid supplementation on dietary utilization of feather meal protein by chicks. Poultry Sci., 40: pp1231-1245

NEWTON, G. L., BOORAM, C. V., BARKER, R. V. & HALE, O. M. (1977). Dried Hermetia illucens larvae meal as a supplement for swine. J. Anim. Sci., 44: pp395-400

NIIMI, A. J. (1972). Total nitrogen, nonprotein nitrogen, and protein content in largemouth bass (Micropterus salmoides) with reference to quantitative protein estimates. Can. J. Zool., 50: pp1607-1610

NOSE, T. (1974). Effects of aminoacid supplement to petroleum yeast on growth of rainbow trout fingerlings. I. A preliminary experiment. Bull. Freshwater Fishr. Res. Laboratory, 24: pp57-63

NOSE, T. (1979). Diet compositions and feeding techniques in fish culture with complete diets. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp191-218

NOSE, T. & ARAI, S. (1979). Recent advances in studies on mineral nutrition of fish in Japan. In: T. V. R. Pillay & W. A. Dill (Editors), Advances in Aquaculture, Fishing News Books, England: pp584-590

NOSE, T. & TOYAMA, K. (1966). Nutritional evaluation of brown meals as a protein source in diets for rainbow trout. Bull. Jpn. Soc. Sci. Fish., 49: pp1083-1087

OGINO, C. (1980). Requirements of carp and rainbow trout for essential amino acids. Bull. Jpn. Soc. Sci. Fish., 46: pp171-174

OGINO, C., TAKASHIMA, F. & CHIOU, J. Y. (1978). Requirement of rainbow trout for dietary magnesium. Bull. Jpn. Soc. Sci. Fish., 44: pp1105-1108

OGINO, C. & TAKEDA, H. (1978). Requirements of rainbow trout for dietary calcium and phosphorus. Bull. Jpn. Soc. Sci. Fish., 44: pp1019-1022

OGINO, C. & YANG, G-Y. (1978). Requirement of rainbow trout for dietary zinc. Bull. Jpn. Soc. Sci. Fish., 44: pp1015-1018

OPSTVEDT, J. (1974). Influence of residual lipids on the nutritive value of fish meal. VI. Effects of fat addition to diets high in fish meal on fatty acid composition and flavour quality of broiler meat. Acta Agric. Scand., 27: pp61-75

ORME, L. E. (1978). The status of coolwater fish diets. Am. Fish. Soc. Spec. Publ., 1: pp167-171

ØRSKOV, E. R. (1977). Nutritional principles and evaluation of by-products, waste products and new feeds for ruminants. Livestock Production Science, 4: pp165-175

PAPOUTSOGLOU, S. E. & PAPAPARASKEVA-PAPOUTSOGLOU, E. G. (1978). Comparative studies on body composition of rainbow trout (Salmo gairdneri R.) in relation to type of diet and growth rate. Aquaculture, 13: pp235-243

PEARSON, D. (1970). General methods. In: J. & A. Churchill (Editors), Chemical Analysis of Food, 6th edition, London: pp16-18

PFEFFER, E. (1977). Studies on the utilization of dietary energy and protein by rainbow trout (Salmo gairdneri) fed either by hand or by an automatic self-feeder. Aquaculture, 10: pp97-107

PFEFFER, E. (1982). Utilization of dietary protein by salmonid fish. Comp. Biochem. Physiol., 72B: pp51-57

PHILLIPS, A. M. (1956). The nutrition of trout. I. General feeding methods. Prog. Fish Cult., 18: pp113-118

PHILLIPS, A. M. & BALZER, G. C. (1957). The nutrition of trout. V. Ingredients for trout diets. Prog. Fish Cult., 19: pp158-167

PHILLIPS, A. M. & BROCKWAY, D. R. (1957). The nutrition of trout. IV. Vitamin requirements. Prog. Fish Cult., 19: pp119-123

PHILLIPS, A. M., LIVINGSTON, Jr., D. L. & POSTON, H. A. (1966). The effect of changes in protein quality, caloric sources and caloric levels upon the growth and chemical composition of brook trout. Fishery Res. Bull., 29: pp6-14

PHILLIPS, A. M. & PODOLIAK, H. A. (1957). The nutrition of trout. Prog. Fish Cult., 19: pp68-75

PHILLIPS, A. M., TUNISON, A. V. & BROCKWAY, D. R. (1948). The utilization of carbohydrates by trout. Fishery Res. Bull., 11: 44pp

PHILLIPSON, J. (1964). A miniature bomb calorimeter for small biological samples. OIKOS 15: pp130-139

POTTER, L. M. & SHELTON, J. R. (1978). Evaluation of hydrolyzed feather meal and zinc bacitracin supplements of various purities in diets of young turkeys. Poultry Sci., 57: pp947-953

RAI, P. S. & NETKE, S. P. (1973). Egg shell as a source of calcium for growing chicks. Ind. J. Poultry Sci., 8: pp7-10

RAILO, E., NIKINMAA, M. & SOIVIO, A. (1985). Effects of sampling on blood parameters in the rainbow trout, Salmo gairderni Richardson. J. Fish. Biol., 26: pp725-732

REFSTIE, T. & AUSTRENG, E. (1981). Carbohydrate in rainbow trout diets. III. Growth and chemical composition of fish from different families fed four levels of carbohydrate in the diet. Aquaculture, 25: pp35-49

REINITZ, G. (Unpublished). Fish Nutrition.

REINITZ, G. (1980). Acceptability of animal fats in diets for rainbow trout at two environmental temperatures. Prog. Fish Cult., 42: pp218-222

REINITZ, G. & HITZEL, F. (1980). Formulation of practical diets for rainbow trout based on desired performance and body composition. Aquaculture, 19: pp243-252

REINITZ, G. L., ORME, L. E., LEMM, C. A. & HITZEL, F. N. (1978a). Differential performance of four strains of rainbow trout reared under standardized conditions. Prog. Fish Cult., 40: pp21-23

REINITZ, G. L., ORME, L. E., LEMM, C. A. & HITZEL, F. N. (1978b). Influence of varying lipid concentrations with two protein concentrations in diets for rainbow trout (Salmo gairdneri). Trans. Am. Fish. Soc., 107: pp751-754

REINITZ, G. L. & YU, T. C. (1981). Effects of dietary lipids on growth and fatty acid composition of rainbow trout (Salmo gairdneri). Aquaculture, 22: pp359-366

ROACH, A. G., SANDERSON, P. & WILLIAMS, D. R. (1967). Comparison of methods for the determination of available lysine value in animal and vegetable proteins sources. J. Sci. Fd. Agric., 18: pp274-278

ROBERTS, P. (1983). The number of replicates and other considerations in the design of field trials. In: Recent Advances in Animal Nutrition: pp3-11

ROBERTS, R. J. (1978). Fish Pathology, Bailliere Tindall: 318pp

RUMSEY, G. L., CACADE, M., ZALL, R. R. & LISK, D. J. (1981). Dairy-processing wastes as a replacement protein source in diets to rainbow trout. Prog. Fish Cult., 43: pp86-88

RUMSEY, G. L., PAGE, J. W. & SCOTT, M. L. (1983). Methionine and cystine requirements of rainbow trout. Prog. Fish Cult., 45: pp139-143

RUNGRUANGSAK, K. & UTNE, F. (1981). Effect of different acidified wet feeds on protease activities in the digestive tract and on growth rate of rainbow trout (Salmo gairdneri Richardson). Aquaculture, 22: pp67-79

RYCHLY, J. (1980). Nitrogen balance in trout. II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. Aquaculture, 20: pp343-350

RYCHLY, J. & SPANNHOF, L. (1979). Nitrogen balance in trout. I. digestibility of diets containing varying levels of protein and carbohydrate. Aquaculture, 16: pp39-46

SAITO, A. & REGIER, L. W. (1971). Pigmentation of brook trout (Salvelinus fontinalis) by feeding dried crustacean waste. J. Fish. Res. Board Can., 28: pp509-512

SALONEN, K. et al. (1976). In: Yurkowski, M. & Tabachek, J. L. (1979). Proximate and aminoacid composition of some natural fish foods. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp435-448

SATIA, B. P. (1974). Quantitative protein requirements of rainbow trout. Prog. Fish Cult., 36: pp80-85

SCHMIDT, P. J. & BAKER, E. G. (1969). Indirect pigmentation of salmon and trout flesh with canthaxanthin. J. Fish. Res. Board Can., 26: pp357-360

