DIGESTIBILITY STUDIES IN RAINBOW TROUT
(Oncorhynchus mykiss)

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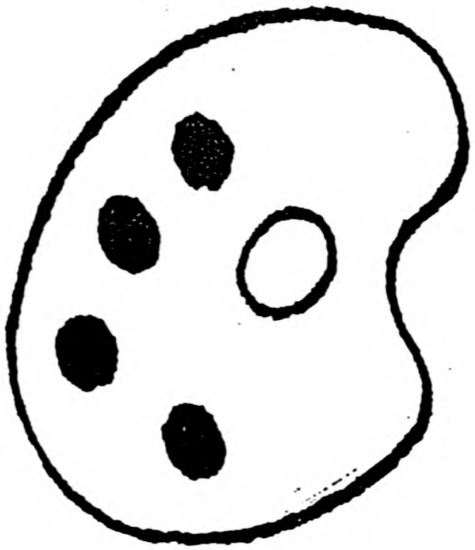

DECLARATION

I hereby declare that this thesis has been composed by myself and is the result of my own investigation. It has never been accepted nor is being submitted for any other degrees. All the sources of information have been duly acknowledged.

Ana Marcia Pimentel Rodrigues

Candidate's signature

Principal Supervisor's signature

Stirling, 29th September 1992

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ABSTRACT

The bioavailability of nutrients in commercial feeds available in Portugal was evaluated in three size groups (40g, 100g and 180g) of rainbow trout (*Oncorhynchus mykiss*) on two private farms. Dietary crude fibre level was used as the digestibility marker. A series of laboratory trials was also carried out to investigate the influence of various biotic and abiotic factors on apparent digestibility coefficients and to provide corroboration for the field survey.

The principal factors influencing apparent digestibility values during the field survey were diet quality, temperature and water-dissolved oxygen. The apparent digestibilities of crude protein, organic matter and dry matter were strongly inversely correlated with dietary fibre level in the three size groups of farmed trout. In addition, significant positive correlations were obtained between lipid level in commercial feeds and the organic matter digestibility coefficients. Under controlled conditions, dietary lipid level (7%, 14% and 21%) had a marked effect on digestibility, the highest digestibility values being obtained with the 21% lipid diet (87.77%, 76.55% and 70.46%) compared to 84.59%, 63.25% and 57.74% with the 7% lipid diet for crude protein, organic matter and dry matter, respectively. A signification correlation was also obtained between all apparent digestibility coefficients and the feed protein level for all size groups of fish studied in the field. In the laboratory, however, only at 21°C was there a marked effect and significantly higher digestibility values were obtained with the higher protein (45%) diet (85.98%, 83.74%, 80.37% and 84.54% for protein, organic matter, dry matter and energy, respectively) as compared to the lower protein (30%) diet (74.16%, 71.66%, 65.54% and 68.70% for protein, organic matter, dry matter and energy, respectively).

General increases in apparent digestibility values of more than 10% were obtained between the lower (10°C or 15°C) and the higher (21°C or 22°C) experimental temperatures, much higher than values previously reported.

Food intake was shown to be directly dependent on dissolved oxygen (D.O.) and for one unit decrease of D.O. food intake decreased by about 0.5% or 0.25% body weight/day whether fish were subjected to an abrupt decrease of water O_2 level or to prolonged hypoxia. Furthermore, apparent digestibility values were significantly increased when fish were subjected to prolonged hypoxia.

Feeding frequency (1, 2 or 4 meals/day) did not influence digestibility, whereas time of day and fish size did have a significant effect.

Mean digestibility values estimated either from chromic oxide or crude fibre analysis were significantly different. The difference was, however, very small and bearing in mind the advantages of an internal marker as compared to an added marker, this study has shown that crude fibre is a useful tool for feed evaluation, especially in practical situations.

The need for, and importance of, digestibility evaluations is discussed and summarised in the form of a conceptual model.

ACKNOWLEDGEMENTS

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1 - INTRODUCTION

World aquaculture production has grown from 10.5 million tons in 1985 to 14.5 million tons in 1988, of which 44.5% and 49.2% were fish, respectively. Further growth is expected and by the end of the century it is projected to be around 23 million tons (FAO, 1988, 1990, 1991). With this increase in intensive culture of many species of fish throughout the world and the consequent increase in environmental impact of aquaculture, the demand for more efficient dry diets is also expanding rapidly. Feed represents the single largest component of the production costs at around 30-60% of the total (Tacon & Jackson, 1985; Kaushik, 1989b; Cho *et al.*, 1989). At the same time feeds potentially present the largest future problem for the industry because of the wastes released into the environment (Cho *et al.*, 1991). Nutritionally or physically faulty feed impairs fish productivity and the impact on the environment may be substantial through excreted waste or wasted feed. Thus, it is more and more important to produce least cost diets and to optimize diet formulations.

In the past, high quality fish meal has been the major source of protein for fish diets, but evidence indicates that this supply will not keep pace with the increased future demand. In view of the high cost of good quality fish meals of relatively constant chemical composition, alternative and less expensive sources of good quality protein have been used in fish diet formulation in recent years. These include meat and bone meal, blood meal, soybean meal, poultry by-products meal, hydrolysed feather meal, dried brewers yeast and corn gluten meal (Tacon & Jackson, 1985). Attempts are now being made to use greater amounts of novel or plant protein sources within compounded fish feeds in order to formulate "least cost" diets for fish, as is done with many domestic animals. Because of this, it will be necessary to know more than just

the proximate analysis of the various diet ingredients. In addition to proximate analysis, efficient chemical analysis for amino acids, fatty acids, and individual mineral elements are now routinely carried out in many laboratories.

In evaluation of a feed or diet, digestibility measures the proportion of nutrients which are assumed to have been absorbed by the gut mucosa by quantifying the nutrients ingested and those voided in the faeces. Thus, digestibility coefficients are used as a general measure of the nutritive value of food.

If the measure of the difference between intake and faecal output takes into account the part of the faeces which is not derived from undigested food residues (such as cellular material abraded from the lining of the gut, bacteria, enzymes, mucoproteins, and other residues of digestive juices secreted into the gut and not reabsorbed), then digestibility measured this way is referred to as true digestibility. This is distinct from the approximate measure of apparent digestibility which is simply a measurement of the proportion of the diet which does not appear in the faeces. This inclusion of the endogenous/metabolic proteins with the dietary protein in the faeces causes a bias of digestibility coefficients towards lower values. In both cases, however, digestibility studies will contribute towards not only the increase in productivity through the optimization of nutrient utilisation, and consequently lower energy losses, but also the reduction of nutrient losses into the immediate aquatic environment, thus contributing to the maintenance and improvement of water quality, which is a requisite for the further expansion of aquaculture. There is a lack of information on the digestibility of the proximate components of feeding stuffs and such data is required for most

efficient diet formulation.

The nutritional usefulness of food for fish populations depends in part on the extent to which it is digested and a significant portion of the information on the digestive function can be inferred from the anatomy of the digestive tract. The digestive system of rainbow trout follows the same pattern as in all higher vertebrates (Fänge & Grove, 1979). The mouth is situated at the anterior end of the head. It is a horizontal opening at the tip which can close. The teeth only assist in the process of food capture but do not serve to comminute the prey or ration. The oesophagus is a short and wide mucus-lubricated entryway into the stomach, which is a distinctly U-shaped stomach with muscular closures at each end - the cardiac sphincter anteriorly, and the pyloric sphincter (pylorus) posteriorly. The lining of the stomach contains many secretory cells which, in contrast to the case in mammals, secrete both hydrochloric acid and the digestive enzymes, in addition to mucus.

The midgut begins immediately posterior to the pylorus and unlike higher vertebrates it has pyloric caeca attached. These are blind tubular outgrowths, closely resembling the intestine in structure, which serve to expand the surface area of the midgut three times (Ezeasor & Stokoe, 1980). In salmonids there is little difference between midgut and hindgut detectable by eye, although histological examination shows a fairly sudden change from a columnar secretory/absorptive epithelium to a squamous one that secretes mostly mucus (Smith, 1982, 1989; Kapoor *et al.*, 1975). Ezeasor & Stokoe (1980) stated that an ileocaecal valve marks the posterior demarcation of the midgut in rainbow trout. The digestive apparatus of rainbow trout is completed by

several other organs, the liver, the gall bladder and a diffused pancreas surrounding the pyloric caeca (Bergot *et al.*, 1975).

Digestion is a combination of the mechanical, chemical and enzymatic processes in the gastrointestinal tract by which ingested food is broken down into simple components (Choubert, 1983) and in the main both digestion and digestive enzymes in fish appear similar to those in mammals. Table 1.1 shows the digestive enzymes and principal sites of their formation in rainbow trout (Fänge & Grove, 1979; Ash, 1985; Hepher, 1988; Smith, 1989).

The mucus secreted in the mouth, pharynx and oesophagus of rainbow trout differs from that of higher vertebrates in that it seems to lack digestive enzymes such as α-amylase. Thus, digestion proper starts in the stomach (Fange & Grove, 1979; Hepher, 1988; Smith, 1989). Rainbow trout digest protein to a considerable extent in the stomach, where the endopeptidase pepsin and hydrochloric acid are at work. The optimum pH range is 2.5-3.5 (Kitamikado & Tachino, 1960b; Hepher, 1988). In the intestine and the pyloric caeca protein splitting proceeds further, but through the activity of tryptic enzymes (trypsin and chymotrypsin) which originate from the pancreas, and various exopeptidases (carboxypeptidase, aminopeptidase, di- and tripeptidases). In the rainbow trout intestine the optimum pH for proteases is in the range 7.6-8.0 (Falge *et al.*, 1978). In contrast to the activity in the stomach, the proteolytic activity in the intestine and pyloric caeca of young fish is slight at first but increases as fish grow (Kitamikado & Tachino, 1960b; Kawai & Ikeda, 1973a). The highest protease activities are reached in the stomach and intestines at fish sizes of

Table 1.1 - Digestion enzymes and sites of enzyme formation in rainbow trout.

Stomach	Intestine	Pancreas
Pepsinogen	Enterokinase	Trypsinogen
(HCl)	Aminopeptidases	Chymotrypsinogen
Lipases	Dipeptidases	Proelastase
Esterases	Tripeptidases	Procarboxipeptidases
α-Amylase	Lipases	Lipases
Chitinase	Esterases	Esterases
Chitobiase	α-Amylases	α-Amylases
Lysozyme	α-Glucosidases (e.g. maltase)	α-Glucosidase (e.g. maltase)
Alkaline and Acid Phosphatases	β-Galactosidase	Chitinase
	Chitobiase	(HCO_3^-)
	Lysozyme	
	Alkaline & Acid Phosphatases	

around 100g live weight, after which a slight decrease occurs (Kitamikado & Tachino, 1960b; Kawai & Ikeda, 1973a).

The specific protease activity in the pyloric caeca of rainbow trout is dependent on the temperature and feed quality (Kawai & Ikeda, 1973a; Falge *et al.*, 1978; Dabrowski *et al.*, 1989). At temperatures of 11°C and 18°C it is higher for a protein-rich diet than for a low protein diet. A temperature effect is observable only for protein-rich diets, and at low protein contents the temperature does not affect the enzyme activity, no effect being recorded at 6°C. Higher dietary protein (60-80% fish meal) contents linked with a low cellulose (0-20% α-cellulose) content generally enhance the proteolytic activity in young rainbow trout, while an inverse relation has the opposite effect (20-40% fish meal and 40-60% cellulose). Elevated starch levels in the feed (30-60% starch, 55-25% protein) cause a fall in protease activity in rainbow trout relative to that for a protein-rich diet (5% starch, 80% protein; Falge *et al.*, 1978). Even a single administration of a diet containing 40% starch results in a significant drop (Falge *et al*, 1978).

In rainbow trout, lipase activity in the stomach is relatively low. To an overriding extent, fat digestion takes place in the intestine with the aid of pancreatic lipases (Kitamikado & Tachino, 1960c). The pyloric caeca also display a high level of lipase activity (Morishita *et al.*, 1967) and even young fish rapidly acquire a very pronounced capability for fat digestion. Lipases hydrolyse neutral fat (triglycerides) into di- and monoglycerides, glycerol and free fatty acids (Fänge & Grove, 1979). Even phospholipids and wax esters are attacked by lipases. However, fish hydrolyse

triglycerides four times faster than wax esters. In fat digestion the liver is of prime importance. The bile it produces is stored in the gall bladder until it is released into the intestine on the arrival of food (Talbot & Higgins, 1982). The bile salts have a high surface activity and these emulsify the fat which effectively increases the surface area of the fat droplets for attack by lipases and esterases. Bile salts also solubilize the products from lipolysis, combining with them to form micelles for subsequent absorption. According to studies of Falge *et al.* (1978) the bile of rainbow trout does not exhibit any enzymatic activity and neither proteases, amylases nor esterases are detectable, which agrees with findings for higher vertebrates. At 5°C the lipolytic effect still remains 70% of the maximum, which occurs at 25°C, and the enzymes show their greatest activity at pH 7.6. A significant reduction in lipase activity, however, occurs if the proportion of starch in the diet is raised to 60% (Falge *et al.*, 1978).

Carbohydrate-digesting enzymes from the pancreas and in the intestinal epithelium transform oligo- and polysaccharides into hexoses and pentoses (Fänge & Grove, 1979). Thus, starch and glycogen are cleaved by α-amylase to give oligosaccharides and maltose, since α-amylase acts on the α-1,4-glucosidic bonds. For the breakdown of di-and oligosaccharides (depending on their structure) there is a range of glucosidases (e.g. maltase), galactosidases and fructosidases. Rainbow trout exhibit amylase activity in the stomach, intestine and pancreas whereas α-glucosidase is not present in the stomach. In rainbow trout, moreover, it was not possible to detect β-glucosidase, α-galactosidase, β-fructosidase or cellulase (Kitamidado and Tachino, 1960a, Lindsay and Harris, 1980). After hatching, the amylase and maltase activities

of rainbow trout fry increase in a similar fashion to that found for peptic and tryptic enzymes (Kawai and Ikeda, 1973a). Increasing the protein content and decreasing the cellulose content of the diet has the effect of raising the total activity of carbohydrases (Kawai and Ikeda, 1973a). The amylase activity increases in response to a rise in temperature and a decrease in the starch content of the feed (Singh and Nose, 1967; Falge *et al.*, 1978). The optimum pH range for amylase and maltase activity in the intestine of rainbow trout is 7.0-8.0 (Hepher, 1988).

The carbohydrases are of special interest in fish since not all fish species digest carbohydrates with the same efficiency. Carnivorous fish such as the salmonids digest some carbohydrates, notably starch, less efficiently than omnivorous or herbivorous fish and controversy still exists on the possible reasons for this. Thus, it was first suggested that in carnivores there was a reduction in gland stimulation and enzyme secretion with increased starch levels in the diet (Falge *et al.*, 1978). However, the work of Spannhof & Plantikow (1983) found that in rainbow trout the maximum volume of intestinal juice produced after feeding with a carbohydrate diet was more than double that produced after feeding a protein rich diet, and that amylase activity was 15 times higher than pre-feeding activity. Then, most authors suggested that in carnivorous fish only a limited amount of amylase was secreted and therefore its activity was restricted to digesting only low concentrations of starch (Kitamikado & Tachino, 1960a; Hofer & Sturmbauer, 1985). This limited amylolytic activity in carnivores could have been explained by a more localised amylase secretion, according to Fish (1960), who states that in *Oreochromis mossambicus*, which is principally herbivorous, the amylase activity was dispersed along the entire intestine, but in the

carnivorous perch (*Perca* sp.) it was concentrated in the pancreas only. Barrington (1957) pointed out that the pancreatic tissue is dispersed in various organs and the "amylolytic activity" along the gut could have been a result of "contamination" by this tissue. Yamane (1973a,b) studied the localisation of amylase activity in common carp, tilapia (*Oreochromis mossambicus*) and other species of fish, histochemically, using a substrate film method instead of the tissue homogenisation procedure. He found that although weak amylolytic activity was detected in various parts of the digestive organs, this activity appeared only on the luminal surface of the tract and not in the mucosa or submucosa. However, strong amylase activity was found in the pancreatic exocrine cells and Yamane's conclusions were that amylases are synthesized and secreted by the cytoplasm of the pancreatic exocrine cells alone. In addition, he considered this to be probably true in both carnivorous and non-carnivorous fish and therefore localisation of amylase secretion could not be a satisfactory explanation for its limited activity in carnivores. Thus, he suggested either a limited enzyme production in the pancreas, or its subsequent inactivation. The results of Onishi *et al.* (1973a,b) support Yamane's suggestions. They found that the activities of amylase and protease in the intestinal contents of common carp increased gradually after feeding, reaching a maximum after five hours. Furthermore, with this increase, amylase levels in the hepatopancreas decreased sharply after feeding, probably due to the secretion of the accumulated enzyme into the intestinal lumen. However, more recently, a different explanation was given for reduced amylase activity in carnivorous fish by Spannhof & Phantikow (1983). These authors found that the amylase activity in the intestinal juice was different when "soluble starch" or crude potato starch were ingested. In the latter case, amylase activity of the intestinal juice was only 28% of

that when "soluble starch" was ingested and by eluting the insoluble chyme residues from fish which received potato starch, about 70% of the amylase activity in the intestinal juices of fish receiving "soluble starch" was recovered. The authors concluded that the amount of amylase secreted was constant, regardless of the form of starch, but that in the case of crude starch the amylase was bonded to the starch and thus rendered inactive. Spannhof & Plantikow (1983) have also shown that *in vitro* amylase was strongly adsorbed by the starch from which it could be eluted by repeated washing. This adsorption of the amylase reduced the activity of the enzyme, which acts as a free mobile enzyme, and thus inhibited the hydrolysis of the starch. A further explanation was given by Hofer & Sturmbauer (1985), who found wheat and some other grains to contain albumins which inhibited the α-amylase of carp and rainbow trout.

In contrast to the digestion of starch, carnivorous fish digest glucose, disaccharides and oligosaccharides such as dextrin, well (Phillips *et al.*, 1948; Singh & Nose, 1967; Kaushik, 1989a).

An extensive survey on the presence of cellulase in fish was conducted by Lindsay & Harris (1980). The majority of the omnivorous and piscivorous fish demonstrated no cellulase activity or only a small amount. They noted that in those fish exhibiting cellulase activity, its level varied within and between species and that it was higher in fishes feeding extensively on invertebrates. From their results they also concluded that the fish examined did not seem to possess an endogenous cellulase or even maintain a stable cellulolytic microflora. The cellulolytic activity, according to

Lindsay & Harris (1980) seems to be the result of cellulase enzymes produced within the invertebrates and bacteria ingested by the fish which become closely associated with the gut wall of fish, and so the presence of cellulases in an organism should not be construed as necessarily conferring any nutritional competence.

The lack of cellulase in the digestive tract of salmonids (Lindsay & Harris, 1980; Kitamikado & Tachino, 1960a) may explain why Buhler & Halver (1961) found a decrease in growth of chinook salmon (*Oncorhynchus tschawytscha*) with the increase in the content of α-cellulose replacing dextrin in the diet. Bergot, F. (1981) found that cellulose was not digested by rainbow trout. More recently, the work of Bromley & Adkins (1984) and of Hilton *et al.* (1983) also in rainbow trout have confirmed Borgot's (1981) results.

A number of other digestive enzymes are also found in the digestive tract of rainbow trout. Chitinase is found in the stomachs of several fish (Micha *et al.*, 1973; Lindsay, 1984a). The latter author found no correlation between chitinase activity and feeding habit of the fish examined. However, he observed that species that were able to disrupt their prey mechanically (e.g. cyprinids) had low activity, while species that ingested their prey whole (e.g. salmonids) had high activity. He concluded that the primary function of gastric chitinase in fish might be to disrupt chemically the chitinase envelope of the prey, allowing access to the soft inner tissues by the digestive juices. Rainbow trout secrete a gastric chitinase, the activity of which is amongst the highest recorded in fish (Micha *et al.*, 1973). Lindsay (1984b) also found other endogenous chitinolytic enzymes in trout such as chitobiase and lysozyme. He

also found that gastric chitinase was able to hydrolyse only the apexes of the chitin microfibrils and that chitinase absorbed into the chitin substrate away from the apical area of the microfibrils, did not find a susceptible substrate for hydrolysis and this resulted in a depressed hydrolytic rate. Lindsay (1984b) also found that lysozyme was rapidly absorbed onto both chitin and cellulose, but chitobiase was not absorbed by any of these. This resembles the effect of starch on the activity of amylase referred to above. The end products of chitin (unbranched homopolymer of β(1-4) linked N-acetylglucosamine residues) hydrolysis resulting from the action of chitinase consist of N-acetylglucosamine residues and chitobiose. These dimers are further hydrolysed to their constituent aminosugars by chitobiase (Lindsay, 1984b).

After the ingested food has been digested, the resulting simpler compounds are absorbed. Thus, absorption is a complex mixture of different processes by which the range of digested constituents are transferred from the lumen of the gastrointestinal tract to the bloodstream and lymphatic system. The processes involved range from simple passive diffusion to active transport of specific molecules to bulk uptake of proteins and lipids (Fange & Grove, 1979; Hepher, 1988; Smith, 1989).

Proteins can be absorbed as whole proteins, peptides or free amino acids (Smith, 1989). Dabrowski and Dabrowska (1981) looked comprehensively at the uptake of amino acids in rainbow trout and found that some amino acids (e.g. phenylalanine, leucine, glycine, glutamic acid, serine) were absorbed through the stomach. But the absorption sites of most amino acids were the anterior intestine (the pyloric caeca region) and the middle segment of the intestine, when fish were fed a fish meal based

diet. However, according to these authors and to Dabrowski (1983), when less digestible diets such as diets based on poultry by-products meal or soybean meal the amino acids' absorption sites were moved backwards at least one segment - to the posterior intestine (anterior part of the rectum) as was the case with arginine, histidine and lysine - although the overall amino acid absorption for both types of diets were similar. Bogé *et al.* (1979, 1981) also studied intestinal absorption in rainbow trout and found glycine transport sites in both the midgut and hindgut. However, transport rates were higher in the midgut than in the hindgut.

For the active transport of amino acids three specific mechanisms are probably used, depending on whether one is dealing with neutral, dibasic or acidic amino acids (Ash, 1985). As in mammals, sodium has been found to be essential for this transport mechanism (Hepher, 1988; Smith, 1989; Fänge & Grove, 1979). Furthermore, amino acid transport is competitive and the transport of one amino acid may be inhibited in the presence of other amino acids or even other substances. Thus, Ingham and Arme (1977) used an isolated intestine preparation from rainbow trout and showed that the uptake of L-leucine was active, sodium dependent and susceptible to inhibition by L-valine and L-methionine; the presence of L-valine, however, did not reduce the uptake of L-methionine. Hokazono *et al.* (1979) showed that the uptake of L-lysine was slowed by the presence of glucose and speeded by ATP, both in the midgut and the hindgut.

Dabrowski (1983) stated that the limiting factor in the utilization of amino acids by fish is not digestion but rather rate of absorption and this may affect the availability

of the amino acids in the proportions required for biosynthesis. In natural conditions, however, this seems to be corrected by the ability of some fish to absorb oligopeptides (Bogé *et al.*, 1981) and even macromolecules (Bogé & Pérès, 1983). This seems to be the case in rainbow trout. Thus, Grabner & Hofer (1985) working with rainbow trout showed that 50-60% of the digestive end products were oligopeptides and only 30-40% were free amino acids. They supported the suggestion that most protein absorption is of oligopeptides in the midgut. Ezeasor & Stokoe (1981) and Georgopoulou *et al.* (1985, 1986) stated that in rainbow trout protein absorption in the posterior intestine takes place by pinocytosis. Some controversy still exists, however, about the importance of this uptake of macromolecules and about its persistence in the adult fish. Watanabe (1981) considered that uptake of protein macromolecules is a larval function which disappears in the adult, whereas Ezeasor & Stokoe (1981), Georgopoulou *et al.* (1986), Ash & McLean (1989) and Sire *et al.* (1992) stated that the cells of the posterior intestine of both juvenile and adult rainbow trout are able to absorb proteins in macromolecular form and to digest them intracellularly. Georgopoulou *et al.* (1986) detected the marker protein horseradish peroxidase (M.W.40000) in the enterocytes of the posterior intestine of both juvenile and adult rainbow trout although ferritin (M.W.460000) could only be detected in the juvenile trout. Furthermore, *in vitro* experiments could give no evidence for the intact absorption of α-casein in trout (Marcotte & de la Noüe, 1984).

To summarize, even if the absorption of protein macromolecules only exists in juveniles, it will increase the total amino acid absorption, especially of those amino acids which are poorly absorbed individually, and will help to meet the high

requirements for proteins of juvenile salmonids (Luquet & Kaushik, 1981).

Lipids are absorbed as fatty acids, mono- and diglycerides (Smith, 1989). The anterior intestine and pyloric caeca have been shown to be the main sites for lipid absorption in young and adult rainbow trout (Bergot & Flechon, 1970a,b; Bergot *et al.*, 1975; Bauermeister *et al.*, 1979; Bergot, P., 1981 and Ezeasor & Stokoe, 1981).

Electron microscopy of enterocytes from the intestine and pyloric caeca regions of rainbow trout revealed the presence of two forms of absorbed fat, namely: fat particles of less than 100nm diameter and fat droplets of a few micrometres' diameter (Smith, 1989). The former resemble the chylomicra and the very low density lipid particles in mammals related to transport of dietary fat, while the latter are used for the temporary storage of fatty acids and are located in the enterocytes' endoplasm outside the endoplasmatic reticulum and never in the intercellular spaces (Bergot & Flechon, 1970a,b; Eseazor & Stokoe, 1981). Furthermore, in rainbow trout these two forms of lipids have been shown to be essentially composed of triglycerides using the Nile blue test (Bergot & Flechon, 1970a,b). Sire *et al.* (1981) fed ^{14}C-labelled linoleic and palmitic acids to rainbow trout and followed the distribution of these fatty acids, both chemically and with electron microscopy. The labelled lipid was slow to appear in the blood (not being detectable until after 4 hrs) in the form of triglycerides. In the epithelial cells of the anterior intestine and pyloric caeca during the same time, they observed the formation of very low density lipid (VLDL) particles which progressively passed through the Golgi apparatus, lamellar structures and out into the intercellular space. From there, VLDL particles passed into the interstitial spaces among the fibres

of the lamina propria, and finally into lymph and blood vessels. Bauermeister *et al.* (1979) obtained similar results when feeding wax ester-rich zooplankton to rainbow trout. They showed that the columnar epithelium had numerous osmiophilic droplets (30-100 nm in diameter) in the cisternae of the endoplasmatic reticulum. As the next step, they saw lipid droplet conglomerates (up to 100 lipid droplets) in the Golgi region and finally they saw chylomicra of less than 400nm in diameter being discharged serosally, into the lamina propria as early as 4 hours after feeding. In their opinion, the wax esters were oxidized, hydrolysed, converted into fatty acids, and assembled into triglycerols by the time they were discharged from the enterocytes into the lymph or blood vessels in a similar fashion to that in mammals.

Much of the carbohydrate transport has been studied by measuring the uptake of glucose, but information about glucose (and other sugars) transport mechanisms in the fish intestines continues to be confusing and sometimes contradictory. It is unclear whether differing results represent methodological differences or incomplete understanding of the processes involved (Smith, 1989; Singh & Nose, 1967).

Stokes & Fromm (1964) studying the intestinal transport of D-glucose in a perfused segment of rainbow trout intestine, found transport sites in both mid-gut and hind-gut, but maximum transport rates were higher (3-4 x) in the mid-gut than in the hind-gut. Di Bennedetto & Farmanfarmaian (1975) found glucose and galactose absorption rates similar in rainbow trout and pointed out that their absorption also occurred primarily in the midgut and by active transport. According to Fänge & Grove (1979) absorption of glucose by the intestinal epithelium occurs by an active mechanism and

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Stokes & Fromm (1964) studying the intestinal transport of D-glucose in a perfused segment of rainbow trout intestine, found transport sites in both mid-gut and hind-gut, but maximum transport rates were higher (3-4 x) in the mid-gut than in the hind-gut. Di Bennedetto & Farmanfarmaian (1975) found glucose and galactose absorption rates similar in rainbow trout and pointed out that their absorption also occurred primarily in the midgut and by active transport. According to Fänge & Grove (1979) absorption of glucose by the intestinal epithelium occurs by an active mechanism and

can take place against considerable gradients. Furthermore, these authors stated that the transport of glucose is associated with electric potentials. Buclon (1974) had found the transport of glucose to be sodium dependent. Furthermore, Stokes & Fromm (1964) and Fänge & Grove (1979) noted that the transport of glucose is affected by temperature, being diminished at lower temperatures. In contrast, Escoubert *et al.* (1974) found no increase in the rate of intestinal absorption in rainbow trout when the water temperature was increased from 11°C to 20°C.

Not all carbohydrates are equally well absorbed by fish and in rainbow trout the relative efficiency of absorption was found to be: monosaccharides (glucose) higher (around 100%) than dissaccharides (sucrose and lactose, 99% and 97% respectively), higher than starches (dextrin being better absorbed - 50% - than raw potato starch - 32%) for a 50% inclusion level (Singh & Nose, 1967). These authors also showed that for the simple carbohydrates the absorption efficiency remained nearly constant regardless of the dietary inclusion level (20-60%) whereas for the more complex carbohydrates there was a decrease with increasing inclusion levels. This suggested that the enzymatic breakdown (digestion of polysaccharides to monosaccharides) in the digestive tract may act as a limiting factor. Considering the higher absorption of mono- and disaccharides in the diet, Singh & Nose (1967) concluded that trout had a reduced ability to convert polysaccharides to disaccharides.

By contrast, Pieper & Pfeffer (1979) also working with rainbow trout found sucrose and gelatinized corn starch to be better utilised than glucose, whereas lactose was utilised inefficiently. All the carbohydrates were incorporated in the diets at 30%.

In view of their observations the authors concluded that the enzymatic breakdown in the digestive tract was not a limiting factor. On the contrary, they suggested that the more even influx of end products of digestion due to slower release of monosaccharides may have had positive effects.

More recently, Spannhof & Plantikow (1983) stated that the degree of polymerization of the carbohydrate had a major influence on absorption. Monosaccharides were absorbed completely, dextrin and starch gum were moderately absorbed, whereas in crude starches absorption was low. This suggests that the different digestibilities of dextrins, starch gums and crude starches were clearly related to the degree of hydrolysis of the carbohydrate. The same authors also observed in rainbow trout that carbohydrate digestion was inversely related to the carbohydrate content of the feed. Absorption first increased in proportion to the amount of starch ingested, reached a maximum which varied depending on the quality of the starch, and finally decreased again as the proportion of the starch in feed further increased. Thus, when the feed contained crude starch the maximum absorption was reached when the starch content of the feed was 20%. When the starch was partially hydrolysed (starch gum) the maximum absorption was not reached until the starch content of the feed was 30-35% (for a 2% body weight/day ration). However, no such depression of starch digestion was observed if the feed contained dextrin (starch product with an advanced degree of hydrolysis).

Firstly, Spannhof & Plantikow (1983) considered the proof that crude starch adsorbed amylase in the chyme to such an extent (20% crude starch bound 70% of the amylase)

that a reduction/decline could be expected in the rate at which the starch in the feed was hydrolysed and later absorbed. The rather better absorption of starch gum compared with crude starch was attributed to a slight degree of amylase adsorption by this form of starch, although not quantified. Secondly, the observation that crude starch accelerated the passage of digesta through the intestine (almost twice as quickly as the control diet) reduced the time available for absorption, partially explaining the lower absorption of crude starch-containing feeds compared to those containing other forms of starch.

In feed evaluation, it is desirable to have a quick and simple method for the determination of nutrient digestibility and, for terrestrial animals, digestion trials are carried out routinely. A digestion trial involves a record of the nutrients consumed and of the amounts of them voided in the faeces. In terrestrial animals evaluation of food intake and uncontaminated faeces collection are quite simple and convenient, using various types of metabolism cages adjusted to the size of the animal in such a way that faeces fall into a properly placed container. A specially designed urine conduit attached to the animal leads the urine down through the cage floor. The feed box is attached to the front of the cage to prevent scattering, or alternatively a collection bag and harness can be attached to the animal (Maynard *et al.*, 1979; Church & Pond., 1982). For fish, however, the aquatic environment makes measurements of feed intake and faeces and urine outputs very difficult due to possible leaching of food and faeces into the water, contamination of the faeces by wasted/uneaten food, or by organic fluids such as urine or ammonia. Absorption *in vivo* has been studied negatively in fish - anything that does not appear in the faeces

being assumed to have been absorbed. Since the lost nutrients are treated as if they are absorbed by the fish, an error is caused in the digestibility coefficients which are biased towards higher values, according to the losses of nutrient in the water. In contrast, errors due to contamination of faeces results in digestibility coefficients biased towards lower values.

Several researchers have developed methods for the collection of faeces for fish digestion studies, either when using direct or indirect quantitative methods, which would in turn give an indication of the amount of nutrient ingested and later egested. The earliest method of faecal collection was used in digestibility studies of brook trout (*Salvelinus fontinalis*) and involved laborious and time-consuming water filtration techniques that required measurements and analysis of all egesta and excretions (Migita *et al.*, 1937 and Tunison *et al.*, 1942, cited by Windell (1978) and Austreng (1978). However, according to Austreng (1978) the method was not very accurate since errors could have arisen because of contamination of faeces by nitrogenous compounds excreted in urine, or across the gills, or through leaching. Phillips *et al.* (1948) dissected the digestive tract of force-fed rainbow trout and analysed their gut contents. The usefulness of this method is limited by the fact that it is necessary to kill the fish, and by the difficulty of estimating how far digestion has proceeded in an individual before it is killed. In addition, the calculation of the digestibility coefficients requires a knowledge of the amounts of feed eaten and faeces produced, which is normally difficult to quantify in fish feeding trials (Austreng, 1978).

Nose (1960) dissected the digestive tract of rainbow trout into several regions.

Hastings (1967) removed faeces by dissection from the lower one third of the intestine of channel catfish (*Ictalurus puntactus*). Smith and Lovell (1972, 1973) compared catfish faeces siphoned from troughs with samples from four separate sections of the digestive tract (stomach, upper and lower intestine and rectum). They showed that siphoned collections gave significantly higher digestibility coefficients than the collections from the rectum, probably due to leaching of nutrients into the water. Inaba *et al.* (1962) stripped the contents of the large intestine using slight pressure and recovered the faeces from the pond with a net. Forster and Gabbot (1971), cited by Cho *et al.* (1976), used a false bottom of plastic mesh in the tank, while Cho & Slinger (1979a,c) used a group of caged fish in a feeding tank during the day which were transferred to a faeces collection tank at night. All these methods suffer from similar disadvantages, such as the samples collected not being representative of naturally defecated material and frequent handling of the fish which is stressful.

By contrast, Smith (1971) employed a direct method of total faecal collection in which yearling size rainbow trout were confined to a metabolism chamber. Using this chamber, collection of gill excretions and urine as well as faeces was possible. However, the test fish were highly stressed and restriction of swimming activity and force-feeding was found to be necessary. Analysis and calculations were also laborious (Austreng, 1978).

In order to completely avoid any leaching from the faeces into the water, and the need to sacrifice a great number of fish, several authors chose to take the fish out of the water and sample the faeces directly by manually stripping the posterior region of the

intestine after anaesthetizing the fish (Nose, 1960, 1961, 1967a,b; Inaba *et al.*, 1962; Singh & Nose, 1967; Austreng, 1978; Tacon & Rodrigues,1984; Vens-Cappell, 1985; Spyridakis *et al.*, 1989). Although this method has its advantages, there remains the technical problem of obtaining a faecal sample which has been completely digested and which is not contaminated by other body fluids such as sexual products, mucus, blood and other endogenous products. Hence it is evident that there is presently no method for faeces collection in fish which is without any inherent errors.

Faecal collection methods still pose a serious technical problem which directly affect the accuracy of reported digestibility results and the standardisation of methods in digestion studies with fish is an urgent and indispensable precondition for the elaboration of feed value tables (Nose,1967a,b; Smith & Lovell, 1972, 1973; Furukawa, 1976; Austreng, 1978; Smith *et al.*, 1980; Furuichi & Takahashi, 1981; Choubert *et al.*, 1979, 1982b; Vens-Capell, 1985 and Spyridakis *et al.*, 1989).

Practical digestibility studies can be carried out using either direct or indirect methods. In the direct method, the animals are fed a diet of known composition over a time period of several days, during which the faeces are collected. Thus, the direct method implies measuring total food intake and total faeces output. In the indirect or indicator method, a reference substance or marker is used, this being an inert material, either intentionally incorporated in the diet or present as a constituent of the diet, which is unaltered by the process of digestion. Knowledge of levels present in the diet together with concentration in the faeces enables the calculation of the digestibility for a given nutrient, obviating quantitative faecal collection and total feed intake problems. In

addition, fish can be maintained under normal culture conditions, and large numbers can be used which will help eliminate the effect of individuals. The indirect method is based on the assumption that the amount of marker or indicator in the feed and egested in the faeces is the same over equal periods of time (Windell *et al.*, 1978a).

Recently, most digestibility experiments with fish have used the indirect method (Nose, 1960; Hastings, 1967; Smith & Lovell, 1973; de la Noüe & Choubert, 1986; Nose, 1989; Kaushik, 1989a; Cho, 1992) and the digestibility coefficients calculated using the formulae of Maynard and Loosli (1969):

$$\text{Apparent Nutrient Digestibility (\%)} = 100 - 100 \times \left[\frac{\%\text{indicator in feed}}{\%\text{indicator in faeces}} \times \frac{\%\text{nutrient in faeces}}{\%\text{nutrient in feed}} \right]$$

$$\text{Apparent Dry Matter Digestibility (\%)} = 100 \left[1 - \frac{\text{wt. indicator/g dry matter in feed}}{\text{wt. indicator/g dry matter in faeces}} \times \frac{\text{1-wt. indicator/g dry matter in faeces}}{\text{1-wt. indicator/g dry matter in feed}} \right]$$

Reference substances of widely differing character can be used to study digestion processes. The indicators may be solid or water soluble, they may constitute a part of the food or be added to the diet. However, it is probable that different types of markers pass through a section of the gut at different rates and thus the usefulness of each may be restricted to the study of particles with similar characteristics (Choubert, 1983).

There is no ideal reference substance for estimating dry matter digestion. However, Hydén (1960) and Waller *et al.* (1980) suggested that, to be accepted as reliable, a

reference substance should possess the following characteristics:

(i) The substance should not influence the normal digestive process of the animal or be unpalatable.

(ii) The substance should not be absorbed to any significant degree and recovery of one hundred percent of the daily dose in the faeces should be possible.

(iii) The reference substance and nutrient under investigation should move together through the digestive tract.

(iv) The substance must be well defined from a chemical point of view and ought to be easily and accurately determinable.

(v) The substance should not be taken up or absorbed, produced or destroyed to any significant degree by micro-organisms or by other parts of the contents in the gut.

In 1874 the German investigator, Eugen Wildt, used silica as a reference substance in sheep and tried to follow the progress of digestion with regard to crude fibre, nitrogen-free extract, crude protein, organic substances, dry matter, water and several of the ash constituents (sodium, potassium, calcium, magnesium, iron, phosphorus, sulphur and chloride). He also studied the net effects of absorption and excretion in the different parts of the alimentary tract and calculated the retention time of the digesta in these sections. In a latter publication, Wildt (1879) presented further data from experiments with this technique (Hyden, 1960). Wildt was therefore the first researcher to use a reference substance which could be determined quantitatively, and thus laid down the principles on which modern studies are based.

However, many years passed until researchers began to use markers in digestion trials again. Cr_2O_3 has been widely used and preferred as an internal marker since it seems to fulfil the characteristics for an "ideal" reference substance, referred to above. It was the Swedish investigator Edin who in 1918 and then in 1926 first used chromic oxide (Cr_2O_3), using a well developed quantitative method for the determination of digestibility in terrestrial animals. The method involved incorporation of the marker into strips of a specially prepared macaroni containing approximately 15% chromic oxide, so as to secure a proper mixture of the Cr_2O_3 with the feed and avoid uneven excretion (Edin *et al.*, 1944).

Several methods have been used to analyse chromic oxide in faeces. These include an acid digestion method (Furukawa and Tsukahara, 1966) and a method where ashed material is fused in a furnace with a flux of potassium nitrate, sodium carbonate and sodium hydroxide (Edin *et al.*, 1944; Schürch *et al.*, 1950). Both of these methods require a photometric determination. Ever since Edin proposed the use of chromic oxide, most indicator trials in domestic animals have been carried out with this marker.

In 1952 Danski and Hill investigated the excretion of Cr_2O_3 in growing chickens. They used chromic oxide incorporated into the experimental diet, either as the pure powder at a one percent level or as a chromic/water based dough. They found that collection of faeces over period of less than 16 hours showed excessive variability of chromic oxide concentration, but that those over periods of 24 hours showed good replication. The authors suggested that the variability that was obtained for chromic

oxide concentration in the faeces was due to the level of food intake the previous day. However, considerable variation was found in total recovery of chromic oxide which averaged approximately 94%. This incomplete recovery of ingested chromic oxide was explained by incomplete collection of faeces.

Dansky & Hill (1952) concluded that there was a diurnal rhythm in the excretion of chromic oxide but that the effects of this could be eliminated by collecting faeces in unit periods of twenty four hours. They suggested that changes in levels of feed were quickly reflected in a change in the level of chromic oxide in the faeces and hence could give considerable variation in daily results.

Knapka *et al.* (1967), working with donkeys, found significantly lower digestion coefficients based on chromic oxide data than those based on polyethylene, ^{144}Cerium and total collection. This was directly attributed to the low recovery (81.5%) of this indicator due either to the loss of faecal material or error in sampling techniques. Recovery of less than 100% of ingested chromic oxide has also been reported in pigs, sheep and in cattle (Kane *et al.*, 1953; Moore, 1957; Elam *et al.*, 1959). Barnicoat (1945) reported incomplete recovery of Cr_2O_3 in an experiment with pigs that had received dietary chromic oxide continuously for seven weeks, and suggested the possibility that the Cr_2O_3 was being retained in the digestive tracts of the experimental animals. There is some support for this in Knapka *et al.*'s (1967) donkey experiment, where one group was confined in stalls and given little exercise whilst the other group had access to unlimited exercise. The exercised group showed a normal faeces/feed equilibrium with Cr_2O_3 after a few days. The other group showed low chromic oxide

recovery rates and it has been suggested that lack of exercise may have affected the passage of this indicator through the gut. Haerlein *et al.* (1966) recovered about 98.4% of the Cr_2O_3 administered to ponies that were subjected to limited exercise and Olssen (1950) quoted by Kotb & Luckey (1972) reported a recovery of 99% with hens kept in batteries and hence also subject to limited exercise.

Diurnal variation in excretion of chromic oxide have been reported by Kane *et al.* (1953), Elam *et al.* (1959) in cattle, Moore (1957) and Haerlein *et al.* (1966) in the pig and pony, respectively, and as already indicated, it has been reported in chickens. It has not, however, been reported in rats and humans and digestion coefficients based on total collection of faeces and chromic oxide gave good correlations (Irwin & Crampton, 1951; Rosenlund & Njaa, 1982).

Nose (1960, 1961) and Inaba *et al.* (1962) were the first to use chromic oxide to investigate digestibility in fish (rainbow trout). Inaba *et al.* (1963) and Kitamikado *et al.* (1964a,b) used the same indicator technique to investigate the digestibility of protein and starch and the effect of starch and oil on protein digestibility. Similar investigations in rainbow trout were carried out by Singh & Nose (1967) and Nose (1967b). Several other authors have also used chromic oxide in digestion studies with fish with apparent success (Cho *et al.*, 1974, 1976; Lall & Bishop, 1976; Austreng, 1978; Lied & Julshamn, 1989). Other authors have questioned the use of chromic oxide for digestibility studies in fish (Bowen, 1978a,b). However, Bowen's experimental diet was prepared as an aqueous suspension in a laboratory blender and only 6 specimens of tilapia (*Sarotherodon mossambicus*) were allowed to consume

"pieces" of the diet. Thus, these technical problems almost certainly affected Bowen's results (Foltz, 1978).

Although to date, Cr_2O_3 has been the most extensively used digestibility indicator in fish feeding trials, other compounds have also been evaluated. Hirao *et al.* (1960) and Yamada *et al.* (1962) used diets labelled with ^{32}P in the form of water-insoluble ammonium phosphomolybdate to determine the digestibility of commercial feeds for rainbow trout. Lied *et al.* (1982) compared titanium oxide (TiO_2) with Cr_2O_3 in digestibility studies with cod (*Gadus morhua*) and concluded that both markers compared well. However, when analytical sensitivities and amounts of work involved were taken into account, it was concluded that Cr_2O_3 was a more convenient indicator than TiO_2. Barash *et al.* (1983) studied the nutrient requirements of tilapia using magnesium ferrite ($MgOFe_2O_3$) coated with paraffin as a dietary marker. The marker concentration was determined by weighing the faeces in the presence and absence of a magnetic field. Other compounds, including polyethylene, polyethylene glycol (PEG), heavy metal nitrates, ^{144}Cerium, ^{131}I, ^{51}Cr, ^{14}C labelled glucose and dysprosium have also been tested as digestibility markers, although to a limited extent in fish (Hiyama & Singh, 1966; Luckey *et al.*, 1975; Waller *et al.*, 1980; Kennelly *et al.*, 1980; Storebakken *et al.*, 1981; Tacon & Rodrigues, 1984).

Those methods which involve labelling food with a radio-isotope are restricted in their application due to problems associated with a safe formulation and disposal of radio-active food. However, in common with radiographic methods used to study gastric evacuation (Ross & Jauncey, 1981; Talbot & Higgins, 1983), the use of isotopes

allows various measurements to be made without killing the fish. A further advantage of this method is that food preparation does not present the health hazards associated with radio-isotope techniques. Nevertheless, the need for specially prepared experimental diets still remains. Thus, the ability to use a substance which exists already in the feed, i.e. an internal marker, is a distinct advantage in digestibility studies as it is less costly, especially in large scale trials. As is the case for an added/external indicator, a naturally occurring marker must be nonabsorbable; it must not affect or be affected by the digestive processes or its microbial population; and it must be easily and accurately determinable.

The acid-insoluble ash (AIA) component of feeds is composed predominantly of silicates and has been discussed by Van Keulen & Young (1977). It has been used as a naturally occurring marker for the determination of digestibility in monogastric animals and, to a lesser extent, in ruminants. McCarthy *et al.* (1974) and Vogtmann *et al.* (1975) confirmed the suitability of AIA as a marker for poultry and swine. Similarly, Shrivastava & Talapatra (1962) and Van Keulen & Young (1977) demonstrated that AIA was a suitable marker for sheep fed several varieties of hay and diets of pelleted hay plus grain, respectively. Thonney *et al.* (1979) have successfully used AIA as a marker in cattle fed various levels of hay plus grain.

All the reports cited above have compared the AIA ratio technique to the conventional total collection digestibility method and obtained almost identical mean digestibility values (Furuichi & Takahashi, 1981). Under the conventional method of determining digestibility, animals are frequently fed restricted amounts to ensure total consumption.

A major application of the AIA marker method is with groups of animals fed *ad libitum* (Block *et al.*, 1981) as is often the case in fish digestibility trials. Some workers (McCarthy *et al.*, 1974; Van Keulen & Young, 1977; Vogtmann *et al.*, 1975) have found it necessary to add celite (brand name for diatomaceous silica) to those feed mixtures which contain only a small amount (<0.5%) of AIA to improve the precision of the analysis without affecting absolute values of digestibility coefficients. Furuichi & Takahashi (1981) concluded that the addition of celite was unnecessary. Van Keulen & Young (1977) reported that there was no evidence of a diurnal variation in AIA excretion in sheep and Furuichi & Takahashi (1981) found a very small variation of AIA in the faeces of rabbits. Van Keulen & Young (1977) obtained a 100% recovery of AIA from sheep.

AIA is believed to be insoluble in the gastrointestinal tract and comparing its excretion behaviour with that of chromic oxide Furuichi & Takahashi (1981) found that the excretion ratios of the two markers were very similar and the distributions of both AIA and Cr_2O_3 in rabbit faeces were almost identical. According to the same authors, these results may be due to the fact that AIA behaves throughout the gastrointestinal tract in a manner similar to that of chromic oxide (the feasibility of Cr_2O_3 as a marker in rabbits has been demonstrated by other authors; Furuichi *et al.*, 1979 and Huang *et al.*, 1954, cited by Furuichi & Takahashi, 1981). Yen *et al.* (1983), in studies with lean and obese pigs, suggested that AIA may be used as a natural marker for estimating apparent nutrient digestibility in pigs, especially when young. The results compared well to those with the total collection method. Penning & Johnson (1983a) used AIA as a marker to estimate herbage (rye-grass and lucerne) digestibility in

sheep as compared to digestibility measured using the total collection method and *in vitro*. While both total collection and *in vitro* estimations of digestibility were similar, digestibility calculated using AIA as a marker was lower, especially for lucerne. According to the authors, it was not clear why AIA gave relatively poorer predictions of digestibility for lucerne as for rye-grass, although the recovery rates of AIA were more variable for lucerne than for rye-grass.

Very little information seems to be available on the use of AIA as a reference substance in fish. Tacon & Rodrigues (1984) used AIA at varying concentrations in digestibility studies with rainbow trout but obtained poor results in comparison to chromic oxide. AIA had erratic and significantly reduced nutrient digestibility coefficients over the range of dietary inclusion levels tested (0.5%, 1% and 2%) suggesting that the marker moved throughout the gastrointestinal tract at a slower rate than the digesta. In contrast, Atkinson *et al.* (1984) also in rainbow trout showed that AIA compared well with chromic oxide, although they suggested the addition of extra AIA in the form of celite (diatomaceous silica) or other to the diet to improve the precision of the analysis.

Different cell wall fractions, such as cellulose, hemicellulose, lignin, cutin and silica or combinations of these, have been examined to determine their suitability as naturally occurring internal markers.

Penning & Johnson (1983a) examined the use of potentially indigestible cellulose (PIC) as an internal marker to estimate organic matter digestibility (OMD) of two

feeds in sheep. They reported that PIC estimated OMD well when compared to the total collection method, but required the use of rumen-fistulated animals and up to 10 days to digest samples. Lignin has been shown by some workers to be partially digestible *in vivo* (Ely *et al.*, 1953; Kane *et al.*, 1953; Elam & Davies, 1961). These workers considered that losses of lignin due to its digestion limited its usefulness as an internal marker in ruminants. However, lignin is often regarded as being indigestible as there appear to be no known anaerobic microbial or mammalian enzymes for lignin degradation (Van Soest, 1982).