SCHULZ, D., HARTFIELD, W. & GREUEL, E. (1982). Verwendung von Nebenprodukten tierischer hertzunft in der Ernährung von Regenbogenforellen, (Salmo gairdneri, R.). II. Einsatz von Blut-Feder-und Geflügelschlachtbfallmehl sowie Gelatine in einer gereinigten Diät. Z-Tierphysiol-Tierernanahrg. U. Futtermittelkde. 48: pp265-275

SCHULZ, E. & OSLAGE, H. J. (1976). Composition and nutritive value of single-cell protein (SCP). Anim. Feed. Sci. Technol., 1: pp9-24

SHANG, Y. C. (1981). Aquaculture Economics: Basic Concepts and Methods of Analysis. Westview Press, Boulder, Colorado: 153pp

SHIBATA, N. (1983). Effect of fishing season on lipid content and composition of antarctic krill. Bull. Jpn. Soc. Sci. Fish., 49: pp259-264

SIMPSON, K. L. & KAMATA, T. (1979). Use of carotenoids in fish feeds. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp416-424

SINGH, R. P. & NOSE, T. (1967). Digestibility of carbohydrates in young rainbow trout. Bull. Freshwat. Fish. Res. Lab. Tokyo, 17: pp21-25

SKREDE, A., KROGDAHL, Å. & AUSTRENG, E. (1980). Digestibility of amino acids in raw fish flesh and meat and bone meal for the chicken, fox, mink and rainbow trout. Tiereznahr Futtermittelk, 43: pp92-101

SLINGER, S. J., RAZZAQUE, A. & CHO, C. Y. (1979). Effect of feed processing and leaching on the losses of certain vitamins in fish diets. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp425-434

SMITH, R. R. (1971). A method for measuring digestibility and metabolizable energy of fish feed. Prog. Fish Cult., 33: pp132-134

SMITH, R. R. & RUMSEY, G. L. (1976). Nutrient utilization by fish. Proceedings, First International Symposium Feed Composition, Animal Nutrient Requirements, and Computerization of Diets, July 11-16, 1976, Utah State University, Logan, Utah, USA: pp320-326

SOEVIK, T., OPSTVEDT, J. & BRAEKKAN, O. R. (1981). Biological availability of iron found in fish meals. J. Sci. Food Agric. 32: pp1063-1068

SOIVIO, A. & NYHOLM, K. (1973). Notes on haematocrit determinations on rainbow trout, Salmo gairdneri. Aquaculture, 2: pp31-35

SPANNHOF, L. & KÜHNE, H. (1977). Untersuchungen zur verwertung verschiedener futtermischungen durch europäische aale (Anguilla anguilla). Arch. Tierernähr, 27: pp517-531

SPANNHOF, L. & PLANTIKOW, H. (1983). Studies on carbohydrate digestion in rainbow trout. Aquaculture, 30: pp95-108

SPINELLI, J. & DASSOW, J. A. (1979). Fish proteins: their modification and potential uses in the food industry. In: Chemistry and Biochemistry of Marine Fish Products: pp13-25

SPINELLI, J., LEHMAN, L. & WIEG, D. (1974). Composition, processing and utilization of red crab (Pleuroncodes planipes) as an aquaculture feed ingredient. J. Fish. Res. Board Can., 31: pp1025-1029

SPINELLI, J., MAHNKEN, C. & STEINBERG, M. (1979). Alternative sources of protein for fish meal in salmonid diets. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp20-23

STAFFORD, E. A. (1984). The use of earthworms as a feed for rainbow trout (Salmo gairdneri). PhD thesis, University of Stirling: 242pp

STAFFORD, E. A. & TACON, A. G. J. (1985). The nutritional evaluation of dried earthworm meal (Eisenia foetida, Savigny, 1826) included at low levels in production diets for rainbow trout, Salmo gairdneri Richardson. Aquaculture and Fisheries Management, 16

STANSBY, M. E. (1983). Properties of fish oils and their application to handling of fish and to nutritional and industrial use. In: Chemistry and Biochemistry of Marine Food Products: pp75-92

STOCKLAND, W. L., MEADE, R. J. & NORDSTROM, J. W. (1978). Lysine, methionine and tryptophan supplementation of a corn-meat and bone meal for growing swine. J. Anim. Sci., 32: pp262-267

SUMMERS, J. D., SLINGER, S. J. & ASHTON, G. C. (1964). Evaluation of meat meal as a protein supplement for the chick. Can. J. Anim. Sci., 44: pp228-234

TACON, A. G. J. (1978/1979). Activated sewage sludge, a potential animal foodstuff. II. Nutritional characteristics. Agric. Environm., 4: pp271-279

TACON, A. G. J. (1979a). The nutritional evaluation of animal and food processing wastes. Third International Conference on Effluent Treatment in the Biochemical Industries, London, 7 November 1979.

TACON, A. G. J. (1979b). The use of activated sludge-single cell protein (ASCP), derived from the treatment of domestic sewage in trout diets. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technolgoy, Vol. 2, H. Heeneman, Berlin: pp249-267

TACON, A. G. J. (1981). The possible substitution of fish meal in fish feeds. Proceedings of the SMBA/HIDB Fish Farming Meeting, Oban, 26-27 February 1981: pp46-56

TACON, A. G. J. (1982a). Utilization of chick hatchery waste: the nutritional characteristics of day-old chicks and egg shells. Agricultural Wastes, 4: pp335-343

TACON, A. G. J. (1982b). Preliminary report presented to Forrest Feeding Quality Meat and Bone Meal, Newarthill, Motherwell, Scotland

TACON, A. G. J. (1983). Replacement of marine fish protein in salmonid diets. Report presented to Ministry of Agriculture, Fisheries and Food: 98pp

TACON, A. G. J. & BEVERIDGE, M. M. (1982). Effects of dietary trivalent chromium on rainbow trout. Nutr. Rep. Int., 25: pp49-56

TACON, A. G. J. & COOKE, D. J. (1980). Nutritional value of dietary nucleic acids to trout. Nutr. Rep. Int., 22: pp631-640

TACON, A. G. J. & DE SILVA, S. S. (1983). Mineral composition of some commercial fish feeds available in Europe. Aquaculture, 31: pp11-20

TACON, A. G. J. & FERNS, P. N. (1976). The use of activated sludge from domestic sewage in trout diets. Nutr. Rep. Int., 13: pp549-562

TACON, A. G. J. & FERNS, P. N. (1978/1979). Activated sewage sludge, a potential animal foodstuff. I. Proximate and mineral content: seasonal variation. Agric. Environm., 4: pp257-269

TACON, A. G. J., HAASTER, J. V., FEATHERSTONE, P. B., KERR, K. & JACKSON, A. J. (1983a). Studies on the utilization of full-fat soybean and solvent extracted soybean meal in a complete diet for rainbow trout. Bull. Jpn. Soc. Sci. Fish., 49: pp1437-1443

TACON, A. G. J. & JACKSON, A. J. (1985). Utilization of conventional and unconventional protein sources in practical fish feeds. In: C. B. Cowey, A. M. Mackie & J. G. Bell (Editors), Nutrition and Feeding in Fish, Academic Press, London: pp119-145

TACON, A. G. J. & RODRIGUES, A. M. P. (1984). Comparison of chromic oxide, crude fibre, polyethylene and acid-insoluble ash as dietary markers for the estimation of apparent digestibility coefficients in rainbow trout. Aquaculture, 43: pp391-399

TACON, A. G. J., STAFFORD, E. A. & EDWARDS, C. A. (1983b). A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture, 35: pp187-199

TACON, A. G. J., WEBSTER, J. L. & MARTINEZ, C. A. (1984). Use of solvent extracted sunflower seed meal in complete diets for fingerling rainbow trout (Salmo gairdneri Richardson). Aquaculture, 43: pp381-389

TAKEUCHI, T. & WATANABE, T. (1976). Nutritive value of ω3 highly unsaturated fatty acids in pollock liver oil for rainbow trout. Bull. Jpn. Soc. Sci. Fish., 42: pp907-919

TAKEUCHI, T. & WATANABE, T. (1977). Effect of eicosapentaenoic acid and docosahexaenoic acid in pollock liver oil on growth and fatty acid composition of rainbow trout. Bull. Jpn. Soc. Sci. Fish., 43: pp947-953

TAKEUCHI, T., WATANABE, T. & OGINO, C. (1978a). Supplementary effect of lipids in a high protein diet of rainbow trout. Bull. Jpn. Soc. Sci. Fish., 44: pp677-681

TAKEUCHI, T., WATANABE, T. & OGINO, C. (1978b). Optimum ratio of protein to lipid in diets of rainbow trout. Bull. Jpn. Soc. Sci. Fish., 44: pp683-688

TAKEUCHI, T., WATANABE, T. & OGINO, C. (1978c). Use of hydrogenated fish oil and beef tallow as a dietary energy source for carp and rainbow trout. Bull. Jpn. Soc. Sci. Fish., 44: pp875-881

TAKEUCHI, T., WATANABE, T., OGINO, C., SAITO, M., NISHIMURA, K., & NOSE, T. (1981). A long-term feeding with rainbow trout by a low protein diet with a high energy value. Bull. Jpn. Soc. Sci. Fish., 45: pp637-643

TAKEUCHI, T., YOKOYAMA, M., WATANABE, T. & OGINO, C. (1978d). Optimum ratio of dietary energy to protein for rainbow trout. Bull. Jpn. Soc. Sci. Fish, 44: pp729-732

TATTERSON, I. N. (1982). Fish silage - preparation, properties and uses. Animal Fd. Sc. Technol., 7: pp153-159

TATTERSON, I. N. & WINDSOR, M. L. (1974). Fish silage. J. Sci. Food Agric., 25: pp369-379

THOMAS, V. M. & BEESON, W. M. (1977). Feather meal and hair meal as protein sources for steer calves. J. Anim. Sci., 46: pp819-825

TIEWS, K., GROPP, J. & KOOPS, H. (1976). On the development of optimal rainbow trout pellet feeds. Arch. Fisch. Wiss., 27: pp1-29

TIEWS, K., KOOPS, H., GROPP, J. & BECK, H. (1979). Compilation of fish meal-free diets obtained in rainbow trout (Salmo gairdneri) feeding experiments at Hamburg. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp219-228

TORRISSEN, O. & BRAEKKAN, O. R. (1979). The utilization of astaxanthin forms by rainbow trout (Salmo gairdneri). In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 2, H. Heeneman, Berlin: pp377-382

TORRISSEN, O., TIDEMANN, E., HANSEN, F. & RAA, J. (1981/1982). Ensiling in acid - a method to stabilize astaxanthin in shrimp processing by-products and improve uptake of this pigment by rainbow trout (Salmo gairdneri). Aquaculture, 26: pp77-83

TOYAMA, K., HOSHI, M., KAMIOKA, Y., OHTAKA, T., YAMAZAKI, M. & AOE, H. (1983). Amino acid composition of fish meals and fish solubles prepared from putrified raw fish. Bull. Jpn. Soc. Sci. Fish., 49: pp1221-1231

TUSÉ, D. (1984). Single-cell protein: current status and future prospectus. CRC Critical Reviews in Fd. Sci. Nutr., Vol.19: pp273-325

WALKER, N. (1977). The composition of processed abattoir blood and its use in finishing-pig diets. Anim. Feed Sci. Technol., 2: pp21-30

WATSON, M. J. (1985). Aspects of aminoacid metabolism in teleost fish. In: C. B. Cowey, A. M. Mackie & J. G. Bell (Editors), Nutrition and Feeding in Fish, Academic Press, London: pp47-67

WATANABE, T. (1982). Lipid nutrition in fish. Comp. Biochem. Physiol., 73B: pp3-15

WATANABE, T., KOBAYASHI, T., UTSUE, O. & OGINO, C. (1974a). Effect of dietary methyl limolenate on fatty acid composition of lipids in rainbow trout. Bull. Jpn. Soc. Sci. Fish., 40: pp393-397

WATANABE, T., NANRI, H., SATOH, S., TAKEUCHI, M. & NOSE, T. (1983). Nutritional evaluation of brown meals as a protein source in diets for rainbow trout. Bull. Jpn. Soc. Sci. Fish., 49: pp1083-1087

WATANABE, T., TAKASHIMA, F. & OGINO, C. (1974b). Effect of dietary methyl limolenate on growth of rainbow trout. Bull. Jpn. Soc. Sci. Fish., 40: pp181-188

WATANABE, T., TAKEUCHI, T. & OGINO, C. (1979). Studies on the sparing effect of lipids on dietary protein in rainbow trout (Salmo gairdneri). In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Heeneman, Berlin: pp113-126

WEATHERLEY, A. H. & GILL, H. S. (1983). Protein, lipid, water and caloric contents of immature rainbow trout, Salmo gairdneri Richardson, growing at different rates. J. Fish Biol., 23: pp653-673

WEDEMEYER, G. A. & NELSON, N. C. (1975). Statistical methods for estimating normal blood chemistry ranges and variance in rainbow trout (Salmo gairdneri), Shasta Strain. J. Fish. Res. Board Can., 32: pp551-554

WESSELS, J. P. H. (1972). Study of the protein quality of different feather meals. Poultry Sci., 51: pp537-541

WILDER, O. H. M. (1953). Feathers supply protein for poultry feed. Poultry Processing MKTNG, 59: pp11+27

WILTON, R. P. (1985). Amino acid and protein requirement of fish. In: C. B. Cowey, A. M. Mackie & J. G. Bell (Editors), Nutrition and Feeding in Fish, Academic Press, London: pp1-16

WINDELL, J. T., FOLTZ, J. W. & SAROKON, J. A. (1978). Methods of faecal collection and nutrient leaching in digestibility studies. Prog. Fish Cult., 40: pp51-55

WINDSOR, M. & BARLOW, S. (1981). Introduction to fishery by-.products. Fishing News Books Ltd., Farnham, England: 187pp

WOJNO, T. & DABROWSKA, H. (1984a). Próba zastosowania maczki z Odpadów drobiowych oraz dabatku wybranych ami nokwasów syntetycznych w zywieniu pstraga reczowego (Salmo gairdneri Rich.). Roczniki Nauk Rolniczych HT100: pp143-156

WOJNO, T. & DABROWSKA, H. (1984b). Badania nad zastosowaniem maczki z Kryla w zywieniu pstraga teczowego (Salmo gairdneri Rich.). Roczniki Nauk Rolniczych 100 z 4

WEATHERLEY, A. H. & GILL, H. S. (1983). Protein, lipid, water and caloric contents of immature rainbow trout, Salmo gairdneri Richardson, growing at different rates. J. Fish Biol., 23: pp653-673

WEDEMEYER, G. A. & NELSON, N. C. (1975). Statistical methods for estimating normal blood chemistry ranges and variance in rainbow trout (Salmo gairdneri), Shasta Strain. J. Fish. Res. Board Can., 32: pp551-554

WESSELS, J. P. H. (1972). Study of the protein quality of different feather meals. Poultry Sci., 51: pp537-541

WILDER, O. H. M. (1953). Feathers supply protein for poultry feed. Poultry Processing MKTNG, 59: pp11+27

WILTON, R. P. (1985). Amino acid and protein requirement of fish. In: C. B. Cowey, A. M. Mackie & J. G. Bell (Editors), Nutrition and Feeding in Fish, Academic Press, London: pp1-16

WINDELL, J. T., FOLTZ, J. W. & SAROKON, J. A. (1978). Methods of faecal collection and nutrient leaching in digestibility studies. Prog. Fish Cult., 40: pp51-55

WINDSOR, M. & BARLOW, S. (1981). Introduction to fishery by-.products. Fishing News Books Ltd., Farnham, England: 187pp

WOJNO, T. & DABROWSKA, H. (1984a). Próba zastosowania maczki z Odpadów drobiowych oraz dabatku wybranych ami nokwasów syntetycznych w zywieniu pstraga reczowego (Salmo gairdneri Rich.). Roczniki Nauk Rolniczych HT100: pp143-156

WOJNO, T. & DABROWSKA, H. (1984b). Badania nad zastosowaniem maczki z Kryla w zywieniu pstraga teczowego (Salmo gairdneri Rich.). Roczniki Nauk Rolniczych 100 z 4

YAMAGUCHI, K., MIKI, W., TORIU, N., KONDO, Y., MURAKAMI, M., KONOSU, S., SATAKE, M. & FUJITA, T. (1983). The composition of carotenoid pigments in the antarctic krill Euphausia superba. Bull. Jpn. Soc. Sci. Fish., 49: pp1411-1415

YU, T. C. & SINNHUBER, R. O. (1976). Growth response of rainbow trout (Salmo gairdneri) to dietary ω3 and ω6 fatty acids. Aquaculture, 8: pp309-317