Penning & Johnson (1983b) examined the use of indigestible acid detergent fibre (IADF) as an internal marker in sheep. Acid detergent fibre (ADF), the most indigestible component of plant cell walls (Van Soest, 1963) and consisting mainly of lignin and cellulose, was isolated from samples of feed and faeces and digested in buffered cellulase solution to obtain the ash-free residue which is indigestible by cellulase (IADF). These authors found that IADF gave more precise estimates of OMD than the *in vitro* technique and also predicted OMD more precisely than the internal markers PIC and AIA.

Ohlde & Becker (1982) also used acid detergent fibre (ADF) to predict organic matter digestibility in tropical and subtropical by-products and concluded that ADF fractions were superior to crude fibre as well as to single fractions of the cell walls such as lignin, cutin and silica. However, they pointed out that the chemical composition of the analysed materials (high lignin, cutin and silica level contents) could be the possible reason for the significantly lower relationship between the crude fibre content and the OMD.

There have been few investigations concerning the ability of fish to hydrolyse cellulose but fish do not seem capable of producing enzymes that can hydrolyse the β,1-4 linkages present in the carbohydrate polymer cellulose (Cowey & Sargent, 1972; Prosser, 1973; Stickney, 1976; Van Dyke & Sutton, 1977). Cellulose digestibility is very low or zero where data is available. Smith & Lovell (1972) reported 0-1.47% cellulose digestion by channel catfish (*Ictalurus punctatus)* fed a high protein diet. Rainbow trout were unable to digest crude fibre (Austreng, 1978; Tacon & Rodrigues, 1984), which consists primarily of cellulose. Neither do fish appear to maintain a symbiotic micro-flora capable for hydrolysing cellulose or other structural hetero-polysaccharides (Austreng, 1978; Bergot, F., 1981; Bromley & Adkins, 1984). The crude fibre fraction of the experimental diets used by these authors was primarily of plant origin (crude fibre being an estimate of the organic fraction of the diet which is resistant to chemical treatment with dilute alkali and acid), consisting largely of cellulose and to a lesser extent of hemicellulose and the aromatic compound lignin. The almost universal occurrence of crude fibre as a natural indigenous marker within pelleted fish feeds thus offers a valuable tool to nutritionists and fish farmers alike for estimating feed digestibility (Tacon & Rodrigues, 1984).

Hydrolysis-resistant organic matter (HROM), chiefly cellulose and chitin, has been used as a reference substance for measurement of digestive efficiency in rainbow trout and different species of tilapia (*Tilapia aurea*, *T. mossambica*, *T. nilotica*) (Buddington, 1980). The author concluded that HROM was an efficient reference material, comparing well with chromic oxide, and was a more accurate marker than ash which was absorbed in significant amounts (7-22%) by all species.

Lied *et al.* (1982) used calcium, iron and zinc as internal reference substances in digestibility studies with Atlantic cod (*Gadus morhua*). Unlike calcium and zinc, calculations based on iron as an indicator resulted in considerably lower estimates of digestibility than when based on titanium (IV)-oxide or chromic oxide. They also estimated protein digestibility with reference to gastric concentrations of calcium and zinc in wild capelin-predating cod and cod fed whole capelin with titanium (IV)-oxide included in the experimental feed. Their results suggested that gastric and intestinal levels of calcium and zinc could be used as indicators to determine protein digestibility in wild cod. As iron is part of the haemoglobin molecule, it was excreted and thus biased the estimation of digestibility when used as an indicator.

Results of feed evaluation research are normally presented by listing ingredients or compounded feeds together with data on composition and digestibilities. The apparent digestibility of a certain nutrient is mostly represented by one value, the apparent digestibility or digestion coefficient. This representation by a single value implies that an apparent digestibility coefficient of a certain nutrient is a constant. However, digestibility depends on three main factors: a) the ingested food and the extent to which it is susceptible to the effects of the digestive juices; b) the activity of the digestive enzymes; c) the length of time the food is exposed to the action of the digestive enzymes. Each of these main factors is affected by a multitude of secondary factors some of which are associated with the fish itself, such as its species, size and physiological condition; some associated with the environmental conditions, such as water temperature; and some are related to the food, such as its composition, particle size, frequency of feeding and amount eaten (Hepher, 1988; Hastings, 1969).

Any factor capable of affecting gut transit times or evacuation rates, digestive enzyme secretion or activity can influence the digestibility coefficients. Table 1.2 summarises those secondary factors which affect digestibility in rainbow trout.

The type and stage of development of the gastrointestinal tract, thus species and age, will primarily influence the digestibility of a given feed. Limited work, however, has been done on these factors and controversy still exists about their influence on digestibility (Table 1.2).

In contrast to endotherms, fish are sensitive to environmental temperature changes and increasing the temperature may increase both digestive enzyme secretion and activity (Kitamikado & Tachino, 1960b), increase the rate of passage of food through the digestive tract, and consequently shorten its exposure time to digestive juices (Brett & Higgs, 1970; Fänge & Grove, 1979; Ross & Jauncey, 1981) and accelerate the rate of transport of digested nutrients through the intestinal wall (Stokes & Fromm, 1964; Escoubert *et al.*, 1974).

There is some disagreement as to whether digestibility in fish and in rainbow trout in particular is affected by the ration level. The stability of digestible coefficients with increased amounts of food might be explained as resulting from: 1 - increased enzyme secretion with increased food uptake into the stomach and intestine, since distension of the stomach wall evokes secretion of gastric juices (Western & Jennings, 1970); 2 - longer retention of food in the gut increasing the feeding level (Beamish, 1972; Jobling *et al.*, 1977; Windell *et al.*, 1969). However, since both increased enzyme secretion and food retention seem not to be linearly related to the amount of food

Table 1.2 - Biotic and abiotic factors affecting digestibility in rainbow trout.

Factor	Effect on digestibility	Author
Fish Size	6g fish lower than 100 g fish	Kitamikado & Tachino (1960b)
	No effect (18g, 207g and 586g) at 11°C and 15°C	Kitamikado *et al.* (1964, a,b)
	Increase between the 18g trout and the large ones at 7°C	Windell *et al.* (1978b)
	Non-significant increase (10g, 50g and 100g)	Gómez-Jarabo *et al.* (1979)
	No effect on the range 2-200g	Watanabe *et al.* (1989)
Temperature	No effect at 10°C or 16-17°C	Luquet & Fauconneau (1979) Possompes (1973)
	Higher nitrogen value at 15°C than at 7°C	Windell *et al.* (1978b)
	No effect on the range 9-15°C. Increase (2-3%) at 18°C	Cho & Slinger (1979a)
	No effect (5°C, 10°C and 15°C)	Watanabe *et al.* (1989)
Feeding level and frequency	Lower total dry matter, carbohydrate and energy values for trout fed 1.6% body weight/day than for trout fed 0.4% and 0.8%. No effect on protein and lipid values.	Windell *et al.* (1978b)
	No effect on protein values.	Storebakken & Austreng (1987)

Table 1.2 (continued)

Factor	Effect on digestibility	Author
Feeding level and frequency	No effect on nitrogen and energy values (0.3%, 1% and 2% body weight/day)	Storebakken *et al.*(1991)
	No effect on nitrogen (1 or 2 meals/day)	Choubert *et al.* (1982a)
	No effect on dry matter, protein and energy (2 to 6 meals/day)	Hudon & de la Noüe (1984)
Food Composition	Higher with sieved than with unsieved fish meal	Kitamikado *et al.*(1964a)
	Higher for extruded food (especially carbohydrates)	Smith (1971), Bergot & Breque (1983), Kaushik & Oliva-Teles (1985)
	Three degrees of extrusion improved energy and starch values, but not protein	Pfeffer *et al.* (1991)
	Decrease protein value with increasing dietary carbohydrate levels	Kitamikado *et al.*(1964b)
	Lower values for lipid with increasing number of carbon atoms in the fatty acid chain. Higher with the number of double bonds.	Nose (1967b)
	Decrease with hydrogenation of fish oils (both total lipids and fatty acids). Decrease for individual fatty acids with increasing chain length up to C18. Higher for longer chain length (up to C22)	Austreng *et al.* (1980)

Table 1.2 (continued)

Factor	Effect on digestibility	Author
Food composition	Increase with increasing dietary lipid level (5% to 23%)	Watanabe *et al.* (1979)
	Reduction (5-10%) with high energy small particle sized formulated feeds compared to natural food organisms	Jobling (1986)
	Higher with increasing dietary energy levels (even when food consists of small particles)	Windell & Norris (1969a,b)
Others (e.g. salinity, hypoxia, stress)	Lower (dry matter, energy and protein) with increasing water salinity (from freshwater to 32.5%)	McLeod (1977)
	Salinity had no effect (protein)	Dabrowski *et al.* (1986)
	Hypoxia (40% saturation) had no effect (protein, gross energy, dry matter). Tendency for higher values (amino acids) and proline, glycine, alanine and tryptophan significantly higher in hypoxic fish.	Pouliot & de la Noüe (1988, 1989), Medale *et al.* (1985, 1987).
	Stress (excessive handling, disease, force feeding, starvation or any environmental factor) may have a disturbing effect.	Elliot (1972); Fänge &Grove (1979); Brett (1979); Ezeazor & Stokoe (1981); Hepher (1988).
	Seasonal variations	Chepik (1964), quoted by Hepher (1988).

eaten, digestibility may become lower when large amounts of food are ingested (Garber, 1983; Jobling *et al.*, 1977; Hepher, 1988).

The physical and chemical composition of food ingested may also affect digestibility. Improving the physical characteristics of the food will make it more susceptible to attack by enzymes. Depending on the chemical composition, the energy of a food changes and increases in dietary energy levels result in a slower gastric emptying time, even when food consists of small particles. This results in longer contact between enzymes and substrate and consequently better digestibility (Windell & Norris, 1969b; Grove *et al.*, 1978).

Although little information is available, some other factors have been shown to affect digestibility itself or to have an influence on gut transit time and enzyme secretion or activity. Those factors are related to the physiological condition of the fish (stress due to handling, disease, force-feeding) or to environmental parameters (salinity, hypoxia).

The main aim of this study was to evaluate the bioavailability of nutrients in commercial feeds in Portugal for rainbow trout over a year and under natural production conditions. Digestibility coefficients for protein, organic matter and dry matter of three size groups of rainbow trout were evaluated using the dietary crude fibre levels as the dietary marker. Parallel with this field investigation, laboratory trials were conducted to investigate the influence of a number of biotic and abiotic factors on digestibility. The laboratory investigations were intended to support the

field survey and provide insight into the factors affecting digestibility in a practical situation.

2 - MATERIALS AND METHODS

I. LONG-TERM DIGESTIBILITY SURVEY OF FARMED RAINBOW TROUT

Two commercial trout farms with different characteristics and at two locations in Portugal were selected to monitor trends in digestibility over a one year period. Since both farms used commercial trout feeds to which no external markers had been added, crude fibre was used as a marker to determine digestibility.

a) Trutorão Trout Farm (Farm A)

Trutorão Trout Farm is located 20-30m above sea level in the centre of Portugal, close to the southern limit for trout distribution in the Northern Hemisphere (fig. 2.1).

As the farm is supplied with water from a spring, the mean temperature variation throughout the year is very small (15.8-20°C; Table 2.1) and is thus around the optimum for rainbow trout (Huet, 1979; Bardach *et al.*, 1972; National Research Council, 1982; Cho & Slinger, 1979b; Sumpter, 1992).

The farm has an annual production of 150t of 220-250g trout. The normal full production cycle is 13-14 months, although the faster growers attain market size in only 8-9 months. Since the farm does not hold a broodstock, it relies on a regular import of eggs from the United States.

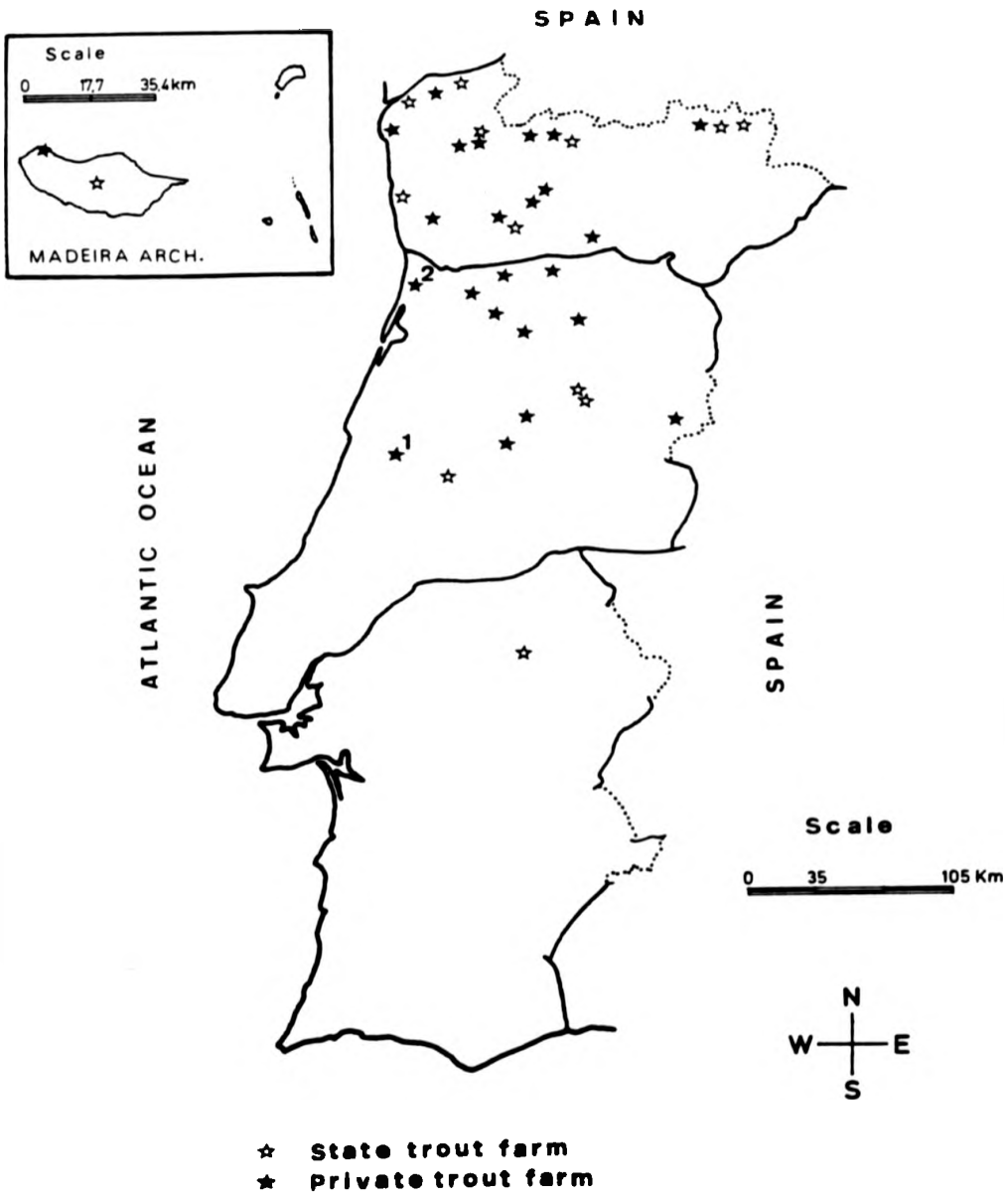

Figure 2.1 - Location of Trout Farms in Portugal
1. Trutorão (Farm A)
2. Inha (Farm B)

Table 2.1 - Average monthly water temperatures at Trutorão Trout Farm between October 1985 and September 1986, recorded at 9.00 a.m. in the ongrowing raceways.

Month	Average Water Temp. (°C) (±S.D.)	Water Temp. at time of faecal collection (°C)
October 1985	16.0 (± 0.20)	15.9
November	16.8 (± 0.25)	16.0
December	16.2 (± 0.51)	16.2
January 1986	15.8 (± 1.22)	16.5
February	15.8 (± 0.62)	16.0
March	17.5 (± 0.28)	16.0
April	16.7 (± 1.13)	16.5
May	18.2 (± 0.75)	17.8
June	18.5 (± 1.82)	18.0
July	19.9 (± 0.95)	17.8
August	19.7 (± 0.46)	18.0
September	19.2 (± 0.42)	17.8

The hatchery has a capacity of 1 million eggs. It contains 28 troughs (5m long x 0.8m wide x 0.4m deep) which are fitted with incubation trays during the incubation and hatching stages. Outdoors the farm consists of 81 raceways. The 40 smallest raceways (11m long x 1.5m wide x 0.8m deep) are for fingerlings, and there are three sizes of raceways available for ongrowing (16 25m x 3m x 1.2m; 13 30m x 4m x 1.2m, and 12 34m x 4m x 1m). There is also a solids settlement basin with a surface area of 1.700m^2 (Plate 2.1).

The farm normally operates on a flow through system as the spring provides sufficient water throughout the year. However, should shortages ever occur, then the farm has been designed so that water from the 16 smaller ongrowing raceways can be directed through the 25 larger ones. In addition, an airline is available to all raceways to supply diffused air from an air compressor should it be required.

The farm has an office block and a warehouse where feeds, tools and vehicles are stored, ice is produced and fish are packed for market. Throughout the farm the fish are fed by hand at a fixed percentage of body weight based on feeding tables. The feeding regime is summarised in Table 2.2.

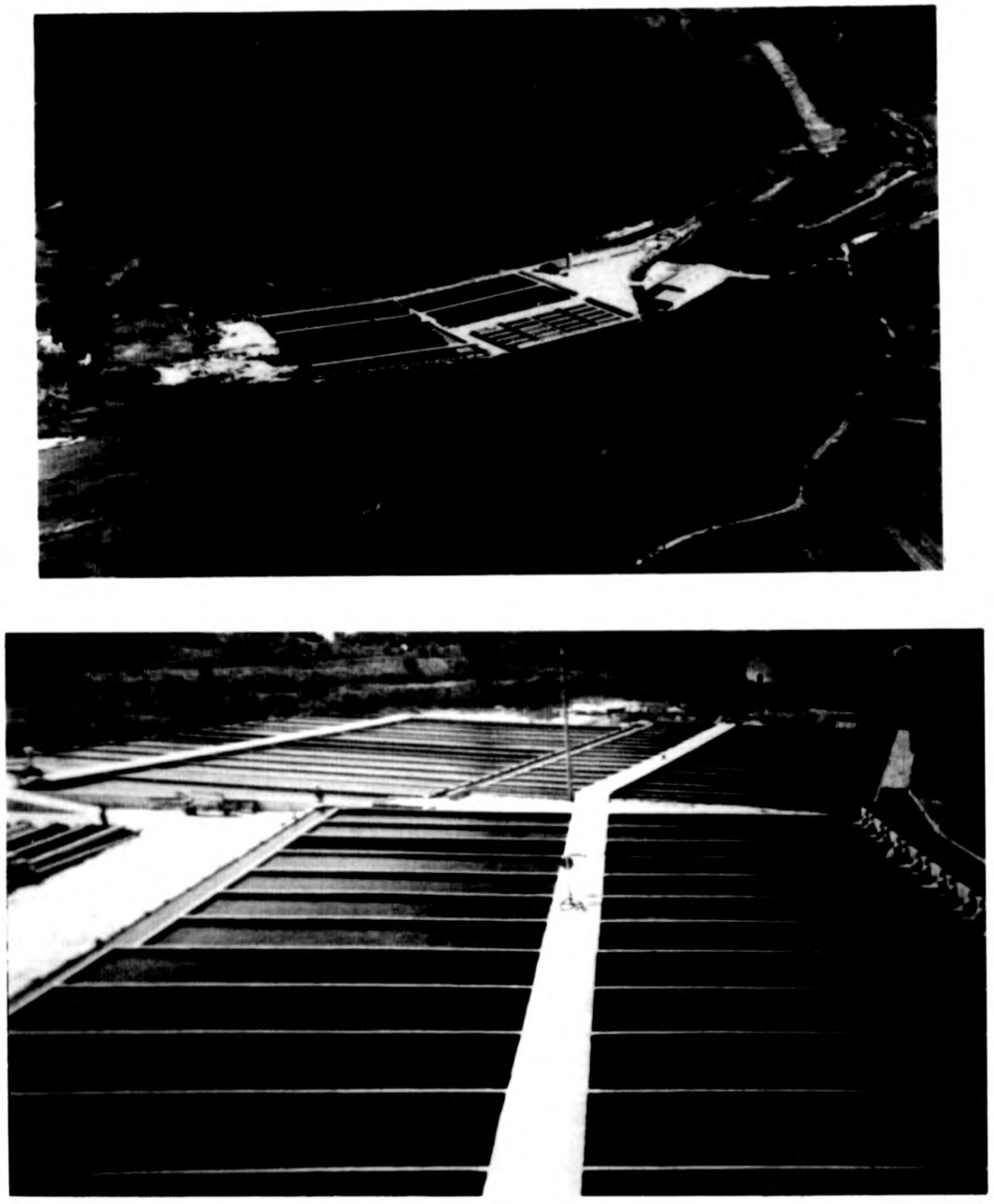

Plate 2.1 - Overall view of Trutorão Trout Farm (Farm A).

Table 2.2 - Feeding regime for rainbow trout at Trutorão Trout Farm (Farm A).

Diet No.	Pellet Size (mm)	Crude Protein Level (%)	Crude Lipid Level (%)	Total Fish Length (cm)	Feeding Frequency (times/day)	% Body Weight fed/day
[1] T1 + T2	crumbs (0.4-0.8)	54	12	<3.5 - 4	6 - 8	4.0
[1] T3	1.0	50	12	4 - 8	4 - 5	4.0
[1] T4	2.0	50	10	8 - 15	3	4.0
[2] T5	3.2	46	10	15 - 17[3]	2	3.5
[2] T7	4.5	46	7	>15	2	2.4
[2] T12	4.5	46	12	>15	2	2.4

1. Imported from BIOTER/BIONA (USA/Spain).
2. Manufactured by SAPROPOR (Portugal), using formulae of Bioter/Biona.
3. Optional, usually fish bigger than 15 cm follow to T7 or T12.

b) Inha Trout Farm (Farm B)

Inha Trout Farm is located 60-70m above sea level in the north of Portugal (Figure 2.1). The farm is supplied by river water and consequently the normal temperature variation throughout the year is quite wide (7-18°C). Occasionally, the temperature can drop as low as 4.5°C in exceptionally cold winters and rise as high as 23°C in very hot summers (Peixoto Correia, Farm Manager, pers.com.). In Table 2.3, the water temperatures recorded on faecal collection days only are presented, since more detailed data was not available for this farm.

The farm has an annual production of 65t of 200-300g trout. The normal production cycle is 14-17 months, although the fastest growing fish attain market size in 10½-12 months. The farm holds a broodstock which supplies all of their egg requirements.

The hatchery has a capacity of 250,000 eggs which are distributed in 20 troughs (5.5m long x 0.55m wide x 0.15m deep), which are fitted with incubation trays during the incubation and hatching stages. Outdoors, the farm consists of 13 raceways (15m long x 1.5m wide x 0.7m deep) and 12 x 4.5m diameter silos with depths ranging from 2m at the edge to 2.8m at the centre. These tanks each have a water capacity of 31m^3 and are laid out in 4 rows of 3 tanks (Plate 2.2). This permits the operation of two alternative water supply systems, depending on the quantity of water available, and also allows pumping costs to be reduced. In addition, the water from the raceways can be re-used in the silos. During the summer, and particularly in July and August, the farm is short of water. At these times up to 70% of the water is recirculated.

Table 2.3 - Water temperature at Inha Trout Farm recorded on faecal collection days during the year 1985-86 at 9 a.m.

Month	Water Temperature (°C)
October 1985	9.5
November	10.0
December	8.0
January 1986	10.0
February	10.0
March	9.0
April	11.0
May	14.0
June	14.0
July	18.0
August	19.0
September	16.0

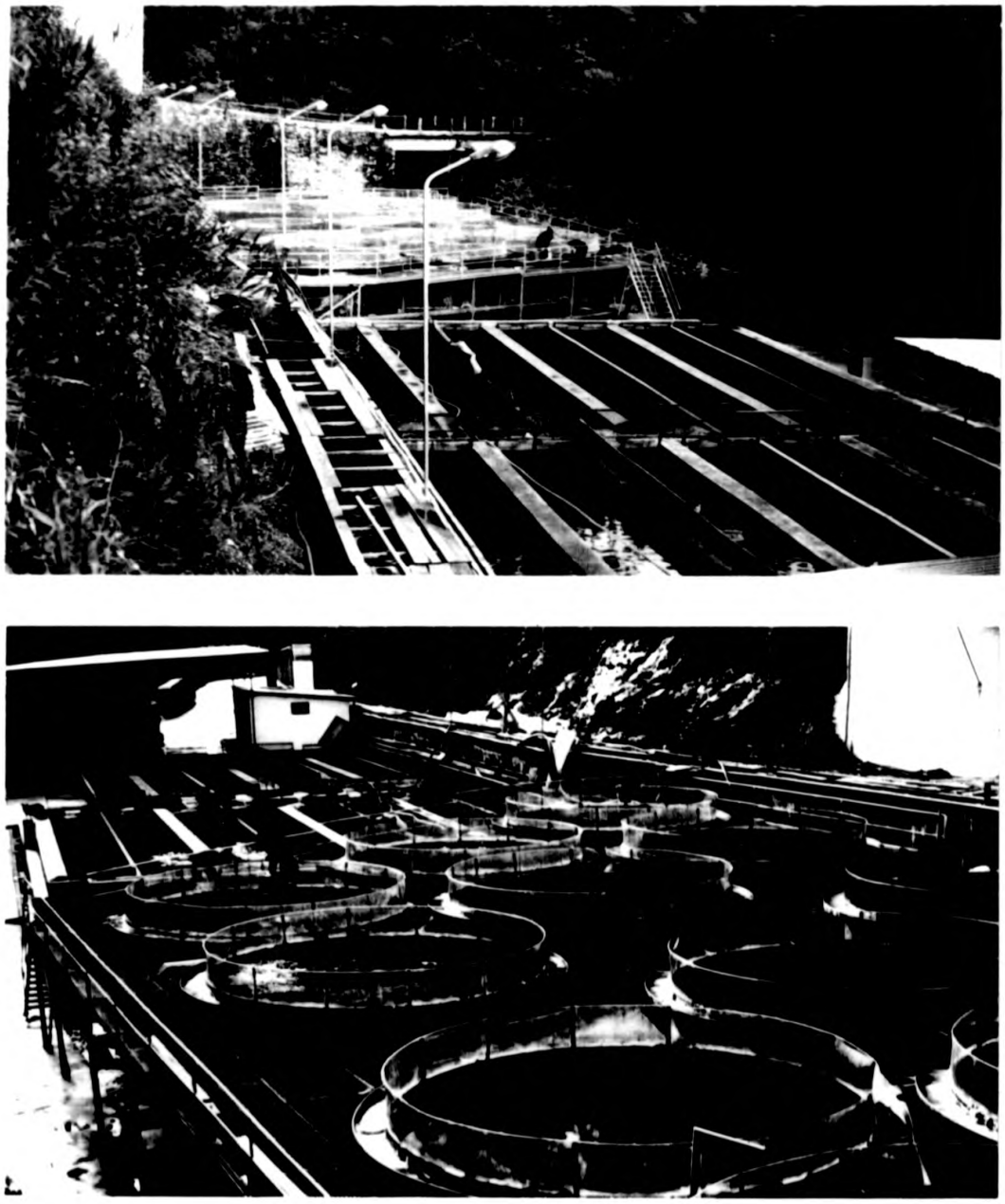

Plate 2.2 - Inha Trout Farm (Farm B).

In order to maintain adequate oxygen levels, the water is aerated when it passes from one silo to the next, and in addition oxygen is supplied through an airline fitted in each silo.

The farm has a warehouse where feeds and tools are stored and for the production of ice and packing of fish for market. Feeding, grading, and harvesting operations are all carried out by hand. The feeding regime on the farm is summarised in Table 2.4.

2. Faecal Collection and Storage

Digestibilities were determined for 3 size groups of fish (14.5-15.5cm - small; 19.5-20.5cm - medium; and 24.5-25.5cm - large, total length) at monthly intervals for a one year period at each of the two trout farms described above.

Fish were categorised by average total length rather than weight, since no suitable balance was available on a regular basis at either farm. However, based on length/weight data collected at the start of the trial, it was established that the relationship between length and weight for fish on both farms was close to that published in BP Nutrition catalogue and Cipasa Nutrition catalogue. Thus, the small, medium and large size groups were equivalent to fish of average weights - 40g, 100g and 180g respectively.

After determining average lengths using a measuring board, fish were anaesthetised using a 3p.p.m. solution of ethylenglycolmonophenylether. In order to avoid contamination of faeces by both water and urine, the fish were gently dried using a

Table 2.4 - Feeding regime for rainbow trout at Inha Trout Farm (Farm B).

[1] Diet No.	Pellet Size (mm)	Crude Protein Level (%)	Crude Lipid Level (%)	Total Fish Length (cm)	Feeding Frequency (Times/Day)	% Body Weight Fed/Day
T0	Crumbs (0.4)	55	10.5	<3	5 - 6	satiation
T01-T04	0.8 - 1.5	55 - 50	10.5	3 - 10	4 - 5	3.5
T2	2	44	8	10 - 12	3	2.5
T3	3.2	40	8	12 - 16	2	2.5
T4	4.5	40	8	>16	2[2]	2.0

1 All feeds were imported from Cipasa (Spain).
2. Only once early in the morning if the temperature is very high.

cloth, and any urine present was voided by applying light pressure in the mid-region, just above the large intestine, towards the anus. Faeces were then obtained by hand-stripping the posterior region of the large intestine of the fish, between the ventral fins and the anus, as described by Austreng (1978).

Faecal samples were collected between 9 a.m. and 1 p.m. and the 3 size groups of fish sampled did not receive the morning meal on collection days.

Each month 260-300, 200-240 and 120-160 fish in the small, medium and large size groups respectively from each farm were stripped and faeces were pooled in glass petri dishes to give sufficient material for two replicate samples in each size group. Immediately after collection, faecal samples were stored at 0-4°C in a refrigerator before being transported back to the nutrition laboratory in a cool box. On arrival at the laboratory, the samples were dried at 105°C for 24 hours, ground into a fine, free-flowing powder in a porcelain mortar using a pestle, and stored in airtight glass containers for subsequent chemical analysis. Moisture, ash, crude protein, and crude fibre levels were determined.

3. Diets

Every month 0.5kg samples of each of the commercially manufactured diets (see Tables 2.2 and 2.4) fed to the three different size groups of rainbow trout sampled were obtained from each farm. On arrival at the Nutrition Laboratory, the diets were stored in airtight plastic containers at 0-4°C in a refrigerator until required for subsequent chemical analysis. Before carrying out approximate analysis, the samples

were ground to a powder in a Moulinex coffee grinder.

4. Analytical Methods

Two replicate samples of each faecal sample and four replicate samples of each commercial diet were subjected to the following chemical analysis:

(i) Moisture

Pre-weighed samples were dried at 105°C (AOAC, 1984) in a Memmert drying oven to constant dry weight and reweighed to the nearest 0.1 mg on a Mettler H10 balance.

(ii) Ash

Ash was determined by incinerating samples in porcelain crucibles at 450°C for 16 hours in a Heraeus M-110 muffle furnace (AOAC, 1984).

(iii) Crude Protein

Total nitrogen was determined by the semi-microkjeldahl technique described by 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 0.2N 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.

(iv) Crude Fibre

Crude fibre was measured as the loss on ignition of the dried residues remaining after digestion of samples with 0.255N H_2SO_4 followed by

0.313N NaOH (AOAC, 1984).

(v) Crude Lipid

Crude Lipid was determined by the method of Korn and Macedo (1973).

5. Trial Analysis

(i) Apparent Food Digestibility

Crude Fibre was used as a marker and apparent digestibility coefficients were calculated using the formula of Maynard and Loosli (1969) employing this naturally occurring dietary marker and then measuring the nutrient levels in food and faeces relative to the indicator.

$$\text{Apparent Digestibilty Coefficient (\%)} = 100 - 100 \times \left[\frac{\%\text{indicator in feed}}{\%\text{indicator in faeces}} \times \frac{\%\text{nutrient in faeces}}{\%\text{nutrient in feed}} \right]$$

$$\text{Apparent Dry Matter Digestibility (\%)} = 100 \left[1 - \frac{\text{wt. indicator/g dry matter in feed}}{\text{wt. indicator/g dry matter in faeces}} \times \frac{\text{1-wt. indicator/g dry matter in faeces}}{\text{1-wt. indicator/g dry matter in feed}} \right]$$

(ii) Statistical Analysis

Statistical evaluation of the data was carried out by analysis of variance (ANOVA) using the Statgraphics Package, and mean differences were determined using Duncan's Multiple Range Test (Duncan, 1955.) Because all data was expressed in percentages, an arcsin transformation of percentages was used to make the data conform to a normal distribution for which these statistical analyses were designed. In some cases the statistical evaluation of the data was also carried out using correlation analysis and multivariate analysis using principal components analysis (PCA) and cluster

analysis. In studies which generate large amounts of information, spread among a great number of variables, multivariate analysis can be useful to emphasise the main trends and possible associations between those variables. The main objective of PCA is to search a group of original variables which characterise a group of objects for a series of axes whose orientation allows the expression of the maximum information in a minimum number of dimensions (Depiereux, 1982).

In this work the objects are the sampling times at the two fish farms during the one year survey period and the variables are the feed composition (levels of moisture, ash, crude protein, crude lipid and crude fibre), the apparent digestibility coefficients for the 3 size groups of fish sampled, and the water temperatures. These variables characterise the objects and create a multi-dimensional space where it is possible to represent those objects.

Without changing the relative position of the objects in the space, PCA selects new coordinates in the direction of the largest, second, third, etc. largest variance among the samples. The larger part of the systematic variance between the samples will be described by these new coordinates, or principal components. The number of axes or principal components equals the number of variables (p), unless the number of objects (n) is smaller than p and then it equals n. However, the major part of the information will be in the two or three first principal components (Legendre & Legendre, 1984). Thus, the graphic representation of the results will be

in a single plane.

The main objective of the cluster analysis is to group objects, which are characterised by a certain number of variables, based on their similarity. Cluster analysis may serve as a useful complement to the PCA (Legendre and Legendre, 1984). In the present work the cluster analysis was based on the euclidian distances between objects calculated from the transformed data matrix and presented graphically as a dendrogram (Sneath and Sokal, 1973).

Data for the field survey was first transformed and then fed into a computer for treatment by the program NTSYS (Rohlf, 1987) which is based on principal components analysis.

II. THE INFLUENCE OF FISH SIZE ON DIGESTIBILITY

The trial was carried out at the Institute of Aquaculture, Stirling. Chromic oxide (Cr_2O_3) and crude fibre were used as dietary markers to compare digestibility values in three size groups of rainbow trout.

1. Fish

Three size groups (40g, 100g and 180g fish) of rainbow trout were obtained from College Mill Trout Farm, Almond Bank, Scotland. Ninety, sixty and thirty rainbow trout of around 40g, 100g and 180g respectively were randomly selected from stock tanks at the farm. The fish were transported to the Institute of Aquaculture in large black plastic bags containing about 30l of water with a stocking density of around 1.5kg of fish per bag. Oxygen from a cylinder was diffused into the water until the bags were completely inflated. Each bag was then sealed using rubber rings.

On arrival at the Institute of Aquaculture, the fish were transferred into the experimental system, where they were acclimatised to the new environment for a period of one week. During this period the fish were fed a commercial high density trout diet (EWOS no. 551 containing 47% crude protein and 9% crude lipid) *ad libitum* twice daily.

2. Experimental System

The 40g fish were held in six 0.6m diameter glass fibre tanks, each with a capacity of 160l of water. Both the 100g and 180g fish were held in 6 square 220l glass fibre

tanks (1m x 1m x 0.5m), four of which were for the 100g fish and the other two for the 180g fish (Plate 2.3).

All of the tanks were part of an outdoor recirculation system. Water was fed to all of the tanks in parallel by gravity from a single header tank. Water entered the tanks tangentially to promote circulation in the tank through a 20cm long 2cm diameter pipe drilled with holes at a rate of 10 l/min. Water left the tanks through a central stand pipe fitted with a collar to promote the removal of solid wastes. The water then passed through a series of 300 l settlement tanks containing filtopak (MASS, Transfer International) to increase the surface area available for settlement of solids. An ABS pump was used to return the water from the sump to the header tank. The system was topped up with fresh mains water at a rate of about 10% per day after removal of chlorine using an activated charcoal carbon filter (ELGA Ltd.)

3. Experimental Diet

A commercial high density diet (EWOS no.551 - Plain) was used in the trial. The manufacturers specified that the diet contained 47% protein, 9% lipid and 2% fibre.

Chromic oxide was incorporated into the diet to act as a digestibility marker by first grinding approximately 10kg of the pelleted ration through a 1mm die plate on a Californian mill. The resulting ground material was weighed to the nearest 0.01g on a Mettler PC 4400 delta range balance and thoroughly mixed in a Hobart A 200 feed mixer with 0.5% of chromic oxide. Sufficient water (20%-30%) was added to produce a paste. The mixture was then passed under pressure through a 2mm die plate of a

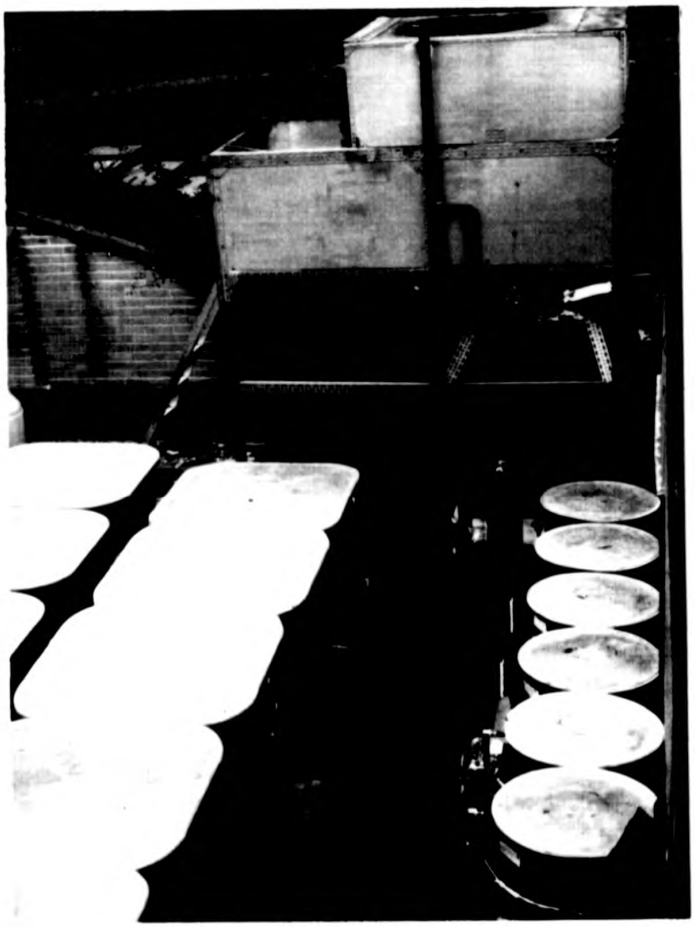

Plate 2.3 - Experimental system at the Institute of Aquaculture, Stirling.

Hobart A200 food mixer. The moist feed pellets were then dried in a convection air drier at 35°C for 16 hours and stored in airtight plastic containers at 0-4°C until fed.

A sample of approximately 100g of the diet was retained for chemical analysis. Moisture, ash, crude protein, amino acids, crude lipid, chromic oxide and crude fibre levels were determined.

4. Experimental Protocol

After acclimatisation to the experimental system, 15 fish were allocated to each tank. For the biggest fish size, each one of two 220l square glassfibre tanks would constitute a replicate, whereas for the other 2 size groups, two 220l square glassfibre tanks for the 100g fish and three 160l circular glassfibre tanks for the 40g fish would be one of the 2 replicates in order to obtain sufficient faecal samples and use the same number of fish per replicate faecal sample, as in the trout farms. Fish were anaesthetised in a 50ppm solution of Benzocaine (Ross & Ross, 1984) and individually weighed to the nearest 0.1g on a Mettler PC 4400 delta range balance. Feeding began the following day. Fish were fed by hand *ad libitum* twice daily at 10 a.m. and 4 p.m., seven days a week.

The trial lasted for a total of four weeks. Faeces were collected on three separate occasions after two, three and four weeks.

On faecal sampling days, the fish were given the morning feed as normal and were then left for a period of four hours. Fish were individually anaesthetized, gently dried

on a paper towel to avoid contamination of faeces by water, and any urine present was voided by applying a light pressure in the mid-region, just above the large intestine, towards the anus. Faeces were then obtained by hand stripping the posterior region of the large intestine of the fish, between the ventral fins and the anus, as described by Austreng (1978).

Faecal samples were collected in glass petri dishes and the samples were pooled for each 1, 2 or 3 tanks sampled, which constituted an experimental replicate for the 180g, 100g and 40g fish. Immediately after collection, the faeces were stored in a freezer at -20°C. The samples were subsequently dried at 105°C in a Memmert drying oven for 24 hours, ground into a fine free-flowing powder in a porcelain mortar using a pestle, and stored in airtight glass containers until required for chemical analysis. Moisture, ash, crude protein, amino acids, crude lipid, crude fibre and chromic oxide levels were determined. The mean water temperature during the 4 week experimental period was 15.2°C (±0.75).

5. Analytical Methods

Moisture, ash, crude protein, crude lipid and crude fibre levels were determined according to the methods described in section I-4 on 2 replicate samples of each faecal sample and 4 replicate samples of the diet. The following methods were used to determine levels of amino acids and chromic oxide in both faeces and diet.

(i) Amino Acids

Amino acids were assayed using a LKB Biochrom 4151 Alpha Plus amino-

acid analyser following hydrolysis with $5cm^3$ of 5.7N HCl (Roach et al. 1967). Levels of all essential amino-acids were determined with the exception of tryptophan.

(ii) Chromic Oxide (Cr_2O_3)

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

6. Trial Analysis

(i) Apparent Nutrient Digestibility

Chromic oxide and crude fibre were used as dietary markers and apparent digestibility coefficients were calculated as described in section I-5.

(ii) Statistical analysis was carried out as described in Section I-5.

III. INFLUENCE OF TEMPERATURE AND DIETARY PROTEIN LEVEL ON DIGESTIBILITY

The trial was carried out at the Marine Station, Oporto, Portugal. Chromic oxide was used as a dietary marker for digestibility evaluation on a double factorial experiment with two levels of protein and at four temperatures.

1. Fish

Rainbow trout in the size range 80-90g were obtained from Inha-Caniçada cage trout farm at Caniçada Reservoir, Portugal. Two hundred and twenty fish were randomly selected from the cages and distributed between two $0.5m^3$ glassfibre tanks. The water was aerated using two portable air pumps fitted with diffusers to supply small air bubbles.

The fish were then transported to the experimental systems at the Marine Station, Oporto, where they were acclimatised to the new environment for a period of one week. During this acclimatisation period, fish were fed a commercial trout diet (ALPIS, Portugal, containing 44% crude protein and 8% crude lipid, size 3.2mm.) *ad libitum*, twice daily.

2. Experimental System

Two indoor pumped recirculation systems (Systems 1 and 2) each consisting of five 230 litre cylindrical (0.70m diameter, 0.60m depth) glassfibre tanks were used (Figure 2.2 and Plate 2.4). Both systems were topped up from the mains at a rate of about 10% of the total water volume per day. Chlorine was removed from the mains water

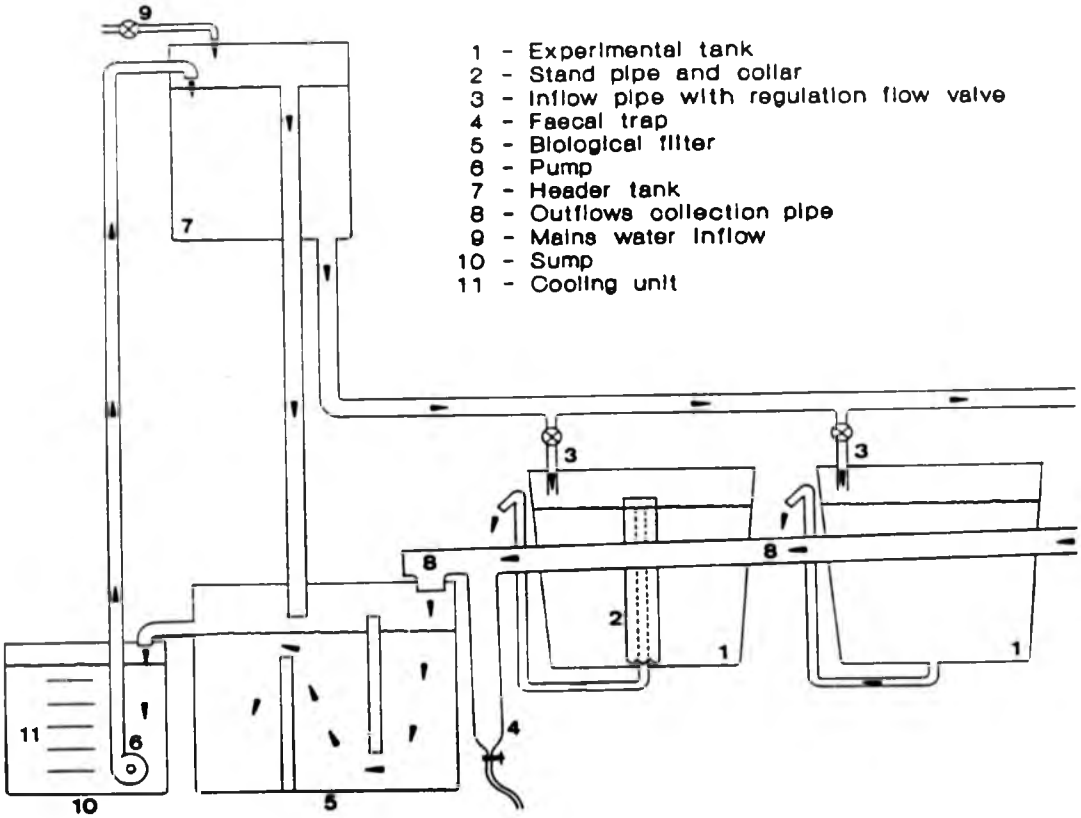

Figure 2.2 - Diagram of the experimental system used at the Marine Station, Oporto (arrows indicate direction of water flow)

Plate 2.4 - Experimental systems used at the Marine Station, Oporto.

using an activated charcoal filter. Water was fed to all the tanks in series by gravity from a header tank. In both systems water was supplied to each tank through a 2cm diameter pipe fitted with a flow regulation valve and a spray bar at a rate of about 9 litres per minute. Water left the tanks through a 3cm diameter central standpipe fitted with a collar to promote the removal of solid wastes. The water was then collected in a 15cm diameter pipe which ran along the side of the tanks and was fitted with a faecal trap consisting of a 10cm diameter column open at the top and fitted with a tap on the bottom, thus allowing drainage of solid wastes. Finally, before the water reached the filter it passed through a bucket containing a 1cm thick layer of sponge which retained any remaining solid wastes. The water then passed through a biological filter which consisted of a 1,000 litre glassfibre tank containing expanded clay to increase the surface area available for bacterial action. A KIHO, GA 8500 pump was used to return the water from the sump to the header tank.

Supplementary aeration of the water was provided in the filter sump, header tank and in each experimental tank from an airline. Oxygen levels and ammonia levels in the tanks were monitored daily using an oxygen meter (WTW OXI91) and a Merck Ammonia Kit (Aquaquent no. 14400) respectively. Oxygen levels at the outflows did not fall below 85% of the saturation level (at each experimental temperature) and NH_4^+ levels in the water never exceeded 0.15mg/l. System 1 was maintained at ambient temperature (21-22°C) while system 2 was fitted with a cooling unit (coil) which allowed the water to be cooled to either 15°C or 10°C.

3. Experimental Diets

3.1 Formulation

Two experimental diets were formulated using brown fish meal as the dietary protein source. The diets were formulated on a near isocaloric basis (Table 3.20) to contain about 45% and 30% protein (Diets 1 and 2 respectively) and 15% lipid. The full dietary formulations are presented in Table 2.5.

Cod liver oil was used to provide a secondary source of dietary lipid 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.*, 1974, Takeuchi and Watanabe, 1976; Yu and Sinnhuber, 1976; Castledine and Buckley, 1980; Reinitz and Yu, 1981; Henderson and Sargent, 1985). Lipid peroxidation of the diets was inhibited by the addition of the anti-oxidant butylated hydroxytoluene (BHT) to the cod liver oil to give a final concentration of 150mg per kg of diet. Whole wheat meal and yellow dextein were used as bulking agents.

Both diets contained 2% vitamin premix (Table 2.6) and 1% mineral premix (Table 2.7). In addition, 1% carboxymethylcellulose was added as a binding agent and 1% chromic oxide was used as an inert marker for digestibility determinations.

3.2 Diet Preparation

Two batches of 10kg of each of the two diets were prepared. In order to obtain a consistent pellet with good binding properties, the diets were prepared using ingredients with a particle size of less than 1mm. Thus, when necessary, ingredients were ground through a 1mm die plate on an Alexanderwerk GKM mill.

Table 2.5 - Formulation of the Experimental Diets (% by Weight).

Ingredient	Crude Protein	Diet 1 45%	Diet 2 30%
Brown Fish Meal[1]		59.3	39.0
Whole Wheat Meal		10.0	10.0
Cod Liver Oil[2]		9.5	11.2
Yellow Dextrin[3]		16.2	34.8
Vitamin Premix[4]		2.0	2.0
Mineral Premix[5]		1.0	1.0
Binder[6]		1.0	1.0
Cr_2O_3		1.0	1.0

1. Brown fish meal - Olfaixe - Produtos de Óleos e Farinhas de Peixe, Ltd., Póvoa de Varzim, Portugal (74% crude protein, 7% lipid).
2. Containing 150mg/kg diet of butylated hydroxytoluene (BDH Chemicals Ltd., Poole, Dorset, England).
3. Yellow corn dextrin - Drogaria Castilho, Porto, Portugal.
4. See Table 2.6.
5. See Table 2.7.
6. Carboxymethylcellulose, disodium salt, high viscosity (BDH Chemicals Ltd., Poole, Dorset, England).