YU, T. C. & SINNHUBER, R. O. (1979). Effect of dietary ω3 and ω6 fatty acids on growth and feed conversion efficiency of coho salmon Oncorhynchus kisutch). Aquaculture, 16: pp31-38

YU, T. C. & SINNHUBER, R. O. (1981). Use of beef tallow as an energy source in coho salmon (Oncorhynchus kisutch) rations. Can. J. Fish. Aquat. Sci., 38: pp367-370

YU, T. C., SINNHUBER, R. O. & PUTNAM, G. B. (1977). Use of swine fat as an energy source in trout rations. Prog. Fish Cult., 39: pp95-97

YURKOWSKI, M. & TABACHEK, J. L. (1979). Proximate and amino acid composition of some natural fish foods. In: J. E. Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Heeneman, Berlin: pp435-448

ZAUGG, W. S. & MCLAIN, L. R. (1969). Inorganic salt effects on growth, salt water adaptation and gill ATP-ase of pacific salmon. In: O. W. Neuhaus and J. E. Halver (Editors), Fish in Research. Academic Press, New York: pp293-306

APPENDICES

APPENDIX I Growth performance, feed utilization efficiency, liver somatic index and blood parameters of rainbow trout replicates (280 fish, 35g) fed the Poultry by-products and hydrolysed feather meal experimental diets after 18 weeks

Diet	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Mean initial weight (g)	34.38	34.97	35.48	35.57	34.16	35.11	34.65	34.28	35.01	35.66	34.78	34.83	35.20	36.55
Mean final weight (g)	61.54	63.24	77.78	81.51	93.86	85.84	124.06	112.87	119.46	123.29	133.26	139.93	127.32	127.38
Weight gain (%)	79.00	80.84	119.22	129.15	174.77	144.49	258.04	229.26	241.22	245.74	283.15	301.75	261.71	248.51
Specific growth rate (%/day)	0.46	0.47	0.62	0.66	0.81	0.71	1.02	0.95	0.98	1.00	1.07	1.11	1.03	1.00
Food intake (mg/day)	1073	990	1046	1094	1142	1156	1331	1268	1307	1397	1394	1454	1377	1418
Protein intake (mg/day)	438	404	428	448	471	477	544	518	545	582	569	593	559	575
Weight gain (mg/day)	217	226	338	368	478	406	715	629	676	701	788	841	737	727
Food conversion ratio	4.94	4.38	3.09	2.97	2.38	2.84	1.86	2.01	1.93	1.99	1.76	1.72	1.86	1.95
Protein efficiency ratio	0.50	0.56	0.79	0.82	1.01	0.85	1.31	1.21	1.24	1.21	1.38	1.42	1.32	1.26
Nitrogen intake (mg/day)	70.08	64.64	68.48	71.68	75.36	76.32	87.04	82.88	87.20	93.12	91.04	94.88	89.44	92.00
Nitrogen deposition (mg/day)	3.09	3.50	4.93	5.13	6.34	5.32	8.21	7.59	7.75	7.53	8.65	8.86	8.24	7.90
Apparent net protein utilization (%)	4.41	5.41	7.20	7.16	8.41	6.97	9.43	9.16	8.89	8.09	9.50	9.34	9.21	8.59
Apparent protein digestibility (%)	82.88	84.46	79.26	83.18	80.08	85.99	82.28	86.65	84.41	87.25	82.97	87.55	85.13	85.87
Apparent lipid digestibility (%)	74.44	75.02	70.77	71.53	73.86	75.11	75.83	76.58	75.94	76.99	77.31	76.95	79.28	80.64
Apparent organic matter digestibility (%)	55.45	60.07	59.33	60.03	59.94	61.86	65.57	69.67	67.50	67.50	60.24	68.39	66.78	59.88
Liver somatic index	0.96	1.05	1.09	1.04	1.20	0.98	1.10	1.03	1.11	1.00	1.18	1.07	1.20	1.25
Haematocrit (%)	23.75	31.25	30.13	33.38	33.38	36.25	35.38	35.38	35.00	37.13	34.63	36.25	31.88	37.75
Glucose in plasma (mg/100 cm^3)	58.46	92.20	102.09	89.04	101.67	95.84	96.37	106.71	81.05	102.82	91.05	106.02	101.12	93.76

APPENDIX II Overall mean weight gain (g) of rainbow trout replicates at successive fortnightly intervals over the Poultry by-product and hydrolysed feather meal experimental test period

Week	1		2		3		4		5		6		7		No feeding
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
0	34.38	34.97	35.48	35.51	34.16	35.11	34.65	34.28	35.01	35.66	34.78	34.83	35.20	36.55	34.25
2	43.50	41.00	41.50	43.82	44.00	41.50	41.75	42.25	44.50	43.25	41.75	43.50	44.50	46.25	27.89
4	45.00	41.50	46.00	47.20	45.40	46.80	47.30	45.80	47.30	48.40	41.50	47.50	48.50	49.80	24.72
6	49.50	45.00	48.50	48.75	50.25	51.05	57.00	50.53	50.50	56.26	54.74	56.00	60.00	55.00	21.33
8	52.90	54.00	49.00	55.31	57.00	58.42	64.50	58.95	63.00	66.47	65.79	65.79	66.25	66.84	20.00
10	57.22	54.71	53.50	61.90	63.00	64.21	74.00	70.56	71.75	79.41	76.32	75.79	78.42	79.44	20.41
12	60.28	54.71	61.84	65.31	68.00	68.95	82.75	78.89	80.00	88.82	87.90	91.18	87.37	88.33	25.00
14	59.17	57.06	67.63	71.88	77.00	76.32	97.22	88.33	93.50	102.35	108.42	108.13	99.47	105.00	21.25
16	61.39	63.44	75.26	78.44	87.90	82.90	108.89	111.67	106.05	114.71	122.78	129.38	115.26	118.82	20.00
18	61.54	63.24	77.78	81.51	93.86	85.84	124.06	112.87	119.46	123.29	133.26	139.93	127.32	127.38	19.15

APPENDIX III Proximate composition and Cr_2O_3 content of faeces of rainbow trout replicates at the end of the Poultry by-product and hydrolysed feather meal experimental test period

Faeces composition (% dry weight)	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Moisture (%)	10.17	10.81	9.16	9.65	9.09	10.73	10.09	12.90	12.18	14.20	12.99	14.88	16.27	12.55
Crude protein (N x 6.25)	15.61	15.79	20.10	16.58	20.28	14.97	20.64	17.66	20.00	16.35	17.37	15.96	18.10	14.27
Lipid (%)	10.10	11.00	12.21	12.10	11.51	12.50	12.31	13.51	13.10	12.53	10.05	12.80	11.00	8.53
Ash (%)	42.26	42.99	43.67	42.90	38.57	37.81	37.60	40.51	36.90	32.94	24.72	28.94	17.21	20.02
Cr_2O_3	1.05	1.17	1.16	1.18	1.21	1.27	1.43	1.62	1.57	1.57	1.30	1.63	1.53	1.27

APPENDIX IV Carcass composition of rainbow trout replicates (280 fish, 35g) at the end of the Poultry by-product and hydrolysed feather meal experimental test period

Proximate composition (% wet weight)	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Moisture (%)	69.07	69.99	67.72	69. 89	67.43	67.65	65.84	66.72	66.70	64.77	64.06	65.52	63.02	63.48
Crude protein (N x 6.25)	19.32	17.50	19.85	17.01	19.08	17.37	17.37	17.39	18.07	16.74	18.52	15.85	17.50	17.19
Lipid (%)	8.28	10.10	9.65	10.31	11.53	12.61	14.16	14.65	12.91	15.93	16.07	16.01	15.87	17.63
Ash (%)	3.79	3.53	3.62	3.64	3.56	3.25	3.31	2.85	3.26	2.97	3.16	2.98	3.26	3.14
	100.57	101.12	101.48	100.85	101.60	100.88	100.68	101.60	100.94	100.41	101.81	100.36	99.70	101.64

APPENDIX V Growth performance, feed utilization efficiency, liver somatic index and blood parameters of rainbow trout replicates (280 fish, 23g) fed the Fish meals experimental diets after 18 weeks