Table 2.6 - Vitamin Premix after Tacon and Ferns, 1976 (to supply/kg of diet).

Vitamin	g per kg of diet
Thiamine HCl	0.50
Riboflavin	0.50
Ca pentothenate	1.00
Niacin	2.00
Pyridoxine HCl	0.40
Biotin	0.60
Folic acid	0.15
B12	0.10
Inositol	20.00
Ascorbic Acid	10.00
Choline Chloride	40.00
Menadione (K3)	0.40
γ aminobenzoic acid	0.50
α tocopherol acetate	0.40
Vitamin A	2000 I.U.
Vitamin D3	1000 I.U.

Table 2.7 - Mineral Premix after Tacon and Beveridge, 1982 (to provide/kg of Diet).

Mineral	g per kg of diet
$MgSO_4.7H_2O$	5.1
KCl	2.0
NaCl	2.4
$FeSO_4.7H_2O$	1.0
$ZnSO_4.7H_20$	0.22
$CuSO_4.5H_20$	0.0314
$MnSO_4.4H_20$	0.1015
$CoSO_4.4H_20$	0.0191
$Ca(IO_3)_2.6H_20$	0.0118
$CrCl_3.6H_20$	0.0051

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 500cm^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 20-30% water per kg of dry diet was added.

The mixture was then passed under pressure through a 2mm die plate on an Alexanderwerk GKM pelletiser. The pellets were dried in a convection air dryer at 35°C for 16 hours. When dry the pellets were sieved through a 1mm sieve to remove dust and stored in airtight plastic containers at 0-4°C until fed.

Approximately 100g samples of each diet were retained for chemical analysis. Levels of moisture, ash, crude protein, amino acids, crude lipid, chromic oxide and crude fibre were determined.

4. Experimental Protocol

In the first experiment (Trial A) the two experimental diets were fed to fish held at 22°C and 15°C while in the second experiment (Trial B) the two diets were fed to fish at 21°C and 10°C.

After acclimatisation to the experimental system at 22°C for one week, 20 fish were allocated to each tank and were maintained on the commercial diet until the appropriate experimental temperatures were reached. This was achieved by lowering the temperature by 1°C every second day.

At the start of each trial, individual fish were anaesthetised in a 3 p.p.m. solution of ethylenglycolmonophenylether (Merck no. 807291) and weighed to the nearest 0.1g on a Mettler PC 4400 delta range balance. Each experimental diet was randomly allocated to two experimental tanks in each of the 2 systems, thus giving one replicate of each diet at each experimental temperature. The extra tank in each system was used for stock fish. Feeding began on the day following the weighing procedure. Diets were fed by hand, *ad libitum*, twice daily at 10 a.m. and 4 p.m., seven days a week.

Each trial lasted for a total of four weeks. Faeces were collected on 4 separate occasions after 2, 3, 3.5 and 4 weeks. On faecal sampling days, the fish were given the morning feed as usual and were then left for a period of four hours. The faecal collection procedure was as described in Section II-4 and faecal samples were pooled for each tank sampled, which constituted an experimental replicate. Immediately after collection, faecal samples were stored in a freezer at -20°C. All samples were then dried, ground and stored for subsequent chemical analysis as described in Section II-4.

Individual fish weights were recorded following faecal stripping after 2 and 4 weeks.

Twice daily, water temperatures were recorded and the average water temperatures were 21.8°C (±0.23) and 14.9°C (±0.09) for Trial A, and 21.1°C (±0.17) and 9.8°C (±0.10) for Trial B.

In both trials, fish were subjected to a 10 hr. photoperiod (08:00h-18:00h).

5. Analytical Methods

Moisture, ash, crude protein, amino acids and chromic oxide levels were determined in 2 replicate samples of each faecal sample and 4 replicate samples of each experimental diet. In addition, crude lipid and crude fibre were also determined in 4 replicate samples of each diet according to the methods described in Sections I-4 and II-5.

The energy content of the faecal samples and diets was measured as follows:

(i) 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). 10-30mg samples were pelleted in a bench press (Briquette press) and weighed to the nearest 0.1mg on a Chan electrobalance. The micro-bomb was calibrated with benzoic acid (BDH Chemicals Ltd.) to produce a calibration curve.

6. Trial Analysis

Apparent digestibility coefficients were calculated and statistical analysis was carried out as described in Section I-5.

IV. INFLUENCE OF DIETARY LIPID LEVEL ON DIGESTIBILITY

The trial was carried out at the Institute of Aquaculture, Stirling. Chromic oxide and crude fibre were both used as dietary markers for digestibility evaluation in a trial comparing dietary lipid levels.

1. Fish

Rainbow trout in the size range 220-250g were obtained from College Mill Trout Farm, Almond Bank, Scotland. Ninety rainbow trout were randomly selected from stock tanks and transported to the Institute of Aquaculture as desribed in Section II-1.

The fish were then transferred to the experimental system where they were acclimatised to the new environment for a period of one week. During this acclimatisation period fish were fed a commercial trout diet (EWOS, pellet size no. 5, containing 47% crude protein and 9% crude lipid), *ad libitum*, twice daily.

2. Experimental System

Six 220-litre $1m^2$ glassfibre tanks were used. These were part of the outdoor recirculation system described in section II-2.

3. Experimental Diets

3.1 Formulation

Three experimental diets were formulated using brown fish meal as the main dietary protein source. The three diets were formulated to contain 50% protein and either 7, 14 or 21% lipid (Diets 1, 2 and 3 respectively). The full dietary formulations are presented in Table 2.8.

Meat and Bone Meal, Soybean Meal and Blood Meal were used as secondary dietary protein sources. Fish Body Oil was used to provide a secondary source of dietary lipid. Wheat Meal was used as a bulking agent. All diets contained 2% vitamin premix (Table 1.6) and 1% mineral premix (Table 2.7). In addition, 1% carboxymethylcellulose was added as a binding agent and 0.5% chromic oxide was used as an inert marker.

3.2 Diet Preparation

About 10kg of each of the three experimental diets were prepared as described in section III-3.2, using a Hobart A 200 feed mixer.

Approximately 100g samples of each diet were retained for subsequent chemical analysis. Levels of moisture, ash, crude protein, amino acids, crude lipid, crude fibre and chromic oxide were determined.

Table 2.8 - Formulation of the Experimental Diets (% by Weight).

		Diet 1	Diet 2	Diet 3
Ingredient	Crude Lipid (%)	7	14	21
Herring meal		40.0	40.0	40.0
Meat and Bone Meal		10.0	10.0	10.0
Soybean Meal		20.0	20.0	20.0
Blood Meal		5.0	5.0	5.0
Wheat Meal		18.0	12.0	6.0
Fish Body Oil		2.5	8.5	14.5
Binder[1]		1.0	1.0	1.0
Vitamin premix[2]		2.0	2.0	2.0
Mineral premix[3]		1.0	1.0	1.0
Chromic oxide		0.5	0.5	0.5

1. Carboxymethylcellulose, disodium salt, high viscosity (BDH Chemicals Ltd.).
2. See Table 2.6
3. See Table 2.7

4. Experimental Protocol

After acclimatisation to the experimental system, 15 fish were allocated to each tank. Fish were anaesthetized in a 50 p.p.m. solution of benzocaine (Ross and Ross, 1984) and individually weighed to the nearest 0.1g on a Mettler PC 4400 delta range balance. The trial began on the following day. Each experimental diet was randomly allocated to 2 tanks, thus giving one replicate of each treatment. The fish were fed by hand, *ad libitum*, twice daily at 10 a.m. and 4 p.m., seven days a week.

The trial lasted for a total of 3 weeks. Faeces were collected on 3 separate occasions after 1, 2 and 3 weeks. On faecal sampling days, the fish were given the morning meal as usual and were then left for a period of four hours. Faecal samples were collected as described in Section II-4 and pooled for each tank sampled which constituted an experimental replicate. Faecal samples were then dried, ground and stored as described in Section II-4 for subsequent chemical analysis.

Daily water temperature was recorded at 11 a.m. during the 3 week experimental period and the overall mean temperature was 14.6°C (±0.50).

5. Analytical Methods

Moisture, ash, crude protein, amino acids, crude lipid, crude fibre and chromic oxide levels were determined in 2 replicate samples of each faecal sample and 4 replicate samples of each experimental diet, according to the methods described in Sections I-4 and II-5.

6. Trial Analysis

Apparent digestibility coefficients were calculated and statistical analysis was carried out as described in Section I-5.

V. INFLUENCE OF TIME AFTER FEEDING ON DIGESTIBILITY

The trial was carried out at the Institute of Aquaculture, Stirling. The aim of this trial was to monitor any fluctuations in digestibility related to time after feeding. Chromic oxide and crude fibre were again used as dietary markers.

1. Fish

Rainbow trout in the size range 180-190g were obtained from College Mill Trout Farm, Almond Bank, Scotland. Ninety fish were randomly selected from stock tanks and transported to the Institute of Aquaculture and acclimatised to the new environment as described in Section II-1.

2. Experimental System

Six 220 litre square (1m x 1m x 0.5m) glassfibre tanks, which were part of the outdoors pumped recirculated system described in Section IV-2, were used.

3. Experimental Diet

The same experimental diet used in the trial described in Section II was used.

4. Experimental Protocol

Six faecal sampling times (24:00h., 04:00h., 08:00h., 12:00h., 16:00h., and 20:00h., respectively 6, 10, 14, 18, 22 and 26 hours after feeding) were chosen to collect faeces from fish in order to evaluate trends in faeces' composition and food digestibility of rainbow trout.

After acclimatisation to the experimental system, 15 fish were allocated to each tank. Fish were anaesthetized in a 50 p.p.m. solution of benzocaine (Ross and Ross, 1984) and individually weighed to the nearest 0.1g on a Mettler PC 4400 delta range balance.

A single faecal collection time was allocated to each experimental tank.

The trial began on the day following weighing. The experimental diet was fed by hand, *ad libitum*, twice daily at 11 a.m. and 6 p.m., seven days a week. On faecal collection days, no food was distributed, thus faecal samples were collected 6, 10, 14, 18, 22 or 26 hours after the last meal had been fed. Faecal samples were collected in accordance with the method described in Section III-4. The trial lasted for a total of three weeks.

Faecal samples were collected on 4 separate occasions after 1, 2, 2.5 and 3 weeks. The samples were then dried, ground and stored for subsequent chemical analysis as described in Section II-4.

Daily water temperature was recorded during the 3 week experimental period and the overall main temperature was 15.2°C (±0.34). Fish were submitted to the natural photoperiod which at the time of the experiment was around 16 hours of daylight.

5. Analytical Methods

Moisture, ash, crude protein, amino acids, crude lipid, crude fibre and chromic oxide levels were determined on 4 replicate samples of each faecal sample according to the methods described in Sections I-4 and II-5.

6. Trial Analysis

Apparent digestibility coefficients were calculated and statistical analysis was carried out as described in Section I-5.

VI. INFLUENCE OF FEEDING FREQUENCY ON DIGESTIBILITY

The trial was carried out at the Marine Station, Oporto, Portugal. The aim of the trial was to investigate the influence of feeding frequency on digestibility. Chromic oxide and crude fibre were again used as dietary markers.

1. Fish

Rainbow trout in the size range 140-160g were obtained from Inha-Caniçada Cage Trout Farm at Caniçada Reservoir, Portugal.

One hundred and forty fish randomly selected from the cages were transported to the Marine Station Oporto as described in Section III-1. On arrival at the Marine Station, they were transferred to the experimental system where they were allowed to acclimatise to the new environment for a period of one week. During this acclimatisation period, the fish were fed a commercial trout ration (ALPIS, Portugal, size 4.5mm, containing 49% crude protein, 12% crude lipid), *ad libitum*, twice daily.

2. Experimental System

The trial was carried out in an indoor pumped recirculation system consisting of six 230 litre cylindrical (0.70m diameter and 0.60m depth) glassfibre tanks. The system was described in Section III-2, the only difference having been the addition of a 6th tank. Temperature and ammonia levels were monitored daily. Ammonia was determined using a Merck Ammonia Kit (Aquaquant no. 14400). Ammonia levels in

the water did not exceed 0.2mg/l. Mean water temperature during the 5.5 week experimental period was 13.4°C (±0.38).

3. Experimental Diet

A commercial trout diet (ALPIS, Portugal, size no 4.5mm) was used in the trial. The manufacturers specified that the diet contained 49% protein,12% lipid and 2% fibre. The inert dietary marker chromic oxide was incorporated into the diet by grinding approximately 30kg of the pelleted ration through a 1mm die plate on a Retfch GM BM, type SR3 59097 hammer mill. The ground diet was weighed to the nearest 0.01g on a Mettler PC 4400 delta range balance and thoroughly mixed in a Alexanderwerk GKM mixer with 1.0% chromic oxide.

The mixture was then passed under pressure through a 3mm die plate of a Californian pellet mill (CPM, California Pellet Mill Company). The feed pellets were allowed to cool and then stored in airtight plastic containers at 0-4°C until fed.

Approximately 100g of the diet was retained for chemical analysis. Moisture, ash, crude protein, crude lipid, crude fibre, chromic oxide and energy levels were determined.

4. Experimental Protocol

After acclimatisation to the experimental system, 20 fish were allocated to each tank. Fish were bulk weighed to the nearest 0.1g on a Mettler PC 4400 delta range balance, using a bucket containing water.

Three feeding frequencies (1 x day^{-1}, 2 x day^{-1}, and 4 x day^{-1}) were compared in replicate tanks (Table 2.9). Fish were fed by hand to satiation seven days a week and feeding levels were recorded.

The trial lasted for a total of 5.5 weeks. Faeces were collected on 4 separate occasions after 3.5, 4.5, 5.0 and 5.5 weeks.

On faecal sampling days the fish were given the 10 o'clock meal as usual and were then left for a period of four hours. Faeces were collected as described in Section II-4 and pooled for each experimental tank sampled. The samples were initially stored in a freezer at -20°C. All faecal samples were subsequently dried, ground and stored for chemical analysis as described in Section II-4.

Individual fish weights were recorded following faecal stripping after 3.5 and 5.5 weeks.

Mean water temperature during the 5.5 week experimental period was 13.4°C (±0.38).

During the experimental period fish were submitted to a 10 hour light:14 hour dark photoperiod.

Table 2.9 - Feeding Frequencies and Meal Times during the experimental period.

Feeding frequency (x day^{-1})	Meal time			
	10:00h.	12:00h.	14:00h.	16:00h.
1	x			
2	x			x
4	x	x	x	x

5. Analytical Methods

Moisture, ash, crude protein, crude fibre, chromic oxide and energy were determined in two replicate samples of each faecal sample and four replicate samples of the experimental diet. In addition, crude lipid was determined in four replicate samples of the experimental diet according to the methods described in Sections I-4, II-5 and III-5.

6. Trial Analysis

Apparent digestibility coefficients were calculated and statistical analysis was carried out as described in Section I-5.

VII. INFLUENCE OF DISSOLVED OXYGEN LEVEL ON DIGESTIBILITY AND FOOD INTAKE

Two trials were carried out at the Institute of Aquaculture, Stirling, to evaluate digestibility trends at different oxygen levels. In addition, the influence of oxygen level on food intake was investigated. Chromic oxide was used as a dietary marker.

1. Fish

Rainbow trout in the size range 98-105g were obtained from Cultenhove Fish Farm, Sauchie Burn, Bannockburn, Scotland. Sixty fish were randomly selected from stock tanks and transported to the Institute of Aquaculture as described in Section II-1.

The fish were then transferred to the experimental system where they were acclimatised to the new environment for a period of one week. During this acclimatisation period, fish were fed a commercial trout diet (EWOS, pellet size no. 6, containing 47% protein and 9% lipid), *ad libitum*, twice daily.

2. Experimental System

Six 160 litre cylindrical (0.60m diameter) fibre glass tanks were used. They were part of the outdoor circulation system described in Section II-2 (Plate 2.5). Of the six tanks in the experimental system, two were used as control tanks, two as experimental tanks and the remaining two as stock tanks (Figure 2.3).

Plate 2.5 - Experimental system at the Institute of Aquaculture, Stirling, used for Trials A and B.

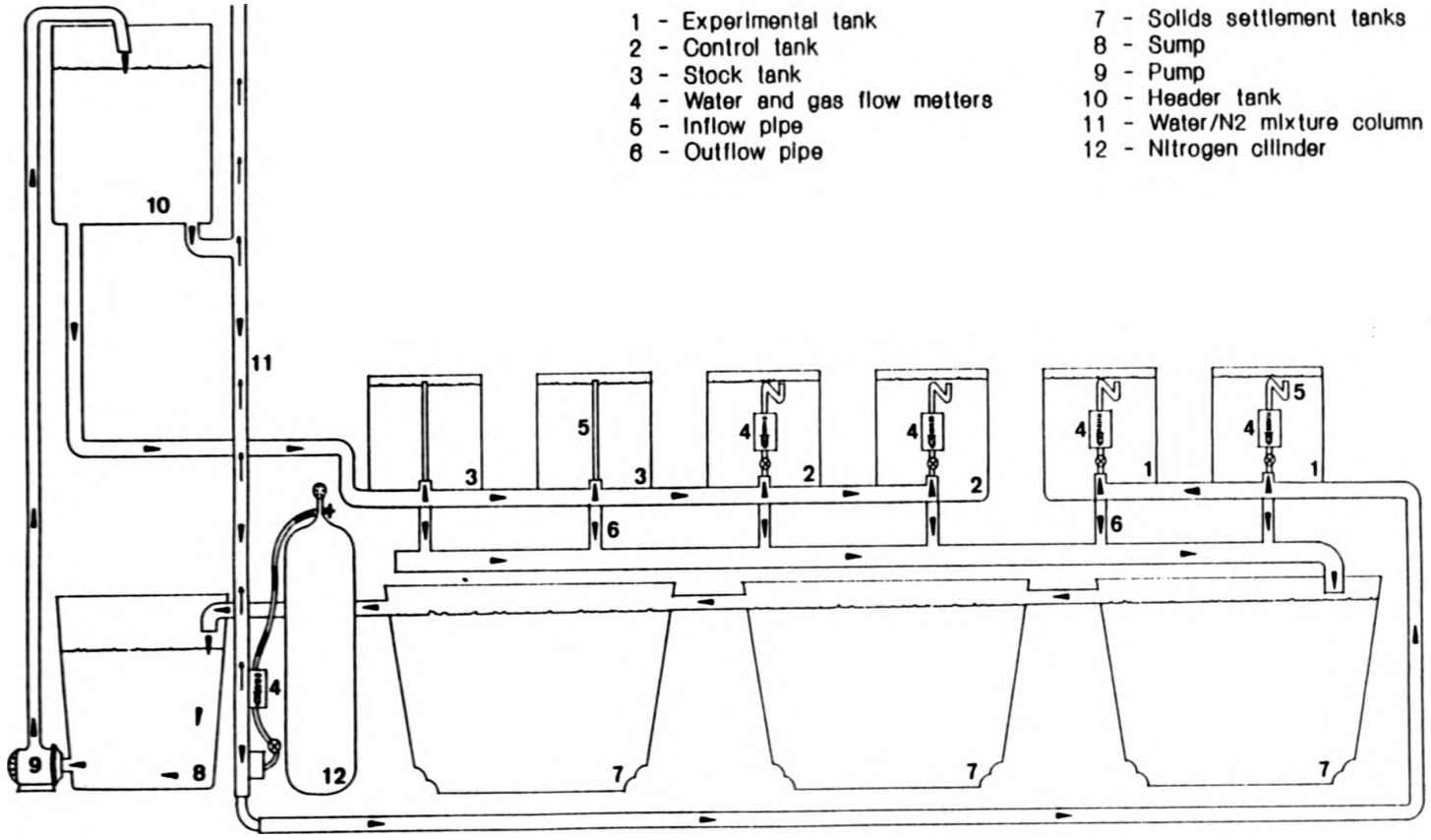

Figure 2.3 - Diagram of the experimental system used for Trials A and B (arrows indicate direction of gas (→) and water (➡) flows).

Water flow meters (G.A. Platon Ltd.) were installed in the inflow pipe of the control and the experimental tanks and the flow rate was set to 2 litres/min. The water entered the tanks just below the water surface to avoid reaeration.

The water was supplied to the control and stock tanks in the experimental system from the same header tank. However, for the two experimental tanks, an independent pipe was derived from the header tank so that the water could flow through an open topped 10cm diameter column. Oxygen-free nitrogen (BOC, Ltd.) could diffuse into the water at a rate controlled by a needle valve and a gas flow meter (G.A. Platon, Ltd.). In this way oxygen levels in the water could be reduced to the required experimental levels before entering the experimental tanks (Figure 2.3 and Plate 2.5).

3. Experimental Diet

A commercial trout diet (EWOS, no. 6) was used in the trial. The manufacturers specified that the diet contained 47% protein, 9% lipid and 2% fibre.

Chromic oxide was incorporated into the diet to act as a digestibility marker by first grinding approximately 10kg of the pelleted ration through a 1mm die plate on a flayel mill (Scot MEC-AYR). The experimental diet was prepared and stored as described in Section VI-3.

A sample of approximately 100g of the diet was retained for subsequent chemical analysis. Moisture, ash, crude protein, amino acid, crude lipid, crude fibre and chromic oxide levels were determined.

4. Experimental Protocol

a) Trial A

In Trial A, digestibility was evaluated and voluntary food intake was recorded at 6 different dissolved oxygen levels.

After acclimatisation to the experimental system, 10 fish were allocated to each tank. Fish were bulk weighed as described in Section VI-4, and feeding on the experimental diet began on the day following the weighing procedure. The diet was fed by hand, *ad libitum*, twice daily at 11 a.m. and 4 p.m., seven days a week.

During the first week the D.O. content of all the tanks was maintained at around 80% saturation (7mg/l at 16°C). Thereafter the D.O. level in the experimental tanks was reduced to approximately 5mg/l. The fish were held at this D.O. level for 3 days, after which faecal samples were collected and D.O. level was again reduced. This continued for a total of 12 days until the D.O. content was 2.3mg/l. (see Table 2.10). When a new D.O. level was being set, oxygen levels were monitored at the inflow and inside each tank using an oxygen meter (Y.S.I., Model 57) continuously for a 15-30min. period and at 2-4 hour intervals during the experimental period. The column worked well and its speed of response was fast when a new D.O. level was set. Once set, the D.O. level was easily maintained constant and steady during the experimental period.

Table 2.10 - Mean Dissolved Oxygen (mg/l) ±S.D. in the inflow water, Nitrogen Flows (cc/min) and faecal collection times for Trial A.

		Experimental Tanks		Control Tanks	
Treatment	Days	N_2 Flow (cc/min)	D.O. Level (mg/l)	D.O. Level (mg/l)	Faecal collection
T1	1-7	off	7.2 (±0.00)	6.9 (±0.17)	once
T2	7-10	60	5.1 (±0.05)	6.7 (±0.11)	once
T3	10-13	100	4.7 (±0.00)	6.9 (±0.05)	once
T4	13-16	140	4.0 (±0.05)	6.6 (±0.05)	once
T5	16-19	200	3.1 (±0.05)	6.5 (±0.05)	once[1]
T6	19-22	260	2.3 (±0.05)	6.5 (±0.17)	once[1]

1. No faecal samples from fish in experimental tanks.

The trial lasted for a total of 3 weeks. Faeces were collected at the end of the first week and thereafter every 3 days. On faecal sampling days, the fish were given the morning feed as usual and were then left for a period of 4 hours. Faecal collection procedure was as decribed in Section II-4 and faecal samples were pooled for each tank sampled, which constituted an experimental replicate. Immediately after collection, faecal samples were stored in a freezer at -20°C. All samples were then dried, ground and stored for subsequent chemical analysis as described in Section II-4. Individual fish weights were recorded following faecal stripping after 2 and 3 weeks. Daily food intake was also recorded for each D.O. level.

Daily water temperature during the experimental period was recorded and the mean temperature was 16.0°C (±0.23), the fish being subjected to a natural 14 hour photoperiod.

b) Trial B

After looking at the influence of an abrupt fall in dissolved oxygen levels on digestibility and food intake a second trial was carried out at 2 reduced D.O. levels which were maintained for one week, in order to evaluate the effect of a more prolonged exposure to reduced D.O. level on digestibility and food intake.

The same number (10) of fish as in Trial A were allocated to each tank after acclimatisation to the experimental system. Weighing and feeding procedures were as described above for Trial A.

Again, during the first week the D.O. content of all the tanks was maintained at around 80% saturation (7mg/l at 15.9°C). At the end of this period the D.O. level in the experimental tanks was reduced to the levels specified in Table 2.11.

This trial also lasted for a total of 3 weeks and faeces were collected twice for each D.O. level. Faecal collection and processing were as described for Trial A. Daily food intake was recorded for each D.O. level. The daily water temperature was recorded and mean temperature was 15.9°C (±0.10). Fish were submitted to a natural 14 hour photoperiod.

5. Analytical Methods

Crude protein and chromic oxide were determined in two replicate samples of each faecal sample and 4 replicate samples of the experimental diet. In addition, ash, crude lipid and crude fibre were also determined in 4 replicate samples of the diet according to the methods described in Section I-4 and II-5. In Trial B, amino acids were determined in each faecal sample and in the experimental diet according to the method described in Section II-5.

6. Trial Analysis

Apparent digestibility coefficients were calculated as described in Section I-5. Statistical analysis was carried out using regression analysis.

Table 2.11 - Mean Dissolved Oxygen (mg/l) ±S.D. in the inflow water, Nitrogen flows (cc/min) and faecal collection times for Trial B.

		Experimental Tanks		Control Tanks	
Treatment	Days	N_2 Flow (cc/min)	D.O. Level (mg/l)	D.O. Level (mg/l)	Faecal Collection
T1	7-14	60	5.1 (±0.17)	6.6 (±0.11)	twice
T2	14-21	140	4.0 (±0.17)	6.8 (±0.23)	twice

3 - RESULTS

I. LONG TERM DIGESTIBILITY SURVEY OF FARMED RAINBOW TROUT

a) Trutorão Trout Farm (Farm A)

1. Commercial Diets

The proximate compositions of the commercial diets used on the farm throughout the one year survey period are shown in Table 3.1. For the three size groups of fish sampled, this farm usually uses T7 or T12 and, according to the manufacturers, the only difference between these diets was the lipid inclusion level (7% and 12%, respectively). However, the farm occasionally used Diet T5 for the smaller fish size (15 cm) and this was the case in August 1986 (Table 3.1 and see Table 2.2).

Although the manufacturers' specifications did not change throughout the one year sampling period, from March onwards a new diet formulation was used (Farm manager, pers.com.). The most significant effect of this alteration in proximate composition was a decrease in crude fibre content. Before the formulation change, crude fibre varied between 1.52% and 2.12%, whereas from March onwards, it did not exceed 0.92%, with a minimum level of only 0.50% in April (Table 3.1).

With the exception of October, November, January and February, the moisture content of diets T12 was lower than the level of 10% specified by the manufacturers (Table 3.1).

The ash content of the T12 diets was higher than the level of 12% specified and varied between 12.16% (May) and 14.63% (June), except in April (11.85%)

Table 3.1 - Proximate composition (n=4, ±S.D.) of the commercial diets used in Farm A (Trutorão) from October 1985 to September 1986.

Nutrient	Month	Oct. 85	Nov.	Dec.	Jan.86	Feb.	Mar.	Apr.	May	June	July	Aug.		Sept.
Content(% dry weight)	Diet No.[1]	T7	T12	T12	T12	T12	T12	T12	T12	T12	T12	T5	T12	T12
Moisture(%)		10.23 (±0.02)	10.63 (±0.03)	9.79 (±0.09)	10.50 (±0.09)	11.20 (±0.07)	9.73 (±0.05)	9.82 (±0.06)	8.91 (±0.04)	8.38 (±0.04)	7.35 (±0.03)	7.54 (±0.05)	8.57 (±0.05)	8.30 (±0.03)
Ash (%)		12.09 (±0.09)	12.66 (±0.22)	13.11 (±0.11)	13.28 (±0.24)	13.33 (±0.32)	12.71 (±0.13)	11.85 (±0.17)	12.16 (±0.37)	14.63 (±0.29)	13.06 (±0.04)	15.56 (±0.21)	13.53 (±0.16)	13.34 (±0.07)
Crude Protein (N x 6.25)		45.66 (±0.04)	47.87 (±0.11)	48.04 (±0.15)	49.78 (±0.20)	47.90 (±0.15)	49.87 (±0.10)	49.27 (±0.11)	49.23 (±0.14)	48.32 (±0.18)	51.56 (±0.17)	43.92 (±0.30)	50.74 (±0.07)	50.62 (±0.17)
Crude Lipid(%)		8.07 (±0.16)	12.58 (±0.43)	12.01 (±0.49)	11.79 (±0.28)	12.16 (±0.27)	12.25 (±0.37)	12.10 (±0.41)	12.39 (±0.43)	13.26 (±0.34)	12.40 (±0.15)	10.48 (±0.33)	12.64 (±0.22)	12.41 (±0.29)
Crude Fibre(%)		1.74 (±0.06)	1.52 (±0.02)	1.87 (±0.06)	2.12 (±0.07)	2.07 (±0.08)	0.52 (±0.02)	0.50 (±0.04)	0.81 (±0.06)	0.92 (±0.05)	0.73 (±0.03)	0.80 (±0.03)	0.71 (±0.01)	0.82 (±0.05)

1. Usually fish bigger than 15cm are fed on T7 or T12. However, T5 is sometimes optionally used for fish sizes of 15-17cm and this was the case in August for the smaller size group sampled. T7 and T12 only differ on the lipid inclusion level, according to the manufacturers (see Table 2.2).

As far as crude protein is concerned, all T12 diets contained more than the specified level of 46%, with values ranging from 47.87% (November) to 51.56% (July).

All T12 diets contained more than the level of 12% crude lipid specified by the manufacturers with the exception of January (11.79%), with values ranging from 12.01% and 13.26% in December and June, respectively (Table 3.1).

Overall, the proximate composition of the T12 diets was quite constant throughout the one year survey period, with the exception of the fibre content.

The moisture (10.23%) and the ash (12.09%) contents of T7 (October 1985) were slightly higher than the specified levels of 10% and 12%, respectively. The protein (45.66%) and the fibre (1.74%) contents were lower than the levels of 46% and 2% specified by the manufacturers, whereas the lipid content (8.07%) was higher than the 7% level specified. In the T5 diet, the moisture (7.54%) and the crude protein (43.92%) contents (August) were lower than the levels of 10% and 46% respectively, specified by the manufacturers, and the ash content (15.56%) was higher than the level of 12% specified. The dietary lipid content (10.48%) was just slightly higher than the level of 10%, whereas its crude fibre content (0.80%) was much lower than the 2% specified level.

2. Apparent Nutrient Digestibility Coefficients

The proximate composition of faeces collected from the three size groups of rainbow trout is presented in Table 3.2. Although there was good replication within treatments,

Table 3.2 - Proximate composition (n=2, ±S.D.) of faeces collected from the 3 size groups of rainbow trout at Farm A (Trutorão).

Nutrient Content (% dry weight)	Month	October 1985			November			December			January		
	Fish Size (cm)	15	20	25	15	20	25	15	20	25	15	20	25
	Replicates												
Ash (%)	A	24.59 (±0.12)	29.70 (±0.00)	23.01 (±0.32)	33.73 (±0.09)	29.40 (±0.80)	27.44 (±0.44)	26.11 (±0.47)	38.79 (±1.00)	33.91 (±0.53)	32.20 (±0.04)	33.81 (±0.42)	33.32 (±0.26)
	B	25.17 (±0.35)	28.19 (±0.13)	24.11 (±0.42)	33.76 (±0.08)	29.45 (±0.63)	30.04 (±0.87)	25.84 (±0.60)	36.87 (±0.48)	35.35 (±0.75)	32.99 (±0.58)	32.18 (±0.14)	32.39 (±0.36)
Crude Protein (Nx6.25)	A	23.77 (±0.07)	20.19 (±0.00)	18.85 (±0.04)	21.60 (±0.05)	21.45 (±0.46)	22.11 (±0.00)	21.14 (±0.09)	19.20 (±0.03)	20.39 (±0.18)	23.53 (±0.18)	22.21 (±0.26)	22.20 (±0.04)
	B	23.86 (±0.00)	20.84 (±0.11)	19.11 (±0.14)	21.43 (±0.07)	21.43 (±0.00)	21.26 (±0.16)	21.43 (±0.05)	19.34 (±0.11)	20.45 (±0.21)	24.22 (±0.04)	23.48 (±0.04)	21.94 (±0.10)
Crude Fibre(%)	A	4.03 (±0.04)	5.20 (±0.02)	5.14 (±0.40)	5.52 (±0.17)	5.67 (±0.20)	4.88 (±0.02)	4.50 (±0.16)	5.37 (±0.32)	4.09 (±0.07)	4.54 (±0.02)	6.68 (±0.00)	4.76 (±0.02)
	B	3.96 (±0.03)	5.94 (±0.50)	5.74 (±0.42)	5.76 (±0.16)	5.27 (±0.28)	5.31 (±0.10)	4.26 (±0.10)	5.83 (±0.29)	4.40 (±0.21)	4.93 (±0.20)	5.02 (±0.00)	5.33 (±0.07)

Table 3.2 (cont.) - Proximate composition (n=2, ±S.D.) of faeces collected from the 3 size groups of rainbow trout at Farm A (Trutorão).

Nutrient Content (% dry weight)	Month	February			March			April			May		
	Fish Size (cm)	15	20	25	15	20	25	15	20	25	15	20	25
	Replicates												
Ash (%)	A	29.54 (±0.90)	31.73 (±0.26)	32.39 (±0.41)	33.54 (±0.38)	32.80 (±0.90)	32.13 (±0.01)	23.36 (±0.09)	38.10 (±0.16)	35.17 (±0.29)	30.96 (±0.72)	35.42 (±2.00)	39.31 (±0.60)
	B	26.68 (±0.62)	33.06 (±0.49)	30.96 (±0.48)	33.39 (±0.77)	30.12 (±0.04)	34.83 (±0.05)	28.04 (±0.09)	42.22 (±0.31)	35.75 (±0.17)	33.01 (±0.90)	30.10 (±1.10)	43.85 (±0.80)
Crude Protein (Nx6.25)	A	20.53 (±0.04)	24.47 (±0.29)	23.38 (±0.18)	18.44 (±0.20)	22.40 (±0.03)	22.10 (±0.09)	20.17 (±0.02)	20.05 (±0.00)	19.84 (±0.15)	17.45 (±0.00)	18.83 (±0.07)	18.89 (±0.41)
	B	21.19 (±0.12)	24.18 (±0.33)	23.22 (±0.12)	18.33 (±0.19)	22.21 (±0.00)	22.88 (±0.00)	20.04 (±0.12)	19.37 (±0.13)	18.71 (±0.03)	17.33 (±0.02)	19.09 (±0.10)	18.51 (±0.24)
Crude Fibre (%)	A	4.14 (±0.12)	5.87 (±0.50)	6.13 (±0.38)	4.71 (±0.00)	3.95 (±0.30)	3.66 (±0.10)	3.65 (±0.19)	3.02 (±0.23)	3.69 (±0.07)	6.18 (±0.06)	7.42 (±0.20)	5.06 (±0.08)
	B	4.60 (±0.02)	5.13 (±0.52)	6.68 (±0.35)	4.74 (±0.02)	3.52 (±0.10)	3.26 (±0.28)	3.92 (±0.21)	3.35 (±0.10)	3.82 (±0.00)	6.00 (±0.09)	7.05 (±0.28)	4.82 (±0.06)

Table 3.2 (cont.) - Proximate composition (n=2, ±S.D.) of faeces collected from the 3 size groups of rainbow trout at Farm A (Trutorão).

Nutrient Content (% dry weight)	Month	June			July			August			September		
	Fish Size (cm)	15	20	25	15	20	25	15	20	25	15	20	25
	Replicates												
Ash (%)	A	38.21 (±1.42)	40.03 (±0.01)	37.62 (±0.15)	45.45 (±0.04)	42.30 (±0.07)	40.90 (±0.10)	39.56 (±0.00)	37.03 (±0.50)	44.67 (±0.04)	42.54 (±0.00)	37.35 (±0.75)	44.87 (±0.90)
	B	37.39 (±0.51)	40.02 (±0.03)	38.08 (±0.40)	45.52 (±0.08)	37.23 (±0.19)	40.50 (±0.09)	38.87 (±0.02)	38.13 (±0.30)	43.71 (±0.10)	41.54 (±0.05)	39.33 (±0.40)	42.15 (±0.87)
Crude Protein (Nx6.25)	A	27.72 (±0.30)	24.40 (±0.03)	24.10 (±0.11)	22.82 (±0.39)	24.07 (±0.00)	22.13 (±0.00)	21.62 (±0.19)	24.66 (±0.00)	21.22 (±0.09)	24.64 (±0.00)	25.02 (±0.14)	22.13 (±0.17)
	B	27.50 (±0.17)	24.04 (±0.04)	23.79 (±0.00)	21.95 (±0.14)	23.56 (±0.06)	22.44 (±0.76)	21.11 (±0.23)	24.46 (±0.21)	20.41 (±0.27)	24.83 (±0.00)	24.34 (±0.04)	22.91 (±0.02)
Crude Fibre (%)	A	4.79 (±0.30)	3.17 (±0.03)	3.53 (±0.05)	3.34 (±0.20)	3.46 (±0.20)	3.02 (±0.10)	1.95 (±0.25)	4.49 (±0.23)	2.72 (±0.02)	2.66 (±0.00)	3.45 (±0.02)	2.45 (±0.10)
	B	4.35 (±0.15)	3.28 (±0.04)	3.46 (±0.03)	3.61 (±0.21)	4.32 (±0.20)	3.72 (±0.02)	2.24 (±0.20)	3.82 (±0.33)	2.58 (±0.04)	3.27 (±0.20)	3.52 (±0.05)	2.69 (±0.15)

throughout the one year survey period there was some change in faeces composition. Thus, with the exception of crude fibre levels in May, from March onwards the crude fibre content of faeces was lower than the levels observed up to March, possibly reflecting the change in diet formulation.

2.1 Apparent Crude Protein Digestibility

Figure 3.1 shows the overall trends of crude protein digestibility values throughout the one year survey period for the three size groups of rainbow trout and the average water temperature during the same period.

Coinciding with the lower temperatures registered during the one year survey period, lower apparent crude protein digestibility values were observed in October 1985, January and February 86 for the 15cm fish, in January 86 and February for the 20cm fish and in December 85 and January 86 for the 25cm fish (Table 3.3 and Figure 3.1).

An increase in temperature in March was followed by an increase in digestibility, however a slight further increase of the temperature (above 18°C) in June caused a general decrease of digestibility for the three size groups of fish. In July the temperature rose to around 20°C and again an improvement of digestibility was recorded for the three size groups sampled, although these values were significantly ($p<0.05$) lower than those obtained in March, April and May for each size group. From July to September the temperature decreased and so did the digestibility values. A specially low digestibility value was observed in August for the 15cm fish (81.31%) compared to the other two groups (around 90%). This difference might be explained

Table 3.3 - Mean apparent digestibility coefficients (n=4, ±S.D.) for the 3 size groups of fish at Trutorão (Farm A).

Apparent Nutrient Digestibility (%)	Fish Size (cm)	Months											
		Oct.85	Nov.	Dec.	Jan. 86	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept
		← a..h											
Crude Protein (N x 6.25)	15	$^{b}77.27_{j}(\pm0.27)$	$^{d}87.87_{k}(\pm0.35)$	$^{f}81.06_{j}(\pm0.74)$	$^{gh}78.50_{k}(\pm0.66)$	$^{g}79.32_{m}(\pm0.87)$	$^{a}95.94_{k}(\pm0.04)$	$^{b}94.60_{k}(\pm0.24)$	$^{ab}95.29_{j}(\pm0.06)$	$^{d}88.47_{k}(\pm0.58)$	$^{c}90.85_{k}(\pm0.62)$	$^{f}81.31_{m}(\pm1.75)$	$^{e}86.34_{j}(\pm1.56)$
	20	klm $^{e}85.91_{k}(\pm0.82)$	$^{de}87.53_{k}(\pm0.53)$	$^{de}86.58_{k}(\pm0.58)$	$^{f}82.96_{k}(\pm3.32)$	$^{f}80.90_{j}(\pm1.25)$	$^{b}93.72_{j}(\pm0.35)$	$^{b}93.69_{j}(\pm0.50)$	$^{a}95.68_{k}(\pm0.15)$	$^{e}85.69_{m}(\pm0.40)$	$^{c}91.21_{k}(\pm1.22)$	$^{e}91.67_{k}(\pm0.74)$	$^{d}88.52_{k}(\pm0.31)$
	25	$^{ef}86.66_{k}(\pm0.74)$	$^{h}82.09_{j}(\pm0.35)$	$^{h}81.24_{j}(\pm0.77)$	$^{h}81.30_{k}(\pm1.35)$	$^{g}84.24_{k}(\pm0.85)$	$^{b}93.19_{j}(\pm0.58)$	$^{a}94.78_{k}(\pm0.28)$	$^{b}93.76_{m}(\pm0.13)$	$^{e}86.95_{j}(0.06)$	$^{c}90.54_{k}(\pm1.08)$	$^{d}89.00_{j}(\pm0.12)$	$^{f}85.78_{j}(0.48)$
Organic Matter	15	$^{f}63.31_{j}(\pm1.05)$	$^{d}79.53_{k}(\pm0.53)$	$^{f}63.59_{m}(\pm1.24)$	$^{f}65.13_{k}(\pm1.89)$	$^{g}60.90_{j}(\pm1.82)$	$^{a}91.48_{k}(\pm0.24)$	$^{b}89.07_{k}(\pm0.59)$	$^{b}89.70_{j}(\pm0.01)$	$^{c}85.29_{k}(\pm0.94)$	$^{c}86.80_{k}(\pm0.67)$	$^{e}72.38_{m}(\pm2.02)$	$^{d}81.32_{k}(\pm2.03)$
	20	$^{efg}74.74_{k}(\pm1.55)$	$^{de}77.51_{k}(\pm0.95)$	$^{ef}76.07_{k}(\pm0.76)$	$^{g}71.37_{k}(\pm5.08)$	$^{fg}73.69_{k}(\pm1.70)$	$^{ab}89.01_{j}(\pm0.98)$	$^{ab}89.29_{k}(\pm1.06)$	$^{a}91.41_{k}(\pm0.64)$	$^{d}79.95_{j}(\pm0.50)$	$^{b}86.89_{k}(\pm1.04)$	$^{b}87.59_{k}(\pm1.02)$	$^{c}83.28_{k}(\pm0.47)$
	25	$^{f}72.07_{k}(\pm2.04)$	$^{e}75.59_{j}(\pm1.71)$	$^{g}66.80_{j}(\pm1.84)$	$^{g}67.38_{k}(\pm1.87)$	$^{ef}74.48_{k}(\pm0.96)$	$^{a}88.59_{j}(\pm0.37)$	$^{a}90.24_{k}(\pm0.24)$	$^{a}89.10_{j}(\pm0.18)$	$^{cd}80.83_{j}(\pm0.21)$	$^{b}84.95_{k}(\pm1.86)$	$^{c}82.69_{j}(\pm0.70)$	$^{d}79.18_{k}(\pm0.54)$
Dry Matter	15	$^{de}57.44_{j}(\pm0.45)$	$^{c}74.56_{k}(\pm0.21)$	$^{de}58.36_{j}(\pm1.37)$	$^{e}56.34_{k}(\pm2.13)$	$^{e}53.61_{j}(\pm2.94)$	$^{a}89.45_{k}(\pm0.04)$	$^{a}87.20_{k}(\pm0.54)$	$^{a}87.40_{j}(\pm0.22)$	$^{b}80.56_{k}(\pm1.13)$	$^{b}79.54_{k}(\pm0.94)$	$^{d}62.12_{m}(\pm3.08)$	$^{c}72.64_{k}\pm3.34)$
	20	$^{ef}69.84_{k}(\pm2.44)$	$^{de}73.29_{k}(\pm1.18)$	$^{ef}67.82_{k}(\pm1.61)$	$^{f}64.38_{k}(\pm6.18)$	$^{f}63.50_{k}(\pm2.99)$	$^{ab}86.48_{k}(\pm0.93)$	$^{ab}84.68_{k}(\pm0.94)$	$^{a}89.52_{k}(\pm0.33)$	$^{de}72.13_{j}(\pm0.56)$	$^{bc}81.59_{k}(\pm2.44)$	$^{b}83.39_{k}(\pm1.61)$	$^{cd}77.10_{k}(\pm0.27)$
	25	$^{de}69.12_{k}(\pm2.07)$	$^{cde}71.19_{k}(\pm1.48)$	$^{f}56.95_{j}(\pm2.92)$	$^{f}59.09_{k}(\pm2.91)$	$^{de}69.05_{k}(\pm1.63)$	$^{a}85.36_{j}(\pm1.01)$	$^{a}87.11_{k}(\pm0.27)$	$^{a}84.27_{m}(\pm0.46)$	$^{bc}74.35_{j}(\pm0.30)$	$^{b}78.67_{k}(\pm2.65)$	$^{cd}73.71_{j}(\pm0.81)$	$^{e}68.58_{k}(\pm1.73)$

Values in same row with same superscript are not significantly ($p<0.05$) different, for each size group within each digestibility coefficient.
Values in same column with same subscript are not significantly ($p<0.05$) different, for each digestibility coefficient.

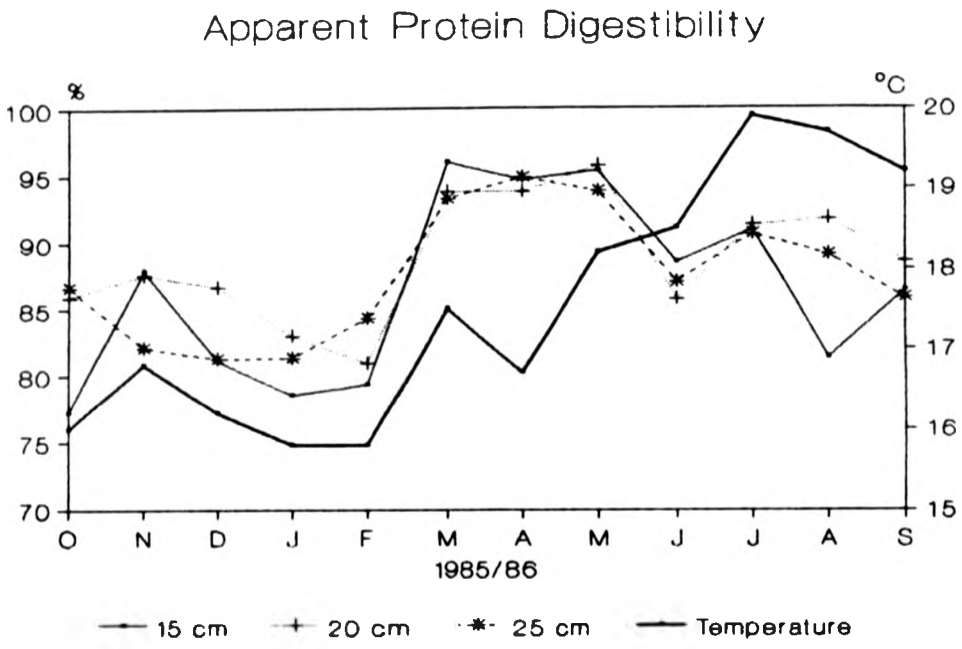

Figure 3.1 - Mean apparent protein digestibility coefficients of the 3 size groups of fish at Trutorão Trout Farm (Farm A) and average monthly water temperatures between October 1985 and September 1986.

by the fact that this size group was fed a different diet (Diet T5).

The change in diet formulation seems to have had a dramatic effect on digestibility from March onwards (Table 3.3 and Figure 3.1). Correlation analysis showed that there was a higher inverse correlation between apparent crude protein digestibility and the crude fibre content of the diets ($r = -0.84$; $r = -0.80$, $r = -0.81$, for the 15cm, 20cm and 25cm fish, respectively). Since the correlation coefficent (r) is a measure of intensity of association between the two variables (Zar, 1984) it can be assumed that the change in formulation, especially the fibre content of the diets, was the main factor influencing digestibility changes throughout the year. The correlation between digestibility values and temperatures, although significant, was low, only around 0.40 (Table 3.4). In late summer-autumn the digestibility decreased, although temperature continued to increase. Thus, there was most probably some other factor influencing digestibility. A natural decline of digestibility related to seasonal variations, a possible reaction to the thermal stress itself or to adversely low levels of dissolved oxygen as temperature increases could have acted in opposition to the temperature effect. In fact, much better correlations between digestibility and temperature were obtained when based only on data between October 85 and June 86, i.e. before temperature rose above 18°C (see Table 3.5). There were high significant correlations ($r = 0.89$, $r = 0.90$, $r = 0.72$ for the 15cm, 20cm and 25cm fish, respectively) between apparent crude protein digestibility and temperature for all fish groups (Table 3.5).

Table 3.4 - Correlation matrix showing the relationships between apparent digestibility coefficients and dietary fibre and temperature at Farm A.

		Apparent Digestibility Coefficients		
	Fish Size (cm)	C. Protein	Dry Matter	Organic Matter
Dietary Fibre	15	-0.84 (48) 0.0000	-0.90 (25) 0.0000	-0.94 (43) 0.0000
	20	-0.80 (45) 0.0000	-0.82 (24) 0.0000	-0.92 (42) O.0000
	25	-0.81 (47) 0.0000	-0.78 (23) 0.0000	-0.90 (41) 0.0000
Temperature	15	0.41 (48) 0.0040	0.55 (25) 0.0050	0.66 (43) 0.0000
	20	0.44 (45) 0.0030	0.45 (24) 0.0290	0.57 (42) 0.0001
	25	0.33 (47) 0.0300	0.29 (23) 0.1770	0.51 (41) 0.0007

Coefficient, (sample size), significance level

Table 3.5 - Correlation matrix showing the relationships between apparent digestibility coefficients and temperature between October 1985 and June 1986 at Farm A.

		Apparent Digestibility Coefficients		
	Fish size (cm)	C. Protein	Organic Matter	Dry Matter
Temperature	15	0.89 (31) 0.0000	0.88 (30) 0.0000	0.88 (16) 0.0000
	20	0.90 (29) 0.0000	0.85 (30) 0.0000	0.91 (16) 0.0000
	25	0.72 (32) 0.0000	0.77 (30) 0.0000	0.75 (16) 0.0000

Coefficent, (sample size), significance level.

2.2 Apparent Organic Matter Digestibility

The overall trends of apparent organic matter digestibility (AOMD) values throughout the survey period for the three size groups of fish and the average water temperatures during the same period are shown in Figure 3.2

As was the case with apparent crude protein digestibility, the lower AOMDs obtained coincided with the lower temperatures registered during the year. Thus, for all size groups, the lower AOMD values were obtained between October 1985 and February 1986 (Table 3.3 and Figure 3.2). Furthermore, AOMD followed the trend in temperature up to May 1986 and then declined (Table 3.3 and Figure 3.2). The intensity of association between AOMD values and temperatures, although significant, was low (Table 3.4) as was the case of apparent crude protein digestibility coefficients. However, when only considering data up to June 1986, much better correlations ($r = 0.88$, $r = 0.85$, $r = 0.77$, for the 15cm, 20cm and 25cm fish, respectively, see Table 3.5) between AOMD and temperature were obtained. Again, this may have been related to dissolved oxygen and good correlations between temperature and AOMD coefficients were obtained when based only on data between October 1985 and June 1986 (Table 3.5).

The change in diet formulation seems to have had a strong influence on digestibility from March onwards (Table 3.3 and Figure 3.2). The correlation between crude fibre and AOMD was around $r = -0.90$ (see Table 3.4) for all size groups of fish.

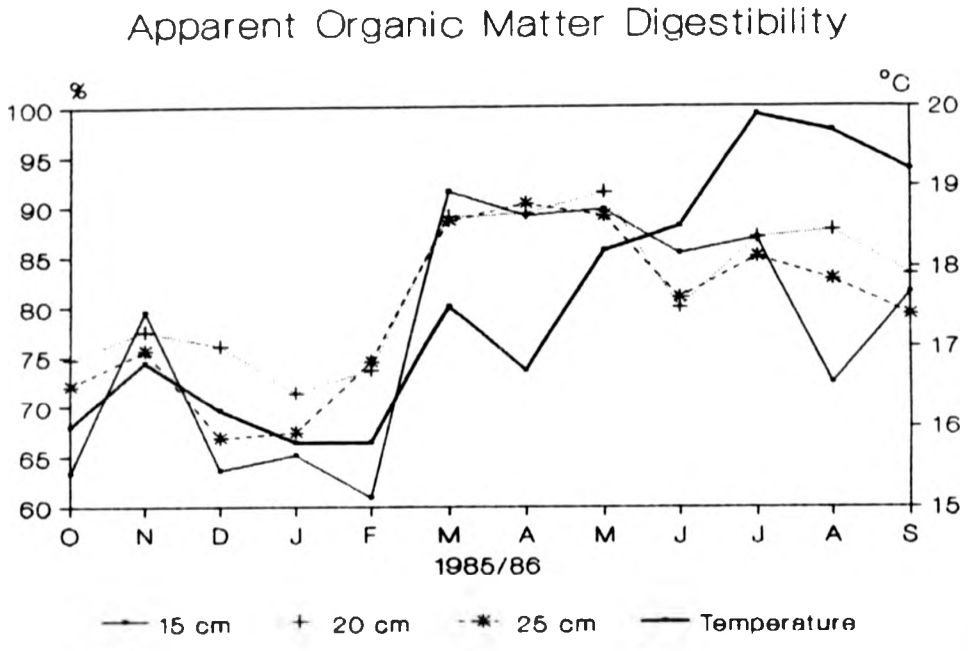

Figure 3.2 - Mean apparent organic matter digestibility coefficients for the 3 size groups of rainbow trout at Trutorão Trout Farm (Farm A) and average water temperature between October 1985 and September 1986.

2.3 Apparent Dry Matter Digestibility

As was seen with the other digestibility coefficients dry matter digestibility followed the trend in temperature up to May 1986, but declined thereafter (Table 3.3 and Figure 3.3). Again, this reduction in digestibility was probably related to lower dissolved oxygen at high temperatures and good correlations between temperature and dry matter digestibility values were obtained when only the data up to June 1986 was considered (Table 3.5).

The change in diet formulation from March 1986 onwards seems once more to have had an important effect on digestibility. In fact, there was high inverse correlations between the apparent dry matter digestibility coefficients and the fibre content of the diets throughout the year for all size groups of fish (Table 3.4).

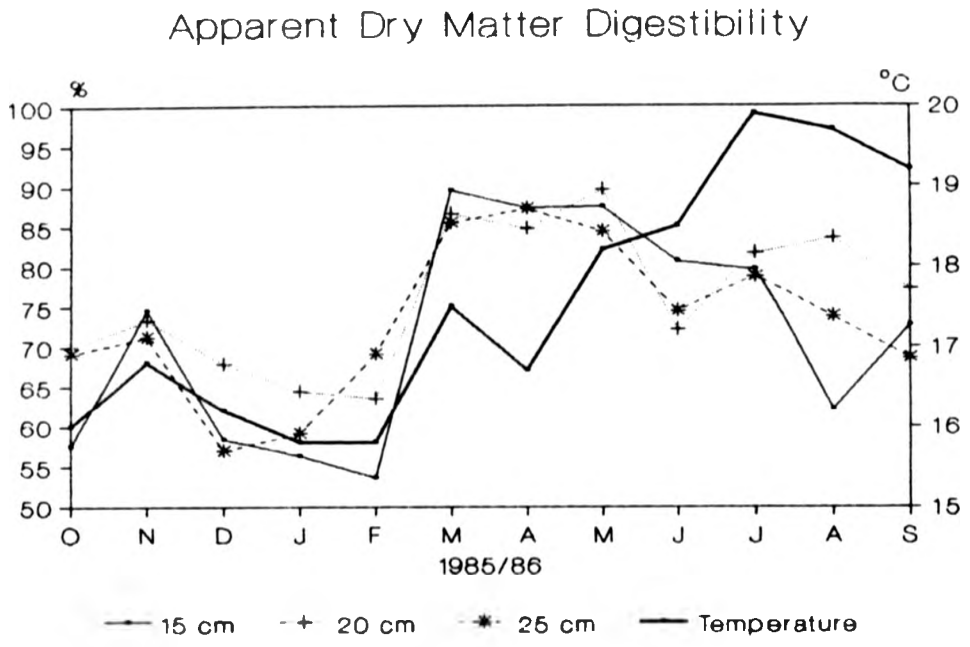

Figure 3.3 - Mean apparent dry matter digestibility coefficients for the 3 size groups of rainbow trout at Trutorão Trout Farm (Farm A) and average water temperatures between October 1985 and September 1986.

b) Inha Trout Farm (Farm B)

1. Commercial Diets

The proximate composition of the commercial diets used on the farm throughout the survey period are shown in Table 3.6. The farm uses a 3.2mm diameter pellet for fish of 12-16cm and a 4.5mm for fish bigger than 16cm (see Table 2.4). Thus, the smaller size (15cm) sampled was fed 3.2mm diets, whereas the other 2 size groups (20cm and 25cm) were fed 4.5mm diets. However, in July 1986 the smaller size group sampled was still receiving diet 2mm, in spite of their size.

In contrast with the diets used in Farm A, those used in this farm showed a wider variation in their proximate composition throughout the year.

The maximum specified level of moisture for both diets (3.2mm and 4.5mm) was 13%, although all diets sampled had a lower moisture content, the variation being between 7.83% (August) and 10.94% (November) for diets 3.2mm and between 8.03% (June) and 11.92% (January) for diet 4.5mm (Table 3.6).

The ash content varied from 18.55% (April) to 12.30% (December) for diets 3.2mm and between 17.41% (March) and 12.65% (January) for diets 4.5mm, 18% being the maximum level of ash specified by the manufacturers.

All diets contained the minimum specified level of 40% crude protein with values ranging from 41.27% (February) to 49.19% (August) and from 43.61% (January) to 49.59% (June) for diets 3.2mm and 4.5mm, respectively.

Table 3.6 - Proximate composition (n+4, ±S.D.) of the commercial diets used at Farm B (Inha) from October 85 to September 86.

	Month	October	November		December		January	February		March
Nutrient Content (% dry weight)	Diet no.	4.5	3.2	4.5	3.2	4.5	4.5	3.2	4.5	4.5
Moisture(%)		10.58 (±0.03)	10.94 (±0.04)	11.28 (±0.11)	9.84 (±0.01)	11.81 (±0.07)	11.92 (±0.05)	9.92 (±0.09)	10.81 (±0.05)	10.22 (±0.03)
Ash (%)		15.11 (±0.05)	14.99 (±0.15)	13.80 (±0.05)	12.30 (±0.28)	13.83 (±0.09)	12.65 (±0.13)	15.43 (±0.44)	15.66 (±0.07)	17.41 (±0.14)
Crude Protein (N x 6.25)		44.13 (±0.05)	44.73 (±0.10)	43.92 (±0.00)	42.95 (±0.08)	43.91 (±0.02)	43.61 (±0.09)	41.27 (±0.17)	44.96 (±0.03)	45.17 (±0.05)
Crude Lipid(%)		7.95 (±0.14)	9.33 (±0.21)	9.58 (±0.12)	11.69 (±0.24)	9.62 (±0.07)	10.46 (±0.05)	8.54 (±0.17)	10.38 (±0.08)	8.73 (±0.23)
Crude Fibre(%)		2.33 (±0.19)	1.45 (±0.22)	1.96 (±0.11)	4.95 (±0.14)	3.59 (±0.09)	5.35 (±0.15)	3.56 (±0.20)	4.00 (±0.16)	3.94 (±0.19)

Table 3.6 (cont)

Nutrient Content (% dry weight)	Month	April		May		June	July		August	September	
	Diet no.	3.2	4.5	3.2	4.5	4.5	2	4.5	3.2	3.2	4.5
Moisture(%)		10.56 (±0.02)	10.05 (±0.03)	10.41 (±0.05)	10.31 (±0.06)	8.03 (±0.01)	8.50 (±0.03)	8.99 (±0.05)	8.83 (±0.03)	7.83 (±0.03)	8.13 (±0.02)
Ash (%)		18.55 (±0.20)	16.77 (±0.03)	18.39 (±0.11)	16.34 (±0.17)	14.46 (±0.19)	12.32 (±0.11)	15.61 (±0.15)	13.46 (±0.04)	13.42 (±0.13)	15.60 (±0.11)
Crude Protein (N x 6.25)		42.71 (±0.11)	45.15 (±0.11)	43.19 (±0.13)	44.43 (±0.08)	49.59 (±0.10)	51.10 (±0.14)	45.73 (±0.09)	49.19 (±0.00)	47.10 (±0.02)	45.55 (±0.03)
Crude Lipid(%)		9.50 (±0.21)	8.34 (±0.16)	6.03 (±0.52)	10.70 (±0.31)	14.43 (±0.30)	10.66 (±0.14)	10.25 (±0.14)	10.55 (±0.27)	11.03 (±0.18)	10.65 (±0.28)
Crude Fibre(%)		2.68 (±0.09)	3.12 (±0.04)	2.64 (±0.05)	2.83 (±0.02)	3.23 (±0.01)	3.12 (±0.16)	2.42 (±0.12)	2.68 (±0.14)	2.19 (±0.12)	2.47 (±0.11)

With the exception of diets 4.5mm in October (7.95) and 3.2mm in May (6.03%) all diets contained the specified minimum of 8% lipid with values ranging from 8.54% (February) to 11.69% (December) for diets 3.2mm and from 8.34% (April) to 14.43% (June) for diets 4.5mm.

With the exception of diet 4.5mm in Janury (5.35%) all diets contained less than the specified maximum of 5% crude fibre with values ranging from 1.96% (November) to 4.00% (February) and from 1.45% (November) and 4.95% (December) for diets 4.5mm and 3.2mm, respectively.

Diet 2mm (July) contained less moisture (8.50%), less ash (12.32%) and less fibre (3.12%) than the maximum specified levels of 10%, 15% and 4%, respectively. It showed to contain more than the minimum specified levels of 44% crude protein and 8% crude lipid (Table 3.6).

2. Apparent Nutrient Digestibility Coefficients

The proximate composition of faeces collected from the three size groups of rainbow trout is presented in Table 3.7. Although there was good replication within treatments, there was a wider variation than in Farm A in nutrient content throughout the year for all fish sizes. Thus, for example the fibre content of faeces varied between around 3% (June) and around 9% (August) for the 15cm fish and between 3% (July) and 8% (February) for the 20cm and the 25cm fish (Table 3.7).

Table 3.7 - Proximate composition (n=2, ±S.D.) of faeces collected from the 3 size groups of rainbow trout at Farm B (Inha).

Nutrient Content (% dry weight)	Month	October			November			December			January		
	Fish Size (cm)	15	20	25	15	20	25	15	20	25	15	20	25
Ash (%)	Replicates A	24.82 (±0.53)	24.65 (±0.04)	24.68 (±0.18)	25.14 (±0.15)	25.35 (±0.15)	24.56 (±0.16)	21.71 (±0.05)	22.69 (±0.00)	21.69 (±0.17)	26.01 (±0.11)	29.12 (±0.13)	24.11 (±0.03)
	B	23.27 (±0.53)	25.55 (±0.63)	25.27 (±0.00)	24.78 (±0.11)	25.68 (±0.14)	23.42 (±0.03)	21.88 (±0.09)	23.44 (±0.12)	22.76 (±0.56)	26.82 (±0.40)	28.18 (±0.14)	24.29 (±0.02)
Crude Protein (Nx6.25)	A	27.87 (±0.08)	26.78 (±0.02)	28.13 (±0.02)	27.56 (±0.11)	26.41 (±0.26)	27.60 (±0.00)	24.23 (±0.00)	28.33 (±0.09)	29.11 (±0.00)	19.25 (±0.07)	18.24 (±0.08)	20.80 (±0.19)
	B	28.10 (±0.13)	27.79 (±0.14)	27.93 (±0.15)	27.63 (±0.06)	25.84 (±0.21)	27.42 (±0.04)	24.26 (±0.06)	27.84 (±0.21)	29.30 (±0.00)	19.93 (±0.02)	18.47 (±0.04)	21.11 (±0.07)
Crude Fibre(%)	A	5.91 (±0.08)	6.94 (±0.40)	5.56 (±0.14)	5.14 (±0.41)	5.97 (±0.03)	4.84 (±0.05)	6.56 (±0.41)	4.28 (±0.02)	3.77 (±0.20)	6.97 (±0.19)	5.86 (±0.03)	8.25 (±0.15)
	B	6.03 (±0.06)	6.36 (±0.20)	5.36 (±0.10)	4.55 (±0.42)	5.87 (±0.01)	4.98 (±0.04)	7.30 (±0.33)	4.22 (±0.03)	4.40 (±0.30)	6.55 (±0.21)	5.95 (±0.05)	8.50 (±0.13)

Table 3.7 (cont.) - Proximate composition (n=2, ±S.D.) of faeces collected from the 3 size groups of rainbow trout at Farm B (Inha)

Nutrient Content (% dry weight)	Month	February			March			April			May		
	Fish Size (cm)	15	20	25	15	20	25	15	20	25	15	20	25
	Replicates												
Ash (%)	A	24.93 (±0.20)	22.53 (±0.15)	21.96 (±0.07)	27.19 (±0.10)	33.36 (±0.00)	30.13 (±0.02)	30.79 (±0.20)	29.85 (±0.14)	29.95 (±0.00)	33.70 (±0.12)	30.57 (±0.10)	31.24 (±0.17)
	B	24.76 (±0.15)	22.11 (±0.14)	21.94 (±0.08)	26.70 (±0.08)	32.93 (±0.15)	32.01 (±0.21)	30.56 (±0.15)	30.19 (±0.19)	29.84 (±0.25)	34.70 (±0.40)	30.21 (±0.15)	30.63 (±0.19)
Crude Protein (Nx6.25)	A	17.74 (±0.00)	21.40 (±0.07)	21.77 (±0.15)	24.66 (±0.19)	19.78 (±0.00)	21.42 (±0.09)	24.97 (±0.00)	20.59 (±0.01)	20.83 (±0.00)	20.12 (±0.07)	19.97 (±0.12)	19.90 (±0.06)
	B	18.94 (±0.10)	21.01 (±0.04)	21.54 (±0.00)	25.17 (±0.23)	19.63 (±0.13)	21.10 (±0.06)	24.74 (±0.14)	20.44 (±0.07)	21.14 (±0.19)	20.67 (±0.29)	19.65 (±0.05)	19.58 (±0.00)
Crude Fibre (%)	A	5.86 (±0.14)	8.09 (±0.20)	8.10 (±0.14)	5.49 (±0.31)	6.49 (±0.40)	5.48 (±0.23)	4.75 (±0.01)	5.55 (±0.20)	5.35 (±0.20)	5.78 (±0.15)	6.63 (±0.12)	6.78 (±0.04)
	B	6.09 (±0.15)	8.41 (±0.19)	7.87 (±0.12)	4.90 (±0.25)	5.77 (±0.20)	6.06 (±0.20)	4.70 (±0.00)	5.19 (±0.30)	5.93 (±0.21)	6.19 (±0.17)	6.85 (±0.11)	6.84 (±0.05)

Table 3.7 (cont.) - Proximate composition (n=2, ±S.D.) of faeces collected from the 3 size groups of rainbow trout at Farm B (Inha).

Nutrient Content (% dry weight)	Month	June			July			August			September		
	Fish Size (cm)	15	20	25	15	20	25	15	20	25	15	20	25
	Replicates												
Ash (%)	A	34.34 (±0.02)	34.93 (±0.16)	36.27 (±0.12)	25.47 (±0.18)	36.49 (±0.00)	36.80 (±0.19)	26.20 (±0.00)	-	-	25.36 (±0.03)	-	32.05 (±0.10)
	B	34.34 (±0.01)	35.14 (±0.12)	36.05 (±0.10)	25.20 (±0.15)	37.46 (±0.40)	37.70 (±0.12)	25.81 (±0.10)	-	-	25.67 (±0.06)	-	31.76 (±0.15)
Crude Protein (Nx6.25)	A	26.55 (±0.24)	26.76 (±0.08)	24.53 (±0.16)	23.28 (±0.07)	25.11 (±0.06)	22.81 (±1.05)	21.24 (±0.00)	-	-	23.38 (±0.05)	-	19.08 (±0.19)
	B	26.07 (±0.01)	26.12 (±0.59)	24.36 (±0.00)	23.05 (±0.19)	24.60 (±0.04)	22.63 (±0.02)	21.28 (±0.00)	-	-	23.81 (±0.14)	-	18.72 (±0.26)
Crude Fibre (%)	A	3.78 (±0.31)	3.44 (±0.08)	3.18 (±0.01)	7.47 (±0.03)	3.16 (±0.13)	2.39 (±0.21)	9.69 (±0.41)	-	-	8.40 (±0.30)	-	6.58 (±0.02)
	B	3.19 (±0.41)	3.18 (±0.10)	3.24 (±0.00)	7.41 (±0.00)	2.93 (±0.07)	2.78 (±0.11)	8.96 (±0.40)	-	-	8.95 (±0.15)	-	6.68 (±0.03)

2.1 Apparent Crude Protein Digestibility

Figure 3.4 shows the overall trends of crude protein digestibility values throughout the one year survey period for the three size groups of trout and the water temperatures during the same period.

The lower digestibility values were obtained in December and June for the 20cm and 25cm fish and in December, March and June for the 15cm fish. The poor digestibility coefficients in December and March coincided with the lowest temperatures registerd during the survey period.

With the exception of the digestibility values in June, in general the increases or decreases in digestibility "seem to agree" with the temperature changes throughout the year (Figure 3.4). The higher digestibility values were obtained in May and September for the 15cm fish and for the 25cm fish. In August, no faecal samples were collected for the 20cm and the 25cm fish and in September no faecal samples were obtained for the 20cm fish. Thus, having no values in these months for the 20cm fish, their best digestibility was obtained in May (Figure 3.4 and Table 3.8). However, when correlation analysis was carried out a significant correlation between temperature and apparent crude protein digestibility was only obtained for the 15cm fish. The same trend was also seen in the correlation between fibre content of the diets and protein digestibility (Table 3.9).

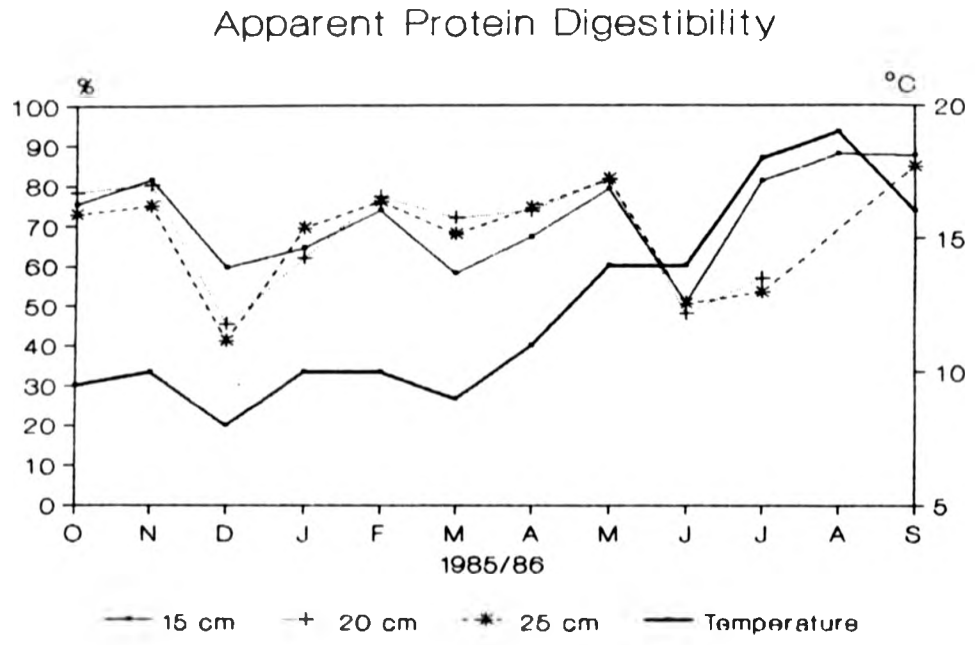

Figure 3.4 - Mean apparent protein digestibility coefficients of the 3 size groups of rainbow trout in Inha Trout Farm (Farm B) and water temperatures between October 1985 and September 1986.

Table 3.8 - Mean apparent digestibility coefficients (n=4, ±S.D.) for the 3 size groups of fish at Inha (Farm B).

Apparent Nutrient Digestibility (%)	Fish Size (cm)	Months											
		Oct. 85	Nov.	Dec.	Jan. 86	Feb.	Mar.	April	May	June	July	Aug.	Sept.
			a..h										
Crude Protein (Nx6.25)	15	$^{c}75.26_{f}(\pm0.16)$	$^{b}81.47_{k}(\pm1.32)$	$^{e}59.55_{k}(\pm2.46)$	$^{d}64.39_{f}(\pm2.00)$	$^{c}73.52_{m}(\pm0.42)$	$^{e}57.97_{f}(\pm3.23)$	$^{d}66.99_{f}(\pm0.10)$	$^{b}79.14_{f}(\pm0,52)$	$^{f}50.51_{k}(\pm4.31)$	$^{b}80.98_{k}(\pm0.09)$	$^{a}87.77$ ±0.90)	$^{a}87.34_{k}(\pm0.33)$
	20	$^{b}78.27_{k}(\pm1.55)$	$^{a}80.31_{k}(\pm0.16)$	$^{f}45.43_{f}(\pm1.24)$	$^{c}61.86_{m}(\pm0.14)$	$^{b}77.11_{k}(\pm0.75)$	$^{c}71.86_{k}(\pm1.79)$	$^{c}73.56_{k}(\pm0.91)$	$^{a}81.27_{k}(\pm0.52)$	$^{e}47.90_{k}(\pm1.77)$	$^{d}56.75_{f}(\pm1.37)$	-	-
	25	$^{c}72.89_{m}(\pm0.46)$	$^{c}74.98_{f}(\pm0.50)$	$^{f}41.21_{f}(\pm5.01)$	$^{d}69.29_{k}(\pm0.31)$	$^{c}75.87_{f}(\pm0.27)$	$^{d}67.76_{k}(\pm2.15)$	$^{c}74.22_{k}(\pm1.32)$	$^{b}81.53_{k}(\pm0.27)$	$^{e}50.38_{k}(\pm0.76)$	$^{e}53.21_{f}(\pm4.48)$	-	$^{a}84.53_{f}(\pm0.34)$
Organic Matter	15	$^{c}65.08_{f}(\pm0.20)$	$^{b}73.47_{k}(\pm1.94)$	$^{e}36.11_{k}(\pm4.01)$	$^{ef}32.08_{f}(\pm2.50)$	$^{d}47.02_{m}(\pm1.12)$	$^{ef}32.68_{f}(\pm4.84)$	$^{d}51.71_{k}(\pm0.49)$	$^{c}64.38_{k}(\pm1.73)$	$^{f}28.34_{k}(\pm7.01)$	$^{c}64.28_{k}(\pm0.32)$	$^{ab}75.38$ (±1.18)	$^{a}78.25_{k}(\pm0.86)$
	20	$^{a}69.03_{k}(\pm1.35)$	$^{a}71.39_{k}(\pm0.26)$	$^{b}20.81_{f}(\pm4.16)$	$^{g}26.00_{m}(\pm0.14)$	$^{c}55.34_{k}(\pm0.90)$	$^{e}47.78_{k}(\pm3.72)$	$^{d}51.01_{k}(\pm1.76)$	$^{b}65.04_{k}(\pm0.56)$	$^{g}25.78_{k}(\pm3.24)$	$^{f}40.12_{f}(\pm1.70)$	-	-
	25	$^{b}62.49_{m}(\pm0.55)$	$^{ab}65.71_{f}(\pm1.32)$	$^{f}20.15_{f}(\pm7.73)$	$^{d}44.63_{k}(\pm0.93)$	$^{c}53.63_{f}(\pm0.78)$	$^{d}42.83_{k}(\pm4.21)$	$^{c}53.20_{k}(\pm2.74)$	$^{ab}65.68_{k}(\pm0.07)$	$^{e}24.90_{k}(\pm0.82)$	$^{e}29.95_{m}(\pm6.68)$	-	$^{a}69.93_{f}(\pm0.20)$
Dry Matter	15	$^{b}62.42_{f}(\pm0.46)$	$^{a}70.99_{k}(\pm2.15)$	$^{d}29.84_{k}(\pm4.64)$	$^{e}21.96_{f}(\pm3.00)$	$^{c}41.89_{f}(\pm1.37)$	$^{de}24.89_{k}(\pm5.19)$	$^{c}44.47_{k}(\pm0.35)$	$^{b}57.35_{k}(\pm1.78)$	$^{f}15.04_{k}(\pm7.90)$	$^{b}59.93_{k}(\pm0.20)$	$^{a}73.17$ (±1.33)	$^{a}76.40_{k}(\pm0.94)$
	20	$^{a}66.44_{k}(\pm1.80)$	$^{a}68.22_{k}(\pm0.32)$	$^{d}18.91_{k}(\pm3.96)$	$^{e}9.92_{m}(\pm0.84)$	$^{b}53.63_{k}(\pm1.14)$	$^{c}36.96_{k}(\pm4.54)$	$^{c}43.18_{k}(\pm2.32)$	$^{ab}59.68_{k}(\pm0.81)$	$^{e}6.31_{k}(\pm2.70)$	$^{d}20.92_{f}(\pm3.55)$	-	-
	25	$^{ab}58.68_{m}(\pm0.92)$	$^{ab}60.67_{f}(\pm1.35)$	$^{e}12.02_{k}(\pm8.16)$	$^{cd}38.14_{k}(\pm1.16)$	$^{abc}51.97_{k}(\pm0.87)$	$^{d}32.83_{k}(\pm4.13)$	$^{bcd}45.96_{k}(\pm3.40)$	$^{ab}60.20_{k}(\pm0.15)$	$^{f}0.97_{f}(\pm0.75)$	$^{e}13.27_{f}(\pm6.91)$	-	$^{a}64.33_{f}(\pm0.33)$

Values in same row with same superscript are not significantly ($p<0.05$) different, for each size group of fish within each digestibility coefficient.
Values in same column with same subscript are not significantly ($p<0.05$) different, for each digestibility coefficient.

Table 3.9 - Correlation matrix showing the relationships between apparent digestibility coefficients and dietary fibre and temperature at Farm B.

		Apparent Digestibility Coefficients		
	Fish size (cm)	C. Protein	Organic Matter	Dry Matter
Dietary fibre	15	-0.62 (48) 0.0000	-0.79 (48) 0.0000	-0.81 (23) 0.0000
	20	-0.30 (40) 0.0595	-0.62 (39) 0.0000	-0.58 (19) 0.0086
	25	-0.18 (44) 0.2399	-0.36 (44) 0.0156	-0.32 (20) 0.1661
Temperature	15	0.59 (48) 0.0000	0.53 (48) 0.0001	0.56 (23) 0.0056
	20	-0.18 (40) 0.2628	-0.04 (39) 0.8207	-0.18 (19) 0.4619
	25	0.08 (44) 0.6082	0.03 (44) 0.8273	0.05 (20) 0.8237

Coefficent (sample size) significance level

With the exception of the 15cm fish, from June onwards a further increase (from 14°C to 18°C) in temperature was not followed by an increase in digestibility. Thus, as had been the case for Farm A, some other factors may be influencing digestibility. Following the same criteria as for Farm A, data was divided and correlation analysis were carried out on samples from October 1985 to June 1986. In fact, much better and significant correlations between temperatures and protein digestibility values were obtained for all size groups (Table 3.10). For the remaining months (June to September) only for the 15cm fish there was a significant ($p<0.01$) correlation between temperature and protein digestibility ($r = 0.79$).

Since in June the fibre content of the diet (Table 3.6) was just slightly higher than that of the faeces of all size groups of fish (Table 3.7) the correlations between dietary fibre contents and protein digestibility values were calculated only considering samples from October 1985 to June 1986. Again, better and significant inverse correlations were obtained for all fish sizes (Table 3.10).

Table 3.10 - Correlation matrix showing the relationships between apparent digestibility coefficients and dietary fibre and temperature between October 1985 and June 1986 at Farm B.

		Apparent Digestibility Coefficients		
	Fish size (cm)	C. Protein	Organic Matter	Dry Matter
Temperature	15	0.69 (32) 0.0000	0.65 (32) 0.0000	0.60 (16) 0.0000
	20	0.61 (32) 0.0000	0.48 (31) 0.0000	0.39 (16) 0.0000
	25	0.73 (32) 0.0000	0.64 (31) 0.0000	0.62 (16) 0.0000
Dietary fibre	15	-0.80 (32) 0.0000	-0.93 (32) 0.0000	-0.93 (16) 0.0000
	20	-0.54 (32) 0.0000	-0.78 (31) 0.0000	-0.84 (16) 0.0000
	25	-0.28 (32) 0.0000	-0.55 (32) 0.0000	-0.51 (16) 0.0000

Coefficient, (sample size), significance level

2.2 Apparent Organic Matter Digestibility

In Figure 3.5 are shown the overall trends of apparent organic matter digestibility coefficients (AOMD) throughout the one year survey period for the three size groups of fish and the water temperatures during the same period.

As had been the case for crude protein digestibility, with the exception of the low digestibility values in June, the lower AOMD values coincided with the lower temperatures registered.

With the exception of the digestibility values in June and February for all size groups of fish, in general the decreases or increases in digestibility seem to follow the changes in temperature during the survey period (Figure 3.5). The higher digestibility values were obtained in September and May 1986 and November 1985 for the 15cm and the 25cm fish and in May 1986 and October and November 1985 for the 20cm fish (no values were obtained in August and September 1986 for this size group). However, only the data for the 15cm fish showed a significant correlation between temperatures and AOMD coefficients (Table 3.9). Furthermore, better and, although low, significant correlations were obtained between the digestibility coefficients of all size groups and the dietary fibre contents (Table 3.9).

Again, as was the case for protein digestibility, significant correlations between temperatures and the AOMD coefficients of all size groups were obtained (Table 3.10) when only considering data from October 1985 to June 1986. For the remaining months there was a significant ($p<<0.01$) correlation ($r= 0.72$) between temperatures

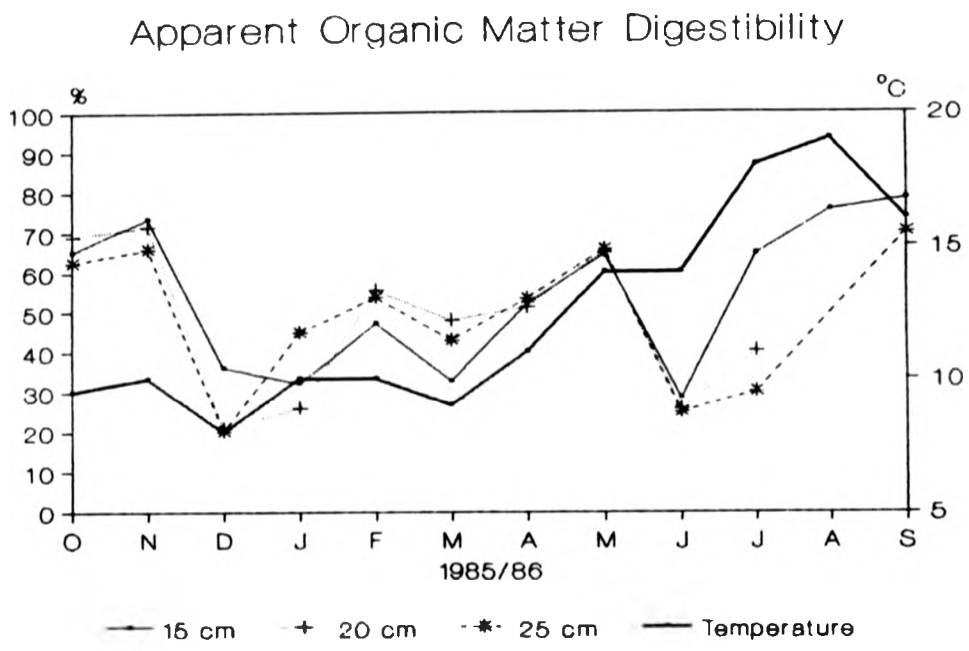

Figure 3.5 - Mean apparent organic matter digestibility coefficients of the 3 size groups of fish at Inha Trout Farm (Farm B) and water temperatures between October 1985 and September 1986

and AOMD values only for the 15cm fish (Table 3.11). In addition, much better correlations between AOMD values and dietary fibre contents were obtained between October and June (Table 3.10).

2.3 Apparent Dry Matter Digestibility

Figure 3.6 shows the overall trends of apparent dry matter digestibility (ADMD) coefficients for the three size groups of trout throughout the one year survey period and the water temperatures during the same period.

With the exception of the digestibility values in June for all size groups and of the digestibility values in January for the 15cm and 20cm fish, the lower ADMD coefficients coincided with the lower temperatures registered and in general the decreases or increases in digestibility seem to follow the changes in temperature throughout the year (Figure 3.6).

As was the case for the other digestibility coefficients the best ADMD values were obtained in September and May 1986 and November 1985 for the 15cm and 25cm fish, and in May 1986 and November 1985 for the 20cm fish.

However, a significant correlation between the ADMD coefficients and temperature was only obtained for the 15cm fish. In addition, inverse and significant correlations were obtained for the 15cm and the 20cm fish between the dietary fibre contents and the ADMD values (Table 3.9). Better and significant correlations between ADMD values and either temperatures or dietary fibre levels for all size groups (Table 3.10) were

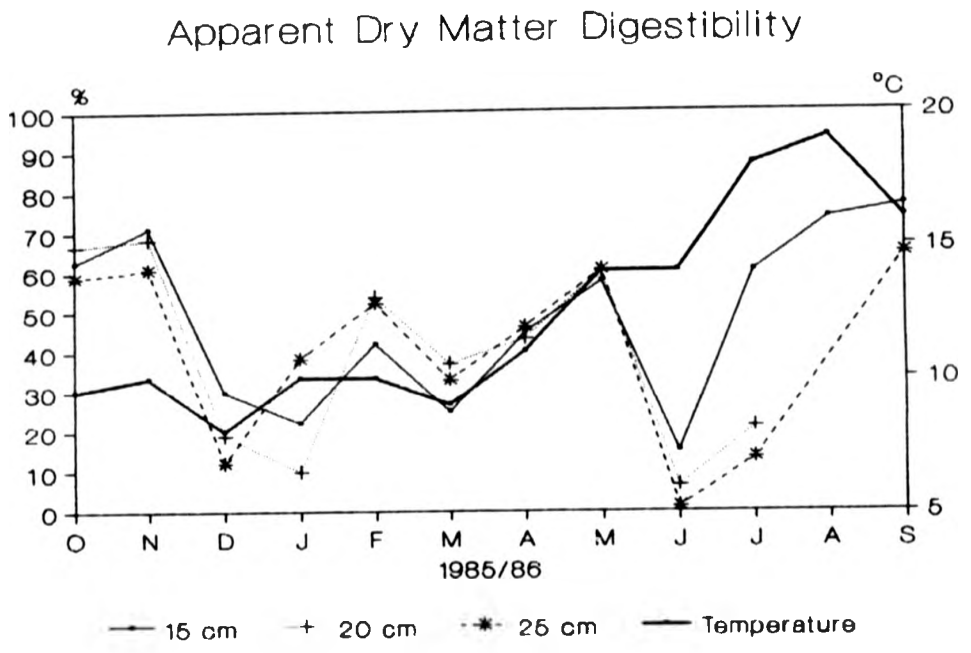

Figure 3.6 - Mean apparent dry matter digestibility coefficients of the 3 size groups of fish at Inha Trout Farm (Farm B) and water temperatures between October 1985 and September 1986.

obtained between October 1985 and June 1986 (Table 3.10). Furthermore, for the remaining months only for the 15cm was there a significant ($p<<0.01$) correlation ($R=0.62$) between ADMD coefficients and temperatures (Table 3.11).

The statistical methods used are to some extent limited because they only allow comparison of the variables in pairs. Since a high number of variables were involved in this study, a more wide-ranging examination of the data seemed preferable in order to investigate which variables had the greatest influence and which would best explain the trends obtained. Consequently, a multivariate approach using cluster analysis and principal components analysis was used to investigate the data further.

Figures 3.7 and 3.8 show the distribution of the samples from both farms during the survey period on the plan of the first and second principal components and on the plan of the first and third principal components, respectively. These three principal components account for 91.9% of the total variation among the samples and the correlation between this reduced matrix and the original data is 0.93 ($p<<0.01$).

From the analysis of the plan of the first and second principal components (Figure 3.7) several features can be discerned. The largest difference is between the two farms, Trutorão Trout Farm to the right and Inha Trout Farm to the left. Trutorão samples fall into only 2 groups (A and B) as shown by the cluster analysis (Figure 3.9). This is made more apparent in the principal components plots by the encircling of the different groups (Figures 3.7 and 3.8).

Table 3.11 - Correlation matrix showing the relationships between apparent digestibility coefficients and dietary fibre and temperature between June and September 1986 for the 15cm fish, at Farm B.

		Apparent Digestibility Coefficients		
	Fish size (cm)	C. Protein	Organic Matter	Dry Matter
Temperature	15	0.79 (16) 0.0000	0.72 (16) 0.0000	0.62 (7) 0.0000
Dieetary fibre	15	-0.69 (16) 0.0000	-0.75 (16) 00000	-0.70 (7) 0.0000

Coefficient, (sample size), significance level

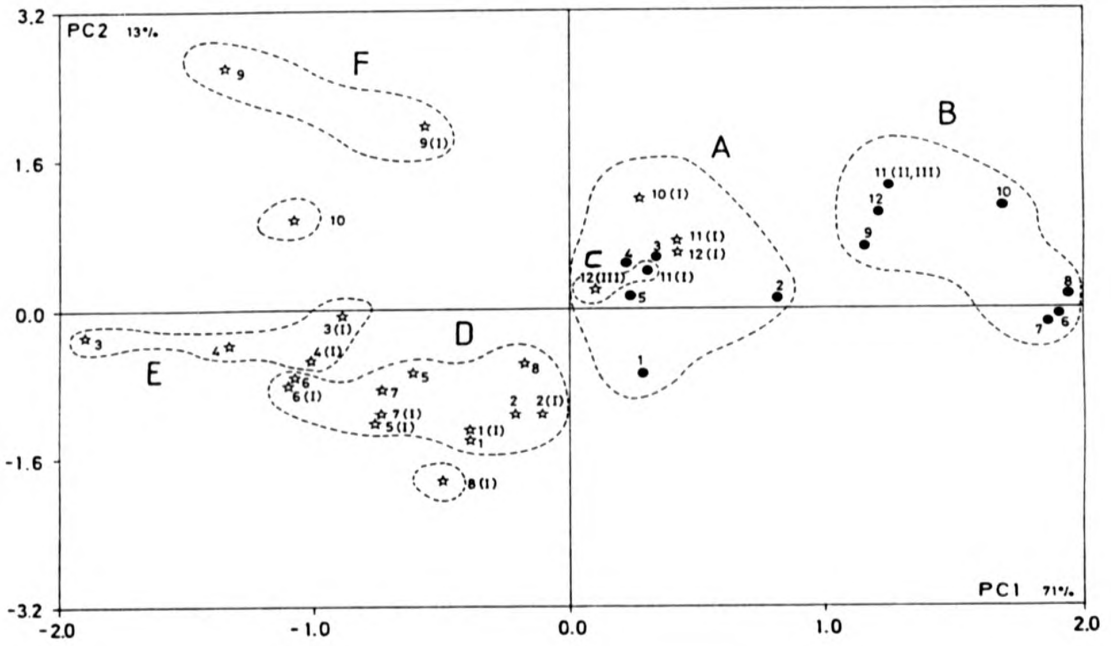

Figure 3.7 - Principal components plot of all samples for Trutorão (●) and Inha (☆) Trout Farms on the plan of the first and second principal components. 1...12 - October 1985 ... September 1986; I, II or III - 15cm, 20cm, or 25cm fish only.

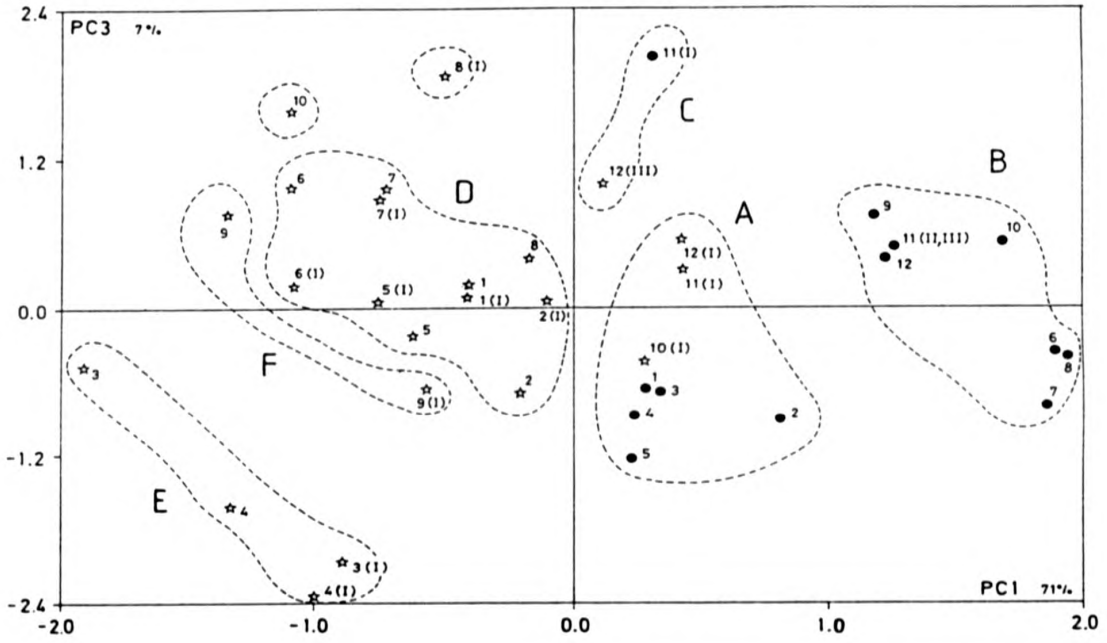

Figure 3.8 - Principal components plot of all samples for Trutorão (●) and Inha (☆) Trout Farms on the plan of the first and third principal components. 1...12 - October 1985 to September 1986; I, II or III - 15cm, 20cm or 25cm fish only.

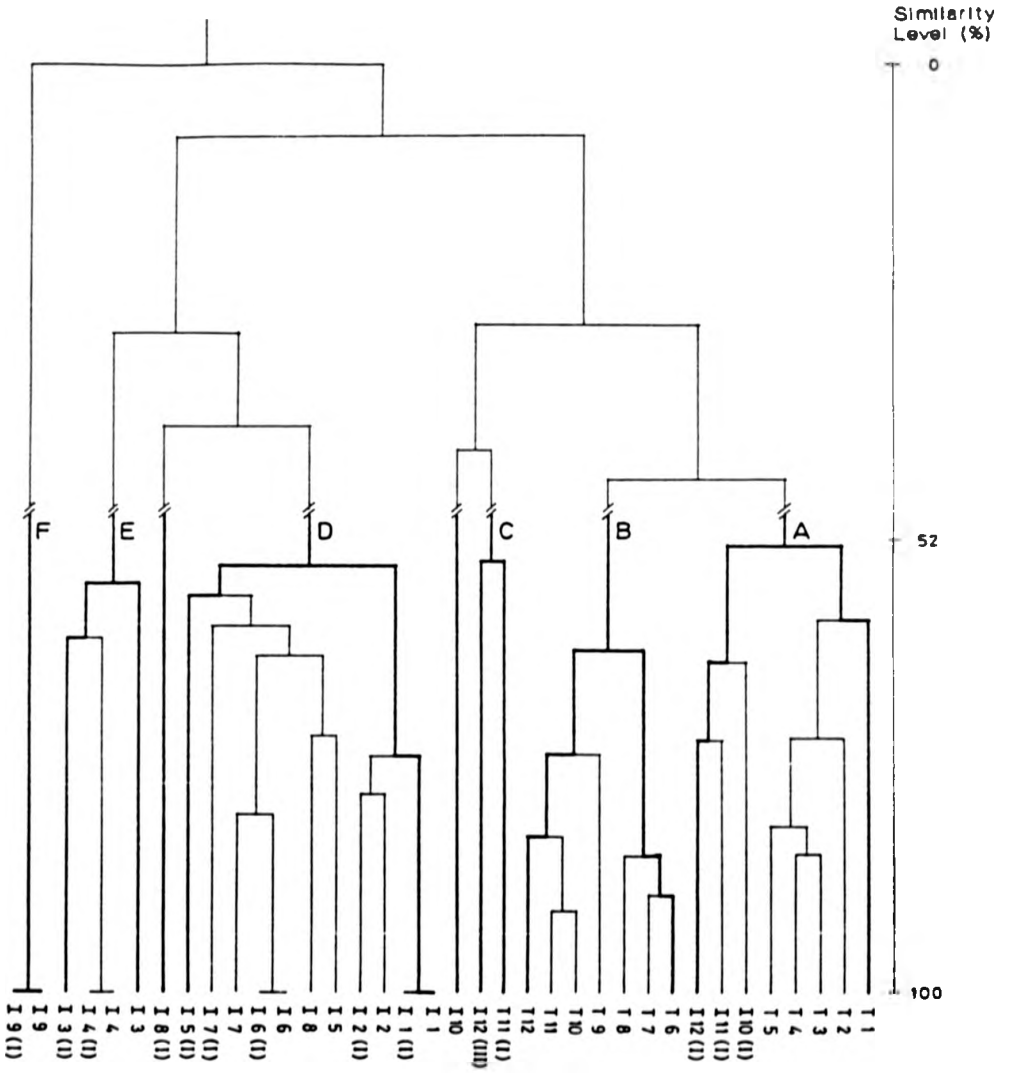

Figure 3.9 - Clusters obtained at the 52% similarity level. T1...T12 and I1...I12 - October 1985 to September 1986 for Trutorão and Inha Trout Farms, respectively. I, II or III - 15cm, 20cm or 25cm fish only.

Cluster B comprises the samples after the formulation of T12 diets changed whereas Cluster A includes those before the change in diet formulation. By contrast, Inha samples show a much wider variation which is evident from the wider distribution of the samples in this plan (Figure 3.7) and supported by the higher number of groups formed on the cluster analysis (Figure 3.9). Within each farm the separation of the samples is also along the first principal component, although some separation also occurs along the second principal component, especially for Inha Trout Farm.

In the plan of the first and third principal components (Figure 3.8) the separation between the two farms is still evident and again Trutorão is to the right and Inha to the left. Inha samples again show a wider variation than Trutorão samples, and consequently it seems that the two farms are separated along the first horizontal axis in the plot, which is the direction of the largest variation among the samples. Again, within each farm the separation of the samples is mainly along the first principal component, although and especially in the case of Inha Trout Farm some separation also occurs along the third principal component (Figure 3.8).