Diet	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Mean initial weight (g)	23.79	23.33	23.20	23.34	23.35	23.64	23.21	23.20	23.25	23.82	23.46	23.05	23.34	23.07
Mean final weight (g)	69.88	77.50	65.47	63.62	68.76	70.43	50.86	54.30	50.35	56.56	55.11	47.78	59.67	51.93
Weight gain (%)	193.73	232.19	182.19	170.00	194.47	197.92	119.12	134.05	116.55	137.44	134.91	107.28	155.65	125.09
Specific growth rate (%/day)	0.86	0.96	0.82	0.79	0.85	0.86	0.62	0.67	0.61	0.68	0.68	0.58	0.74	0.65
Food intake (mg/day)	905	967	894	865	909	1169	757	788	733	826	827	781	875	853
Protein intake (mg/day)	375	401	370	358	379	487	310	323	304	343	338	320	361	352
Weight gain (mg/day)	366	430	336	320	360	371	219	247	215	260	251	196	288	229
Food conversion ratio	2.47	2.25	2.66	2.70	2.53	3.15	3.46	3.19	3.41	3.18	3.29	3.98	3.04	3.73
Protein efficiency ratio	0.98	1.07	0.91	0.89	0.95	0.76	0.71	0.77	0.71	0.76	0.74	0.61	0.80	0.65
Nitrogen intake (mg/day)	60.00	64.16	59.20	57.28	60.64	77.92	49.60	51.68	48.64	54.88	54.08	51.20	57.76	56.32
Nitrogen deposition (mg/day)	6.10	6.70	5.68	5.59	5.94	4.76	4.42	4.78	4.42	4.74	4.64	3.83	4.99	4.07
Apparent net protein utilization (%)	10.17	10.44	9.60	9.76	9.79	6.11	8.91	9.25	9.11	9.64	8.58	7.48	8.64	7.23
Apparent protein digestibility (%)	85.09	87.45	77.05	76.37	70.43	74.17	75.41	73.73	77.70	78.37	75.81	76.38	78.40	77.78
Apparent lipid digestibility (%)	91.18	90.86	72.86	75.80	72.43	79.36	85.85	86.39	77.49	79.02	90.38	83.17	61.21	58.73
Apparent organic matter digestibility (%)	68.54	71.81	52.22	56.46	42.49	46.81	56.06	54.77	52.37	54.64	53.60	54.00	59.54	56.95
Liver somatic index	1.45	1.23	1.44	1.38	1.42	1.45	1.28	1.06	1.06	1.05	1.27	1.25	1.15	1.37
Haematocrit (%)	31.50	34.50	39.60	37.50	34.60	34.60	34.50	33.70	36.00	34.50	40.50	33.50	33.20	33.20
Haemoglobin (g/100 cm^3)	5.73	5.19	5.78	5.65	5.47	5.47	5.61	4.70	5.96	4.99	5.75	3.88	5.00	5.00

APPENDIX VI Overall mean weight gain (g) of rainbow trout replicates at successive fortnightly intervals over the Fish meals experimental test period

Week	1		2		3		4		5		6		7		No feeding
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
0	23.79	23.33	23.20	23.34	23.35	23.64	23.21	23.20	23.25	23.28	23.46	23.05	23.34	23.07	23.44
2	34.00	30.25	30.50	28.75	29.25	29.25	26.50	27.25	27.00	28.50	29.25	26.50	26.50	27.00	26.25
4	37.50	34.50	35.50	34.00	35.00	34.50	31.00	32.00	33.00	34.50	35.25	31.25	33.68	31.75	26.84
6	39.75	36.75	40.50	39.00	39.50	39.50	34.50	35.50	35.50	39.00	37.50	33.50	38.33	34.50	26.94
8	42.50	39.25	44.00	43.00	43.00	42.25	36.50	37.75	36.75	42.25	40.25	36.25	41.11	36.00	25.88
10	50.00	45.78	48.50	47.00	48.68	47.00	40.00	42.89	39.75	46.25	44.25	40.00	43.33	39.21	24.70
12	54.70	52.22	54.21	53.42	51.87	51.84	44.50	46.31	43.50	49.75	48.94	43.75	49.41	42.77	23.53
14	60.58	60.00	63.68	56.84	63.43	56.06	46.25	51.05	47.89	52.00	52.77	46.00	52.94	47.22	22.00
16	66.00	67.77	61.11	58.75	65.66	60.00	46.17	50.58	47.64	48.00	53.52	46.84	57.91	55.00	22.00
18	69.88	77.50	65.47	63.62	68.76	70.43	50.86	54.30	50.35	56.56	55.11	47.78	59.67	51.93	17.97

APPENDIX VII Proximate composition and Cr_2O_3 content of faeces of rainbow trout replicates at the end of the Fish meals experimental test period

Faeces composition (% dry weight)	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Moisture (%)	10.30	7.63	3.22	4.71	7.08	5.97	5.35	4.25	4.90	3.28	2.98	6.22	5.93	3.05
Crude protein (N x 6.25)	17.48	18.31	19.79	22.38	21.44	20.23	22.78	20.08	19.25	19.58	21.04	21.21	21.81	21.13
Lipid (%)	3.29	3.80	18.26	17.12	13.65	14.38	15.43	16.38	9.37	9.32	7.61	5.32	10.99	11.01
Ash (%)	19.45	18.18	40.77	45.79	54.63	46.90	42.86	42.29	46.38	50.03	50.02	52.26	46.04	45.72
Cr_2O_3	1.58	1.76	1.02	1.12	0.87	0.94	1.13	1.10	1.02	1.07	1.00	1.01	1.20	1.13

APPENDIX VIII Carcass composition of rainbow trout replicates (280 fish, 23g) at the end of the Fish meals experimental test period

Proximate composition (% wet weight)	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Moisture (%)	69.30	68.45	70.84	71.32	69.58	71.95	74.65	72.74	74.12	73.91	74.68	73.68	72.51	74.11
Crude protein (N x 6.25)	16.70	17.12	18.13	16.93	18.67	19.09	17.68	16.64	16.21	17.50	15.99	16.80	16.70	16.73
Lipid (%)	11.01	11.58	7.27	7.92	7.89	6.11	4.11	6.73	5.37	5.60	4.55	5.39	6.74	5.70
Ash (%)	4.03	3.30	3.70	3.58	4.11	3.63	3.56	3.69	3.81	3.67	4.17	3.94	3.52	3.87
	101.04	100.45	99.94	99.75	100.25	100.78	100.00	99.80	99.51	100.33	99.39	99.81	99.47	100.41

APPENDIX IX Growth performance, feed utilization efficiency, liver somatic index and blood parameters of rainbow trout replicates (240 fish, 36g) fed the Meat and bone meal experimental diets after 13 weeks

Diet	1		2		3		4		5		6	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B
Mean initial weight (g)	36.28	36.43	36.77	36.23	36.73	36.14	36.28	36.47	36.52	36.13	36.05	36.82
Mean final weight (g)	136.12	140.83	138.33	132.52	125.80	123.88	136.69	126.07	123.95	117.42	101.24	97.86
Weight gain (%)	275.19	286.58	276.20	265.77	242.50	242.78	276.76	245.68	239.40	224.99	180.33	165.78
Specific growth rate (%/day)	1.45	1.48	1.45	1.43	1.36	1.35	1.46	1.36	1.34	1.30	1.13	1.07
Food intake (mg/day)	1362	1373	1378	1331	1284	1247	1343	1290	1306	1319	1168	1174
Protein intake (mg/day)	634	639	631	610	585	568	605	582	584	590	531	533
Weight gain (mg/day)	1097	1147	1116	1061	979	964	1103	985	961	893	716	671
Food conversion ratio	1.24	1.20	1.23	1.25	1.31	1.29	1.22	1.31	1.36	1.48	1.63	1.75
Protein efficiency ratio	1.73	1.79	1.77	1.74	1.67	1.70	1.82	1.69	1.64	1.51	1.35	1.26
Nitrogen intake (mg/day)	101.44	102.24	100.96	97.60	93.60	90.88	96.80	93.12	93.44	94.40	84.96	85.28
Nitrogen deposition (mg/day)	10.81	11.22	11.05	10.87	10.46	10.61	11.39	10.58	10.28	9.46	8.43	7.87
Apparent net protein utilization (%)	10.66	10.97	10.94	11.14	11.17	11.67	11.77	11.36	11.00	10.02	9.92	9.23
Apparent protein digestibility (%)	79.83	82.02	76.93	78.71	74.86	74.89	72.73	75.45	69.01	67.55	67.41	64.29
Apparent lipid digestibility (%)	92.63	80.46	86.25	77.15	83.77	71.04	75.83	67.30	73.55	51.12	63.16	54.63
Apparent organic matter digestibility (%)	55.72	55.09	50.80	51.12	52.14	52.09	52.78	54.97	53.27	52.87	55.52	53.99
Liver somatic index	0.95	0.95	0.87	0.91	0.97	0.90	1.06	0.97	1.04	1.09	1.12	1.08
Haematocrit (%)	39.83	44.33	41.67	44.83	41.83	44.83	43.33	44.50	43.83	45.83	44.33	46.67
Haemoglobin (g/100 cm^3)	8.32	9.46	8.89	9.56	8.92	9.56	9.24	9.49	9.35	9.78	9.46	9.96

APPENDIX X Overall mean weight gain (g) of rainbow trout replicates at successive fortnightly intervals over the Meat and bone meal experimental test period

Week	1		2		3		4		5		6		No feeding
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	
0	36.28	36.43	36.77	36.23	36.73	36.14	36.28	36.47	36.52	36.13	36.05	36.82	36.02
2	42.75	46.50	45.50	44.25	45.00	44.25	44.50	44.00	43.00	44.75	43.00	44.50	35.50
4	58.16	55.25	55.75	59.00	51.50	53.00	54.00	55.75	53.75	54.75	50.75	49.75	35.00
6	66.58	68.25	68.00	65.00	64.00	58.25	65.50	65.00	65.75	65.50	58.75	60.75	34.50
8	80.79	84.00	85.50	81.50	79.25	75.25	82.75	78.50	79.25	79.25	70.00	70.25	34.00
10	102.63	103.75	104.50	98.75	94.50	92.75	101.00	92.25	95.75	97.25	80.00	80.25	33.75
12	119.47	127.75	127.00	118.25	114.25	112.25	113.50	112.00	115.00	116.25	94.75	91.50	32.26
13	136.12	140.83	138.33	132.52	125.80	123.88	136.69	126.07	123.95	127.42	101.24	97.86	31.31

APPENDIX XI Proximate composition and Cr_2O_3 content of faeces of rainbow trout replicates at the end of the Meat and bone meal experimental test period

Faces composition (% dry weight)	1		2		3		4		5		6	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B
Moisture (%)	0.74	0.82	0.48	0.92	0.52	0.97	0.32	1.17	0.80	1.10	0.65	0.95
Crude protein (N x 6.25)	21.75	18.51	21.36	19.84	23.80	22.36	24.35	24.42	26.69	27.72	28.64	28.61
Lipid (%)	2.17	5.62	3.54	5.29	4.35	7.30	6.56	9;70	6.75	10.86	11.02	13.38
Ash (%)	22.52	23.79	22.58	24.17	17.76	19.99	19.02	19.66	19.41	17.66	16.86	16.77
Cr_2O_3	1.14	1.11	1.09	1.09	1.10	1.03	1.01	1.12	1.05	1.04	0.99	0.96

APPENDIX XII Carcass composition of rainbow trout replicates (240 fish, 36g) at the end of the Meat and bone meal experimental test period

Proximate composition (% wet weight)	1		2		3		4		5		6	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B
Moisture (%)	70.45	71.49	68.24	72.06	72.65	72.71	69.35	72.77	71.72	72.06	71.97	72.19
Crude protein (N x 6.25)	17.63	17.69	19.09	17.62	17.39	17.17	18.66	17.73	17.45	17.35	17.42	16.39
Lipid (%)	8.67	7.45	7.83	7.17	6.51	6.06	8.41	6.17	8.16	7.89	8.71	8.76
Ash (%)	3.42	3.30	4.42	3.85	3.54	3.57	3.77	3.52	3.95	4.20	3.87	2.55
	100.17	99.93	99.58	100.70	100.09	99.51	100.19	100.19	101.28	101.50	101.97	99.89

APPENDIX XIII Growth performance, feed utilization efficiency, liver somatic index and blood parameters of rainbow trout replicates (280 fish, 19g) fed the PBHFm and the Meat and bone meal experimental diets after 12 weeks

Diet	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Mean initial weight (g)	19.56	18.83	19.24	19.00	19.89	19.00	19.41	19.78	18.74	19.16	19.49	19.02	19.24	19.26
Mean final weight (g)	81.66	90.98	89.53	85.91	58.05	47.85	65.83	55.99	40.31	58.35	68.76	66.90	78.64	65.79
Weight gain (%)	317.49	383.16	365.33	352.16	191.86	151.84	239.16	183.06	115.10	204.54	252.80	251.74	308.73	241.59
Specific growth rate (%/day)	1.70	1.88	1.82	1.80	1.27	1.11	1.46	1.24	0.92	1.33	1.50	1.50	1.67	1.46
Food intake (mg/day)	1087	1109	1103	1097	880	813	925	828	727	838	910	916	1043	968
Protein intake (mg/day)	449	458	458	455	365	337	386	346	304	351	376	379	426	396
Weight gain (mg/day)	739	859	837	796	454	343	553	431	257	466	586	570	707	554
Food conversion ratio	1.47	1.29	1.32	1.38	1.94	2.36	1.67	1.92	2.83	1.79	1.55	1.61	1.47	1.75
Protein efficiency ratio	1.64	1.87	1.83	1.75	1.24	1.02	1.43	1.24	0.84	1.33	1.56	1.50	1.66	1.40
Nitrogen intake (mg/day)	71.84	73.28	73.28	72.80	58.40	53.92	61.76	55.36	48.64	56.16	60.16	60.64	68.16	63.36
Nitrogen deposition (mg/day)	10.29	11.72	11.42	10.95	7.77	6.38	8.95	7.78	5.28	8.31	9.76	9.40	10.37	8.74
Apparent net protein utilization (%)	14.32	15.99	15.58	15.04	13.30	11.83	14.49	14.05	10.85	14.80	16.22	15.50	15.21	13.79
Apparent protein digestibility (%)	83.18	84.94	83.99	82.74	69.91	68.49	71.81	78.13	76.17	77.49	80.78	80.44	84.95	83.63
Apparent lipid digestibility (%)	86.77	77.08	87.59	83.98	67.50	71.04	75.38	77.87	81.18	83.10	86.01	87.26	86.91	92.98
Apparent organic matter digestibility (%)	59.79	63.83	65.99	65.87	45.04	43.24	46.70	54.61	53.24	56.92	63.09	59.43	66.93	61.73
Liver somatic index	1.35	1.38	1.50	1.51	1.62	1.54	1.26	1.26	1.29	1.18	1.54	1.32	1.35	1.09
Haematocrit (%)	44.00	38.00	35.75	39.00	38.75	33.00	37.75	39.25	39.00	42.00	37.00	44.00	93.25	40.75
Haemoglobin (g/100 cm^3)	9.39	8.11	7.83	8.42	8.26	7.56	8.06	8.38	8.32	8.96	8.04	9.39	9.23	8.70

APPENDIX XIV Overall mean weight gain (g) of rainbow trout replicates at successive fortnightly intervals over the PBHFm and Meat and bone meal experimental test period

Week	1		2		3		4		5		6		7		No feeding
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
0	19.56	18.83	19.24	19.00	19.89	19.00	19.41	19.78	18.74	19.16	19.49	19.02	19.24	19.26	18.40
2	23.75	23.25	25.53	25.83	22.50	21.84	22.37	22.00	20.25	21.32	23.95	22.75	25.83	22.50	15.25
4	31.25	26.84	31.58	30.83	26.84	24.73	27.89	26.00	22.75	23.16	26.84	26.84	29.44	28.50	12.63
6	39.50	40.00	40.00	39.72	31.05	30.53	33.42	27.00	26.50	28.68	31.58	33.89	38.06	36.58	12.00
8	49.75	53.67	48.95	49.44	36.67	35.00	40.53	36.00	29.50	37.56	40.00	40.00	46.11	43.89	13.33
10	62.75	68.00	64.71	63.89	49.06	40.79	50.79	44.0	35.50	45.59	50.29	52.37	59.44	49.48	15.00
12	81.66	90.98	89.53	85.91	58.05	47.85	65.83	55.99	40.31	58.35	68.76	66.90	78.64	65.79	15.00

APPENDIX XV Proximate composition and Cr_2O_3 content of faeces of rainbow trout replicates at the end of the PBHFm and Meat and bone meal experimental test period