The principal components vector plots in Figures 3.10 and 3.11 show how the different variables (apparent digestibility coefficients for all size groups of fish sampled, water temperatures registered and proximate composition of feeds throughout the year) influenced the distribution of the samples in the corresponding principal components plots in Figures 3.7 and 3.8, respectively. The higher the value a variable has along a principal components axis, positive or negative, the more it influences the distribution of the samples along that axis. Since the differences between the two farms and

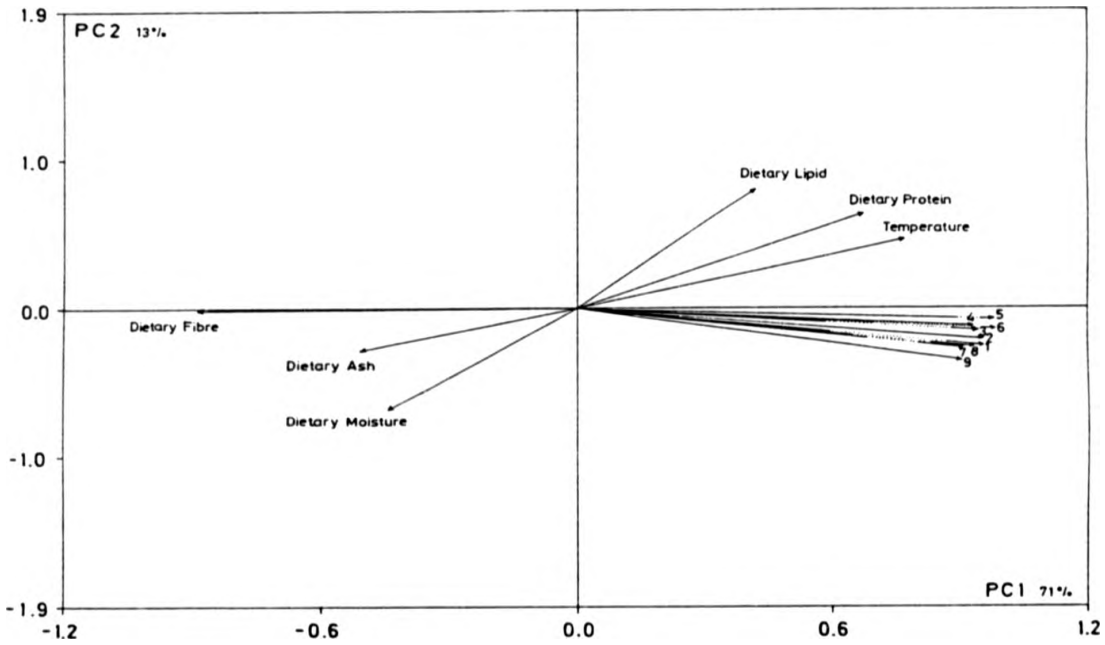

Figure 3.10 - Principal components plot of all the variables on the plan of the first and second principal components. 1...9 - digestibility coefficients (1, 2, 3 - crude protein for the 15cm, 20cm and 25cm fish, respectively; 4, 5, 6 - organic matter for the 15cm, 20cm and 25cm fish, respectively; 7, 8, 9 - dry matter for the 15cm, 20cm and 25cm fish, respectively).

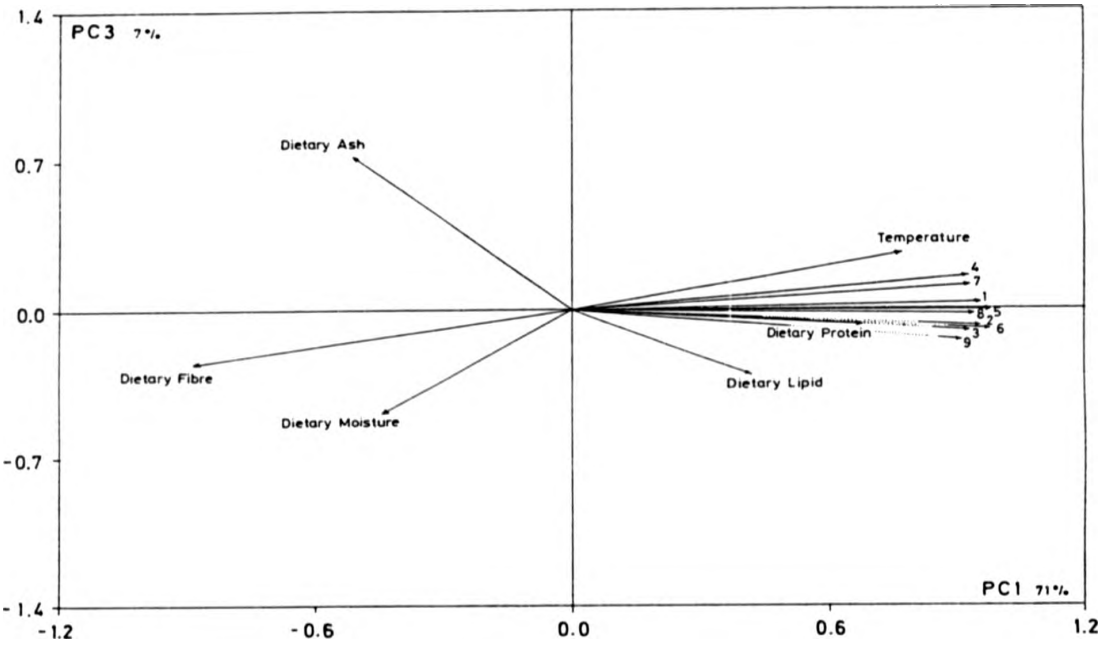

Figure 3.11 - Principal components plot of all the variables on the plan of the first and third principal components (for legend see Figure 3.10).

between the different clusters within each farm seem to be mainly along the first principal component, the variables responsible for this separation will be those to the far right and far to the left in the variables plots (Figures 3.10 and 3.11). Thus, the temperature and the digestibility coefficients with high positive values and the dietary level of crude fibre with high negative values are the main variables responsible for the distribution of the samples. In fact, these variables are highly correlated with the first principal component ($r = 0.95$, $r = 0.77$ and $r = -0.89$ for the digestibility coefficients of all size groups, for the temperature and for the dietary crude fibre level, respectively).

In general, Trutorão Trout Farm has higher digestibility coefficients and diets with lower levels of crude fibre than Inha Trout Farm. Only the digestibility values for the 15cm fish between July and September 1986 and the digestibility values of the 25cm fish in September in Inha were similar to the lower digestibility values obtained in Trutorão (Figures 3.7 and 3.8, cluster A). In Trutorão the bigger differences in digestibility and dietary crude fibre levels are between samples in cluster B (Figures 3.7 and 3.8), with higher digestibility values and lower levels of dietary fibre, and samples in cluster A with lower digestibility values and higher levels of dietary fibre. In Inha the largest differences in digestibility and dietary crude fibre content are for the 15cm fish between samples in cluster A with higher digestibility values and lower levels of dietary crude fibre and samples in clusters F and E with lower digestibility coefficients and higher levels of dietary fibre (Figures 3.7 and 3.8) and for the 20cm and 25cm fish between samples in cluster D and those in clusters F and E.

As mentioned earlier, the second and third principal components and consequently the variables associated with these axes also had some influence on the distribution of the samples, especially within Inha Trout Farm, on the principal components plots in Figures 3.7 and 3.8. Thus, the dietary levels of lipid and moisture which are significantly ($p<0.01$) correlated to the second principal component ($r= 0.74$ and $r= -0.49$), respectively) contributed to the separation of the samples along these axis. Thus, on the plan of the first and second principal components (Figure 3.7) samples in cluster F with very low digestibility values and high dietary fibre contents were the samples with the higher levels of lipids (14%) and lower moisture (8%) content (see also Table 3.6) and so displaced further upwards along the second axis.

On the plan of the first and third principal components (Figure 3.8) cluster C, which in Figure 3.7 (first and second principal components plot) seemed to overlap cluster A, is separated mainly due to the higher ash content of the samples which are grouped (around 15% in contrast to around 13% ash for samples in cluster A). However, it is again within Inha Trout Farm that a wider distribution of the samples occurs. Within cluster D there is an increase of the ash content of the diets from October-November 1985 (around 15% ash) to April 1986 (around 16% ash). Samples I10 and I8(I) had similar ash contents to values in April. Within clusters E and F the increase in ash content is also observed from the right to the left of each cluster. Besides the higher levels of crude fibre and low digestibility values, the high moisture (around 12%) content of the diets (see also Table 3.6) would have also contributed to separate the samples in cluster E to a lower position along the third principal component.

The increasing trend of dietary ash observed in Inha, from the right to the left within each cluster, follows the increase of the level of crude fibre of the diets and the decrease in digestibility values.

From the above description it is apparent that the contribution of the dietary parameters on the distribution of the samples along the second and third principal components were more important in Inha than in Trutorão, indicating a wider variation of the proximate composition of the feeds used throughout the year in Inha. Thus, this multivariate approach combining all the variables seems to reveal relationships such as the influence of the dietary ash content on the digestibility coefficients which were not demonstrated as being significant with the univariate approach. In fact, significant ($p<0.05$) or highly significant ($p<0.01$) inverse correlations were obtained between the apparent digestibility coefficients and the dietary ash content, in addition to the highly significant correlations between the digestibility coefficients and temperatures and crude fibre content of diets (Tables 3.12, 3.4, 3.5, 3.9 and 3.10). In some cases significant correlations between the digestibility coefficients and the dietary levels of protein and lipid were also obtained (Table 3.12).

The multivariate approach strengthened those indications obtained by the univariate method that T12 diets were different before and after the formulation change. The method revealed that the samples from Trutorão fell into two distinct groups as displayed in the principal components plots (Figures 3.7 and 3.8) corresponding to samples before and after the formulation of diets had changed. It is also clear that diet T5 fed to the 15cm fish in August in Trutorão was different from all the other diets

used (Figure 3.8, cluster C). It was, however, less different from T12 diets before the change in diet formulation than from T12 diets after the referred change.

Table 3.12 - Correlation matrix showing the relationships beteween apparent digestibility coefficients and temperature and dietary parameters, for all size groups of fish, obtained from the principal components analysis.

	Apparent Digestibility Coefficients								
Fish size (cm)	15			20			25		
	Protein	Organic Matter	Dry Matter	Protein	Organic matter	Dry matter	Protein	Organic matter	Dry matter
Temperature	0.72**	0.73**	0.67**	0.62**	0.69**	0.58**	0.63**	0.66**	0.51**
Dietary Moisture	-	-0.35*	-	-	-0.41*	-	-	-0.33*	-
Dietary Ash	-0.42**	-0.34*	-0.34*	-0.47**	-0.48**	-0.43**	-0.47**	-0.52**	-0.46**
Dietary C. Protein	0.53**	0.54**	0.49**	0.57**	0.62**	0.52**	0.55**	0.58**	0.40**
Dietary C. Lipid	-	-	-	-	0.34*	-	-	0.36*	-
Dietary C. Fibre	-0.83**	-0.90**	-0.85**	-0.81**	-0.89**	-0.84**	-0.76**	-0.83**	-0.72**

n = 36
* $p<0.05$
** $p<0.01$
\- non-significant

II. INFLUENCE OF FISH SIZE ON DIGESTIBILITY

1. Experimental Diet

The crude protein (52.83%) and the crude lipid (10.64%) contents of the commercial diet were 9% and 12% respectively greater than the minimum levels of 47% crude protein and 9% crude lipid specified by the manufacturers (Table 3.13). Crude fibre content was close to the specified level of 2%. From Table 3.14 it is apparent that the diet contained adequate levels of all the essential amino acids.

It had been intended that the diet should contain 0.5% chromic oxide. However, from the analysis it is clear that only around 50% of this level was actually present. It is assumed that this must have been due to an error during diet preparation.

2. Food Intake and Fish Performance

All three size groups of rainbow trout accepted the experimental diet readily and fed aggressively throughout the experimental period. Food intakes on a percent body weight per day basis for the duration of the three week experimental period were 1.74% (±0.03), 1.80% (±0.06) and 1.75% (±0.02) for the 40g (40.35g ±2.44), 100g (98.50g ±3.50), and 180g (183.00g ±2.70) size groups of fish respectively. Intake levels were thus slightly higher than the levels of 1.3-1.6% recommended by the manufacturers in their rainbow trout feeding guides (Ewos Ltd., Alpis Ltd., BP Nutrition Ltd.).

Table 3.13 - Proximate composition and marker content of the experimental diet (values expressed as % dry weight).

Nutrient Content (% dry weight)	Manufacturer Specifications	Experimental Diet (± S.D.)
Moisture (%)	9.0	6.12 (± 0.24)
Crude Protein (N x 6.25)	> 47.0	52.83 (± 0.09)
Crude Lipid (%)	> 9.0	10.64 (± 0.51)
Ash (%)	13.0	16.54 (± 0.63)
Crude Fibre (%)	2.0	1.98 (± 0.17)
Cr_2O_3	-	0.23 (± 0.01)

Table 3.14 - Amino acid profile of the experimental diet and amino acid requirement of rainbow trout (g/100g dry weight).

Amino Acid	Diet (g/100g dry weight)	Requirements of Rainbow Trout (% diet)
Arginine	3.80	1.40, 40%[1] (Ogino, 1980)
Histidine	1.48	0.64, 40% (Ogino, 1980)
Isoleucine	2.03	0.96, 40% (Ogino, 1980)
Leucine	5.07	1.76, 40% (Ogino, 1980)
Lysine	4.02	2.12, 40% (Ogino, 1980)
Methionine	1.53	0.55-0.75, 35% (Rumsey *et al.*, 1983)
Phenylalanine	2.81	1.24, 40% (Ogino, 1980)
Threonine	2.47	1.36, 40% (Ogino, 1980)
Valine	3.60	1.24, 40% (Ogino, 1980)
Alanine	3.97	
Aspartic Acid	6.18	
Cystine	0.50	0.30, 35% (Rumsey *et al.*, 1983)
Glutamic Acid	9.14	
Glycine	4.51	
Proline	4.49	
Serine	3.21	
Tyrosine	1.85	0.84, 40% (Ogino, 1980)
TOTAL	60.66	

1. Percentage of crude protein in the diet.

3. Apparent Nutrient Digestibility Coefficients

The proximate composition, marker content and amino acid profiles of faeces are shown in Tables 3.15 and 3.16. In most cases there was good replication within treatments with the exception of the fibre content of the faeces of the 180g fish and of the crude lipid content of faeces of all three size groups of fish. Of the two markers determined, Cr_2O_3 exhibited the best replication.

3.1 Apparent Crude Protein Digestibility

A small variation in apparent crude protein (ACP) digestibility with fish size was found when Cr_2O_3 was used as dietary marker (81.74% to 82.74%) and a slightly wider variation was observed when ACP digestibility coefficients were calculated using crude fibre (80.76% to 83.44%). Using both markers, there was a small but nevertheless significant ($p<0.05$) fall in digestibility coefficients between the 40g and 100g fish size groups. The subsequent increase in digestibility coefficients (82.55% for Cr_2O_3 and 83.44% for crude fibre) for 180g fish group was only significant ($p<0.05$) when crude fibre was used as a marker (Table 3.17).

3.2 Apparent Organic Matter Digestibility

An inverse relationship between fish size and apparent organic matter digestibilty was observed when both Cr_2O_3 and crude fibre were used as dietary markers (Table 3.17). When based on Cr_2O_3, the apparent organic matter (AOM) digestibility showed a slightly wider variation (between 65.54% for the 40g fish and 60.47% for the 180g fish) than when based on crude fibre (between 64.00% and 62.95% for the 40g and the 180g fish, respectively). Nevertheless, the differences in digestibilities between fish size groups were significant ($p<0.05$) for both markers.

Table 3.15 - Proximate composition and marker content of faeces collected from the three size groups of rainbow trout (% dry weight).

Faeces Composition (% dry weight)	Fish Size	40g (± S.D.)	100g (± S.D.)	180g (± S.D.)
	Replicates[1]			
Crude Protein (N x 6.25)	A	22.30 (±0.03)	22.70 (±0.40)	23.65 (±0.23)
	B	22.15 (±0.20)	23.85 (±0.03)	22.40 (±0.70)
Crude Lipid (%)	A	6.18 (±0.44)	4.80 (±2.76)	4.83 (±0.32)
	B	5.37 (±0.00)	4.12 (±0.61)	5.77 (±0.81)
Ash (%)	A	30.05 (±0.05)	30.36 (±0.52)	30.69 (±0.74)
	B	29.92 (±0.03)	28.63 (±0.54)	29.52 (±0.15)
Crude Fibre (%)	A	4.56 (±0.19)	4.49 (±0.03)	5.46 (±1.03)
	B	4.67 (±0.57)	4.58 (±0.06)	4.98 (±0.12)
Cr_2O_3 (%)	A	0.56 (±0.01)	0.55 (±0.02)	0.57 (±0.01)
	B	0.56 (±0.00)	0.56 (±0.01)	0.58 (±0.01)

1. Replicates A and B indicate replicate tanks for each size group of fish (each individual value represents the mean value of 2 replicate analyses).

Table 3.16 - Amino acid profile of faeces collected from the 3 size groups of rainbow trout during the experimental period(g/100g dry weight).

Amino Acid	Replicates[1]	40g fish		100g fish		180g fish	
		A	B	A	B	A	B
Arginine		1.12	1.00	1.04	1.05	0.83	1.04
Histidine		0.36	0.37	0.38	0.37	0.31	0.33
Isoleucine		0.85	0.83	0.69	0.63	0.62	0.57
Leucine		1.51	1.59	1.30	1.29	1.15	1.13
Lysine		0.87	0.85	0.90	0.94	0.80	0.82
Methionine		0.39	0.38	0.21	0.25	0.25	0.20
Phenylalanine		1.23	1.11	1.12	1.04	1.19	1.00
Threonine		0.95	0.91	1.02	0.95	0.88	0.83
Valine		1.17	0.98	0.98	1.16	0.86	0.85
Alanine		1.17	1.01	1.20	1.21	0.99	1.08
Aspartic Acid		2.83	2.98	2.92	2.78	2.54	2.56
Cystine		0.35	0.31	0.18	0.17	0.19	0.18
Glutamic Acid		2.61	2.52	2.65	2.63	2.42	2.34
Glycine		1.74	1.70	1.98	1.91	1.70	1.81
Proline		1.31	1.09	1.42	1.55	1.61	1.53
Serine		1.29	1.11	1.31	1.30	1.19	1.18
Tyrosine		0.75	0.70	0.63	0.68	0.58	0.61
TOTAL		20.50	19.44	19.93	19.91	18.11	18.06

1. Replicates A and B indicate replicate tanks for each size group of fish.

Table 3.17 - Mean apparent digestibility coefficients (n=4, mean ±S.D.) for the three size groups of rainbow trout.

Apparent Nutrient Digestibility (%)	Fish Size (g)	Marker	
		Chromic Oxide	Crude Fibre
Crude Protein (Nx6.25)	40	[a1]82.71 (±0.11)	[b]81.94 (±0.33)
	100	[b] 81.74 (±0.38)	[c]80.76 (±0.38)
	180	[ab]82.55 (±0.79)	[a]83.44 (±0.48)
Organic Matter	40	[a]65.54 (±0.04)	[a]64.00 (±0.45)
	100	[b]64.99 (±0.25)	[b]63.12 (±0.25)
	180	[c]60.47 (±0.56)	[c]62.95 (±4.54)
Dry Matter	40	[a]59.05 (±0.73)	[ab]58.10 (±3.29)
	100	[a]59.22 (±1.12)	[b]57.44 (±0.62)
	180	[a]60.47 (±0.56)	[a]62.95 (±4.54)
Crude Lipid	40	[b]71.35 (±11.56)	[b]70.12 (±11.78)
	100	[a]82.61 (±6.65)	[a]81.68 (±7.02)
	180	[b]80.07 (±2.62)	[ab]80.98 (±3.47)

1. For each digestibility coefficient, values in same column with same superscript are not significantly different at p = 0.05.

3.3 Apparent Dry Matter Digestibility

When apparent dry matter digestibility values were based on chromic oxide, there was a small but insignificant ($p<0.05$) increase from 59.05% for the 40g fish to 60.47% for the 180g fish. By contrast, the variation in digestibility values with size when crude fibre was used as a marker was much wider (between 58.10% and 62.95% for the 40g and 180g fish, respectively). Furthermore, the 180g fish showed a significantly ($p<0.05$) higher digestibility than the 100g fish (Table 3.17).

3.4 Apparent Crude Lipid Digestibility

The highest apparent crude lipid (ACL) digestibility values were obtained for the 100g fish (82.61% and 81.68% for Cr_2O_3 and crude fibre, respectively). Furthermore, these values are significantly ($p<0.05$) higher than those obtained for the 40g fish using both markers (71.35% for Cr_2O_3 and 70.12% for crude fibre). Although the digestibility values obtained for the 180g fish appear to be much higher than those of the 40g fish (Table 3.17), the differences were not significant. This is probably a reflection of the high degree of variability encountered between replicates when carrying out crude lipid analysis.

3.5 Apparent Amino Acid Digestibility

With the exception of proline, there was a general trend of increasing amino acid digestibility coefficients with increasing fish size (Table 3.18). However, in the cases of arginine, phenylalanine, valine, alanine and serine for both markers, these differences were not significant ($p<0.05$). The most marked increase was for cystine where the digestibility coefficient increased from around 72% for the 40g fish to approximately 85% for both the 100g and 180g fish using both markers.

Table 3.18 - Mean apparent amino acid digestibility coefficients for the three size groups of rainbow trout (n=2, mean±S.D.)

Apparent Amino Acid Digestibility (%)	Fish Size	Marker					
		Cr_2O_3			Crude Fibre		
		40g	100g	180g	40g	100g	180g
Arginine		[a1]88.54 (±0.92)	[a]88.60 (±0.07)	[a]90.17 (±1.44)	[a]88.02 (±1.16)	[a]87.99 (±0.08)	[a]90.60 (±2.09)
Histidine		[b]89.87 (±0.20)	[b]89.49 (±0.33)	[a]91.35 (±0.28)	[b]89.42 (±0.03)	[b]88.93 (±0.36)	[a]91.76 (±0.90)
Isoleucine		[b]83.00 (±0.25)	[a]86.51 (±1.04)	[a]88.27 (±0.83)	[c]82.24 (±0.59)	[b]85.79 (±1.11)	[a]88.88 (±0.06)
Leucine		[c]87.44 (±0.46)	[b]89.41 (±0.19)	[a]91.00 (±0.22)	[c]86.88 (±0.26)	[b]88.84 (±0.22)	[a]91.45 (±0.44)
Lysine		[b]91.21 (±0.15)	[c]90.52 (±0.17)	[a]91.94 (±0.04)	[b]90.81 (±0.30)	[b]90.01 (±0.17)	[a]92.33 (±0.63)
Methionine		[b]89.66 (±0.19)	[a]93.77 (±0.69)	[a]94.11 (±0.99)	[b]89.20 (±0.38)	[a]93.44 (±0.71)	[a]94.43 (±0.52)
Phenylalanine		[a]82.90 (±1.24)	[a]84.06 (±1.04)	[a]84.40 (±2.11)	[a]82.12 (±1.60)	[a]83.21 (±1.12)	[a]85.24 (±0.86)
Threonine		[b]84.53 (±0.47)	[b]83.46 (±1.04)	[a]86.14 (±0.74)	[b]83.84 (±0.76)	[b]82.58 (±1.12)	[a]86.86 (±0.31)
Valine		[a]87.73 (±1.53)	[a]87.69 (±1.31)	[a]90.50 (±0.20)	[a]87.17 (±1.82)	[a]87.03 (±1.36)	[a]90.97 (±0.52)
Alanine		[a]88.72 (±1.17)	[a]87.42 (±0.08)	[a]89.57 (±0.52)	[a]88.20 (±1.42)	[a]86.74 (±0.11)	[a]90.07 (±1.26)
Aspartic Acid		[b]80.69 (±0.71)	[b]80.88 (±0.90)	[a]83.49 (±0.11)	[b]79.83 (±0.40)	[b]79.85 (±0.98)	[a]84.31 (±1.10)
Cystine		[b]72.89 (±2.33)	[a]84.94 (±0.00)	[a]85.72 (±0.00)	[b]71.65 (±2.91)	[a]84.12 (±0.00)	[a]85.69 (±0.00)
Glutamic Acid		[b]88.47 (±0.29)	[b]88.02 (±0.22)	[a]89.58 (±0.37)	[b]87.95 (±0.50)	[b]87.38 (±0.25)	[a]90.11 (±0.41)
Glycine		[a]84.33 (±0.26)	[b]82.12 (±0.69)	[a]84.43 (±0.50)	[b]83.63 (±0.54)	[ab]81.16 (±0.74)	[a]85.18 (±1.62)
Serine		[a]84.64 (±1.63)	[a]83.15 (±0.31)	[a]85.23 (±0.27)	[a]83.94 (±1.97)	[a]82.24 (±0.35)	[a]85.97 (±0.83)
Proline		[a]89.02 (±1.42)	[a]86.29 (±0.67)	[a]86.01 (±0.70)	[a]88.52 (±1.68)	[a]85.56 (±0.69)	[a]86.72 (±0.39)
Tyrosine		[b]83.90 (±0.78)	[a]85.33 (±0.61)	[a]87.13 (±0.30)	[b]83.18 (±1.10)	[ab]84.54 (±0.61)	[a]87.76 (±1.23)
Average Digestibility (%)		85.74	86.58	88.18	85.69	85.85	88.72

1. For each marker, values in same row with some superscript are not significantly different at p = 0.05.

Digestibilities for all of the other amino acids only varied by a maximum of around 5% between the 40g fish and the 180g fish.

Proline was the only amino acid where the trend was for digestibility values to decrease with increasing fish size (between 89.02% for the 40g fish and 86.01% for the 180g fish when based on chromic oxide and between 88.52% and 86.72% for the 40g and the 180g fish, respectively when based on crude fibre). However, these differences for both markers were not significant ($p<0.05$).

4. Comparison Between Markers

With the exception of the 180g fish group, all apparent nutrient and amino acid digestibility coefficients calculated using Cr_2O_3 were slightly higher than those calculated using crude fibre as a marker (Tables 3.17 and 3.18 and Figure 3.12). The converse was true for the 180g fish. When the performances of the two markers were compared statistically, there were no significant ($p<0.05$) differences between the digestibility coefficients for both the apparent dry matter and the apparent crude lipid values. By contrast, there was interaction between fish size and marker when apparent crude protein and apparent organic matter digestibility coefficients were calculated. Thus the two dietary markers behaved differently when used to calculate these digestibility coefficients. However, as can be seen in the analysis of variance tables (Table 3.19) most of the variability is "explained" by the main effects (marker and fish size) and of these fish size carries the biggest share, whereas the variation caused by the marker is relatively small.

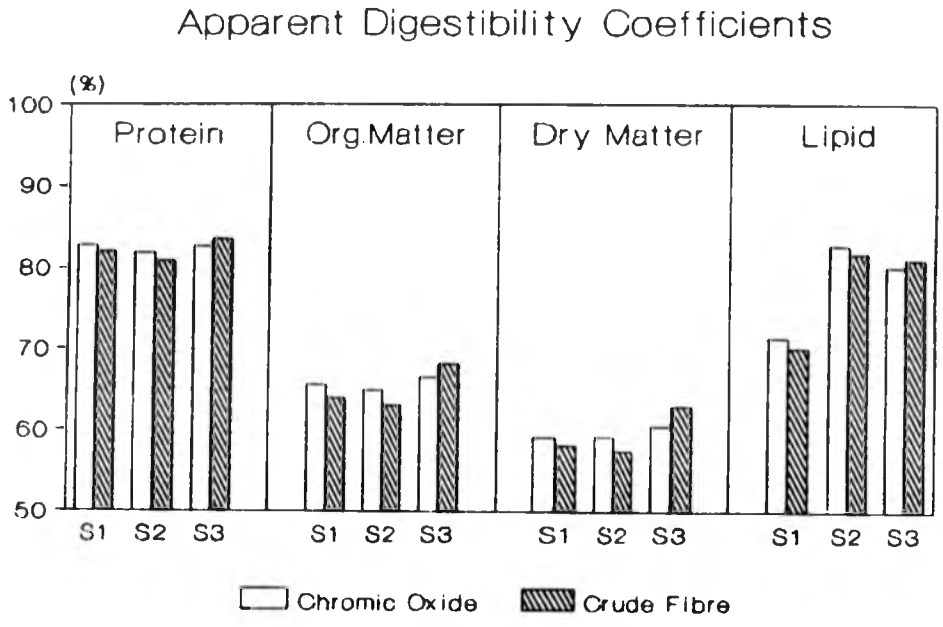

Figure 3.12 - Apparent digestibility coefficients (%) of the 3 size groups of rainbow trout (S1-40g fish, S2-100g fish and S3-180g fish) fed the experimental diet using chromic oxide (Cr_2O_3) and crude fibre as dietary markers (individual values represent the mean of four replicates)

Table 3.19 - Two-way analysis of variance for the protein (A) and organic matter (B) digestibility coefficients of the 3 size groups of Rainbow Trout based on Cr_2O_3 and crude fibre as dietary markers.

(A)

Source of Variation	Sum of squares	d.f.	Mean square	F-ratio	p
MAIN EFFECTS	7.1423750	3	2.3807917	20.986	.0000
Marker	0.2562667	1	0.2562667	2.259	.1502
Fish Size	6.8861083	2	3.4430542	30.350	.0000
2-FACTOR INTERACTIONS	2.3907583	2	1.1953792	10.537	.0009
Marker x Fish Size	2.3907583	2	1.1953792	10.537	.0009
RESIDUAL	2.0420000	18	0.1134444		
TOTAL (CORR.)	11.575133	23			

(B)

MAIN EFFECTS	18.174629	3	6.0582097	21.914	.0000
Marker	0.717604	1	0.7176042	2.596	.1245
Fish Size	17.457025	2	8.7285125	31.574	.0000
2-FACTOR INTERACTIONS	5.7001583	2	2.8500792	10.310	.0010
Marker x Fish Size	5.7001583	2	2.8500792	10.310	.0010
RESIDUAL	4.9760750	18	0.2764486		
TOTAL (CORR.)	28.850863	23			

III. INFLUENCE OF TEMPERATURE AND DIETARY PROTEIN LEVEL ON DIGESTIBILITY

1. Experimental Diets

The proximate composition, marker and energy contents of the experimental diets are shown in Table 3.20. The crude lipid content of diet 2B (11.43%) was lower than the formulated level of 15% and as a consequence this diet had a lower energy content (4.30 kcal/g) compared with 4.57 kcal/g to 4.79kcal/g for the other 3 diets. Tables 3.21 and 3.22 show the amino acid profiles of the experimental diets. Diets 1A and 1B seem to contain adequate levels of the essential amino acids, whereas diets 2A and 2B are slightly deficient in threonine. Furthermore, diets 2A and 2B have lower levels of tyrosine (0.74% and 0.80%, respectively) than the required level of 0.84% (Ogino, 1980) and diet 2B also has a lower level of cystine (0.12%) than the 0.30% level indicated for rainbow trout (Rumsey *et al.*, 1983).

2. Food Intake and Fish Performance

All fish accepted the experimental diets readily and fed aggressively throughout the two experimental periods (Trials A and B). Food intake on a percent body weight per day basis for both trials is presented in Table 3.23.

In Trial A, fish held at 22°C consumed consistently more of the higher protein diet than of the lower, with overall mean daily intake levels of 2.11% and 1.60% respectively. Furthermore, these differences were significantly different ($p<0.05$). The converse was the case in Trial B, with overall intake levels of 1.79% for fish fed the higher protein diet and 2.13% for those fed the lower protein diet. However, the difference was not

Table 3.20 - Proximate composition, marker and energy contents of the experimental diets (values expressed as % dry weight ±S.D.) used in Trials A and B.

		Trial A (15°C + 22°C)		Trial B (10°C + 21°C)	
	Diet No.	1A	2A	1B	2B
Nutrient Content ((%) dry weight)	Crude Protein (%)	45	30	45	30
Moisture (%)		5.66 (±0.02)	6.85 (±0.10)	6.23 (±0.05)	5.49 (±0.02)
Crude Protein (N x 6.25)		44.19 (±0.34)	29.86 (±0.21)	43.94 (±0.30)	29.56 (±0.26)
Crude Lipid (%)		14.27 (±0.31)	14.76 (±0.28)	13.84 (±0.35)	11.43 (±0.06)
Ash (%)		12.76 (±0.03)	9.45 (±0.16)	12.89 (±0.06)	9.56 (±0.00)
Crude Fibre (%)		0.24 (±0.02)	0.31 (±0.03)	0.22 (±0.04)	0.41 (±0.05)
Cr_2O_3 (%)		1.06 (±0.00)	1.03 (±0.02)	1.04 (±0.01)	1.08 (±0.01)
Energy (kcal/g)		4.79 (±0.01)	4.57 (±0.08)	4.69 (±0.08)	4.30 (±0.08)

Table 3.21 - Amino acid profiles of the experimental diets and amino acid requirements of rainbow trout (g/100g dry weight) - Trial A.

Amino Acid	Diet 1A	Diet 2A	Requirements of Rainbow Trout (% diet)
Arginine	2.85	1.67	1.40, 40%[1] (Ogino, 1980)
Histidine	1.19	0.82	0.64, 40% (Ogino, 1980)
Isoleucine	2.61	1.27	0.96, 40% (Ogino, 1980)
Leucine	4.25	2.57	1.76, 40% (Ogino, 1980)
Lysine	3.99	2.21	2.12, 40% (Ogino, 1980)
Methionine	1.25	0.80	0.55-0.75, 35% (Rumsey *et al.*, 1983)
Phenylalanine	2.31	1.48	1.24, 40% (Ogino, 1980)
Threonine	2.06	1.28	1.36, 40% (Ogino, 1980)
Valine	3.19	2.13	1.24, 40% (Ogino, 1980)
Alanine	3.17	1.69	
Aspartic Acid	5.18	2.88	
Cystine	0.80	0.45	0.30, 35% (Rumsey *et al.*, 1983)
Glutamic Acid	6.94	3.60	
Glycine	3.10	1.82	
Proline	2.23	1.63	
Serine	1.57	0.98	
Tyrosine	1.16	0.74	0.84, 40% (Ogino, 1980)
TOTAL	47.85	28.12	

1. Percentage of crude protein in the diet.

Table 3.22 - Amino acid profile of the experimental diets and amino acid requirements of rainbow trout (g/100g dry weight) - Trial B.

Amino Acid	Diet 1B	Diet 2B	Requirements of Rainbow Trout (% diet)
Arginine	2.39	2.25	1.40, 40%[1] (Ogino, 1980)
Histidine	1.13	0.83	0.64, 40% (Ogino, 1980)
Isoleucine	2.25	1.73	0.96, 40% (Ogino, 1980)
Leucine	3.45	2.69	1.76, 40% (Ogino, 1980)
Lysine	3.14	3.80	2.12, 40% (Ogino, 1980)
Methionine	0.59	0.86	0.55-0.75, 35% (Rumsey *et al.*, 1983)
Phenylalanine	2.01	1.53	1.24, 40% (Ogino, 1980)
Threonine	1.81	1.34	1.36, 40% (Ogino, 1980)
Valine	2.67	2.03	1.24, 40% (Ogino, 1980)
Alanine	2.76	1.98	
Aspartic Acid	4.30	3.21	
Cystine	0.37	0.12	0.30, 35% (Rumsey *et al.*, 1983)
Glutamic Acid	5.62	4.26	
Glycine	2.63	2.04	
Proline	1.65	-	
Serine	1.43	1.06	
Tyrosine	1.15	0.80	0.84, 40% (Ogino, 1980)
TOTAL	39.35	30.53	

1. Percentage of crude protein in the diet.

Table 3.23 - Food intake(% body weight/day ±S.D.) over the four week experimental period for Trials A and B.

	Trial A				Trial B			
Temperature	15°C		22°C		10°C		21°C	
Diet No.	1A	2A	1A	2A	1B	2B	1B	2B
% Crude Protein	45	30	45	30	45	30	45	30
Week 1	2.66[a] (±0.09)	2.61[a] (±0.10)	2.46[a] (±0.23)	1.67[a] (±0.05)	2.22[a] (±0.00)	1.94[a] (±0.27)	2.33[a] (±0.06)	2.50[a] (±0.36)
Week 2	2.38[a] (±0.09)	2.32[a] (±0.26)	2.13[a] (±0.23)	1.62[a] (±0.10)	1.90[a] (±0.03)	1.99[a] (±0.16)	1.78[a] (±0.09)	2.08[a] (±0.28)
Week 3	2.27[a] (±0.04)	2.34[a] (±0.10)	1.94[b] (±0.06)	1.71[b] (±0.03)	1.16[c] (±0.07)	1.48[b] (±0.01)	1.52[b] (±0.06)	1.89[a] (±0.12)
Week 4	2.42[a] (±0.09)	2.18[a] (±0.25)	1.93[ab] (±0.17)	1.38[b] (±0.01)	1.29[b] (±0.01)	1.58[ab] (±0.20)	1.53[b] (±0.07)	2.07[a] (±0.22)
Overall (±S.D.)	2.43[a] (±0.16)	2.36[ab] (±0.18)	2.11[b] (±0.25)	1.60[c] (±0.15)	1.64[b] (±0.47)	1.75[ab] (±0.20)	1.79[ab] (±0.38)	2.13[a] (±0.26)

For each Trial, values in same row with same superscript are not significantly different at p = 0.05.

significant. Dietary protein content did not have a significant ($p<0.05$) effect on food intake at either 10°C or 15°C.

In Trial A, food intake at each of the dietary protein levels was significantly ($p<0.05$) higher at 15°C than at 22°C. In the second trial (Trial B), although the lowest overall food intakes were recorded for fish held at 10°C, the differences in intakes between fish fed the same protein level at the two temperatures were not significant.

The trials were not intended to be full scale growth trials, but even after 4 weeks temperature effects on growth and food utilisation efficiency were apparent (Tables 3.24 and 3.25).

In Trial A, final weights and specific growth rates (S.G.R.) of fish at 15°C were significantly ($p<0.05$) higher than those of fish held at 22°C. Furthermore, food conversion ratios (FCR) of fish at 22°C were very poor (3.38 and 3.24 for diets 1A and 2A respectively).

In Trial B, the best growth and food utilisation were obtained at 10°C, and these differences were significant. No significant ($p<0.05$) differences in performance were obtained between protein levels at each experimental temperature. However, in all cases the final mean weight was slightly greater when fish were fed the higher protein level diets than the lower protein level diets. Again, FCR's of fish at 21°C were poor (3.02 and 4.89 for diets 1B and 2B, respectively). It is therefore possible that these trends may have developed into significant differences in longer trials.

Table 3.24 - Growth and food utilisation of rainbow trout fed the two experimental diets during Trial A

Temperature	15°C		22°C	
Diet No.	1A	2A	1A	2A
% Crude Protein	45	30	45	30
Initial Weight (g)	91.50[a] (±1.55)	91.40[a] (±1.41)	91.90[a] (±2.97)	91.50[a] (±2.40)
Final Weight (g)	125.12[a] (±1.63)	124.50[a] (±0.01)	109.32[b] (±2.89)	103.45[b] (±0.45)
Weight Gain (g/day)	1.20[a] (±0.10)	1.18[a] (±0.09)	0.62[b] (±0.15)	0.43[b] (±0.10)
S.G.R. (%/day)	1.12[a] (±0.10)	1.10[a] (±0.15)	0.62[b] (±0.05)	0.44[b] (±0.10)
F.C.R.	2.13[b] (±0.05)	2.01[b] (±0.07)	3.38[a] (±0.10)	3.24[a] (±0.15)

For each Trial, values in same row with same superscript are not significantly different at p = 0.05.

Table 3.25 - Growth and food utilisation of rainbow trout fed the two experimental diets during Trial B

	Temperature	10°C		21°C	
	Diet No.	1B	2B	1B	2B
	% Crude Protein	45	30	45	30
Initial Weight (g)		90.31^{a} (±1.34)	89.75^{a} (±3.00)	90.30^{a} (±2.35)	90.48^{a} (±3.08)
Final Weight (g)		121.03^{a} (±0.26)	118.40^{a} (±3.87)	105.80^{b} (±2.49)	101.73^{b} (±2.40)
Weight Gain (g/day)		1.10^{a} (±0.10)	1.02^{a} (±0.20)	0.55^{b} (±0.07)	0.40^{b} (±0.15)
S.G.R. (%/day)		1.04^{a} (±0.05)	0.99^{a} (±0.09)	0.57^{b} (±0.10)	0.42^{b} (±0.10)
F.C.R.		1.39^{b} (±0.10)	1.55^{b} (±0.15)	3.02^{a} (±0.05)	4.89^{a} (±0.11)

For each Trial, values in same row with same superscript are not significantly different at p = 0.05.

3. Apparent Nutrient Digestibility Coefficients

The proximate composition, marker and energy contents of faeces for Trials A and B are shown in Tables 3.26 and 3.27. In general there was good replication within treatments. The amino acid profiles of faeces from Trials A and B are shown in Tables 3.28 and 3.29, respectively. In general, the amino acid content of faeces was higher in Trial A than in Trial B, irrespective of the diet.

3.1 Apparent Crude Protein Digestibility

In Trial A, differences in dietary protein level had no significant ($p<0.05$) effect on crude protein digestibility values either at 15°C or 22°C. By contrast, temperature had a marked effect on digestibility values, with significantly ($p<0.05$) higher apparent crude protein digestibilities at 22°C for both diets when compared to the values obtained at 15°C (Table 3.30).

In Trial B, digestibility values at the two temperatures were again significantly ($p<0.05$) different (Table 3.31). In contrast to Trial A, however, there was a significant $p<0.05$) difference between diets. Thus, at each experimental temperature, fish fed Diet 1 (45% crude protein) showed higher digestibility values (73.71% at 10°C and 85.98% at 21°C) than fish fed Diet 2 containing 30% crude protein (70.46% at 10°C and 74.16% at 21°C).

Table 3.26 - Proximate composition, marker and energy contents (% dry weight ±S.D.) of faeces collected from fish at 15°C and 22°C in Trial A.

Faeces composition (% dry weight)	Temperature	15°C		22°C	
	Diet No.	1A	2A	1A	2A
	% C. Protein	45	30	45	30
	Replicates[1]				
Crude Protein (N x 6.25)	A	35.42 (±0.17)	23.24 (±0.05)	29.50 (±0.01)	22.64 (±0.01)
	B	35.64 (±0.80)	22.92 (±0.01)	29.89 (±0.08)	22.68 (±0.00)
Ash (%)	A	32.91 (±0.30)	22.05 (±0.11)	33.28 (±0.03)	27.19 (±0.00)
	B	32.27 (±0.45)	21.89 (±0.10)	33.23 (±0.05)	27.20 (±0.01)
Chromic Oxide (%)	A	2.37 (±0.01)	2.23 (±0.00)	3.10 (±0.04)	3.39 (±0.05)
	B	2.31 (±0.04)	2.24 (±0.01)	2.90 (±0.06)	3.33 (±0.02)
Energy (kcal/g)	A	3.52 (±0.03)	3.35 (±0.06)	3.34 (±0.03)	3.92 (±0.14)
	B	3.64 (±0.16)	3.40 (±0.25)	3.56 (±0.02)	3.71 (±0.17)

1. Replicates A and B indicate replicate tanks for each treatment (each individual value represents the mean value of two replicate measurements).

Table 3.27 - Proximate composition, marker and energy contents (% dry weight ±S.D.) of faeces collected from fish at 10°C and 21°C in Trial B.

Faeces composition (% dry weight)	Temperature	10°C		21°C	
	Diet No.	1B	2B	1B	2B
	% C. Protein	45	30	45	30
	Replicates[1]				
Crude Protein (N x 6.25)	A	26.56 (±0.50)	20.92 (±0.11)	29.64 (±0.18)	20.99 (±0.00)
	B	27.65 (±0.11)	21.49 (±0.06)	30.03 (±0.04)	22.48 (±0.01)
Ash (%)	A	27.72 (±0.11)	18.15 (±0.69)	31.73 (±0.40)	27.36 (±0.30)
	B	27.87 (±0.10)	17.17 (±0.70)	31.11 (±0.42)	26.96 (±0.29)
Chromic Oxide (%)	A	2.47 (±0.02)	2.67 (±0.01)	4.91 (±0.00)	2.96 (±0.03)
	B	2.39 (±0.01)	2.59 (±0.04)	5.12 (±0.02)	3.20 (±0.01)
Energy (kcal/g)	A	3.22 (±0.00)	3.06 (±0.07)	3.26 (±0.00)	3.68 (±0.15)
	B	3.07 (±0.11)	3.43 (±0.04)	3.39 (±0.04)	3.98 (±0.06)

1. Replicates A and B indicate replicate tanks for each treatment (each individual value represents the mean value of two replicate measurements).

Table 3.28 - Amino acid profile of faeces collected from rainbow trout fed diets containing 45% and 30% crude protein at 15°C and 22°C - Trial A.

	Temperature	15°C				22°C			
	Diet No.	1A		2A		1A		2A	
	% C. Protein	45		30		45		30	
Amino Acid	Replicates[1]	A	B	A	B	A	B	A	B
Arginine		0.74	0.90	0.68	0.60	0.92	0.77	0.63	0.51
Histidine		0.53	0.59	0.44	0.39	0.42	0.42	0.37	0.35
Isoleucine		1.25	1.42	0.85	0.74	0.94	0.99	0.75	0.78
Leucine		1.82	2.05	1.20	1.09	1.40	1.57	1.09	1.13
Lysine		1.35	1.54	0.80	0.74	0.98	1.10	0.76	0.74
Methionine		0.47	0.52	0.26	0.33	0.30	0.22	0.15	0.26
Phenylaline		1.21	1.10	0.61	0.53	0.95	0.91	0.74	0.77
Threonine		1.14	1.32	0.82	0.76	0.95	0.96	0.56	0.80
Valine		1.42	1.47	0.98	0.89	1.14	1.32	0.93	0.60
Alanine		1.40	1.59	0.99	0.94	0.91	1.31	0.93	0.91
Aspartic Acid		3.08	3.27	2.10	1.97	2.48	2.65	1.99	2.02
Cystine		0.35	0.40	0.16	0.11	0.14	0.14	0.14	0.10
Glutamic Acid		3.53	3.98	1.86	1.38	2.26	2.01	1.44	1.73
Glycine		1.90	2.12	1.34	1.27	1.59	1.78	1.12	1.19
Proline		1.36	1.74	1.07	0.96	1.21	1.38	0.79	1.04
Serine		0.97	1.14	0.72	0.63	0.85	0.74	0.57	0.70
Tyrosine		0.65	0.74	0.49	0.43	0.58	0.52	0.56	0.59
TOTAL		23.17	25.89	15.37	13.76	18.02	18.79	13.52	14.22

1 Replicates A and B indicate 2 replicate tanks per treatment.

Table 3.29- Amino acid profile of faeces collected from rainbow trout fed diets containing 45% and 30% crude protein at 10°C and 21°C - Trial B.

	Temperature	10°C				21°C			
	Diet No.	1B		2B		1B		2B	
	% C. Protein	45		30		45		30	
Amino Acid	Replicates[1]	A	B	A	B	A	B	A	B
Arginine		0.63	0.77	0.58	0.55	0.61	0.55	0.45	0.57
Histidine		0.46	0.54	0.37	0.38	0.48	0.44	0.33	0.37
Isoleucine		0.80	1.01	0.67	0.68	0.96	0.91	0.67	0.80
Leucine		1.34	1.41	0.95	1.03	1.35	1.32	0.98	1.06
Lysine		0.77	1.01	0.56	0.71	0.83	0.81	0.43	0.55
Methionine		0.16	0.21	0.26	0.27	0.23	0.23	0.25	0.22
Phenylaline		0.76	0.92	0.62	0.74	0.95	1.02	0.67	0.76
Threonine		0.88	0.86	0.59	0.58	0.83	0.79	0.56	0.60
Valine		0.96	1.08	0.81	0.93	1.09	1.05	0.81	0.95
Alanine		0.98	1.10	0.79	0.96	1.31	1.27	0.89	0.97
Aspartic Acid		1.97	2.09	1.67	1.82	2.07	2.10	1.69	1.72
Cystine		0.16	0.15	0.10	0.09	0.21	0.19	0.11	0.12
Glutamic Acid		1.71	1.79	1.43	1.57	1.69	1.47	1.51	1.73
Glycine		1.73	1.74	1.12	1.17	1.71	1.72	1.16	1.19
Proline		1.20	1.11	0.77	0.79	1.20	1.18	0.80	0.90
Serine		0.54	0.58	0.48	0.53	0.66	0.63	0.50	0.55
Tyrosine		0.47	0.45	0.29	0.30	0.68	0.67	0.30	0.33
TOTAL		15.52	16.82	12.06	13.10	16.86	16.35	12.11	13.39

1 Replicates A and B indicate 2 replicate tanks per treatment.

Table 3.30 - Mean apparent digestibility coefficients (n=2, mean ±S.D.) for rainbow trout fed diets containing 45% and 30% crude protein at 15°C and 22°C - Trial A.

Apparent Nutrient Digestibility (%)	Diet No.	Crude Protein (%)		Temperature (°C) 15	22
				a,b	
Crude Protein (N x 6.25)	1A	45		$^{b}63.70\ (\pm0.82)_{c}$	$^{a}76.30\ (\pm1.09)_{c}$
	2A	30		$^{b}64.50\ (\pm0.38)_{c}$	$^{a}77.82\ (\pm0.26)_{c}$
Organic Matter	1A	45	c,d	$^{b}65.12\ (\pm0.86)_{c}$	$^{a}73.04\ (\pm1.29)_{c}$
	2A	30		$^{b}60.44\ (\pm0.07)_{d}$	$^{a}75.66\ (\pm0.05)_{c}$
Dry Matter	1A	45		$^{b}55.48\ (\pm0.80)_{c}$	$^{a}65.44\ (\pm1.51)_{d}$
	2A	30		$^{b}54.60\ (\pm0.18)_{c}$	$^{a}70.14\ (\pm0.44)_{c}$
Energy	1A	45		$^{b}66.25\ (\pm1.51)_{c}$	$^{a}74.55\ (\pm1.93)_{c}$
	2A	30		$^{b}66.07\ (\pm1.57)_{c}$	$^{a}74.52\ (\pm1.06)_{c}$

For each digestibility coefficient, values in same row with same superscript are not significantly different at p = 0.05.

For each digestibility coefficient, values in same column with same subscript are not significantly different at p = 0.05.

3.2 Apparent Organic Matter Digestibility

Temperature again had a marked effect on digestibility values with significantly ($p<0.05$) higher values recorded at the higher temperature in each trial (Tables 3.30 and 3.31).

In Trial A, dietary protein inclusion level did not have a significant ($p<0.05$) effect on apparent organic matter digestibility at 22°C, whereas at 15°C the level recorded for fish fed the 45% crude protein diet (Diet 1A) was significantly higher (65.12%) than that of the fish fed the 30% crude protein diet (60.44%). By contrast, in Trial B, the digestibility recorded for fish fed the 45% crude protein diet (Diet 1B) was significantly ($p<0.05$) higher than that of fish fed the 30% crude protein diet (83.74% and 71.66% for Diets 1B and 2B, respectively) at 21°C, whereas at 10°C there was no significant ($p<0.05$) difference between dietary treatments.

3.3 Apparent Dry Matter Digestibility

Again, digestibility coefficients were significantly ($p<0.05$) higher at the higher experimental temperatures (22°C and 21°C for Trials A and B, respectively) than at the lower temperatures (15°C and 10°C) within each treatment (Tables 3.30 and 3.31).