Faeces composition (% dry wt)	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Moisture (%)	2.97	2.25	2.70	2.17	2.61	2.26	3.01	3.42	2.43	8.15	2.50	3.32	3.48	3.70
Crude protein (N x 6.25)	17.34	17.07	19.35	20.79	22.63	22.95	21.99	20.00	21.21	21.73	21.36	19.80	18.42	17.35
Lipid (%)	4.03	7.67	4.96	6.38	7.45	6.43	5.98	6.30	5.40	5.26	5.19	4.31	5.43	2.52
Ash (%)	25.47	25.30	20.50	22.28	53.59	53.95	48.83	53.38	55.16	58.63	43.72	43.76	35.90	39.65
Cr_2O_3	1.22	1.34	1.46	1.45	0.89	0.86	0.93	1.09	1.04	1.13	1.29	1.17	1.50	1.30

APPENDIX XVI Carcass composition of rainbow trout replicates (280 fish, 19g) at the end of the PBHFm and Meat and bone meal experimental test period

Proximate composition (% wet weight)	1		2		3		4		5		6		7	
Replicate	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Moisture (%)	68.43	68.55	69.40	69.64	69.57	69.89	68.61	69.46	70.92	69.02	68.58	69.76	70.39	69.27
Crude protein (N x 6.25)	18.10	17.58	18.15	17.43	17.61	16.92	17.58	17.05	17.23	17.23	18.19	17.21	17.59	17.42
Lipid (%)	9.27	9.98	8.95	9.04	8.83	9.03	10.10	9.46	8.04	9.88	8.90	8.61	8.71	9.69
Ash (%)	4.43	4.20	3.73	4.22	4.15	4.44	4.19	4.35	4.07	4.09	4.55	4.49	3.69	3.72
	100.23	100.31	100.23	100.33	100.16	100.28	100.48	100.32	100.26	100.22	100.22	100.07	100.38	100.10

APPENDIX XVII Monthly particle size analysis of samples of Brown fish meal A between March 1984 and February 1985

Mesh size (mm)	March (%)	April (%)	May (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)	December (%)	January (%)	February (%)
>4	-	-	-	-	3.78	-	-	-	-	-	-	-
<4>3	-	1.21	0.27	0.06	1.30	-	-	-	-	-	-	-
<3>2	4.78	6.31	5.20	1.30	11.24	0.10	0.14	0.82	1.32	3.18	0.78	
<2>1	11.25	7.28	12.43	12.32	18.28	0.78	2.60	21.62	15.21	10.18	16.40	
<1>0.5	31.26	47.28	27.28	31.36	39.00	66.28	61.68	62.76	61.37	52.56	19.68	
<0.5	52.64	37.92	54.82	54.96	26.40	32.88	35.58	14.80	22.10	34.08	63.14	

APPENDIX XVIII Monthly particle size analysis of samples of Brown fish meal B between March 1984 and February 1985

Mesh size (mm)	March (%)	April (%)	May (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)	December (%)	January (%)	February (%)
> 4	1.18	-	0.26	1.80	2.20	1.60	0.64	0.10	1.15	4.56	-	-
< 4>3	2.10	-	1.08	2.84	3.62	0.98	2.04	0.76	0.97	0.20	0.86	0.04
< 3>2	10.76	-	4.64	13.78	12.22	3.44	5.74	5.04	10.98	3.26	4.52	2.00
< 2>1	19.08	3.24	14.80	25.38	19.76	7.46	12.26	17.00	11.25	6.52	13.72	12.04
< 1>0.5	37.16	58.60	25.00	37.78	40.44	27.14	57.56	37.72	37.29	21.06	14.86	47.00
< 0.5	29.72	38.16	54.22	18.42	21.76	59.38	21.76	39.38	38.36	64.40	66.04	38.92

APPENDIX IXX Monthly particle size analysis of samples of Brown fish meal C between March 1984 and February 1985

Mesh size (mm)	March (%)	April (%)	May (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)	December (%)	January (%)	February (%)
> 4	2.81	0.34	0.47	2.68	1.84	0.68	0.12	0.80	0.15	2.34	-	1.91
< 4>3	1.90	0.56	1.21	2.36	2.22	1.26	0.42	1.32	2.21	1.17	1.32	2.51
< 3>2	6.37	2.92	8.37	10.78	10.22	3.64	0.88	4.64	7.97	8.34	3.32	9.87
< 2>1	11.14	8.52	11.58	21.98	26.70	9.94	3.14	12.06	5.25	17.27	3.14	9.84
< 1>0.5	9.02	12.90	22.03	25.50	33.54	69.50	89.50	61.50	25.03	38.94	39.76	57.28
< 0.5	68.76	74.76	56.34	36.70	25.48	14.98	5.94	19.68	59.39	31.94	52.46	18.59

APPENDIX XX Monthly particle size analysis of samples of Brown fish meal D between March 1984 and February 1985

Mesh size (mm)	March (%)	April (%)	May (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)	December (%)	January (%)	February (%)
> 4	-	-	0.08	-	-	-	-	-	-	-	0.01	-
< 4>3	0.40	0.12	0.12	-	0.02	-	-	-	0.04	-	0.30	0.12
< 3>2	8.02	5.42	12.68	5.07	5.64	0.66	1.18	3.94	5.92	4.06	8.23	5.36
< 2>1	18.24	16.70	23.42	21.91	19.64	5.26	16.02	16.70	23.96	7.80	15.75	18.72
< 1>0.5	12.32	28.62	42.48	47.28	61.04	10.28	59.82	28.14	51.42	20.46	47.28	54.36
< 0.5	61.02	49.14	21.22	25.74	13.66	83.80	22.98	51.22	18.66	67.68	28.43	21.44

APPENDIX XXI Monthly particle size analysis of samples of Brown fish meal E between March 1984 and February 1985

Mesh size (mm)	March (%)	April (%)	May (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)	December (%)	January (%)	February (%)
> 4	1.08	0.38	0.68	0.45	0.92	0.04	-	-	0.12	-	0.08	1.21
< 4>3	2.24	0.12	3.22	1.12	1.94	0.70	3.98	0.22	0.48	0.70	0.10	3.47
< 3>2	4.32	1.72	13.16	7.63	12.72	4.42	7.70	3.24	3.58	3.32	2.48	11.95
< 2>1	15.22	7.92	17.80	19.81	20.82	10.48	11.30	10.82	2.90	6.52	2.26	15.47
< 1>0.5	11.36	12.68	39.22	45.40	59.60	49.38	47.64	65.30	26.04	39.18	40.98	48.73
< 0.5	65.78	77.18	25.92	25.59	4.00	34.98	29.38	20.42	66.88	50.28	54.10	19.17

APPENDIX XXII Monthly particle size analysis of samples of Brown fish meal F between March 1984 and February 1985

Mesh size (mm)	March (%)	April (%)	May (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)	December (%)	January (%)	February (%)
>4	0.20	-	-	-	-	-	-	-	-	-	-	-
<4>3	1.20	0.73	0.35	0.08	0.08	0.02	-	-	-	0.29	1.20	0.80
<3>2	5.22	6.47	2.31	7.02	4.80	2.84	2.24	2.20	4.50	6.24	3.25	
<2>1	13.28	13.43	15.21	17.34	17.62	10.96	10.10	2.26	10.73	9.26	12.21	
<1>0.5	18.30	28.25	23.24	31.88	13.62	22.46	17.62	12.04	31.25	20.23	25.05	
<0.5	61.80	51.12	58.89	43.68	63.88	63.72	70.04	83.50	53.23	63.07	58.69	

APPENDIX XXIII Monthly particle size analysis of samples of Poultry by-product and hydrolysed feather meal between May 1983 and April 1984

Mesh size (mm)	May (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)	December (%)	January (%)	February (%)	March (%)	April (%)
>4	1.96	0.62	1.16		0.92	0.95	1.15	0.97	0.98	0.92	1.22	0.93
<4>3	1.30	0.70	2.84		0.70	1.15	2.74	0.85	0.83	0.76	0.88	0.78
<3>2	13.76	7.22	8.16		7.52	8.74	10.21	11.25	9.25	7.52	9.46	7.32
<2>1	41.54	21.06	31.18		28.86	24.25	27.43	38.25	39.26	29.96	41.76	29.96
<1>0.5	28.28	58.80	51.12		55.20	51.27	47.28	41.25	41.25	55.20	41.84	55.20
<0.5	13.16	11.60	5.54		6.76	13.64	11.19	7.43	8.43	5.64	4.84	5.40

APPENDIX XXIV Monthly particle size analysis of samples of Meat and bone meal between December 1984 and November 1985

Mesh size (mm)	December (%)	January (%)	February (%)	March (%)	April (%)	May (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)
>4	0.52	-	0.31	-	0.53	-	-	-	-	0.42	0.20	0.48
<4>3	0.32	0.41	0.57	-	0.83	0.29	-	-	0.51	0.73	0.28	0.18
<3>2	12.66	1.96	2.21	0.98	1.47	0.98	1.50	1.73	2.02	1.97	2.96	2.18
<2>1	2.36	10.25	8.47	11.25	10.41	21.25	7.66	12.43	15.25	17.27	23.70	16.50
<1>0.5	79.30	60.25	69.26	71.25	78.23	68.45	73.52	65.95	69.26	70.27	51.80	63.54
<0.5	4.84	27.13	19.18	16.52	8.53	9.03	17.32	19.89	12.96	9.34	21.06	17.06

APPENDIX XXV Cost of experimental diets per Kilogramme weight gain of Poultry by-product and hydrolysed feather meal (PBHFm) trial. (Prices refer to December 1985; 1 Sterling pound = Escudos 230.00)

Percentage of protein replacement	Dietary treatments 1 0%	2 20%	3 40%	4 60%	5 80%	6 90%	7 100%
Brown fish meal	49.042	39.184	29.388	19.592	9.796	4.898	-
PBHFm	-	7.050	14.050	21.100	28.100	31.600	35.100
Corn starch	6.525	8.265	10.005	13.485	16.965	20.445	23.925
Yellow dextrin	5.520	6.720	8.400	11.280	13.920	16.800	19.680
Cod liver oil	23.275	21.025	18.024	13.704	9.384	5.064	0.744
Vitamin premix	46.000	46.000	46.000	46.000	46.000	46.000	46.000
Mineral premix	27.375	27.375	27.375	27.375	27.375	27.375	27.375
Carboxymethyl-cellulose	59.850	59.850	59.850	59.850	59.850	59.850	59.850
Potassium sorbate	240.000	240.000	240.000	240.000	240.000	240.000	240.000
Butylated hydroxytoluene	1.500	1.500	1.500	1.500	1.500	1.500	1.500
Cr_2O_3	39.00	39.000	39.000	39.000	39.000	39.000	39.000
Electricity	112.962	112.962	112.962	112.962	112.962	112.962	112.962
Water	0.027	0.027	0.027	0.027	0.027	0.027	0.027
Total cost/Kg Escudo ($)	611.076	608.958	606.581	605.976	604.879	605.521	606.163
Total cost/Kg Pound (£)	2.6568	2.6476	2.6373	2.6347	2.6230	2.6327	2.6355
Cost/Kg weight gain Escudo ($)	2835.393	1845.143	1571.045	1169.532	1185.563	1053.606	1151.710
Cost/Kg weight gain Pound (£)	12.328	8.0224	6.8306	5.0849	5.1546	4.5810	5.0074

APPENDIX XXVI Cost of experimental diets per Kg and Kg/weight gain of Brown fish meals trial (Prices refer to December 1985; 1 Sterling pound = Escudos 230.00)

Ingredients	Dietary treatments 1	2	3	4	5	6	7
Brown fish meal	-	46.500	58.032	49.600	51.460	55.180	49.600
"Pruteen"	100.340	-	-	-	-	-	-
Corn starch	18.705	13.775	0.580	12.760	7.395	5.365	11.745
Yellow dextrin	15.600	11.280	0.480	10.560	6.000	4.320	9.840
Cod liver oil	24.275	15.025	2.525	5.025	11.525	1.775	7.525
Vitamin premix	46.00	46.000	46.000	46.000	46.000	46.000	46.000
Mineral premix	23.275	23.275	23.275	23.275	23.275	23.275	23.275
Carboxymethyl-cellulose	59.850	59.850	59.850	59.850	59.850	59.850	59.850
Potassium sorbate	240.000	240.000	240.000	240.000	240.000	240.000	240.000
Butylated hydroxytoluene	1.500	1.500	1.500	1.500	1.500	1.500	1.500
Cr_2O_3	39.000	39.000	39.000	39.000	39.000	39.000	39.000
Electricity	112.962	112.962	112.962	112.962	112.962	112.962	112.962
Water	0.027	0.027	0.027	0.027	0.027	0.027	0.027
Total cost/Kg Escudo ($)	681.534	609.194	584.231	600.559	598.994	589.254	601.324
Total cost/Kg Pound (£)	2.9632	2.6487	2.5401	2.6111	2.6043	2.5620	2.614
Cost/Kg weight gain Escudo ($)	1601.605	1632.640	1659.216	1987.850	1970.690	2121.314	2002.409
Cost/Kg weight gain Pound (£)	6.9635	7.0984	7.2140	8.6428	8.5682	9.2231	8.706

APPENDIX XXVII Cost of experimental diets per Kg and Kg/weight gain of Meat and bone meal trial. (Prices refer to December 1985: 1 Sterling pound = Escudos 230.00)

% of protein replacement	Dietary treatments 1 0%	2 20%	3 40%	4 60%	5 80%	6 100%
Brown fish meal	40.548	32.488	24.366	16.244	8.122	-
Meat and bone meal	-	5.720	11.440	17.160	22.880	28.600
Corn starch	20.445	20.735	21.170	21.605	22.040	22.330
Yellow dextrin	17.040	17.280	17.520	17.760	18.000	18.480
Cod liver oil	21.525	17.525	13.525	9.525	5.525	1.525
Vitamin premix	46.000	46.000	46.000	46.000	46.000	46.000
Mineral premix	27.375	27.375	27.375	27.375	27.375	27.375
Carboxymethyl-cellulose	59.850	59.850	59.850	59.850	59.850	59.850
Potassium sorbate	240.000	240.000	240.000	240.000	240.000	240.000
Butylated hydroxytoluene	1.500	1.500	1.500	1.500	1.500	1.500
Cr_2O_3	39.00	39.000	39.000	39.000	39.000	39.000
Electricity	112.962	112.962	112.962	112.962	112.962	112.962
Water	0.027	0.027	0.027	0.027	0.027	0.027
Total cost Escudo ($)	626.272	620.462	614.735	609.008	603.281	597.649
Total cost Pound (£)	2.7229	2.6977	2.6728	2.6479	2.6229	2.5985
Cost/Kg weight gain Escudo ($)	764.052	769.372	799.155	767.350	850.626	1010.027
Cost/Kg weight gain Pound (£)	3.3220	3.3451	3.4746	3.3363	3.6984	4.3914

APPENDIX XXVIII Cost of experimental diets per Kg and Kg/weight gain of Poultry by-product and hydrolysed feather meal (PBHFm) and Meat and bone meal (MBm) trial. (Prices refer to December 1985; 1 Sterling pound = Escudos 230.00)

	Dietary treatments						
% protein PBHFm replacement MBm	1 0% 0%	2 50% 0%	3 0% 60%	4 30% 40%	5 20% 50%	6 30% 30%	7 Commercial diet
Brown fish meal	38.812	19.406	15.500	11.656	11.656	27.156	
PBHFm	-	19.800	-	11.850	7.950	11.900	
MBm	-	-	24.820	16.320	20.400	12.240	
Corn starch	22.475	22.040	5.655	11.020	8.265	13.775	
Yellow dextrin	18.480	18.240	4.560	9.120	6.720	11.520	
Cod liver oil	23.525	3.775	7.775	1.275	2.525	3.775	
Vitamin premix	46.000	46.000	46.000	46.000	46.000	46.000	
Mineral premix	27.375	27.375	27.375	27.375	27.375	27.375	
Carboxymethyl-cellulose	59.850	59.850	59.850	59.850	59.850	59.850	
Potassium sorbate	240.000	240.000	240.000	240.000	240.000	240.000	
Butylated hydroxytoluene	1.500	1.500	1.500	1.500	1.500	1.500	
Cr_2O_3	39.000	39.000	39.000	39.000	39.000	39.000	
Electricity	112.962	112.962	112.962	112.962	112.962	112.962	
Water	0.027	0.027	0.027	0.027	0.027	0.027	
Total cost Escudo ($)	630.006	609.975	584.724	586.680	584.230	607.080	80.000
Total cost Pound (£)	2.7391	2.6521	2.5423	2.5508	2.5401	2.6395	0.3478
Cost Kg/weight gain Escudo ($)	869.408	853.965	1257.156	1050.157	1320.360	959.186	
Cost Kg/weight gain Pound (£)	3.7800	3.7129	5.4659	4.5659	5.7407	4.1704	