In Trial A, a significant ($p<0.05$) difference in apparent dry matter digestibility was found between fish fed the 45% crude protein diet (Diet 1A) and fish fed the 30% crude protein diet (65.44% and 70.14% for Diets 1A and 2A, respectively) at 22°C, whereas at 15°C there was no significant ($p<0.05$) difference between diets (Table 3.30).

Table 3.31 - Mean apparent digestibility coefficients (n=2, mean ±S.D.) for rainbow trout fed diets containing 45% and 30% crude protein at 10°C and 21°C - Trial B.

Apparent Nutrient Digestibility (%)	Diet No.	Crude Protein (%)		Temperature (°C) 10	Temperature (°C) 21
				a,b	
Crude Protein (N x 6.25)	1B	45		$^{b}73.71\ (\pm1.14)_{c}$	$^{a}85.98\ (\pm0.24)_{c}$
	2B	30		$^{b}70.46\ (\pm0.98)_{d}$	$^{a}74.16\ (\pm0.18)_{d}$
Organic Matter	1B	45	c,d	$^{b}64.68\ (\pm0.77)_{c}$	$^{a}83.74\ (\pm0.37)_{c}$
	2B	30		$^{b}62.53\ (\pm1.12)_{c}$	$^{a}71.66\ (\pm1.45)_{d}$
Dry Matter	1B	45		$^{b}58.11\ (\pm0.86)_{c}$	$^{a}80.37\ (\pm0.36)_{c}$
	2B	30		$^{b}59.56\ (\pm0.73)_{c}$	$^{a}65.54\ (\pm1.63)_{d}$
Energy	1B	45		$^{b}69.85\ (\pm0.66)_{c}$	$^{a}84.54\ (\pm0.13)_{c}$
	2B	30		$^{a}68.89\ (\pm2.65)_{c}$	$^{a}68.70\ (\pm0.84)_{d}$

For each digestibility coefficient, values in same row with same superscript are not significantly different at p = 0.05.

For each digestibility coefficient, values in same column with same subscript are not significantly different at p = 0.05.

In Trial B, again at the lower experimental temperature (10°C) there were no significant ($p<0.05$) differences between diets. However, at 21°C fish fed Diet 1B showed a significantly ($p<0.05$) higher apparent dry matter digestibility (80.37%) than fish fed Diet 2B (65.54%).

3.4 Apparent Amino Acid Digestibility

In Trial A, differences in dietary protein level had no significant effect on digestibility values for most amino acids, either at 15°C or 22°C (Table 3.32) as was shown for the apparent crude protein digestibility. Digestibility values for isoleucine and phenylalanine at 22°C were however significantly ($p<0.05$) higher for diet 1 (45% crude protein). By contrast, temperature had an important effect on digestibility values. Thus, with the exception of arginine digestibility for diet 1 and of phenylalanine, cystine, glutamic acid and tyrosine digestibilities for Diet 2, the apparent amino acid digestibility coefficients at 22°C for both diets were significantly ($p<0.05$) higher than the values obtained at 15°C (Table 3.32).

In Trial B, temperature had again a marked effect on amino acid digestibility (Table 3.33). Thus, with the exception of isoleucine and methionine for diet 1 (45% crude protein) and of phenylalanine, valine, cystine, glutamic acid and serine for diet 2 (30% crude protein) all the other apparent amino acid digestibility values were significantly ($p<0.05$) higher at 21°C than at 10°C. In contrast to Trial A, however, there was an influence of the dietary protein level on digestibility. Thus, with the exception of arginine, lysine and methionine at 21°C, fish fed diet 1B showed significantly higher digestibility values (around 90% average digestibility) than fish fed diet 2B (around

Table 3.32 - Mean apparent amino acid digestibility coefficients (n=2, mean ±S.D.) for rainbow trout fed diets containing 45% and 30% crude protein at 15°C and 22°C - Trial A.

		Amino Acid Digestibility (%)			
	Temperature	15°C		22°C	
	Diet no.	1A	2A	1A	2A
Amino Acid	% C. Protein	45	30	45	30
Arginine		87.00^{a}_{a} (±2.03)	82.40^{a}_{a} (±1.28)	89.58^{a}_{a} (±0.34)	89.59^{a}_{b} (±1.01)
Histidine		78.75^{a}_{a} (±2.00)	76.76^{a}_{a} (±2.05)	87.57^{a}_{b} (±0.17)	86.60^{a}_{b} (±0.26)
Isoleucine		76.90^{a}_{a} (±2.49)	71.26^{a}_{a} (±2.59)	86.96^{a}_{b} (±0.59)	81.60^{b}_{b} (±0.28)
Leucine		79.44^{a}_{a} (±2.10)	79.55^{a}_{a} (±1.05)	87.67^{a}_{b} (±1.24)	86.81^{a}_{b} (±0.50)
Lysine		83.64^{a}_{a} (±1.60)	84.01^{a}_{a} (±0.42)	90.80^{a}_{b} (±0.70)	89.64^{a}_{b} (±0.05)
Methionine		82.12^{a}_{a} (±1.28)	83.08^{a}_{a} (±1.78)	92.71^{a}_{b} (±0.78)	92.15^{a}_{b} (±2.34)
Phenylalanine		77.45^{a}_{a} (±0.62)	82.32^{a}_{a} (±1.63)	85.83^{a}_{b} (±0.23)	84.42^{b}_{a} (±0.20)
Threonine		73.03^{a}_{a} (±3.20)	71.67^{a}_{a} (±1.28)	83.66^{a}_{b} (±0.40)	83.75^{a}_{b} (±3.25)
Valine		79.55^{a}_{a} (±0.38)	79.85^{a}_{a} (±1.03)	86.38^{a}_{b} (2.10)	89.05^{a}_{b} (±2.21)
Alanine		78.70^{a}_{a} (±2.29)	73.78^{a}_{a} (±0.54)	87.59^{a}_{b} (±3.02)	83.37^{a}_{b} (±0.05)
Aspartic Acid		72.32^{a}_{a} (±1.40)	67.56^{a}_{a} (±1.23)	82.53^{a}_{b} (±1.34)	78.74^{a}_{b} (±0.12)
Cystine		78.83^{a}_{a} (±2.38)	86.22^{a}_{a} (±3.20)	93.84^{a}_{b} (±0.29)	91.87^{a}_{a} (±1.65)
Glutamic Acid		75.37^{a}_{a} (±2.78)	79.34^{a}_{a} (±2.39)	89.18^{a}_{a} (±0.35)	86.54^{a}_{a} (±1.52)
Glycine		70.72^{a}_{a} (±2.50)	67.09^{a}_{a} (±0.91)	80.81^{a}_{b} (±2.40)	80.62^{a}_{b} (±0.58)
Proline		68.58^{a}_{a} (±3.01)	71.41^{a}_{a} (±2.27)	79.50^{a}_{b} (±2.02)	82.84^{a}_{b} (±2.52)
Serine		69.64^{a}_{a} (±3.00)	68.38^{a}_{a} (±3.07)	82.20^{a}_{b} (±0.41)	80.20^{a}_{b} (±2.11)
Tyrosine		72.94^{a}_{a} (±2.56)	71.46^{a}_{a} (±2.52)	83.33^{a}_{b} (±0.50)	76.27^{a}_{a} (±0.70)
Average Digestibility (%)		76.76	76.24	86.48	84.94

For each temperature, values in same row with same superscript are not significantly different at p = 0.05.

For each diet, values in same row with same subscript are not significantly different at p = 0.05.

85% in average). Furthermore at the lower temperature only the digestibility values for cystine, glycine and tyrosine were significantly lower for both diets (Table 3.33).

3.5 Apparent Energy Digestibility

With the exception of fish fed Diet 2B, containing 30% crude protein in Trial B, temperature had a significant ($p<0.05$) effect on energy digestibility (Tables 3.30 and 3.31).

Also, no significant ($p<0.05$) effect of dietary protein inclusion level was observed except at 21°C (Trial B) when there was again a marked effect (Table 3.31 and Figures 3.13 and 3.14).

Table 3.33 - Mean amino acid digestibility coefficients (n=2, mean ±S.D.) for rainbow trout fed diets containing 45% and 30% crude protein at 10°C and 21°C - Trial B.

		Amino Acid Digestibility (%)			
	Temperature	10°C		21°C	
	Diet no.	1B	2B	1B	2B
Amino Acid	% C. Protein	45	30	45	30
Arginine		87.50^{a}_{b} (±2.05)	89.66^{a}_{b} (±0.16)	94.98^{a}_{a} (±0.52)	92.06^{a}_{a} (±0.39)
Histidine		81.21^{a}_{b} (±2.56)	81.39^{a}_{b} (±0.28)	91.59^{a}_{a} (±0.77)	85.18^{b}_{a} (±0.07)
Isoleucine		82.83^{a}_{a} (±3.21)	83.93^{a}_{b} (±0.13)	91.42^{a}_{a} (±0.57)	85.09^{b}_{a} (±0.55)
Leucine		83.01^{a}_{b} (±1.00)	84.83^{a}_{b} (±0.70)	92.01^{a}_{a} (±0.37)	86.67^{b}_{a} (±0.01)
Lysine		87.88^{a}_{b} (±2.60)	93.11^{a}_{b} (±0.83)	94.60^{a}_{a} (±0.25)	95.48^{a}_{a} (±0.14)
Methionine		86.62^{a}_{a} (±2.86)	87.12^{a}_{b} (±0.06)	91.95^{a}_{a} (±0.24)	90.35^{a}_{a} (±0.98)
Phenylalanine		82.17^{a}_{b} (±2.81)	81.67^{a}_{a} (±2.67)	89.89^{a}_{a} (±0.21)	83.59^{b}_{a} (±0.16)
Threonine		79.53^{b}_{b} (±0.14)	82.02^{a}_{b} (±0.17)	90.76^{a}_{a} (±0.60)	84.78^{b}_{a} (±0.09)
Valine		83.71^{a}_{b} (±1.77)	82.33^{a}_{a} (±2.10)	91.72^{a}_{a} (0.47)	84.78^{b}_{a} (±0.38)
Alanine		83.93^{a}_{b} (±1.68)	81.78^{a}_{b} (±0.80)	90.34^{a}_{a} (±0.50)	83.49^{b}_{a} (±0.00)
Aspartic Acid		79.88^{a}_{b} (±1.31)	77.60^{a}_{b} (±1.70)	89.99^{a}_{a} (±0.20)	81.31^{b}_{a} (±0.32)
Cystine		82.16^{a}_{b} (±0.40)	67.42^{b}_{a} (±1.48)	88.83^{a}_{a} (±1.12)	66.31^{b}_{a} (±0.21)
Glutamic Acid		86.73^{a}_{b} (±0.73)	85.49^{a}_{a} (±0.80)	94.18^{a}_{a} (±0.74)	86.65^{b}_{a} (±0.15)
Glycine		71.89^{b}_{b} (±0.30)	76.88^{a}_{b} (±0.73)	86.54^{a}_{a} (±0.35)	79.73^{b}_{a} (±0.28)
Proline		70.20_{b} (±0.95)	-	85.10_{a} (±0.61)	-
Serine		83.31^{a}_{b} (±1.23)	80.36^{a}_{a} (±1.60)	90.68^{a}_{a} (±0.58)	82.59^{b}_{a} (±0.21)
Tyrosine		82.96^{b}_{b} (±0.13)	84.81^{a}_{b} (±0.24)	88.38^{a}_{a} (±0.22)	86.16^{b}_{a} (±0.16)
Average Digestibility (%)		82.08	82.53	90.76	84.64

For each temperature, values in same row with same superscript are not significantly different at p = 0.05.

For each diet, values in same row with same subscript are not significantly different at p = 0.05.

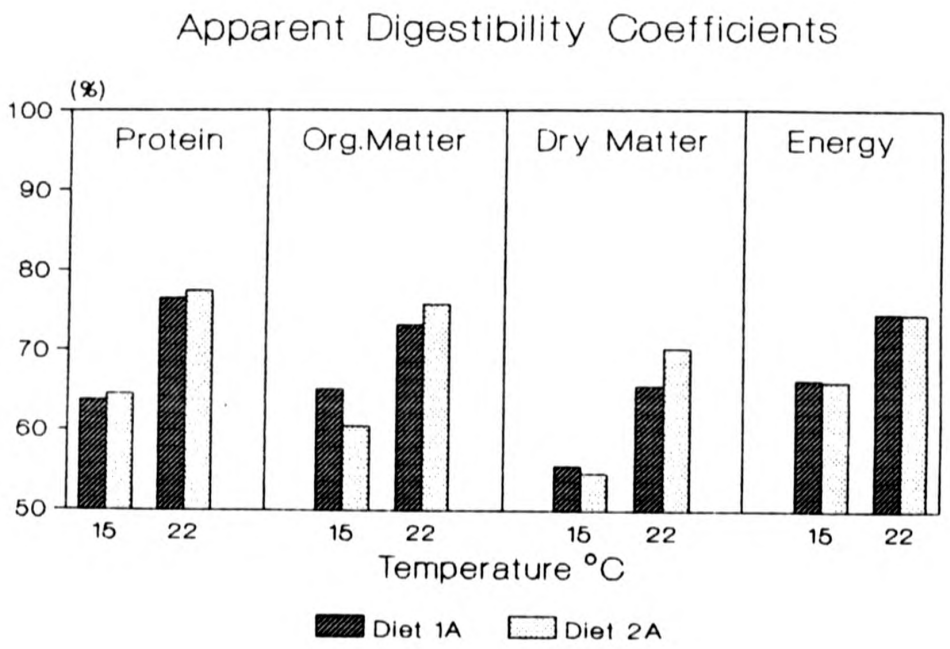

Figure 3.13 - Apparent digestibility coefficients of rainbow trout held at 15°C and 22°C and fed diets containing either 45% crude protein (Diet 1A) or 30% crude protein (Diet 2A)

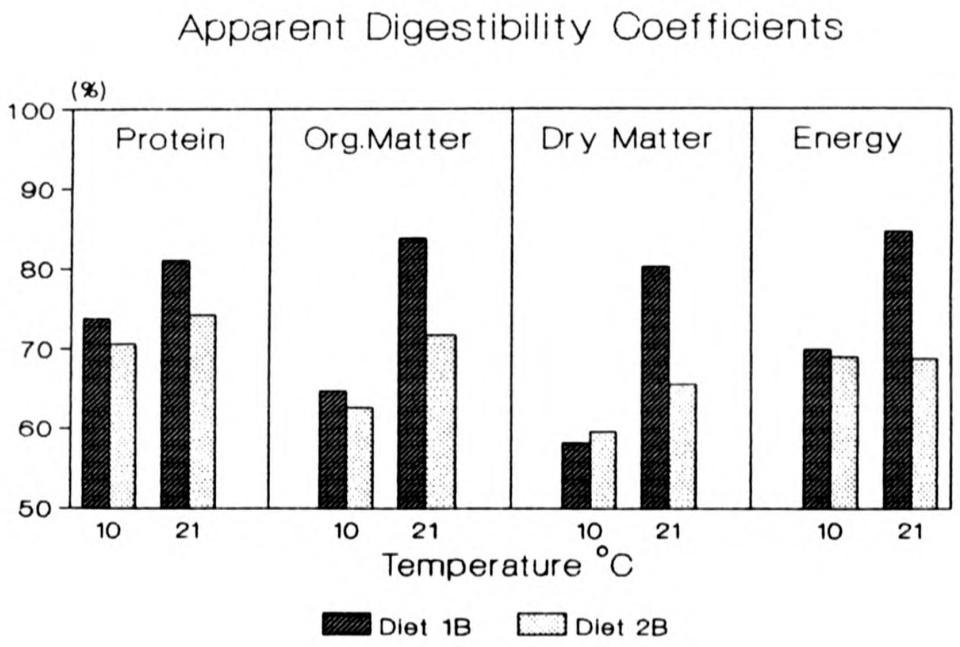

Figure 3.14 - Apparent digestibility coefficients of rainbow trout held at 10°C and 21°C and fed diets containing either 45% crude protein (Diet 1B) or 30% crude protein (Diet 1B)

IV. INFLUENCE OF DIETARY LIPID LEVEL ON DIGESTIBILITY

1. Experimental Diets

The crude protein contents of the 3 experimental diets (Table 3.34) were all slightly higher than the formulated level of 50%. The crude lipid levels (Table 3.34) were all close to the formulated levels of 7%, 14% and 21% for Diets 1, 2 and 3, respectively. Again, it had been intended that the diets should contain 0.5% chromic oxide. However, from the analysis it is clear that only around 50% of this level was actually present. It is assumed that this must have been due to an error during diet preparation (Table 3.34). From Table 3.35, it is apparent that the diets contained adequate levels of all the essential amino acids.

2. Food Intake

All the fish accepted the experimental diets readily and fed aggressively throughout the experimental period.

Food intakes on a percent body weight per day basis for the duration of the 3 week experimental period were 1.60% (±0.04), 1.58% (±0.02), and 1.56% (±0.02) for fish fed Diets 1, 2 and 3, respectively. Intake levels were thus all higher than the level of around 1.1% recommended in the rainbow trout feeding guides (Ewos Ltd., BP Nutrition Ltd.) for the temperature and fish size used.

Table 3.34 - Proximate composition and marker content of the 3 experimental diets ±S.D. (values expressed as % dry weight).

Nutrient Content (% dry weight)	Diets		
	1	2	3
Moisture (%)	5.99 (±0.08)	5.33 (±0.10)	4.96 (±0.13)
Crude Protein (N x 6.25)	52.14 (±0.12)	52.23 (±0.39)	51.60 (±0.02)
Crude Lipid (%)	7.58 (±0.32)	14.12 (±0.21)	20.54 (±0.22)
Ash (%)	15.42 (±0.27)	13.92 (±0.20)	13.89 (±0.34)
Crude Fibre (%)	2.08 (±0.09)	1.70 (±0.12)	2.10 (±0.12)
Chromic Oxide (%)	0.24 (±0.01)	0.25 (±0.03)	0.23 (±0.01)

Table 3.35 - Amino acid profile of the experimental diets (g/100g dry weight).

Amino Acid	Diet 1	Diet 2	Diet 3	Requirements of Rainbow Trout (% diet)
Arginine	3.89	4.06	4.30	1.40, 40%[1] (Ogino, 1980)
Histidine	1.77	1.55	1.41	0.64, 40% (Ogino, 1980)
Isoleucine	2.15	2.02	1.89	0.96%, 40% (Ogino, 1980)
Leucine	4.32	4.20	4.41	1.76, 40% (Ogino, 1980)
Lysine	3.95	4.08	3.97	2.12, 40% (Ogino, 1980)
Methionine	1.39	1.43	1.46	0.55-0.75, 35% (Rumsey *et al.*, 1983)
Phenylalanine	2.68	2.70	2.69	1.24, 40% (Ogino, 1980)
Threonine	2.34	2.33	2.36	1.36, 40% (Ogino, 1980)
Valine	3.54	3.99	2.83	1.24, 40% (Ogino,1980)
Alanine	3.57	3.14	3.36	
Aspartic Acid	5.92	5.81	5.69	
Cystine	-	-	0.50	0.30, 35% (Rumsey *et al.*, 1983)
Glutamic Acid	7.34	7.33	7.31	
Glycine	3.74	3.51	3.26	
Proline	4.09	4.18	4.13	
Serine	2.66	2.49	2.35	
Tyrosine	1.86	1.72	1.83	0.84, 40% (Ogino, 1980)
TOTAL	55.21	54.54	53.75	

1. Percentage of crude protein in the diet.

3. Apparent Nutrient Digestibility Coefficients

The nutrient and marker content of faeces from rainbow trout fed the 3 experimental diets are shown in Table 3.36. In most cases there was good replication within treatment, with the exception of the crude lipid content of faeces and of crude fibre content of faeces from fish fed Diets 1 and 3.

The amino acid profile of faeces from fish fed the 3 experimental diets is shown in Table 3.37.

3.1 Apparent Crude Protein Digestibility

Variation in apparent crude protein (ACP) digestibility values with dietary crude lipid level was greatest when Cr_2O_3 was used as a dietary marker (83.98% to 87.77%) compared with a range of only 85.46% to 86.29% for crude fibre (Table 3.38). In both cases the highest digestibility values were obtained for fish fed the diet containing 21% crude lipid (87.77% and 86.29% for Cr_2O_3 and crude fibre, respectively), although when crude fibre was used as a marker the difference between values for the 7% and 21% crude lipid diets were not significant ($p<0.05$). Using both markers the digestibility coefficients obtained from fish fed the 21% crude lipid diet were significantly ($p<0.05$) higher than those for the 14% crude lipid diet (Table 3.38).

Table 3.36 - Proximate composition and marker content of faeces collected from rainbow trout fed the 3 experimental diets containing 7%, 14% and 21% crude lipid (% dry weight, n=2, mean ±S.D.).

Faeces Composition (% dry weight)	Diet no.	1	2	3
	Lipid Level (%)	7	14	21
	Replicates[1]			
Crude Protein (N x 6.25)	A	18.65 (±0.17)	20.56 (±0.23)	21.24 (±0.17)
	B	19.10 (±0.02)	21.60 (±0.11)	21.26 (±0.20)
Crude Lipid (%)	A	3.60 (±0.34)	2.08 (±0.15)	4.61 (±0.54)
	B	3.80 (±0.24)	2.88 (±0.50)	4.62 (±0.60)
Ash (%)	A	26.08 (±0.37)	30.56 (±0.19)	31.52 (±0.51)
	B	27.80 (±0.22)	30.49 (±0.40)	32.43 (±0.54)
Crude Fibre (%)	A	5.17 (±0.05)	4.66 (±0.11)	6.10 (±0.49)
	B	5.40 (±0.52)	4.78 (±0.25)	6.53 (±0.51)
Cr_2O_3 (%)	A	0.54 (±0.01)	0.62 (±0.03)	0.77 (±0.01)
	B	0.59 (±0.01)	0.64 (±0.01)	0.78 (±0.03)

1. Replicates A and B indicate replicate tanks for each treatment.

Table 3.37 - Amino acid profile of faeces collected from rainbow trout fed the 3 experimental diets containing 7%, 14% and 21% crude lipid (g/100g dry weight).

	Diet no.	1		2		3	
	Lipid level (%)	7		14		21	
Amino Acid	Replicates[1]	A	B	A	B	A	B
Arginine		0.77	0.73	0.89	0.82	0.86	1.04
Histidine		0.28	0.32	0.41	0.40	0.41	0.36
Isoleucine		0.62	0.61	0.68	0.63	0.60	0.67
Leucine		1.15	1.07	1.29	1.15	1.13	1.24
Lysine		1.06	1.07	1.09	1.03	1.07	1.01
Methionine		0.28	0.31	0.30	0.31	0.34	0.30
Phenylalanine		1.05	1.05	1.19	1.16	2.06	1.37
Threonine		0.84	0.92	0.96	1.18	0.84	0.87
Valine		0.84	0.85	1.49	1.07	1.04	0.98
Alanine		1.05	0.96	1.57	1.48	1.01	1.10
Aspartic Acid		3.06	3.12	3.56	3.22	3.05	3.05
Cystine		-	-	-	-	-	-
Glutamic Acid		2.36	2.14	2.62	2.61	2.27	2.45
Glycine		1.71	1.75	2.36	1.80	1.79	1.77
Proline		0.87	0.84	0.96	0.87	1.06	1.27
Serine		0.93	0.97	1.01	1.26	0.93	0.96
Tyrosine		0.63	0.64	0.79	0.71	-	0.66
TOTAL		17.50	17.35	21.17	20.04	18.48	17.73

[1] Replicates A and B indicate replicate tanks for each treatment.

Table 3.38 - Mean apparent digestibility coefficients (n=4, ±S.D.) for rainbow trout fed diets containing 7%, 14% and 21% crude lipid using crude fibre and chromic oxide as dietary markers.

Apparent Nutrient Digestibility (%)	Diet No.	Lipid Level (%)	Marker	
			Cr_2O_3	Crude Fibre
Crude Protein (N x 6.25)	1	7	^{b}84.59 (±0.58)	^{ab}85.74 (±0.17)
	2	14	^{b}83.98 (±0.20)	^{b}85.46 (±0.22)
	3	21	^{a}87.77 (±0.13)	^{a}86.29 (±0.54)
Organic Matter	1	7	^{c}63.25 (±2.40)	^{c}66.08 (±1.20)
	2	14	^{b}67.96 (±0.64)	^{b}71.03 (±0.41)
	3	21	^{a}76.55 (±0.44)	^{a}73.69 (±1.51)
Dry Matter	1	7	^{b}57.74 (±2.02)	^{b}62.08 (±2.86)
	2	14	^{b}60.41 (±1.78)	^{ab}65.85 (±2.76)
	3	21	^{a}70.46 (±0.72)	^{a}68.02 (±2.60)
Crude Lipid	1	7	^{b}79.23 (±1.29)	^{b}80.78 (±1.22)
	2	14	^{a}93.39 (±1.21)	^{a}94.01 (±1.12)
	3	21	^{a}93.33 (±1.80)	^{a}92.51 (±2.78)

For each digestibility coefficient, values in same column with same superscript are not significantly different at p = 0.05.

3.2 Apparent Organic Matter Digestibility

Using both digestibility markers, there was a significant ($p<0.05$) increase in organic matter digestibility with increasing dietary crude lipid level (Table 3.38). However, as had been the case for the apparent crude protein digestibility results, the range of values obtained when Cr_2O_3 was used as a marker was wider (63.25% to 76.55%) than those calculated using crude fibre (66.08% to 73.69%).

3.3 Apparent Dry Matter Digestibility

Again, a wider variation in apparent dry matter (ADM) digestibility values was observed when Cr_2O_3 was used as a dietary market (57.74% to 70.46%) compared to a range of only (62.08% to 68.02%) when crude fibre was used as a marker (Table 3.38). In both cases the highest digesibility values were obtained for fish fed the diet containing 21% crude lipid (70.46% for Cr_2O_3 and 68.02% for crude fibre), although when crude fibre was used as a marker the difference between values for the 14% and 21% crude lipid diets was not significant ($p<0.05$). Using both markers, the digestibility coefficients obtained from fish fed the 21% crude lipid diet were significantly ($p<0.05$) higher than those from fish fed the 7% crude lipid diet (Table 3.38).

3.4 Apparent Crude Lipid Digestibility

The highest crude lipid digestibility coefficients were obtained when fish were fed the 14% and 21% crude lipid diets. Furthermore, there were no significant ($p<0.05$) differences between these dietary treatments (Table 3.38). By contrast, the digestibility values for fish fed the 7% crude lipid diet were only 79.23% (Cr_2O_3) and 80.78% (crude fibre) compared with values of 92-94% at the higher lipid inclusion levels, and

these differences were highly significant ($p<0.001$). Again, there was a trend for the range of values obtained using Cr_2O_3 as a marker to be wider than those for crude fibre, although this was less marked than had been the case for the other nutrient digestibility values.

3.5 Apparent Amino Acid Digestibility

When chromic oxide was used as marker, with the exception of histidine and valine, amino acid digestibilities for the 21% crude lipid diet (Diet 3) were higher than those from fish fed the 7% crude lipid diet (Diet 1). However, only in the cases of lysine, methionine, threonine, aspartic acid, glutamic acid, glycine and serine were these differences significant ($p<0.05$). Furthermore, with the exception of lysine, methionine, aspartic acid and proline, digestibilities of individual amino acids by fish fed the 14% crude lipid diet (Diet 2) were lowest, although only in the cases of histidine, threonine and alanine were digestibility values significantly ($p<0.05$) different from those at the 7% and 21% crude lipid inclusion levels (Table 3.39).

Overall, when Cr_2O_3 was used as a marker, individual amino acid digestibilities varied between 77.79% (aspartic acid) and 92.81% (histidine) for the 7% crude lipid diet compared with a range of 83.79% (glycine) to 93.45% (arginine) for the 21% crude lipid diet (Table 3.39).

When crude fibre was used as a marker only the digestibility values for lysine, threonine and aspartic acid were higher for the 21% crude lipid diet (Diet 3) than for the 7% crude lipid diet (Diet 1). Furthermore, with the exception of lysine, methionine,

phenylalanine, valine, aspartic acid and proline, the digestibility of individual amino acids by fish fed the 14% crude lipid diet (Diet 2) were lowest, although only in the case of valine was there a significant ($p<0.05$) difference between fish fed Diet 2 (90.46%) and fish fed Diet 3 (88.10%).

Overall, when crude fibre was used as a marker, the range of digestibility values was similar to that for chromic oxide, and individual amino acid digestibilities varied between 79.45% (aspartic acid) and 93.30% (histidine) for the 7% crude lipid diet compared with a range of 81.82% (glycine) to 92.68% (arginine) for the 21% crude lipid diet.

For both markers, the digestibility of aspartic acid was the lowest (between 77.79% and 84.09% for Cr_2O_3 and between 79.45% and 82.15% for crude fibre) and most consistently the highest digestibility values were for arginine (91.78% to 93.45% and 92.40% to 92.68% for Cr_2O_3 and crude fibre, respectively).

4. Comparison between markers

With the exception of fish fed the 21% crude lipid diet (Diet 3), all apparent nutrient and amino acid digestibility coefficients using Cr_2O_3 were slightly lower than those calculated using crude fibre as a marker (Tables 3.38 and 3.39 and Figure 3.15). Converse was the case for the 21% crude lipid diet.

When the performances of the two markers were compared statistically, there were no significant ($p<0.05$) differences between the digestibility coefficients for apparent crude

lipid digestibility. However, there was interaction between dietary lipid inclusion level and marker when apparent crude protein, apparent organic matter and apparent dry matter digestibility coefficients were compared. Thus Cr_2O_3 showed a wider variation in digestibility with the lipid inclusion level (particularly for Diet 3) than crude fibre. However, analysing the two-way analysis of variance tables (Table 3.40) for these digestibility coefficients, it is evident that most of the variability is explained by the main effects (marker and lipid inclusion level) and of these, lipid inclusion level carries the biggest share whereas the variation caused by the marker is relatively small.

Table 3.39 - Mean apparent amino acid digestibility coefficients for fish fed the 3 experimental diets (n=2, mean ±S.D.)

		Marker					
		Cr_2O_3			Crude Fibre		
	Diet no.	1	2	3	1	2	3
Apparent Amino Acid Digestibility (%)	Lipid Level (%)	7	14	21	7	14	21
Arginine		^{a}91.78 (±0.83)	^{a}91.63 (±0.67)	^{a}93.45 (±0.82)	^{a}92.40 (±0.52)	^{a}92.41 (±0.58)	^{a}92.68 (±0.63)
Histidine		^{a}92.81 (±0.23)	^{b}89.62 (±0.42)	^{a}91.89 (±0.82)	^{a}93.33 (±0.42)	^{b}90.58 (±0.33)	^{ab}90.89 (±1.27)
Isoleucine		^{ab}87.82 (±0.90)	^{b}87.12 (±0.98)	^{a}90.03 (±0.69)	^{a}88.73 (±0.47)	^{a}88.31 (±0.84)	^{a}88.83 (±0.33)
Leucine		^{ab}89.04 (±1.24)	^{b}88.45 (±1.19)	^{a}92.03 (±0.45)	^{a}89.87 (±0.83)	^{a}89.52 (±1.04)	^{a}91.07 (±0.15)
Lysine		^{b}88.52 (±0.64)	^{b}89.68 (±0.64)	^{a}92.22 (±0.39)	^{b}89.38 (±0.26)	^{ab}90.63 (±0.54)	^{a}91.27 (±0.78)
Methionine		^{b}90.99 (±0.08)	^{b}91.53 (±0.01)	^{a}93.49 (±0.64)	^{a}91.65 (±0.35)	^{a}92.32 (±0.04)	^{a}92.68 (±1.00)
Phenylalanine		^{a}83.32 (±1.04)	^{a}82.72 (±0.70)	^{a}84.98 (±0.00)	^{a}84.57 (±0.47)	^{a}84.32 (±0.56)	^{a}83.62 (±0.00)
Threonine		^{b}84.03 (±0.03)	^{c}83.39 (±0.01)	^{a}89.25 (±0.17)	^{b}85.21 (±0.49)	^{b}84.97 (±0.00)	^{a}87.94 (±0.28)
Valine		^{a}89.84 (±0.55)	^{a}89.52 (±0.00)	^{a}89.40 (±0.54)	^{a}90.60 (±0.21)	^{a}90.46 (±0.00)	^{b}88.10 (±1.07)
Alanine		^{a}87.99 (±1.50)	^{b}80.71 (±1.24)	^{a}90.68 (±0.47)	^{a}88.90 (±1.04)	^{b}82.50 (±1.05)	^{a}89.56 (±0.13)
Aspartic Acid		^{b}77.79 (±1.08)	^{b}78.35 (±0.00)	^{a}84.09 (±0.14)	^{b}79.45 (±0.35)	^{ab}80.29 (±0.00)	^{a}82.15 (±0.86)
Cystine		-	-	-	-	-	-
Glutamic Acid		^{b}86.92 (±1.72)	^{b}85.84 (±0.35)	^{a}90.42 (±0.42)	^{a}87.91 (±1.21)	^{a}87.15 (±0.27)	^{a}89.26 (±0.06)
Glycine		^{b}80.32 (±0.91)	^{b}79.97 (±0.00)	^{a}83.79 (±0.28)	^{a}81.79 (±0.27)	^{a}81.76 (±0.00)	^{a}81.82 (±1.02)
Proline		^{a}91.10 (±0.78)	^{a}91.30 (±0.80)	^{a}92.33 (±0.00)	^{a}91.76 (±0.46)	^{a}92.11 (±0.69)	^{a}91.16 (±0.00)
Serine		^{b}84.81 (±0.30)	^{b}83.64 (±0.00)	^{a}88.06 (±0.16)	^{a}86.72 (±1.09)	^{a}85.20 (±0.00)	^{a}86.62 (±0.34)
Tyrosine		^{ab}85.47 (±0.74)	^{b}83.87 (±0.01)	^{a}89.36 (±0.00)	^{ab}86.58 (±0.27)	^{b}85.32 (±0.00)	^{a}88.40 (±0.01)
Average Digestibility (%)		87.03	86.08	89.71	88.05	87.37	88.50

For each marker, values in same row with same superscript are not significantly different at p = 0.05.

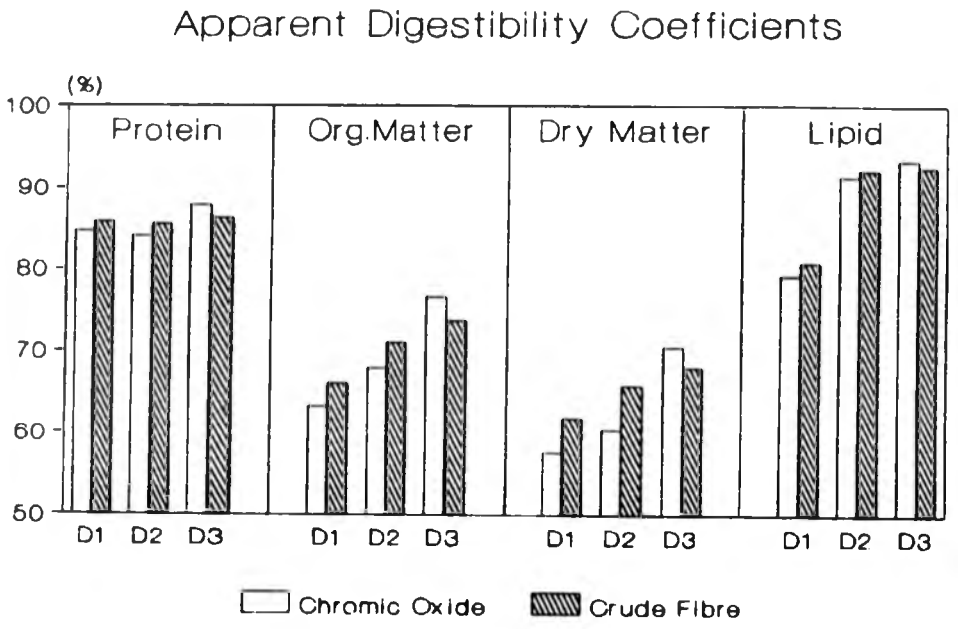

Figure 3.15 - Apparent digestibility coefficients (%) of fish fed the 3 experimental diets containing either 7% crude lipid (Diet 1), 14% crude lipid (Diet 2) or 21% crude lipid (Diet 3) using chromic oxide and crude fibre as dietary markers (individual values represent the mean of four replicates).

Table 3.40 - Two-way analysis of variance for the protein (A) organic matter (B) and dry matter (C) digestibility coefficients of rainbow trout fed the 3 experimental diets containing 7%, 14% and 21% crude lipid, when based on Cr_2O_3 and crude fibre as dietary markers

(A)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	p
MAIN EFFECTS	18.801758	3	6.2672528	26.460	.0000
Marker	0.936150	1	0.9361500	3.952	.0622
Lipid Level	17.865608	2	8.9328042	37.714	.0000
2-FACTOR INTERACTIONS	8.4150250	2	4.2075125	17.764	.0001
Marker x Lipid Level	8.4150250	2	4.2075125	17.764	.0001
RESIDUAL	4.2634000	18	0.2368556		
TOTAL (CORR.)	31.480183	23			

(B)

MAIN EFFECTS	123.18181	3	41.060604	63.654	.0000
Marker	5.69245	1	5.692445	8.825	.0101
Lipid Level	117.48937	2	58.744684	91.068	.0000
2-FACTOR INTERACTIONS	10.907768	2	5.4538838	8.455	.0039
Marker x Lipid Level	10.907768	2	5.4538838	8.455	.0039
RESIDUAL	9.0308750	14	0.6450625		
TOTAL (CORR.)	143.12046	19			

(C)

MAIN EFFECTS	141.287461	3	47.095820	26.792	.0000
Marker	0.58664	1	10.586636	6.022	.0252
Lipid Level	126.69806	2	63.349031	36.038	.0000
2-FACTOR INTERACTIONS	25.828948	2	12.914474	7.347	.0050
Marker x Lipid Level	25.828948	2	12.914474	7.347	.0050
RESIDUAL	29.883575	17	1.7578574		
TOTAL (CORR.)	196.99998	22			

V. INFLUENCE OF TIME AFTER FEEDING ON DIGESTIBILITY

1. Experimental Diet

The experimental diet was the same as that used in Section II, thus the proximate composition, marker and amino acid contents are shows in Tables 3.13 and 3.14.

2. Food Intake and Fish Performance

All fish accepted the experimental diet readily and fed aggressively throughout the three week experimental period.

Food intakes on a percent body weight per day basis for the duration of the experimental period were 1.99% (±0.09), 2.02% (±0.10), 1.85% (±0.02), 1.85% (±0.07), 1.89% (±0.02) and 1.97% (±0.04) for the 6 faecal sampling times (24:00h, 04:00h, 08:00h, 12:00h, 16:00h and 20:00h, respectively).

3. Apparent Nutrient Digestibility Coefficients

The proximate composition, marker content and amino acid profiles of faeces are shown in Tables 3.41 and 3.42.

3.1 Apparent Crude Protein Digestibility

There was a significant ($p<0.05$) decrease in apparent crude protein (ACP) digestibility with time after the last meal when both Cr_2O_3 and crude fibre were used as dietary markers (Table 3.43). The fall was more pronounced when crude fibre was used (from 90.61% after 6h to 82.71% after 26h) than when digestibility was based on Cr_2O_3 (from 84.36% after 6h to 82.05% after 26h).

Table 3.41 - Proximate composition and marker content of faeces collected between 6h and 26h after feeding (values expressed as % dry weight, n=4, mean ±S.D.).

	Time of day	24:00h	04:00h	08:00h	12:00h	16:00h	20:00h
Faeces Composition	Hrs. after feeding	6	10	14	18	22	26
Crude Protein (N x 6.25)		22.75 (±0.02)	22.81 (±0.13)	22.28 (±0.09)	22.94 (±0.11)	21.74 (±0.12)	20.82 (±0.03)
Crude Lipid (%)		9.48 (±0.00)	7.56 (±0.99)	6.39 (±0.35)	7.23 (±0.74)	2.89 (±0.30)	2.42 (±1.08)
Ash (%)		32.46 (±0.01)	33.02 (±0.77)	31.00 (±0.08)	30.83 (±0.83)	34.66 (±0.37)	40.57 (±0.71)
Crude Fibre (%)		7.89 (±0.41)	7.22 (±0.35)	5.93 (±0.41)	5.73 (±0.46)	4.59 (±0.44)	3.96 (±0.17)
Cr_2O_3 (%)		0.64 (±0.01)	0.62 (±0.03)	0.61 (±0.02)	0.59 (±0.01)	0.55 (±0.01)	0.51 (±0.01)

Table 3.42 - Amino acid profile of faeces collected from rainbow trout between 6h and 26h after feeding (g/100g dry weight).

	Time of Day	24:00h	04:00h	08:00h	12:00h	16:00h	20:00h
Amino Acid	Hrs. after feeding	6	10	14	18	22	26
Arginine		1.29	1.58	1.64	1.18	1.38	1.77
Histidine		0.34	0.53	0.30	0.37	0.41	0.39
Isolencine		0.59	0.87	0.60	0.68	0.84	0.81
Leucine		1.12	1.80	1.09	1.26	1.54	1.53
Lysine		0.85	1.41	0.78	0.87	1.00	1.11
Methionine		0.30	0.41	0.30	0.55	0.50	0.48
Phenylalamine		1.42	1.58	1.10	1.03	1.73	1.25
Threonine		0.93	0.98	0.77	0.85	0.97	0.80
Valine		0.85	0.93	0.98	1.53	1.35	1.53
Alanine		0.90	1.54	0.85	1.13	1.21	1.47
Aspartic Acid		2.57	2.57	2.21	2.68	3.25	2.02
Cystine		0.14	-	-	-	0.18	0.16
Glutamic Acid		2.35	2.44	2.10	2.32	2.70	2.75
Glycine		1.37	2.68	1.28	2.02	2.24	2.49
Proline		0.87	1.55	0.78	1.37	1.60	1.46
Serine		1.17	1.39	1.04	1.16	1.36	1.08
Tyrosine		0.67	0.84	0.68	0.52	0.81	0.75
TOTAL		17.73	24.09	16.50	19.52	23.07	21.69

Table 3.43 - Mean apparent digestibility coefficients for rainbow trout between 6h and 26h after feeding using chromic oxide and crude fibre as dietary markers (% dry weight basis, mean ±S.D.)

	Marker	Cr_2O_3						Crude Fibre					
	Time of Day	24:00h	04:00h	08:00h	12:00h	16:00h	20:00h	24:00h	04:00h	08:00h	12:00h	16:00h	20:00h
Apparent Nutrient Digest-ibility(%)	Hrs. after last meal	6	10	14	18	22	26	6	10	14	18	22	26
Crude Protein (N x 6.25)		^{a}84.36 (±0.01)	^{b}83.82 (±0.09)	^{b}83.94 (±0.07)	^{c}82.90 (±0.08)	^{d}82.62 (±0.10)	^{e}82.05 (±0.02)	^{a}90.61 (±0.13)	^{b}89.61 (±0.06)	^{c}87.64 (±0.05)	^{d}86.84 (±0.06)	^{e}84.42 (±0.09)	^{f}82.71 (±0.02)
Organic Matter		^{a}71.11 (±0.01)	^{ab}70.43 (±0.34)	^{bc}69.04 (±0.04)	^{c}67.91 (±0.85)	^{c}67.49 (±0.18)	^{c}68.10 (±1.45)	^{a}82.48 (±0.00)	^{b}81.01 (±0.22)	^{c}76.18 (±0.03)	^{c}75.29 (±0.65)	^{d}70.86 (±0.16)	^{d}69.28 (±1.40)
Dry Matter		^{a}64.41 (±0.28)	^{a}63.01 (±1.70)	^{a}62.72 (±1.29)	^{a}61.48 (±0.46)	^{b}58.30 (±1.07)	^{c}55.01 (±1.26)	^{a}79.53 (±1.15)	^{ab}77.50 (±1.16)	^{bc}72.21 (±2.04)	^{c}71.08 (±2.46)	^{d}63.45 (±3.65)	^{e}57.51 (±1.89)
Crude Lipid		^{c}66.00 (±0.00)	^{bc}72.01 (±3.66)	^{b}75.01 (±1.31)	^{bc}71.85 (±2.88)	^{a}87.94 (±1.24)	^{a}89.13 (±4.86)	^{b}79.37 (±0.00)	^{ab}82.02 (±2.35)	^{ab}82.08 (±0.16)	^{b}78.33 (±2.23)	^{a}89.19 (±1.11)	^{a}89.53 (±4.69)

For each marker, values in same row with same superscript are not significantly different at p = 0.05.

3.2 Apparent Organic Matter Digestibility

For both markers there was a general trend of decreasing apparent digestibility with time after feeding (Table 3.43). As had been the case for apparent crude protein digestibility, the fall was more pronounced when crude fibre was used as dietary marker with digestibility decreasing significantly ($p<0.05$) from 82.48% 6 hours after feeding to 69.28% after 26 hours. When based on Cr_2O_3 there was a statistically significant ($p<0.05$) fall from only 71.11% after 6 hours to 68.01% after 26 hours.

Although the initial digestibility values using crude fibre were much higher than those calculated using Cr_2O_3, there was very little difference between values after 26 hours.

3.3 Apparent Dry Matter Digestibility

Again using both markers there was a significant ($p<0.05$) trend of decreasing digestibility with time after feeding (Table 3.43). Furthermore, the fall was again more pronounced when values were based on crude fibre (from 79.53% after 6h to 57.51% after 26h) than when Cr_2O_3 was the dietary marker (from 64.41% after 6h to 55.01% after 26h).

3.4 Apparent Crude Lipid Digestibility

In contrast with the other digestibility coefficents, there was a significant ($p<0.05$) increase in digestibility with time after feeding using both dietary markers (Table 3.43). Again in contrast with the foregoing digestibility coefficients, the increase when using chromic oxide (66.00% after 6h to 89.13% after 26h) was more marked than when crude fibre was the dietary marker (from 79.37% after 6h to 89.53% after 26h).

3.5 Apparent Amino Acid Digestibility

All apparent amino acid digestibility values were from a single analysis, so it was not possible to compare them statistically.

As had been the case for crude protein digestibility, there was an overall general trend of decreasing amino acid digestibility values with time after feeding for both markers. However, that change was much more pronounced for some amino acids such as proline, alanine, glycine and valine than for others such as phenylalanine, histidine and aspartic acid (Table 3.44).

Furthermore, after 6h the maximum digestibility values were obtained for proline (93.04% and 95.14% for Cr_2O_3 and crude fibre respectively) and the minimum digestibility values were obtained for phenylalanine (81.84% for Cr_2O_3 and 87.32% for crude fibre). After 26h for both markers the maximum digestibility coefficients were those of histidine (88.12% for Cr_2O_3 and 86.82% for crude fibre) compared to the minimum digestibility values of 75.10% (Cr_2O_3) and 72.39% (crude fibre) obtained for glycine. Thus, the overall range of change was higher for crude fibre than for chromic oxide as had been the case for crude protein digestibility.

4. Comparison Between Markers

With the exception of amino acid values after 22h and 26h, both nutrient and amino acid digestibility coefficients were consistently higher when based on crude fibre as a

dietary marker than when calculated from Cr_2O_3 levels (Tables 3.43 and 3.44 and Figure 3.16). The differences were much more pronounced after the initial 6h sampling, whereas after 26 hours there were virtually no differences.

When the performances of the two markers were compared statistically, there were no significant ($p<0.05$) differences between the digestibility coefficients for apparent crude lipid digestibility values. However, there was interaction between marker and time of faecal collection when apparent crude protein, apparent organic matter and apparent dry matter digestibility coefficients were compared. Thus, as it can be seen from the analysis of variance data (Table 3.45), both markers performed differently when used to calculate these digestibility coefficients. The variability is explained mainly by the main effects and both marker and time after feeding contribute virtually to the same extent to that interaction.

Table 3.44 - Apparent amino acid digestibility values for rainbow trout between 6h and 26h after feeding, using Cr_2O_3 and crude fibre as dietary markers (% dry weight basis).

	Marker	Cr_2O_3						Crude Fibre					
	Time of Day	24:00	04:00	08:00	12:00	16:00	20:00	24:00	04:00	08:00	12:00	16:00	20:00
Apparent Amino Acid Digestibility (%)	Hrs after feeding	6	10	14	18	22	26	6	10	14	18	22	26
Arginine		87.80	84.57	83.73	87.89	84.81	78.99	91.48	88.60	85.59	89.27	84.33	76.71
Histidine		91.74	86.71	92.36	90.25	88.41	88.12	94.23	90.18	93.23	91.36	88.05	86.82
Isoleucine		89.55	84.10	88.86	86.94	82.70	82.00	92.71	88.25	90.13	88.42	82.15	80.05
Leucine		92.06	86.83	91.89	90.31	87.30	86.39	94.45	90.26	92.82	91.41	86.90	84.91
Lysine		92.40	86.99	92.68	91.56	89.60	87.55	94.69	90.38	93.52	92.52	89.27	86.19
Methionine		92.95	90.06	92.61	85.99	86.33	85.85	95.08	92.65	93.45	87.58	85.90	84.31
Phenylalanine		81.84	79.14	85.24	85.71	74.25	79.94	87.32	84.58	86.93	87.33	73.44	77.76
Threonine		86.47	85.28	88.25	86.58	83.58	85.39	90.55	89.12	89.59	88.11	83.06	83.80
Valine		91.51	90.42	89.74	83.43	84.32	80.83	94.07	92.91	90.91	85.31	83.82	78.75
Alanine		91.85	85.61	91.93	88.90	87.25	83.30	94.31	89.36	92.85	90.16	86.85	81.49
Aspartic Acid		85.05	84.57	86.52	83.09	78.01	85.26	89.56	88.60	88.06	85.01	77.31	83.66
Cystine		89.94	-	-	-	84.94	85.57	92.97	-	-	-	84.47	84.00
Glutamic Acid		90.76	90.10	91.34	90.10	87.65	86.43	93.55	92.68	92.33	91.23	87.26	84.96
Glycine		89.08	77.96	89.30	82.54	79.23	75.10	92.38	83.70	90.52	84.52	78.57	72.39
Proline		93.04	87.19	93.45	88.10	85.10	85.34	95.14	90.53	94.20	89.46	84.63	83.74
Serine		86.90	83.94	87.78	85.91	82.28	84.83	90.85	88.12	89.18	87.51	81.72	83.18
Tyrosine		86.98	83.36	86.14	89.04	81.69	81.72	90.91	87.70	87.73	90.29	81.11	79.73
Average Digestibility (%)	89.41	89.41	85.43	89.49	87.27	83.97	83.68	92.60	89.23	90.69	88.72	83.46	81.91

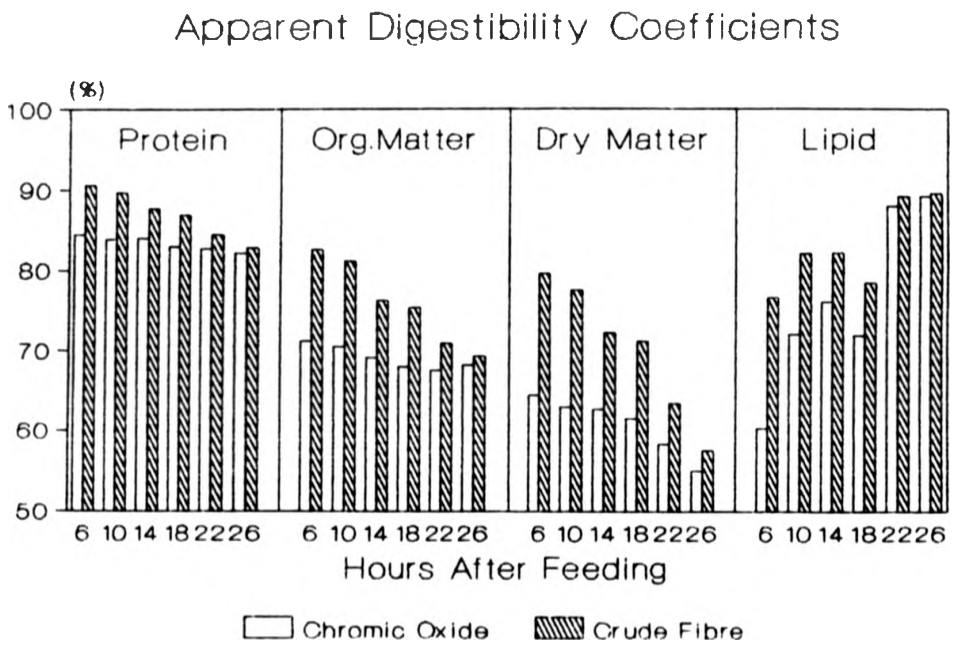

Figure 3.16 - Mean apparent digestibility coefficients (%) for rainbow trout between 6h and 26h after feeding using chromic oxide and crude fibre as dietary markers.

Table 3.45 - Two-way analysis of variance for the protein (A), organic matter (B) and dry matter (C) digestibility coefficients of rainbow trout at 6 different times after feeding when Cr_2O_3 and crude fibre were the dietary markers.

(A)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	p
MAIN EFFECTS	108.65315	6	18.108858	1000.00	.0000
Marker	56.54940	1	56.549400	1000.00	.0000
Time	52.10375	5	10.420750	1000.00	.0000
2-FACTOR INTERACTIONS Marker x time	18.111550	5	3.6223100	735.494	.0000
	18.111550	5	3.6223100	735.494	.0000
RESIDUAL	0.0591000	12	0.0049250		
TOTAL (CORR.)	126.82380	23			

(B)

MAIN EFFECTS	219.59450	6	36.59908	209.957	.0000
Marker	121.68007	1	121.68007	698.040	.0000
Time	97.91443	5	19.58289	112.341	.0000
2-FACTOR INTERACTIONS Marker x time	37.726883	5	7.5453767	43.285	.0000
	37.726883	5	7.5453767	43.285	.0000
RESIDUAL	2.0918000	12	0.1743167		
TOTAL (CORR.)	259.41318	23			

(C)

MAIN EFFECTS	467.84523	6	77.97421	67.833	.0000
Marker	204.28335	1	204.28335	177.715	.0000
Time	263.56188	5	052.71238	45.857	.0000
2-FACTOR INTERACTIONS Marker x time	53.090500	5	10.618100	9.237	.0008
	53.090500	5	10.618100	9.237	.0008
RESIDUAL	13.794000	12	1.1495000		
TOTAL (CORR.)	534.72973	23			

VI. INFLUENCE OF FEEDING FREQUENCY ON DIGESTIBILITY

1. Experimental Diet

The crude protein (48.88%) and the crude fibre (1.91%) contents of the commercial diet were close to the levels of 49% and 2%, respectively, specificied by the manufacturers (Table 3.46), whereas the crude lipid content (13.27%) was 10% higher than the minimum specified level of 12%.

The diet had been formulated to contain 1.0% chromic oxide and from the analysis it is clear that 95% of that level was actually present (Table 3.46).

2. Food Intake

All fish accepted the experimental diet readily and fed quite aggressively throughout the 5.5 week experimental feeding period.

Food intake by fish fed 4 times per day was consistently higher than for those fed the other 2 feeding regimes (Table 3.47). Thus, food intake varied between 1.76% and 2.41%, with an average overall intake of 2.14% (Table 3.47). The lowest food intake was by fish fed once daily (overall mean intake of 1.72%), although the mean food intake by fish fed twice daily (1.79%) was only slightly greater.

3. Apparent Nutrient Digestibility Coefficients

The proximate composition, marker and energy contents of faeces are presented in Table 3.48. In general, there was good replication within treatments.

Table 3.46 - Proximate composition marker and energy contents of the experimental diet (values expressed as % dry weight, n=4, mean ±S.D.).

Nutrient Content (% dry weight)	Manufacturer's specifications	Diet	± S.D.
Moisture (%)	<13.00	9.59	±0.03
Crude Protein (N x6.25)	49.00	48.88	±0.60
Crude Lipid (%)	12.00	13.27	±0.30
Ash (%)	<18.00	16.07	±0.03
Crude Fibre (%)	2.00	1.91	±0.14
Chromic Oxide (%)		0.95	±0.01
Energy (kcal/g)		4.56	±0.01

Table 3.47 - Food intake (% body weight/day) over the experimental period for fish fed either once, twice or four times daily.

Week No.	Mean Water Temperature (°C) at 11.00 a.m.	Feeding Frequency (x day $^{-1}$)		
		1	2 (±S.D.)	4
1	13.8 (±0.40)	1.51 (±0.03)	2.09 (±0.03)	2.41 (±0.26)
2	13.8 (±0.24)	2.18 (±0.06)	2.23 (±0.37)	2.64 (±0.11)
3	13.5 (±0.10)	1.29 (±0.01)	1.60 (±0.05)	1.76 (±0.01)
4	13.0 (±0.20)	1.83 (±0.11)	1.51 (±0.17)	1.94 (±0.04)
5	13.1 (±0.22)	1.74 (±0.09)	1.64 (±0.05)	1.96 (±0.21)
6	13.0 (±0.24)	1.80 (±0.10)	1.69 (±0.06)	2.15 (±0.09)
Overall Food intake		1.72 (±0.30)	1.79 (±0.29)	2.14 (±0.32)

Table 3.48 - Proximate composition, marker and energy content of faeces collected from rainbow trout fed either once, twice or four times daily (values expressed as % dry weight, n=2, mean ±S.D.).

Faeces Composition (% dry weight)	Replicates[1]	Feeding regime (x day^{-1})		
		1	2	4
Crude Protein (N x 6.25)	A	30.81 (±0.55)	29.17 (±0.32)	29.58 (±0.37)
	B	28.89 (±0.11)	29.23 (±0.09)	30.14 (±0.36)
Ash (%)	A	34.69 (±0.60)	34.58 (±0.75)	33.93 (±0.27)
	B	35.55 (±0.50)	33.52 (±0.58)	34.31 (±0.60)
Crude Fibre (%)	A	5.53 (±0.25)	6.11 (±0.00)	5.83 (±0.49)
	B	5.46 (±0.21)	5.97 (±0.01)	5.91 (±0.41)
Cr_2O_3 (%)	A	2.88 (±0.01)	2.57 (±0.01)	2.81 (±0.06)
	B	2.89 (±0.06)	2.83 (±0.07)	2.71 (±0.06)
Energy (kcal/g)	A	3.41 (±0.06)	3.54 (±0.03)	3.52 (±0.01)
	B	3.38 (±0.07)	3.28 (±0.16)	3.02 (±0.03)

1. Replicates A and B indicate 2 replicate tanks for each treatment.

3.1 Apparent Crude Protein Digestibility

When Cr_2O_3 was used as a marker, crude protein values for all three feeding frequences were around 79% and were not significantly different ($p<0.05$). By contrast, when digestibilty coefficients were calculated using crude fibre as a marker, values for all 3 feeding regimes were significantly different ($p<0.05$). The highest value (81.10%) was for fish fed twice daily and the lowest (78.43%) for those fed only once per day (Table 3.49).

3.2 Apparent Organic Matter Digestibility

As had been the case with apparent crude protein digestibility, when crude fibre was used as a marker, the maximum digestibility (75.14%) was for fish fed 2 times per day and the minimum (73.13%) for those fed only once daily. These values were significantly different at the 5% level of significance (Table 3.49).

By contrast, when organic matter digestibilities were calculated using Cr_2O_3 as a marker, the lowest value was obtained for fish fed 2 times daily (72.30%) and the highest for fish fed once daily (74.54%). However, these differences were not significant ($p<0.05$).

3.3 Apparent Dry Matter Digestibility

Maximum digestibility using crude fibre as the dietary marker was again observed for fish fed twice daily (68.98%) and the lowest (66.48%) for fish fed only once per day (Table 3.49). However, in this case differences were not significant ($p<0.05$). By contrast, the minimum apparent dry matter digestibility value using Cr_2O_3 was for fish

Table 3.49 - Mean apparent digestibility coefficients (±S.D.) and apparent energy digestibility (±S.D.) for rainbow trout fed either once, twice or four times daily calculated using chromic oxide and crude fibre as dietary markers.

	Marker	Cr_2O_3			Crude Fibre		
Apparent Nutrient Digestibility (%)	F. Regime	1 x day $^{-1}$	2 x day $^{-1}$	4 x day $^{-1}$	1 x day $^{-1}$	2 x day $^{-1}$	4 x day $^{-1}$
Crude Protein (N x 6.25)		^{a}79.56 (±0.47)	^{a}78.93 (±1.15)	^{a}78.96 (±0.70)	^{c}78.43 (±0.33)	^{a}81.10 (±0.30)	^{b}80.12 (±0.21)
Organic Matter		^{a}74.54 (±0.30)	^{a}72.30 (±1.57)	^{a}72.97 (±0.59)	^{b}73.13 (±0.01)	^{a}75.14 (±0.68)	^{ab}74.45 (±0.35)
Dry Matter		^{a}67.86 (±0.42)	^{b}65.38 (±2.00)	^{ab}66.19 (±0.93)	^{a}66.48 (±1.26)	^{a}68.98 (±0.51)	^{a}68.67 (±2.13)
Energy		^{a}75.50 (±0.44)	^{a}73.62 (±2.75)	^{a}75.36 (±1.64)	^{a}74.13 (±0.43)	^{a}76.40 (±1.00)	^{a}76.68 (±2.22)

For each marker, values in same row with same superscript are not significantly different at p = 0.05.

fed twice daily (65.38%) and the maximum for those fed once a day (67.86%). Furthermore, this difference was significant ($p<0.05$).

3.4 Apparent Energy Digestibility

There was no significant ($p<0.05$) variation of energy digestibility with feeding frequency when both Cr_2O_3 and crude fibre were used as dietary markers (Table 3.49).

4. Comparison Between Markers

For each digestibility coefficient, values obtained when fish were fed both twice and four times daily were consistently higher when crude fibre was used as a marker than when Cr_2O_3 was used. The converse was the case when fish were fed only once daily (Table 3.49 and Figure 3.17).

When the performance of the two markers were compared statistically, there were no significant ($p<0.05$) differences between the energy digestibility coefficients. By contrast, there was interaction between feeding frequency and marker when apparent crude protein, apparent organic matter and apparent dry matter digestibilty coefficients were calculated. Thus the two dietary markers behaved differently when used to calculate these digestibility coefficients (Table 3.50).

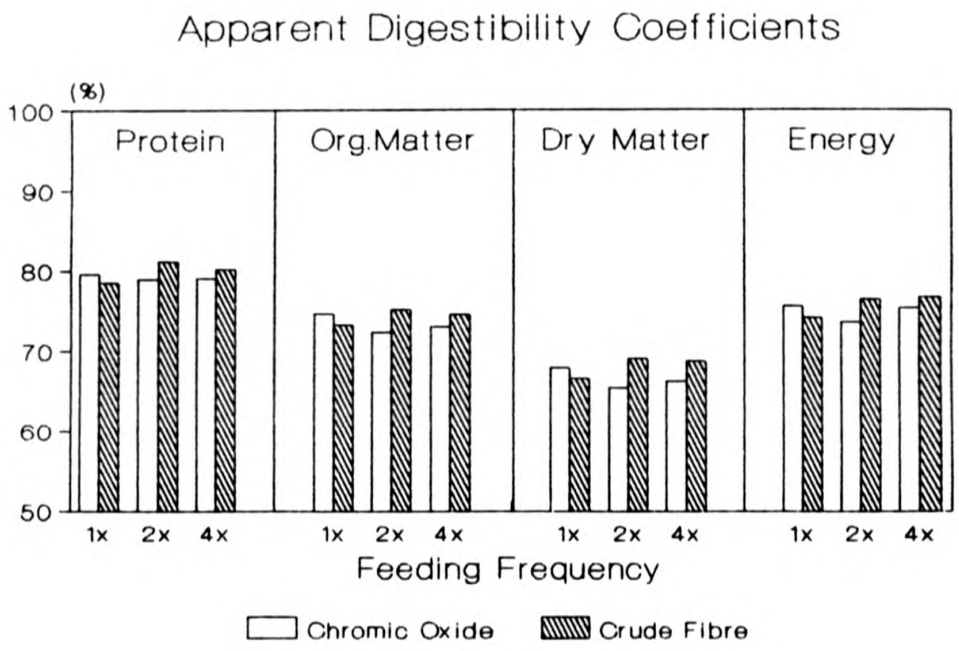

Figure 3.17 - Apparent digestibility coefficients of rainbow trout fed either once, twice or four times daily.

Table 3.50 - Two-way analysis of variance for protein (A), organic matter (B) and dry matter (C) digestibility coefficients for rainbow trout fed 3 different feeding frequencies when based on Cr_2O_3 and crude fibre as dietary markers.

(A)

Source of Variation	Sum of Squares	d.f.	Mean Square	F-ratio	p
MAIN EFFECTS	3.8152792	3	1.2717597	6.661	.0032
Marker	1.5862042	1	1.5862042	8.309	.0099
F. Frequency	1.2290750	2	1.1145375	5.838	.0111
2-FACTOR INTERACTIONS	5.6538583	2	2.8269292	14.807	.0002
Marker x F. Frequency	5.6538583	2	2.8269292	14.807	.0002
RESIDUAL	3.4364520	18	.1909125		
TOTAL	12.905563	23			

(B)

MAIN EFFECTS	1.1517833	3	0.3839278	1.630	.2791
Marker	1.1408333	1	1.1408333	4.844	.0700
F. Frequency	0.0109500	2	0.0054750	0.023	.9771
2-FACTOR INTERACTIONS	4.0768167	2	2.0384083	8.656	.0171
Marker x F. Frequency	4.0768167	2	2.0384083	8.656	.0171
RESIDUAL	1.4130000	6	.2355000		
TOTAL (CORR)	6.6416000	11			

(C)

MAIN EFFECTS	4.2177205	3	1.4059068	1.794	.1888
Marker	3.3390750	1	3.3390750	4.261	.0556
F. Frequency	0.4631017	2	0.2315508	0.295	.7481
2-FACTOR INTERACTIONS	8.9475250	2	4.4737625	5.709	.0134
Marker x F. Frequency	8.9475250	2	4.4737625	5.709	.0134
RESIDUAL	12.537850	16	.7836156		
TOTAL	25.703095	21			

VII. INFLUENCE OF DISSOLVED OXYGEN LEVEL ON DIGESTIBILITY AND FOOD INTAKE

1 - Experimental Diet

The crude protein (53.32%) and the crude lipid (12.77%) contents of the commercial diet exceeded the minimum levels of 47% and 9%, respectively quoted by the manufacturers (Table 3.51). In addition, the crude fibre (2.61%) content was 30% greater than the maximum level of 2% specified.

Table 3.51 shows that the formulated 1% Cr_2O_3 inclusion level was present in the diet. From Table 3.52 it is apparent that the diet contained adequate levels of all the essential amino acids.

2 - Food Intake

Tables 3.53 and 3.54 show a summary of the voluntary food intake on a percent body weight per day basis for the duration of the two 3 week experimental periods (Trials A and B).

In the experimental tanks the mean food intake varied between 2.52% per day for treatment 1 (7.2mg O_2/l) and 0.22% per day for treatment 6 (2.3mg O_2/l) in Trial A (Table 3.53). The intake level for T1 was significantly ($p<0.05$) higher than the intake levels of all the other treatments. However, the food intake (1.05%) during treatment 3 (4.7mg O_2/l) was not significantly different from the intake levels during the 5.1mg O_2/l (1.60%) and the 4.0mg O_2/l (0.68%) treatments. Only the difference between the intake levels of treatments 2 and 4 was significant. A further significant decrease of food intake was obtained for the 3.1mg O_2/l and the 2.3mg O_2/l treatments (Table 3.53).

Table 3.51 - Proximate composition and marker content of the experimental diet used in Trials A and B (values expressed as % dry weight).

Nutrient Content (% dry weight)	Manufacturers specifications (%)	Diet	±S.D.
Moisture (%)		7.55	±0.20
Crude Protein (N x 6.25)	≥ 47	53.32	±0.28
Crude Lipid (%)	≥ 9	12.77	±0.50
Ash (%)		15.02	±0.06
Crude Fibre (%)	≤ 2	2.61	±0.07
Cr_2O_3 (%)		0.98	±0.00

Table 3.52 - Amino acid profile of the experimental diet and amino acid requirements of rainbow trout (g/100g dry weight).

Amino Acid	Diet (g/100g dry weight)	Requirements of rainbow trout (% diet)
Arginine	3.06	1.40, 40%[1] (Ogino, 1980)
Histidine	1.85	0.64, 40% (Ogino, 1980)
Isoleucine	2.04	0.96, 40% (Ogino, 1980)
Leucine	4.21	1.76, 40% (Ogino, 1980)
Lysine	3.19	2.12, 40% (Ogino, 1980)
Methionine	1.66	0.55-0.75, 35% (Rumsey *et al.* 1983)
Phenylalanine	2.38	1.24, 40% (Ogino, 1980)
Threonine	2.89	1.36, 40% (Ogino, 1980)
Valine	3.15	1.24, 40% (Ogino, 1980)
Alanine	3.16	
Aspartic Acid	4.65	
Cystine	0.54	0.30, 35% (Rumsey *et al.*, 1983)
Glutamic Acid	7.14	
Glycine	3.65	
Proline	3.28	
Serine	2.13	
Tyrosine	1.16	0.84, 40% (Ogino, 1980)
TOTAL	50.14	

1. Percentage of crude protein in the diet.

Table 3.53 - Mean food intake (% body weight per day ±S.D.) and mean dissolved oxygen (mg/l ±S.D.) in tanks for the abrupt decrease of water oxygen level trial (Trial A)

	Dissolved Oxygen (mg/l)		Food intake[1] (% body wt./day)	
Treatment	Experimental Tanks	Control Tanks	Experimental Tanks	Control Tanks
T1	7.2 (±0.00)	6.9 (±0.17)	2.52^{a}_{a} (±0.46)	2.41^{a}_{a} (±0.20)
T2	5.1 (±0.05)	6.7 (±0.11)	1.60^{b}_{a} (±0.21)	2.18^{a}_{a} (±0.51)
T3	4.7 (±0.00)	6.9 (±0.05)	1.05^{bc}_{b} (±0.22)	2.12^{a}_{a} (±0.32)
T4	4.0 (±0.05)	6.6 (±0.05)	0.68^{cd}_{b} (±0.23)	2.08^{a}_{a} (±0.11)
T5	3.1 (±0.05)	6.5 (±0.05)	0.32^{d}_{b} (±0.14)	1.95^{a}_{a} (±0.21)
T6	2.3 (±0.05)	6.5 (±0.07)	0.22^{d}_{b} (±0.02)	2.13^{a}_{a} (±0.01)

1. Each individual value represents the average of 2 replicate tanks

Values in same row with same subscript are not significantly different at p = 0.05.

Values in same column with same superscript are not significantly different at p = 0.05.

Table 3.54 - Mean food intake (% body weight per day ±S.D.) and mean dissolved oxygen(mg/l±S.D.) in tanks for the prolonged hypoxia trial (Trial B)

	Dissolved Oxygen (mg/l)		Food Intake[1] (% body wt./day)	
Treatment	Experimental Tanks	Control Tanks	Experimental Tanks	Control Tanks
T0	7.4 (±0.00)	7.2 (±0.05)	1.44^{a}_{a} (±0.04)	1.57^{a}_{a} (±0.20)
T1	5.1 (±0.17)	6.6 (±0.11)	1.14^{b}_{b} (±0.02)	1.64^{a}_{a} (±0.14)
T2	4.0 (±0.17)	6.8 (±0.23)	0.69^{c}_{b} (±0.02)	1.62^{a}_{a} (±0.02)

1. Each individual value represents the average of two replicate tanks.

Values in same row with same subscript are not significantly different at p = 0.05.

Values in same column with same superscript are not significantly different at p = 0.05.

In the control tanks there was a slight but non-significant decrease of food intake coinciding also with a slight decrease of the D.O. level. This D.O. decrease was probably temperature related since the temperature varied between 15.8°C in the first week and 16.3°C in the third week of the experimental period (Table 3.55). There were no significant differences in the food intake level between experimental and control tanks for the 2 first treatments, i.e. down to 5.1mg O_2/l, whereas for all other treatments food intake was significantly higher in the control tanks.

A significant ($p<0.05$) decrease of food intake (from 1.44% to 0.69%) was observed in the experimental tanks in Trial B as the dissolved oxygen level was decreased from 7.4mg O_2/l to 4.0mg O_2/l whereas in the control tanks there were no significant differences among treatments (Table 3.54).

Furthermore, with the exception of the 7.2-7.4mg O_2/l treatment the food intake levels in the control tanks were significantly higher than those in the experimental tanks (Table 3.54).

The decrease in food intake is clearly related to dissolved oxygen level in both trials, and highly significant correlation coefficients ($r= 0.95$ and $r= 0.89$, $p<0.001$) were found for Trials A and B, respectively. These relationships are shown in Figure 3.18.

3 - Apparent Nutrient Digestibility Coefficients

The protein and marker contents of faeces collected from fish during Trials A and B are shown in Tables 3.56 and 3.57, respectively. No faecal samples were obtained from

Table 3.55 - Water temperatures (°C) registered over the experimental periods - Trial A(A) and Trial B(B).

A

Day	Week No.	1	2	3
1		15.8	16.0	16.0
2		15.7	16.2	16.3
3		15.7	16.5	16.0
4		15.8	15.9	16.3
5		15.9	15.8	16.6
6		15.8	15.7	16.5
7		15.9	16.0	16.1
Mean ± S.D.		15.8 (±0.08)	16.0 (±0.26)	16.3 (±0.23)
Overall Mean ±S.D.			16.0 (±0.23)	

B

Day	Week No.	1	2	3
1		16.0	15.9	16.0
2		16.0	15.9	16.1
3		15.9	16.0	16.0
4		15.9	16.0	15.8
5		15.8	15.8	15.8
6		15.9	16.0	15.8
7		15.9	16.0	15.9
Mean ± S.D.		15.9 (±0.07)	15.9 (±0.08)	15.9 (±0.12)
Overall Mean ±S.D.			15.9 (±0.10)	

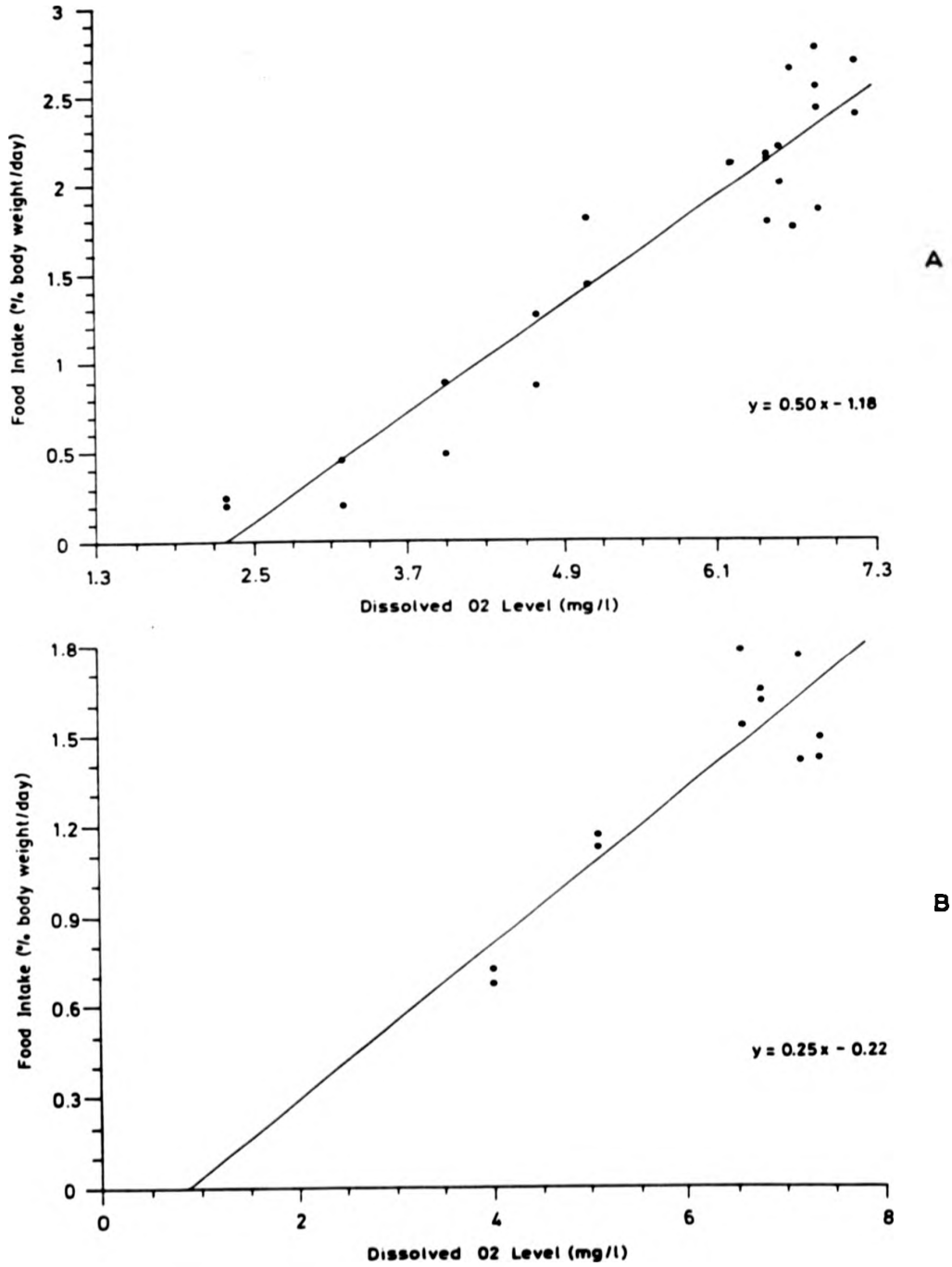

Figure 3.18 - Regression of food intake on dissolved oxygen level during the abrupt decrease of the water O_2 level (A) - Trial A and during prolonged hypoxia (B) - Trial B.

Table 3.56 - Protein and marker contents of faeces collected from rainbow trout during the abrupt decrease of dissolved oxygen level trial -Trial A (n=2, mean±S.D.).

	Treatment no.	T1		T2		T3		T4		T5	
	Dissolved O_2 level (mg/l)	7.2	6.9	5.1	6.7	4.7	6.9	4.0	6.6	3.1	6.5
Faeces composition (% dry weight)	Tank	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control
	Replicates[1]										
Crude Protein (N x 6.25)	A	26.79 (±0.02)	28.88 ±0.04)	25.27 (±0.00)	26.12 (±0.10)	25.84 (±0.05)	27.56 (±0.00)	25.16 (0.15)	26.98 (±0.00)	-	25.14 (±0.09)
	B	27.28 (±0.06)	27.98 (±0.03)	24.77 (±0.03)	27.86 (±0.07)	24.41 (±0.10)	27.85 (±0.02)	24.59 (±0.09)	27.99 (±0.07)	-	25.65 (±0.10)
Cr_2O_3 (%)	A	2.68 (±0.02)	2.33 (±0.03)	2.98 (±0.01)	2.97 (±0.00)	2.92 (±0.00)	2.98 (±0.02)	2.89 (±0.00)	3.33 (±0.04)	-	3.06 (±0.00)
	B	2.50 (±0.00)	2.90 (±0.01)	3.05 (±0.02)	2.71 (±0.05)	2.98 (±0.00)	3.03 (±0.03)	3.31 (±0.00)	2.64 (±0.02)	-	2.86 (±0.01)

[1] Replicates A and B indicate 2 replicate tanks for each treatment.

Table 3.57 - Protein and marker contents of faeces collected from fish subjected to prolonged hypoxia - Trial B (n=2, mean±S.D.).

Faeces Composition (% dry weight)	Treatment no.	T1		T2	
	Dissolved O_2 level (mg/l)	5.1	6.6	4.0	6.8
	Tank	Exp.	Control	Exp.	Control
	Replicates [1]				
Crude Protein (N x 6.25)	A	23.08 (±0.00)	25.30 (±0.03)	21.35 (±0.03)	25.47 (±0.04)
	B	23.18 (±0.02)	25.59 (±0.01)	21.52 (±0.04)	25.14 (±0.02)
Cr_2O_3 (%)	A	2.66 (±0.01)	2.55 (±0.01)	2.66 (±0.00)	2.51 (±0.01)
	B	2.70 (±0.00)	2.50 (±0.02)	2.66 (±0.01)	2.60 (±0.00)

[1] Replicates A and B indicate 2 replicate tanks for each treatment.

fish in the experimental tanks for the 3.1mg O_2/l and the 2.3mg O_2/l treatments in Trial A because food intake was practically zero and hence no faeces were produced. The amino acid profiles of faeces for Trial B are presented in Table 3.58.

Trial A - The influence of an abrupt decrease of water oxygen level

3.1 - Apparent Crude Protein Digestibility

In all the treatments the apparent crude protein digestibility (ACPD) values were higher in the experimental tanks than in the control tanks. However, Duncan's Multiple Range Test showed that these differences were not significant (Table 3.59). There was an increase in digestibility as D.O. decreased but only the ACPD value at 7.2mg O_2/l was significantly ($p<0.05$) different (Table 3.59). Although not significant, an increase in ACPD with time was also observed in the control tanks, suggesting that there may be another factor influencing digestibility since the dissolved oxygen level only varied between 6.9 and 6.5mg/l. It is possible that the slight increase in digestibility with time was temperature related since the water temperature increased from 15.8°C in the first week to 16.3°C in the third week of the experimental period (Table 3.55).

3.2 - Apparent Dry Matter Digestibility

All the apparent dry matter digestibility (ADMD) coefficients were slightly higher in the experimental tanks than in the control tanks with the exception of values for the 4.7mg O_2/l treatment (Table 3.59). However, these differences were not significant ($p<0.05$). As was the case for the crude protein digestibility, an increase in ADMD was observed in both experimental and control tanks. Again, only the ADMD value for the 7.2mg O_2/l treatment was significantly lower than the digestibility values for the other treatments (Table 3.59).

Table 3.58 - Amino acid profile of faeces collected from fish subjected to prolonged hypoxia - Trial B (g/100g dry weight).

		Control Tanks				Experimental Tanks			
	Treatment no.	T1		T2		T1		T2	
	D.O. level (mg/l)	6.6		6.8		5.1		4.0	
Amino Acid	Replicates [1]	A	B	A	B	A	B	A	B
Arginine		0.92	0.89	0.85	0.89	0.68	0.76	0.71	0.72
Histidine		0.33	0.32	0.39	0.33	0.32	0.32	0.31	0.30
Isoleucine		0.76	0.78	0.79	0.75	0.68	0.68	0.67	0.65
Leucine		1.38	1.37	1.43	1.30	1.23	1.24	1.22	1.23
Lysine		0.77	0.79	0.83	0.73	0.72	0.71	0.69	0.66
Methionine		0.66	0.70	0.73	0.65	0.66	0.64	0.60	0.62
Phenylalanine		0.80	0.80	0.81	0.79	0.70	0.73	0.70	0.72
Threonine		0.89	0.84	0.88	0.86	0.81	0.82	0.84	0.73
Valine		1.15	1.10	1.15	1.14	1.02	1.01	0.96	0.95
Alanine		1.17	1.14	1.20	1.13	0.93	1.02	0.95	0.96
Aspartic Acid		2.33	2.21	2.33	2.31	2.20	2.31	2.12	2.07
Cystine		0.30	0.29	0.31	0.30	0.26	0.27	0.28	0.24
Glutamic Acid		2.42	2.38	2.44	2.36	2.22	2.25	2.19	2.17
Glycine		2.14	2.07	2.11	2.09	1.98	1.91	1.46	1.48
Proline		1.63	1.76	1.69	1.78	1.56	1.55	1.51	1.52
Serine		1.07	1.10	1.03	1.19	0.95	1.10	1.03	1.01
Tyrosine		0.42	0.44	0.44	0.43	0.38	0.42	0.36	0.41
TOTAL		19.14	18.98	19.41	18.99	17.30	17.74	16.60	16.44

[1] Replicates A and B indicate 2 replicate tanks per treatment.

Table 3.59 - Mean apparent digestibility coefficients (±S.D.) for rainbow trout subject to an abrupt decrease of the dissolved oxygen level - Trial A.

		Apparent Crude Protein Digestibility (%)			
		Control Tanks		Experimental Tanks	
Days	Treatment	mg O_2/l	Dig.	mg O_2/l	Dig.
6	T1	6.9	79.73^{a}_{a} (±2.92)	7.2	80.79^{b}_{a} (±0.96)
3	T2	6.7	82.48^{a}_{a} (±1.57)	5.1	84.74^{a}_{a} (±0.30)
3	T3	6.9	83.04^{a}_{a} (±0.05)	4.7	84.33^{a}_{a} (±0.68)
3	T4	6.6	82.78^{a}_{a} (±2.35)	4.0	85.15^{a}_{a} (±1.34)
3	T5	6.5	84.18^{a} (±0.80)	3.1	-

		Apparent Dry Matter Digestibility (%)			
		Control Tanks		Experimental Tanks	
Days	Treatment	mg O_2/l	Dig.	mg O_2/l	Dig.
6	T1	6.9	62.67^{a}_{a} (±4.84)	7.2	62.73^{b}_{a} (±1.52)
3	T2	6.7	66.08^{a}_{a} (±1.83)	5.1	68.17^{a}_{a} (±0.42)
3	T3	6.9	68.02^{a}_{a} (±0.30)	4.7	67.41^{a}_{a} (±0.36)
3	T4	6.6	67.32^{a}_{a} (±4.40)	4.0	68.87^{a}_{a} (±2.47)
3	T5	6.5	67.45^{a} (±1.32)	3.1	-

Values in same row with same subscript are not significantly different (for each digestibility coefficient) at p = 0.05.

Values in same column with same superscript are not significantly different (for each digestibility coefficient) at p = 0.05.

Trial B - The influence of prolonged hypoxia

3.1 - Apparent Crude Protein Digestibility

As in Trial A, the apparent crude protein digestibility (ACPD) values were higher in the experimental tanks than in the control tanks (Table 3.60). However, in contrast with Trial A, these differences were significant ($p<0.05$). A slight increase in ACPD was observed when the dissolved oxygen level was decreased and this increase was significant. Regression analysis showed a highly significant ($p<0.001$) inverse correlation between D.O. and protein digestibility ($r= -0.97$) Figure 3.19 and between protein digestibility and food intake ($r= -0.95$) Figure 3.20.

3.2 - Apparent Dry Matter Digestibility

Apparent dry matter digestibility (ADMD) coefficients were higher in the experimental tanks than in the control tanks (Table 3.60) but were only significant in the 5.1mg O_2/l treatment. No significant differences between treatments were found both for the control and the experimental tanks (Table 3.60).

ADMD coefficients were significantly ($p<0.01$) correlated with D.O. level and food intake ($r= -0.82$ and $r= -0.82$, respectively) Figures 3.21 and 3.22.

3.3 - Apparent Amino Acid Digestibility

The apparent amino acid digestibility (AAAD) values were higher in the experimental tanks than in the control tanks with average amino acid digestibility values of around 87% and 85% respectively (Table 3.61). For Treatment 1, with the exception of the digestibility values for methionine, aspartic acid and serine those differences were

Table 3.60 - Mean apparent digestibility coefficients (±S.D.) for rainbow trout subjected to prolonged hypoxia - Trial B.

		Apparent Crude Protein Digestibility (%)			
		Control Tanks		Experimental Tanks	
Days	Treatment	mg O_2/l	Dig.	mg O_2/l	Dig.
7	T1	6.6	81.46^{a}_{b} (±0.41)	5.1	84.12^{b}_{a} (±0.09)
7	T2	6.8	81.77^{a}_{b} (±0.62)	4.0	85.18^{a}_{a} (±0.08)

		Apparent Dry Matter Digestibility (%)			
		Control Tanks		Experimental Tanks	
Days	Treatment	mg O_2/l	Dig.	mg O_2/l	Dig.
7	T1	6.6	61.75^{a}_{b} (±0.55)	5.1	64.03^{a}_{a} (±0.39)
7	T2	6.8	62.21^{a}_{a} (±0.47)	4.0	63.75^{a}_{a} (±0.00)

For each digestibility coefficient, values in same row with same subscript are not significantly different at p = 0.05.

For each digestibility coefficient, values in same column with same superscript are not significantly different at p = 0.05.

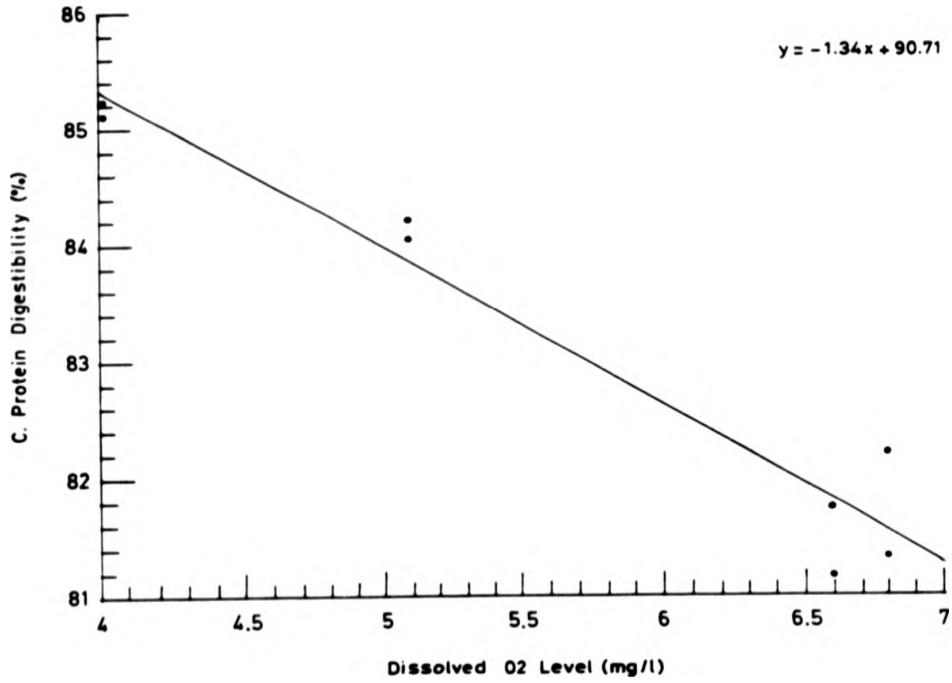

Figure 3.19 - Regression of protein digestibility on D.O. level during prolonged hypoxia - Trial B.

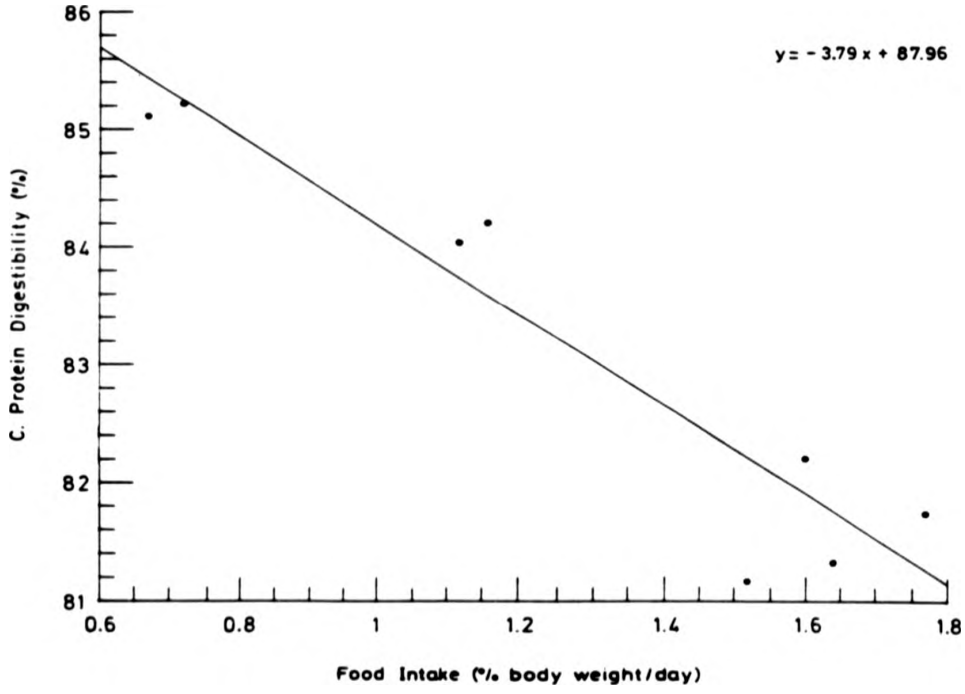

Figure 3.20 - Regression of protein digestibility on food intake during prolonged hypoxia - Trial B.

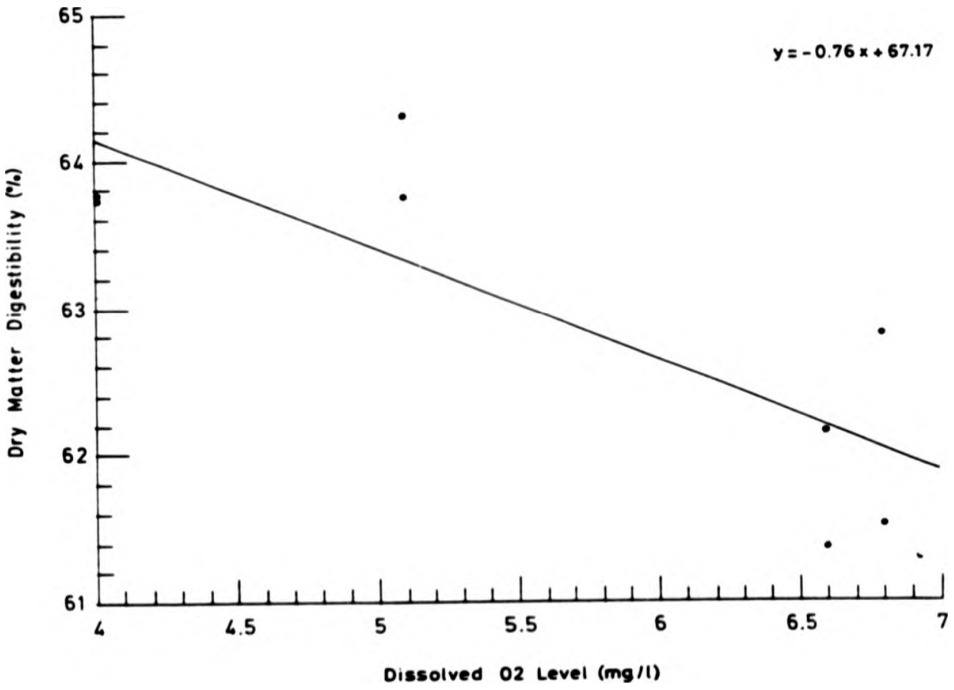

Figure 3.21 - Regression of dry matter digestibility on D.O. level during prolonged hypoxia - Trial B.

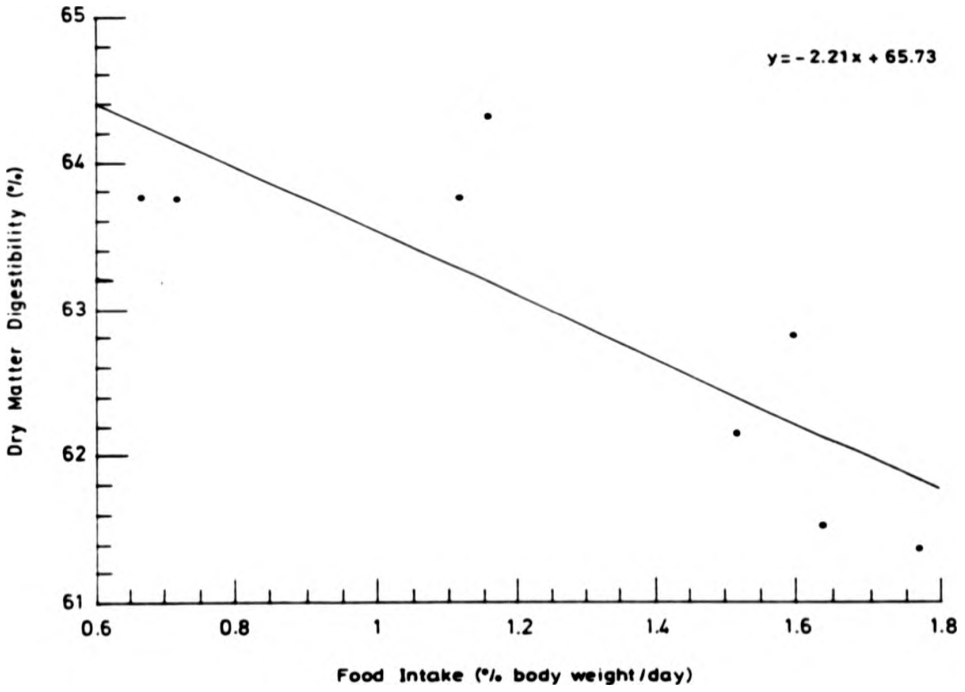

Figure 3.22 - Regression of dry matter digestibility on food intake during prolonged hypoxia - Trial B.

Table 3.61 - Mean apparent amino acid digestibility coefficients (n=2, mean ±S.D.) for rainbow trout subjected to prolonged hypoxia - Trial B.

		Apparent Amino Acid Digestibility (%)			
		Control Tanks		Experimental Tanks	
	Treatment no.	T1	T2	T1	T2
Amino Acid	D.O. level (mg/l)	6.6	6.8	5.1	4.0
Arginine		88.51^{b}_{a} (±0.11)	89.09^{b}_{a} (±0.08)	91.39^{a}_{a} (±0.16)	91.38^{a}_{a} (±0.08)
Histidine		93.18^{b}_{a} (±0.05)	92.52^{a}_{a} (±1.07)	93.67^{a}_{a} (±0.00)	93.92^{a}_{a} (±0.14)
Isoleucine		85.34^{b}_{a} (±0.47)	85.50^{b}_{a} (±0.90)	87.80^{a}_{a} (±0.01)	88.07^{a}_{a} (±0.25)
Leucine		87.31^{b}_{a} (±0.11)	87.54^{a}_{a} (±1.15)	89.27^{a}_{a} (±0.00)	89.27^{a}_{a} (±0.05)
Lysine		90.50^{b}_{a} (±0.30)	90.60^{a}_{a} (±1.08)	91.80^{a}_{a} (±0.01)	92.20^{a}_{a} (±0.25)
Methionine		84.08^{a}_{a} (±0.39)	84.02^{a}_{a} (±1.70)	85.67^{a}_{a} (±0.45)	86.45^{a}_{a} (±0.31)
Phenylalanine		86.94^{b}_{a} (±0.18)	87.09^{b}_{a} (±0.55)	89.01^{a}_{a} (±0.02)	89.00^{a}_{a} (±0.22)
Threonine		88.37^{b}_{a} (±0.31)	88.44^{a}_{a} (±0.47)	89.68^{a}_{a} (±0.02)	89.98^{a}_{a} (±0.49)
Valine		86.13^{b}_{a} (±0.24)	86.04^{b}_{a} (±0.43)	88.21^{a}_{b} (±0.02)	88.82^{a}_{a} (±0.08)
Alanine		85.80^{b}_{a} (±0.06)	85.84^{b}_{a} (±0.95)	88.71^{a}_{a} (±0.19)	88.86^{a}_{a} (±0.07)
Aspartic Acid		81.04^{a}_{a} (±0.44)	80.84^{b}_{a} (±0.59)	82.25^{a}_{b} (±0.09)	83.39^{a}_{a} (±0.27)
Cystine		78.78^{b}_{a} (±0.21)	78.30^{a}_{a} (±1.04)	82.04^{a}_{a} (±0.29)	82.25^{a}_{a} (±1.86)
Glutamic Acid		86.94^{b}_{a} (±0.03)	87.09^{b}_{a} (±0.62)	88.54^{a}_{a} (±0.00)	88.74^{a}_{a} (±0.07)
Glycine		77.60^{b}_{a} (±0.21)	77.91^{b}_{a} (±0.70)	80.49^{a}_{b} (±0.25)	85.15^{a}_{a} (±0.14)
Proline		79.91^{b}_{a} (±1.37)	79.69^{b}_{a} (±0.24)	82.65^{a}_{b} (±0.03)	82.97^{a}_{a} (±0.08)
Serine		80.20^{a}_{a} (±0.66)	80.01^{a}_{a} (±1.54)	82.40^{a}_{a} (±1.33)	82.34^{a}_{a} (±0.24)
Tyrosine		85.60^{a}_{a} (±0.67)	85.60^{a}_{a} (±0.58)	87.38^{a}_{a} (±0.29)	87.76^{a}_{a} (±0.62)
Average Digestibility (%)		85.07	85.06	87.12	87.68

For each treatment, values in same row with same superscript are not significantly different at p = 0.05.

For each type of tank, values in same row with same subscript are not significantly different at p = 0.05.

significant ($p<0.05$). For the second treatment (6.8mg O_2/l and 4.0mg O_2/l for the control and the experimental tanks, respectively) only the digestibility values of arginine, isoleucine, phenylalanine, valine, alanine, aspartic acid, glutamic acid, glycine and proline were significantly ($p<0.05$) higher in the experimental tanks than in the control tanks (Table 3.61).

A slight general increase in AAAD was observed in the experimental tanks when the dissolved oxygen level was decreased. However, only in the case of the AAAD values for valine, aspartic acid, glycine and proline was the increase significant. In the control tanks no significant differences in AAAD were observed between treatments.

4 - DISCUSSION

Rainbow trout, probably the first intensively cultured fish, is still increasing in importance (from around 300 thousand tons in 1985 to around 500 thousand tons in 1989, FAO, 1991). This growth has been boosted in recent years due to the decrease of coastal fishery resources and depletion of wild fish stocks. In order to support this growing industry, and also to address the problems of pollution from commercial farms, there is a need for cost-effective diets which will give maximum growth and minimum waste. Despite this, to date there has been no long term study on the bioavailability of nutrients from commercial feeds.

The net energy of a food is the amount available for maintenance, activity and growth and depends on the amount lost in faeces and on its heat increment or "specific dynamic action". These losses form part of an energy budget, the components of which are well established in terrestrial animal nutrition (Petrusewicz and MacFayden, 1970). In animal feeding tables, energy requirements most commonly refer to net energy. The most useful measure of food energy value would thus seem to be net energy, since requirements and supply can then be directly equated. However, most tables report feed energy as digestible energy (i.e. gross energy minus faecal energy) or metabolisable energy (i.e. gross energy minus faecal and non-faecal excreted energy) mainly because it is easier to measure than net energy. The principal complication, however, is that net energy can vary depending upon the function (maintenance, growth, lactation) for which metabolisable energy is being used.

In fish, digestible energy (DE) seems to be the most convenient measure of energy availability, mainly because of the difficulty of determining metabolisable energy

(ME) at zero nitrogen balance. ME determination in fish requires collection from the water of both kidney and gill excretions. Although metabolism chambers have been constructed which are capable of doing this and ME values have been published for a limited number of trout diets (Smith, 1971, 1979; Smith *et al.*, 1980), the procedure is time-consuming and fish are stressed as a result of being force-fed and confined in narrow tubes. Physiological stress affects the nitrogen balance of fish (Smith, 1971) and this in turn affects ME. ME is affected by level of feeding and also varies according to the amount of amino acids retained for protein synthesis and the amount deaminated and excreted as ammonia and urea. Several authors have suggested that ME should be abandoned and DE preferentially used as a measure of the availability of food energy in nutrition and production studies with fish (Cho *et al.*, 1982, Jobling, 1983; Kaushik, 1989a). Digestibility is the single most important factor determining the availability of energy from dietary nutrients since the energy availability of a nutrient depends more on the amount that is absorbed (i.e. its digestibility) than the amount that is subsequently metabolised (Hastings, 1969; Cho and Slinger, 1979a; Austreng, 1978; Smith, 1979; Smith *et al.*, 1980).

Based on this background, the present study was developed to investigate feed digestibility in rainbow trout, both in the laboratory and in long-term monitoring of farm practices. Two commercial trout farms with different characteristics and at two locations in Portugal were selected to monitor trends in digestibility over a one year period. Trutorão Trout Farm in the centre of Portugal close to the southern limit for trout distribution in the Northern hemisphere (Figure 2.1) is supplied with water from a spring and has a very small mean temperature variation throughout the year (Table

2.1). Inha Trout Farm, located in the north of Portugal (Figure 2.1) is supplied by river water and consequently the normal temperature variation throughout the year is quite wide (Table 2.3).

Both farms used available commercial feeds to which no marker had been added, and so dietary crude fibre, inherently present as a natural component of the feeds, was used as the digestibility marker to measure the apparent digestibilities of crude protein, organic matter and dry matter of three size groups of rainbow trout. Since under a practical situation little control is possible of those factors affecting digestibility, laboratory trials were conducted to investigate the influence of various factors on digestibility and to corroborate the field work. Because the laboratory trials mirrored many of the field observations, the results are discussed together considering the important influencing factors on digestibility.

In the field survey, fish were categorised by average total length rather than weight as no suitable balance was available on a regular basis at either farm. Length/weight data collected at the start of the field survey on both farms were close to those published by BP Nutrition and Cipasa Nutrition. Thus, the small, medium and large size groups sampled were equivalent to fish of average weights - 40g, 100g and 180g, respectively. These size classes cover the typical range of cultured trout. Any smaller size class would have presented technical problems in obtaining faecal samples big enough to allow proximate analysis of the constituents.

From Tables 3.3 and 3.8 and Figures 3.1 to 3.6 it is clear that the digestibility

coefficients for the three size classes of fish follow the same trend, within each farm. Although there are some significant differences within each digestibility coefficient for the three size groups (Tables 3.3 and 3.8), the influence of fish size on digestibility is neither clear nor consistent, possibly reflecting the interactions of other factors. In order to determine whether there was any real difference in digestibility with fish size, three different size groups (40g, 100g and 180g) of fish were studied under controlled laboratory conditions. A significant increase in digestibility was observed with fish size for the majority of the amino acids. For protein, dry matter and lipid, the trend also was for digestibility to increase, and this increase was significant when crude fibre was taken as the dietary marker although there was a non-significant inverse relationship between fish size and organic matter and proline digestibilities (Tables 3.17 and 3.18). Generally, these size-related increases or decreases were small (1-5%) except for lipid digestibility (around 10%) and for cystine digestibility (higher than 10%).

The explanation for size-related change in digestibility may relate to the development of enzymatic activity with fish age. Proteolytic and amylolytic activities of rainbow trout in its first developmental stages are lower than in latter stages (Kitamikado *et al*, 1964 a,b) and this may therefore affect digestibility coefficients. Kitamikado and Tachino (1960b) and Kitamikado *et al.* (1964a,b) working with rainbow trout at several growth stages found that digestibilities of casein and protein from fish meal and beef liver were much lower for fish under 6g body weight than for larger fish (100g). Windell *et al.* (1978b) designed a 3 x 3 factorial experiment with rainbow trout to ascertain the effects of body weight (18g, 207g and 586g) and water

temperature (7°C, 11°C, and 15°C) on the digestibility of a dry pelleted diet (39% crude protein and 8% lipid). They showed that, in spite of a slight increase of the digestibility coefficients for total dry matter, crude protein, crude lipid and gross energy with fish size within each experimental temperature, no significant differences were apparent with body size at 11°C and 15°C. However, at the lowest temperatures their results show a difference in digestibility between small rainbow trout (18g) and larger ones, substantiating the results of Kitamikado *et al.* (1964a,b), and those in this study.

Gomez-Jarabo *et al.* (1979) looked at age and weight as factors in the utilization of protein by rainbow trout using three different size groups (10g, 50g and 100g) at a constant temperature (12°C). They concluded that slight increases in apparent digestibility values with fish size, for the protein level tested (37.5% casein) were not significant.

Ferraris *et al.* (1986) attributed the increase in digestibility with fish size (60g and 175g) for casein, fish meal and soybean meal in milkfish to the relative increase in intestinal length, thus prolonging the time food is exposed to the action of the digestive enzymes. Ulla and Gjedrem (1985) found a positive correlation between fat and protein digestibilities and the length of the intestine of rainbow trout. Rajamani and Job (1976) working on *Tilapia mossambica* also noted that absorption efficiency was directly related to fish size. Recently Watanabe *et al.* (1989) determined the digestible crude protein of various feedstuffs and compounded diets with carp, tilapia, ayu and rainbow trout of different sizes and concluded that digestibility of protein was

not greatly influenced by fish size in the range 2-200g. Several researchers (Jobling *et al.*, 1977; Flowerdew and Grove, 1979) reported that gastric evacuation rate varies exponentially with fish weight. However, larger fish take more time to evacuate a given meal (as % body weight) than smaller fish, thus, allowing more contact time between enzymes and substrate and consequently influencing digestibility.

There is variation in the literature, but the general trend and the data from this study suggests that digestibility does increase with fish size. In the fish size range used in this study, fish do not go through any major morphological or physiological transformations. However, any change in both enzyme activity and food residence time in the gut due either to an increased transit time or longer intestine would explain the increases in indigestibility with fish size.

As mentioned earlier, the bigger increases in digestibility obtained were for lipids and for the amino acids, especially that of cystine. According to Dabrowski and Dabrowska (1981) the amino acid pattern of faeces is determined primarily by the excretion of endogenous nitrogen and the average amino acid composition of faeces can be used with a minimal error to calculate true digestibility. Thus, the around 5% general significant increase in amino acid digestibilities with fish size seems to indicate a decrease of endogenous nitrogen excretion. If so, this would also explain differences in digestibility of protein with fish size. Three size groups of fish were used simultaneously, allowing comparison of the different digestibility coefficients under the same controlled laboratory conditions. However, further studies on the influence of fish size on digestibility should consider following the same cohort of fish

through time and thus evaluate digestibility as fish grow. This would better mirror a practical situation. A better digestibility with fish size means more energy available for any function, such as growth, as fish become older. Since growth rate decreases as fish grow, further adjustments could be made to currently used feeding tables to allow for better digestibility and thus reduce food distributed and consequently reduce production costs, and also solid wastes and their negative impact on the environment.

During the farm trials, the formulation of the diet changed and this was especially reflected in the lower levels of dietary crude fibre from March onwards (Table 3.1). Significant relationships were obtained in this study between the digestibility coefficients and the crude fibre level (Tables 3.4, 3.10, 3.11 and 3.12). The digestibility coefficients obtained at Inha Trout Farm in general were lower than those at Trutorão Trout farm, especially after the change in feed formulation (Tables 3.3 and 3.8 and Figures 3.7 and 3.8), possibly also reflecting the higher levels of crude fibre of the feeds used at Inha Trout Farm (Table 3.6).

The literature on fish nutrition provides some evidence of a relationship between digestibility and dietary fibre content. Thus, increasing the level of fibre in diets was found by several authors to reduce feed digestibility (and growth rates) in several species of fish (Buhler and Halver, 1961; Hilton *et al.*, 1983; Hilton and Slinger, 1983, 1986; Bromley and Adkins, 1984; Anderson, 1985; Anderson *et al.*, 1991; Hanley, 1987; Dade *et al.*, 1990). Crude fibre is the organic insoluble material, mainly cellulose and to a lesser extent hemicellulose and lignin, that remains after treating the samples with dilute sulphuric acid and dilute sodium hydroxide. Even though fibre

does not represent any definite chemical fraction, it is the officially required measurement in many countries and thus it is used in the existing feed tables.

The cell walls of plants contain cellulose, hemicellulose and lignin and are resistant to the alimentary canal secretions of fish (Van Dyke and Sutton, 1977; Stickney and Shumway, 1974; Stickney, 1976; Lindsay and Harris, 1980). In contrast, plant cell contents (which include soluble carbohydrates, organic acids, lipids, protein and starch) are more digestible. The dry matter digestibility of plant material would therefore appear to depend on the amount of refractile material present, the ability of fish to rupture plant cell walls, and possibly the processing methods used in feed manufacture. For example, starch is more available in extruded pellets than in the same diet when steam pelleted (Hilton *et al.*, 1981). This is because more heat is generated when expanded pellets are manufactured by extrusion, and the starch is cooked to a certain extent. Cooked starch is partially hydrolysed and as such is more digestible than raw starch: 80% versus 38% (Nose, 1967b).

With carbohydrates, digestibility depends largely on the size and complexity of the molecule, monosaccharides being readily absorbed across the gut and having digestibilities of 92-99%, whilst a complex polysaccharide such as raw starch may have a much lower digestibility of 38% (Philips *et al.*, 1948).

The relative proportions of carbohydrates, protein and fat in a diet affects digestibility because interactive effects may occur. Thus, Kitamikado *et al.* (1964b) showed that

the protein digestibility of diets fed to trout depends on the level of potato starch present in the diet. This has been supported by other workers and it is now generally accepted that the total digestibility of a diet is negatively correlated with its starch content (Inaba *et al.*, 1963b; Singh and Nose, 1967; Beamish and Thomas, 1984, Kim and Kaushik, 1989). The level of a nutrient in a diet can also affect its own digestibility. Thus, in diets fed to trout, the digestibilities of starch and dextrin decreased with increasing levels of dietary inclusion (Singh and Nose, 1967).

In general, feeds based on animal products are more efficiently digested by fish than those of plant origin. Kitamikado *et al.* (1964a) observed that raw meat (fish, beef liver) had protein digestibilities of 91-97%, whilst the value of soybean meal was only 70%. In a later study of 55 feedstuffs for rainbow trout, protein digestibilities were reported to range as widely as 32-91% (Smith, 1980).

From the foregoing, it can be seen that the change in formulation which occurred during the field survey which had an important effect on digestibility values of all groups of fish sampled at Trutorão Trout Farm (Figures 3.1 to 3.3) could have been based on a change of the feed processing methods, or change in the source or type of carbohydrates used, or even a change towards using more animal products in the formulation as compared to plant products. As expected, the feed manufacturers were using a undivulged formula and thus no information could be obtained about their feed constituents or changes introduced to their formulations. However, it is suspected that they used a different type of carbohydrate after the publication of a paper comparing a Portuguese commercial diet to two high protein diets incorporating 30% of either

gelatinized or natural starch (Kaushik and Oliva-Teles, 1985). The authors concluded that the incorporation of 30% gelatinized starch in the diet enhanced nitrogen and energy retention by trout mainly through its effect on digestible energy. Thus, a protein sparing effect was achieved and consequently a reduction in nitrogenous metabolic wastes, thus increasing the utilization of protein for growth rather than for energy purposes.

Rainbow trout can utilise high levels of dietary fat very efficiently as a source of energy (Austreng, 1979; Austreng *et al.*, 1979). Many factors have been described as influencing fat digestibility and fatty acid chain length and number of double bonds, the relationship between saturated and unsaturated fatty acids, fat intake and the content of other nutrients in the feed are among them.

Nose (1967b) showed that digestibility of lipids depends on their composition and saturation level; it decreased with increases in number of carbon atoms in the fatty acid chain and increased with the number of double bonds. Also working with rainbow trout, Austreng *et al.* (1980) showed that digestibility of total lipids and fatty acids decreased with increasing melting point, thus, soybean oil, cod liver oil and capelin oil were efficiently digested, while hydrogenation of capelin oil resulted in decreased digestibility. The digestibility of saturated and unsaturated fatty acids was similarly influenced by hydrogenation. They also observed a decrease in digestibility of individual fatty acids with increasing chain length, but only up to C18 and longer chain length (up to C22) were more digestible.

Watanabe *et al.* (1979) showed that an increase in dietary lipid level from 5 to 23% increased the total digestibility of the diet by rainbow trout. They attributed this increase to an increase in the digestibilities of protein (from 98.4% to 98.9%) of the carbohydrates (from 50.7% to 58.5%) and also of the lipids themselves (from 74.7% to 87.5%).

In the field survey a significant positive correlation was obtained in some cases between the dietary lipid level and the organic matter digestibility (Table 3.12) coefficients. Under controlled conditions, dietary lipid levels (7%, 14% and 21%) had a marked effect on all digestibility coefficients (Table 3.38) and the best digestibility values were obtained with the 21% lipid diet.

Diets with increased fat levels (higher than 15%) were shown to decrease gastric evacuation rate in rainbow trout (Windell *et al.*, 1969, 1972; Jobling, 1980). These authors found that percentage of gastric evacuation, expressed as ash-free dry weight, varied considerably for different kinds of experimental meals fed to rainbow trout. Twelve hours after feeding, 65% of a commercial diet (28% protein, 6% fat) had been evacuated from the stomach, with corresponding values of 50.8% for gelatin, 47.3% for a gelatin and corn oil mixture (75%:25%), 42.6% for corn oil and 28.8% for pure fat (lard). Values for the energy content of each of these diets were not reported, but from the known calorific values for fat, carbohydrate and protein, it is apparent that the rate of evacuation of the various diets is inversely linked to their energy content. In fact, Grove *et al.* (1978) diluted a commercially available diet with known quantities of kaolin and determined the gastric evacuation time by X-radiography in

rainbow trout. They observed a decrease in gastric emptying time with increasing levels of dilution, which led to an increased frequency of feeding. Thus any reduction in evacuation rate will increase the time of contact between enzymes and substrate and thus favour better digestibilities.

The presence of dietary fat may delay gastric emptying possibly due to the release from the intestinal wall of a hormone similar to enterogastrone which inhibits gastric mobility in mammals (Windell, 1967, 1978; Brett and Higgs, 1970) or changes in activity of extrinsic nerves which supply the gut (Fänge and Grove, 1979).

There was no difference between the apparent crude lipid digestibility coefficients of fish fed Diet 2 (14% lipid) and Diet 3 (21% lipid), although there was still a general improvement in other digestibility coefficients with increasing dietary lipid level from 14% to 21% (Table 3.38). This seems to support the work of Watanabe *et al.* (1979) who explained the improvement on total digestibility of a diet with increasing lipid level by the improvement of the digestibility of the different individual nutrients. In addition, Takeuchi *et al.* (1978a) did not find significant growth differences among fish receiving diets containing more than 10% lipid (in a range of 5-20%) and suggested that the addition of approximately 10% lipid to high protein (54% casein) diets was sufficient to maintain normal fish growth. Furthermore, growth has been shown to be linearly correlated to digestible energy (Anderson, 1985).

In contrast to carbohydrates which are poorly digested by carnivorous fish, protein and fat are very well digested (Cho and Slinger, 1979a; Austreng and Refstie, 1979;

Rychly and Spannhof, 1979; Cho and Kaushik, 1985). Dietary protein is both a "protein-yielding" and "energy-yielding" nutrient and its digestibility determines the potentially available amount of amino acids and energy for utilization by the fish. However, if a feed contains an excessive amount of protein in relation to the amount of energy (35% protein and 15-20% lipid were found to be the optimum ratio for rainbow trout diets, Takeuchi *et al.*, 1978a,b; Watanabe *et al.*, 1979), the excess protein will be used as an energy source instead of for growth, which is certainly neither the most rational from a fish producer's point of view, since protein is the most costly factor in the formulation of feeds, nor from an ecological viewpoint, because it increases the amount of ammonia excreted to the surrounding water. Cho *et al.* (1976) stated that although very high protein levels are used, especially in experimental diets for rainbow trout, they confirm that limitations are not imposed by any inability to assimilate the presented protein.

Low, but significant correlations (r=40-62) were obtained between all the digestibility coefficients and the dietary protein level for all the three size groups of fish in the year survey (Table 3.12). In the laboratory trials, however, only at 21°C (Trial B) was there a marked effect of dietary protein level on the apparent digestibility coefficients with significantly higher digestibility values for fish fed the higher (45%) protein diet (Table 3.31). These results corroborate the work of many workers who found increased protein digestibility with increasing dietary protein level (Inaba *et al.*, 1962; Nose and Mamiya, 1963; Nose, 1967b; Austreng and Refstie, 1979; Kaushik, 1980; Beamish and Thomas, 1984). They considered that the proportion of metabolic faecal nitrogen in faeces would be greater in fish fed low protein diets than from fish fed

high protein diets. Furthermore, the protein quality was improved and the content of nitrogen-free extracts was decreased with increasing protein content of the diet and thus, both these factors would also lead to a higher digestibility. However, Oliva-Teles and Rodrigues (1991) obtained a lower non-faecal nitrogen excretion both at 15°C and 21.5°C with a low protein diet (30% crude protein) which resulted in a better apparent nitrogen retention in trout fed the lower protein diet as compared to a higher protein diet (45% crude protein). By contrast, other authors have found no differences in digestibility with dietary protein inclusion level (Jauncey, 1982; Ash, 1985; Mundheim and Opstved, 1989) as in general has happened during this study in Trial A (15°C and 22°C, Table 3.30) and at 10°C in Trial B (Table 3.31). In Trial A, the protein digestibility values (Table 3.30) were surprisingly low, especially when compared to the values obtained at 10°C (Table 3.31) for both diets and with the total amino acid digestibility values within the same trial (Table 3.32). Low digestibility values have been reported for diets which during the drying process were subjected to elevated temperatures as well as for diets containing fish meal in which oxidative deterioration of oil has occurred. This oxidised meal had decreased unsaturated fatty acids. Also oxidised oils were shown to react with the protein in fish meal (Nose and Toyama, 1966; Nomura *et al.*, 1972).

It is not known if any anomaly occurred during the drying process of the experimental diet and/or during the storage of the dietary ingredients prior to formulation and manufacturing which would lead to the lower digestibilities obtained.

Feeding frequency (1,2 or 4 meals per day) did not seem to influence digestibility

(Table 3.49) and this is supported by the work of Hudon and de la Noüe (1984) and Choubert *et al.* (1984) who also used a commercial diet to which chromic oxide was added as a digestibility marker. They demonstrated that feeding frequency (1,4 or 6 meals per day) had no effect on the digestibility values for protein, organic matter or energy. They attributed their results to a constant gastric evacuation rate, irrespective of feeding frequency. Hudon and de la Noüe (1984) used a fixed feeding rate (2% body weight) at all the feeding frequencies in contrast to the present study, where fish were fed *ad libitum* and thus meal size (% body weight) was not the same for all the feeding frequencies (1,2 or 4 times/day) tested (Table 3.47). In fact, a slight increase in daily meal size was registered with feeding frequency (Table 3.47), although Possompes *et al.* (1975) and Grayton and Beamish (1977) only obtained increased feeding levels up to two meals daily when fish were fed *ad libitum*. At higher frequencies (3 for the former authors and 3,5 and 6 meals per day for the latter authors) no differences in total daily food intake were observed. Nevertheless, recently Storebakken *et al.*,(1991) found no tendency for reduced apparent digestibilities of nitrogen and energy with increase feeding levels (0.3%, 1% and 2% of body weight).

The possible effect of feeding level on digestibility may originate from the digestion process. The stability of digestibility coefficients with increased amounts of food obtained in this study and by others (Storebakken *et al.* 1991; Storebakken and Austreng, 1987) might result from two factors: a) increased enzyme secretion with increased food uptake into the stomach and intestine (Western and Jennings, 1970); b) longer retention of food in the gut with increasing feeding levels (Windell *et al.*, 1969, 1978b; Jobling *et al.*, 1977). However, both increased enzyme secretion and

food retention seem not to be linearly related to the amount of food eaten, thus digestibility may become lower when large amounts of food are ingested (Hepher, 1988) as observed by Windell *et al.* (1978b) who found that fish fed 1.6% of their body weight per day showed significantly lower digestibility values for dry matter, carbohydrates and energy than fish fed 0.4% or 0.8% body weight.

The time of day at which faecal samples were collected influenced digestibility and a general significant decrease in digestibility was observed between 6 hours and 22-26 hours after the last meal was fed. Daily changes in the composition of the faeces have been reported which consequently imply changes in the ratio of nutrients in the faeces to those in the diet. Furthermore, because of the temperature dependent gut transit time faeces collected at the beginning or at the end of the digesta residence time may be different in composition (Choubert, 1983). These points considered with the bell shaped excretion curve for fish fed two or more meals per day obtained by Possompes *et al.* (1975) could explain the differences in digestibility related to time after feeding. Thus, when reporting digestibility results faecal collection time relative to meal time should be referred to to allow better comparison of results.

Most organisms will tolerate a certain range of temperatures, above and below which temperature becomes lethal or sub-lethal, the organism becomes "disorientated", normal activities are inhibited and it dies after a shorter or longer period of time. One of the most widely adopted methods for comparing the magnitude of the effect of temperature on the velocity of different rate processes such as chemical reactions or physical or biological processes is the Q10 approximation, which is the factor by

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which the velocity of a rate process is increased for a rise in temperature of 10°C (Duncan and Klekowski, 1975; Fry, 1978; Hepher, 1988).

The effect of temperature is complex. On one hand it affects enzyme activity and transport across the intestinal wall, while on the other hand it is balanced by the effect on the time the food stays in the digestive tract (Kitamikado & Tachino, 1960b; Morishita *et al.*, 1964; Stokes & Fromm, 1964; Brett & Higgs, 1970; Escoubert *et al.*, 1974; Fange & Grove, 1979; Ross & Jauncey, 1981; Hepher, 1988). The rate of food passage through the digestive tract increases with increasing temperature, thus decreasing the exposure time to the digestive enzymes. When the temperature approaches the upper physiological limit of the species that trend may cease or even reverse. Possompes *et al.* (1975) found that in rainbow trout the mean transit time (MTT) and thus total retention time decreased from 35h to 17h when the temperature increased from 9°C to 18°C. However, a further increase in temperature to 20°C caused an increase of the MTT to 20.5 hours. So a longer time for enzyme action was available at high temperatures, thus favouring higher digestibility values.

Figures 3.1 to 3.6 clearly show that digestibility increased as temperature rose. However, the clear seasonal trend observed until June was less clear when the water temperature increased above the optimum of 15°C for rainbow trout (NRC, 1982; Cho & Slinger, 1979b; Sumpter, 1992) in July. In these conditions, digestibility was depressed and the most obvious factor in this is the low levels of dissolved oxygen associated with the high temperatures.

In the two separate laboratory trials conducted to examine the effects of temperature and dietary protein level, an increase in digestibility of around 10% or more was obtained between the lower (10°C or 15°C) and the higher (21°C or 22°C) experimental temperatures (Tables 3.23 and 3.24). These increased digestibility values at the higher temperatures contrast with the more modest values found in the literature, which show slight increases in digestibility of only about 1-3% (Choubert *et al.*, 1982; Cho & Slinger, 1979a; Watanabe *et al.*, 1989).

The digestive processes (digestion, transit, absorption) can be stimulated by either an abrupt or prolonged increase in temperature (Choubert *et al.*, 1982a). However, the consequences seem variable and very few systematic studies have been carried out on the effect of temperature on digestibility coefficients. Early papers by Krazinkin (1935, 1952) quoted by Choubert *et al.* (1982a), referred to an increase of 18% of the dry matter digestibility when the temperature increased from 10°C to 13°C in common carp, *Cyprinus carpio*, although the digestibility of protein remained unchanged. In contrast, Windell *et al.* (1978b) found no difference in the dry matter and energy digestibilities of rainbow trout at 7°C and 15°C but observed a higher nitrogen digestibility at 15°C than at 7°C. Cho & Slinger (1979a) working with rainbow trout showed that the digestibility coefficients for dry matter, energy, fat and protein of a compounded diet did not vary within the temperature range of 9-15°C but did increase slightly (2-3%) at 18°C. Possompes (1973) and Luquet & Fauconneau (1979) found no differences in trout protein digestibility between 10°C and 16-17°C. Watanabe *et al.* (1989) determined the digestible crude protein of various feedstuffs and compounded diets with carp, tilapia, ayu and rainbow trout at three temperatures (5°C,

10°C and 15°C, for rainbow trout) and within each species they found a slight increase (1-2%) in protein digestibility with temperature. Nevertheless, they concluded that the digestibility of protein was little affected by water temperature.

Luquet & Fauconneau (1979) reported a higher endogenous nitrogen excretion at high temperature than at low temperature, which might explain why some of the above cited studies did not find a significant difference of protein digestibility at different temperatures.

During the field monitoring, water quality parameters did not vary greatly with the exception that at Inha Trout Farm, especially in the silos where the 20cm and 25cm fish were weaned, the suspended solids were very high. The solids were also kept in suspension continuously due to the flow regime in the tanks, where water entered the silos from the bottom and exited through the top (personal observations). This high suspended solids load would certainly increase stress in the fish (Ross *et al.*, 1985). The smaller fish were grown in raceways and from direct observations the water was considerably cleaner. The suspended solids load and the higher temperatures in the summer/autumn could have worked together to depress digestibility, especially in the 20cm and 25cm fish, which had significantly lower digestibility coefficients than the 15cm fish between July and September (Table 3.8).

The lowering of the oxygen content of water is known to be an important stressor for fish, and rainbow trout become stressed when D.O. is below 6mg/l (Pouliot and de la Noüe, 1988, 1989; Medale *et al.*, 1987). Intensive fish farms which seek maximal

biomass production are especially prone to experience this type of stress during the summer. Nevertheless, very few studies have been published on the influence of hypoxia on nutrient digestibility in fish. In this study, both the influence of an abrupt fall in dissolved oxygen levels (Trial A) and the influences of a prolonged hypoxia (Trial B) on digestibility and food intake were studied.

In both cases, in general there was a significant decrease in food intake with decreasing D.O. level (Tables 3.53 and 3.54 and Figure 3.18). Our results corroborate those of Pouliot & de la Noüe (1988, 1989) and those of Medale *et al.* (1985, 1987) who found that feed intake was lower in hypoxic conditions (40% saturation and 5.3mgO_2/l at 15°C, respectively for the former authors and the latter authors) as compared to normoxic fish (89% and 8.4mgO_2/l at 15°C, respectively). Medale *et al.* (1987) showed that the food consumption of hypoxic fish (5.3mgO_2/l at 15°C) dropped to about half that of the control fish (8.4mgO_2/l at 15°C) which agrees well with the results for equivalent D.O. levels in this study (Table 3.53).

When fish were subjected to an abrupt reduction in D.O. level (Trial A) they showed slightly higher digestibility coefficients than normoxic fish (Table 3.59). However, the differences were not significant. Further decreasing the D.O. level caused a slight increase in digestibility, although the differences were only significant between treatment 1 (7.2mgO_2/l) and the other treatments (Table 3.59). These results contrast with those of Pouliot & de la Noüe (1988, 1989) who did not find differences in the apparent digestibility coefficients for protein, gross energy or dry matter between the

experimental hypoxia level (40% saturation) used and the control level (89% saturation).

In contrast, when fish were subjected to prolonged hypoxia (Trial B), they had significantly higher digestibility coefficients for protein, dry matter (Table 3.60) and the majority of the amino acids (Table 3.61) than control fish, with the exception of dry matter digestibility for the lower ($4mgO_2/l$) experimental D.O. level. It is possible that the enzymatic and absorptive processes involved in digestion and absorption could be impaired by decreased D.O. level and as a consequence lead to depressed digestibilities. However, Nikinmaa & Soivio (1982) showed that hypoxia led to an increase in haemoglobin affinity to oxygen in rainbow trout and that the total quantity of oxygen in the blood would be only slightly lower than in normoxic conditions. Consequently, anaerobiosis is unlikely to be the principal explanation of impaired absorption.

Medale *et al.*'s (1985) work, studying the effect of long term (8 weeks) hypoxia on the ammonia excretion in rainbow trout, found that the hypoxic trout would not begin to eliminate ammonia until three hours after feeding, whereas the ammonia excretion in normoxic trout increases immediately after ingestion of a meal. The authors attributed the difference to a slower gut transit time under hypoxia, giving a longer contact time between enzymes and substrate and consequently better digestibility coefficients. In Medale *et al.* (1985), no relationship between food intake level (meal size) and gut transit time was established. However, the influence of meal size (as % body weight) on gut transit time of the digesta through the gastrointestinal tract has

been demonstrated by many researchers (Beamish, 1972; Jobling *et al.*, 1977; Windell *et al.*, 1969; Hepher, 1988) and ingestion of large meals has been shown to impair digestibility (Windell *et al.*, 1978b).

This seems to have been the case in this study, since significant high inverse correlations between food intake and the digestibility coefficients were obtained when fish were subjected to prolonged hypoxia (Figures 3.20 and 3.22).

The acceptance of indicator methods has considerably reduced the effort involved with digestibility studies and since, in 1918, Edin (Edin *et al.*,1944) proposed the use of chromic oxide as an inert reference compound in digestibility determinations with livestock, this indicator has been commonly and successfully used for digestibility studies with fish (Nose, 1960; Inaba *et al.*, 1962; Smith & Lovell, 1973; Cho *et al.*, 1974, 1976; Lall & Bishop, 1976; Austreng, 1978; Windell *et al.*, 1978a,b; Jauncey, 1982; Lied *et al.*, 1982; Tacon & Rodrigues, 1984; Nose, 1989). Nevertheless, the validity of using chromic oxide as an indicator has been questioned (Bowen, 1978a, Hanley, 1987) in herbivorous fish. The use of markers in digestibility studies implies the need for specially prepared experimental diets. Thus, the ability to use a substance which already exists in the feed, i.e. an internal marker, is a distinct advantage in digestibility studies as it is less costly, especially in large scale trials.

The almost universal occurrence of crude fibre as a natural indigenous marker within pelleted fish feeds offers a valuable tool to nutritionists and fish farmers alike for estimating feed digestibility. Thus, during the long term evaluation of digestibility

coefficients in this study, crude fibre was the dietary marker used and generally both chromic oxide and crude fibre were compared as dietary markers in the laboratory studies. In general, when compared statistically, both markers behaved differently. However, the variation was not consistent and in some cases digestibility values were higher when based on chromic oxide, whilst in other cases they were higher when based on crude fibre (Tables 3.17, 3.18, 3.38, 3.39, 3.43, 3.44 and 3.49 and Figures 3.12, 3.15, 3.16 and 3.17).

Due to the relationships between the components of Maynard and Loosli's (1969) equations to calculate digestibility, the higher the concentration of the indicator in the faeces relative to that of the feed, the greater is the estimation of the apparent digestibility. Hence if faecal indicator concentrations are artificially enhanced by factors unrelated to the digestive process, then estimations of digestibility will also be artificially enhanced. Thus, high digestibility values can be due to:

(i) a faster or slower transit time of the marker compared with the nutrient in the digesta. For example, different particulate fractions within the digesta may pass through the gastrointestinal tract at different rates, depending on their physical and chemical characteristics, including density, surface area, particle size and affinity for water (Hydén, 1960; Kionka & Windell 1972; Mugdal *et al.*, 1982; De Silva & Owoyemi, 1983).

(ii) nutrient and marker selection by fish on ingestion (Bowen, 1978a; Block *et al*, 1981; Hanley, 1987)

(iii) variation in the excretion pattern of the marker through the gut (daily and/or seasonal variations) Furuichi & Takahachi (1981).

There may be some support for this from the results obtained during this study looking at the influence of time after feeding, where all nutrient and amino acid digestibility coefficients were lower when based on chromic oxide than on crude fibre, except the amino acid digestibility values after 22 and 26 hours (Tables 3.43 and 3.44 and Figure 3.16). Possompes *et al.* (1973, 1975) showed that the concentration of chromic oxide in the faeces of rainbow trout when receiving only one meal per day was maximal 14h after feeding, and this level was maintained for about 24 hours, whereas in fish fed two or three meals per day the maximal concentration of chromic oxide in the faeces was recovered 16 and 12 hours after feeding the first meal, but the excretion curve was bell shaped. Thus the marker concentration reaches a maximum and then decreases within a day. On the other hand, faecal emission was not influenced by feeding frequency. Thus the ratio between marker and nutrient in faeces will vary with feeding frequency and consequently the estimation of digestibility changes. Although in this study faecal samples were collected four hours after the last meal, the differences in chromic oxide concentrations with feeding frequency might explain why nutrient digestibility coefficients for fish fed once a day (Table 3.49 and Figure 3.17) were higher when based on chromic oxide than on crude fibre as dietary markers. For fish fed more frequently, the converse was the case.

Very little is known about the physiological role of dietary fibre in aquatic species (Bromley & Adkins, 1984, Shiau, 1989). The presence of a "filler" is known to speed up the rate at which food is evacuated from the stomach (Grove *et al.*, 1978, Flowerdew & Grove, 1979; Hilton *et al*, 1983).

Shiau (1989) reported that tilapia fed diets containing 10% of different types of dietary fibre (cellulose, guar gum, agar, carboxymethylcellulose) showed slower carbohydrate absorption rates in the gut as compared to the control fish fed dextrin diets (30%). The same author also referred to the binding capability of the dietary fibre in the gut. In fact, in rats fibre has been reported to bind nutrients, including protein, lipid (Shah *et al.*,1982) and minerals (Ward & Reichert, 1986) resulting in low digestibility estimates. Another mechanism by which cellulose has been reported to be responsible for is that of physically obstructing enzyme action and diluting nutrients (Leary & Lovell, 1975).

However, any of the mentioned physiological effects of fibre, assuming they occur in the rainbow trout's gastrointestinal tract, would influence either the digestive/absorption processes or the time food stays in the gut, thus, as a consequence they should influence digestibility estimates either when based on crude fibre or when based on chromic oxide as dietary markers.

Using two-way ANOVA, it was shown that, in general, chromic oxide and crude fibre behaved differently when used as dietary digestibility markers (Tables 3.19, 3.40, 3.45 and 3.50) since there were significant interactions between the markers and the biotic or abiotic factors studied, for the majority of the digestibility coefficients. However, bearing in mind the advantages of being able to use a specific part of the feed as a digestibility marker, it is important to know the magnitude of those differences. For example, the confidence intervals for the difference in the means for the apparent protein digestibility for the 40g, 100g and 180g fish estimated using chromic oxide or

crude fibre were smaller than 0.9%, 1.2% and 1.5%, respectively (Table 4.1). In addition, in the case of the 180g fish, the difference between the two means was not significant.

The confidence intervals for the difference in means for the apparent protein digestibility for other trials is also presented in Table 4.1. The differences between mean digestibility values based on Cr_2O_3 and crude fibre in general are of a similar magnitude as those obtained for the three size groups of fish. The same was the case for the other digestibility coefficients. Thus, it seems clear that the differences between the two dietary markers are small and thus crude fibre should be considered a useful tool for the estimation of feed digestibility, especially under practical farming conditions.

The fact that fish farms have an impact on the environment has been clear for a long time and the pollution of water bodies by fish farms effluents has been widely studied in recent years (Choubert, 1983; Beveridge, 1984; Merican & Phillips, 1985; Persson, 1988; Cho *et al.*, 1991; Kaushik, 1990; Pike *et al.*, 1990; Lall, 1991; Beveridge *et al.*, 1991). Interest has been focussed on the feed as a source of environmental influence and most authorities responsible for the regulation of fish farm effluents as well as some fish farmers base their assessment of pollution potential on measurements of the levels of certain compounds in the effluents. However, the concentration of a nutrient or element in a farm effluent at any given time is not particularly important or informative, since what is critical for the natural body of water which the farm

Table 4.1 - Confidence intervals (95%) for the difference in crude protein digestibility means between the two dietary markers (data normalised by arcsin transformation).

Trial		Cr_2O_3 $x(s^2)$	C. Fibre $x(s^2)$	Confidence Interval	t	d.f	p	
Fish size								
	40g	65.44 (0.01)	64.86 (0.05)	0.3-0.9	4.787	6	0.003	(s)
	100g	64.71 (0.09)	63.99 (0.08)	0.2-1.2	3.519	6	0.012	(s)
	180g	65.99 (0.13)	65.31 (0.33)	0-1.5	2.025	6	0.089	(n.s.)
Lipid level								
	7%	66.51 (1.12)	67.82 (0.02)	0-2.6	2.446	6	0.050	(n.s.)
	14%	66.40 (0.02)	67.56 (0.03)	0.9-1.4	10.284	6	0.000	(s)
	21%	69.54 (0.01)	68.26 (0.22)	0.7-1.9	5.346	6	0.002	(s)
Feeding Frequency								
	1x	63.11 (0.11)	62.32 (0.06)	0.3-1.3	3.791	6	0.009	(s)
	2x	62.69 (0.66)	64.23 (0.05)	0.5-2.5	3.634	6	0.011	(s)
	4x	62.71 (0.24)	63.51 (0.02)	0.2-1.4	3.143	6	0.020	(s)

effluent is entering is the total loading per unit time which will affect the progress of eutrophication (Cho *et al.*, 1991).

Quantification of waste output from fish culture operations can also be estimated by biological and nutritional procedures instead of the limnological and chemical analysis of effluent samples. The biological and nutritional approach is based on measurements of apparent digestibility coefficients (ADC) of feeds, ingredients or nutrients, nutrient retention efficiencies (NRE), particularly of nitrogen (N) and phosphorus (P) and the quantity of feed waste.

The first measurements (ADC) provide data on total solid waste (including suspended solids) and undigested nutrients excreted in the faeces, the second (NRE) estimates soluble wastes excreted through the gills and urine from growth trials and comparative carcass analysis, thus obviating direct and technically difficult collection of gill and urinary wastes. Non-faecal nitrogen excretion is not considered by some to be important on an individual basis, since average metabolic losses only represent about 2-7% of digestible energy (Rychly and Marina, 1977; Rychly 1980; Sayer and Davenport, 1987; Ross *et al.*, 1988; Kaushik, 1989a) depending on the diet fed. Nevertheless, bearing in mind the total feed input at an intensive farm site, non-faecal nitrogen output into the environment can be considerable. Feed waste in the form of uneaten food is another source of "man-made" waste which cannot be estimated accurately in the aquatic environment. Fortunately, it can be minimised by aiming at optimum rather than maximum production, by applying sensible feeding practices and by using palatable and well manufactured pellets of high water stability. Thus, undigested and to a lesser extent unutilised and wasted feed becomes the sole source

of aquaculture waste. Polluted water (especially ammonia and suspended solids) caused by fish farm effluents might also be detrimental to fish, causing stress. Stressed fish will in turn waste more food, causing further pollution. Furthermore, during the breakdown of dissolved and particulate organic matter and other waste materials, dissolved oxygen is consumed due to the biological oxygen demand (BOD) and the chemical oxygen demand (COD) from oxidation of ammonia to nitrate. Thus the higher the waste output the higher will be the BOD and COD and thus, lower D.O. will be available for fish respiration. In addition, low levels of D.O. directly impair food consumption and consequently uptake of energy, which will further impair growth.

From the foregoing, it is clear that a good understanding of the possible factors which influence digestibility will lead to increased, more soundly-based feed formulation and manufacture, resulting in better fish production with reduced impact on the surrounding environment. Figure 4.1 summarises the environmental and biotic factors which affect digestibility and hence waste output.

In this study, environmental factors, especially water temperature, were shown to have a marked effect on food intake and digestibility. The temperature effect on digestibility was most probably indirect, i.e. due to the temperature influence on digestive enzymes secretion and activity, gut mobility and uptake rate. Water dissolved oxygen (D.O.) was highly correlated to food intake and for one unit decrease of D.O. food intake decreased by about 0.5% or 0.25% body weight/day whether fish were subject to an abrupt decrease of water O_2 level or to prolonged hypoxia.

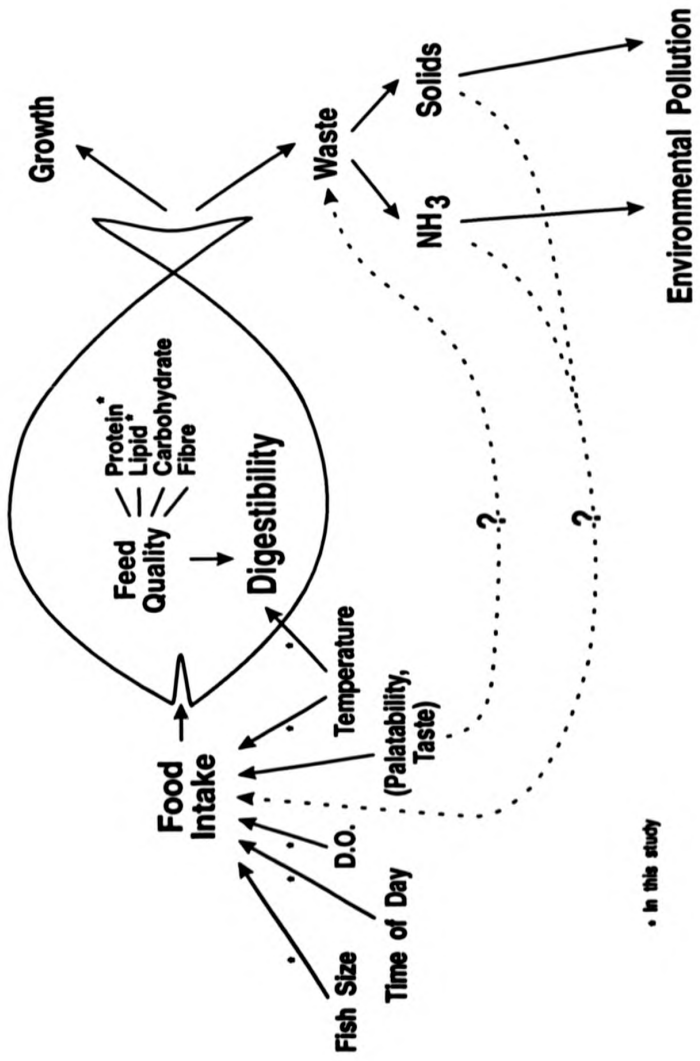

Figure 4.1 - Diagram of a biological and nutritional approach to the environmental impact of a fish farm.

Apparent digestibility was not influenced by feeding frequency. However, digestibility significantly decreased with time of day after the last meal was fed.

Apparent digestibility values increased as fish size increases (from 40g to 180g).

Feed quality in general affects digestibility and significantly higher apparent digestibility coefficients were obtained with increasing dietary lipid level (from 7% to 21%). Furthermore, highly significant inverse correlations were obtained between the apparent digestibility coefficients and the dietary crude fibre level in both forms.

Cohabitation of sustainable fish culture and cleaner waters can be enhanced by a number of strategies including, re-examining feeding strategies and techniques, introduction or redesigning of settling ponds. However, it is clear that adjustments in dietary formulation (quantitative and qualitative) which enhance digestibility also have major effects (Kaushik & Oliva-Teles, 1985; Cho *et al.*, 1989).

A fish feed is always a compromise between nutritional requirements, technological constraints and economy. The first step in making low pollution feeds is a more critical screening of raw materials for high digestibility. The biggest reduction in faeces can be achieved by utilising cooked instead of raw cereals (starch) which increases the digestibility of the carbohydrate fraction leading to a reduction in faecal output by up to 60%, according to Choubert 1983 and Dekker, 1986. Reducing the nitrogen component of the effluent can be achieved by increasing the availability of the amino acids and optimising the balance of available amino acids and the

protein/energy ratio in the diet. A significant reduction in phosphorus, apart from an increase in availability, can only be achieved by utilising deboned fish meals and animal proteins, since feedstuffs of vegetable origin contain phytates which are unavailable for most finfish because of the lack of an endogenous enzyme (phytase) in the gastrointestinal tract that catalyzes phytic acid (Lall, 1991).

Table 4.2 shows estimates of the nutrient flow and waste production in two different situations illustrating the effect of dietary quality. Both are based on nutritionally-sound dietary formulations and assume a food conversion ratio based on commercially realistic data.

As a function of diet quality, the amount of total solid waste output can vary considerably. In fact, due to an assumed 10% increase in digestibility, the total solid waste was drastically reduced to half. Although generalisations of waste production cannot be done without knowing the husbandry and management strategies of a fish farm, improved digestibility due to feed formulation manipulation will not only be benefical to the surrounding environment as it reduces solids load into the effluent water, but also it will reduce production costs. Cost of production is reduced by about £70/t of fish produced when feed digestibility is improved by 10% (Table 4.2). Further savings on transport and storage of less feed can also be expected. Thus a highly digestible diet is a very desirable product.

Thus in order to achieve optimum growth and lower waste outputs nutritionists, feed manufacturers and farms must select the highest possible digestible ingredients, aim

Table 4.2 - Comparison of two possible cases of nutrient flow and waste production as a function of feed quality.

Diet	A	B
Apparent digestibility (%)	95	85
Feed input (kg)	100	100
Solid wastes:		
feed waste[1]	5	5
faecal waste	5	15
Total waste	10	20
Kg waste/t fish produced[2]	120	240
Cost of fish (£/kg)[3]	0.63	0.70

1. Best estimate of 5% feed waste
2. Food conversion ration 1.2:1
3. £500/t feed

for well balanced ingredients in the feeds while supplying the least possible amount, i.e. aiming for optimum instead of maximum production. There are however many variables governing farm effluents quality including method and intensity of culture, species, fish size, temperature and feed quality, among others. Thus, balance studies which provide a deep insight into the way the ingested feed is utilised, and consequently about the expected waste production, should be encouraged. Although techniques are currently available for such quantitative assessment of the balance sheet and in particular of digestibility, there is still need for much further work given the variety of species and farming practices as well as the variety of factors which influence digestibility. Digestibility studies will then contribute towards not only increase in productivity through optimization of nutrient utilization, but also the reduction of nutrient losses into the immediate aquatic environment. Thus, nutritionists have an important role in feed optimization and in the maintenance and improvement of water quality which is a requisite for the further expansion of aquaculture.

5 - BIBLIOGRAPHY

Anderson, J., 1985. Digestible energy and carbohydrates in the nutrition of tilapia (*Oreochromis niloticus* Linn.). *Ph.D. thesis*, University of Aston, Birmingham, U.K., June 1985.

Anderson, J., Capper, B.S. and Bromage, N.R., 1991. Measurement and prediction of digestible energy values in feedstuffs for the herbivorous fish tilapia (*Oreochromis niloticus* Linn.). *British Journal of Nutrition*, 66, pp 37-48.

AOAC , 1984. *Official methods of analysis*, 12th edition. AOAC (Association of Official Analytical Chemists) Washington, D.C.

Ash, R., 1985. Protein digestion and absorption. In: Cowey, C.B., Mackie, A.M. and Bell, J.G., (Eds.), 1985. *Nutrition and Feeding in Fish*. Academic Press, London, pp 69-93.

Ash, R. and McLean, E., 1989. Protein digestion and absorption of intact proteins in fish. *Roche Res. Prize for Animal Nutrition 1989*. Nov. 9th, 1989, pp 49-69.

Atkinson, J.L., Hilton, J.W. and Slinger, S.J., 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat.Sci.*, vol.41, pp 1384-1386.

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture, 13*, 265-272.

Austreng, E., 1979. Fat levels and fat sources in dry feeds for salmonid fish. In: Halver, J.E., and Tiews, K. (Eds.), *World Symposium on finfish nutrition and fishfeed technology*, Vol. II, H. Heeneman, Berlin.

Austreng, E., and Refstie, T., 1979. Effect of varying dietary protein level in different families of rainbow trout. *Aquaculture*, 18, pp 145-156.

Austreng, E., Skrede, A. and Eldegard, A., 1979. Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. *Acta Agriculture Scandinavica*, 29, pp 119-126.

Austreng, E., Skrede, A. and Eldegard, A., 1980. Digestibility of fat and fatty acids in rainbow trout and mink - short communication. *Aquaculture, 19*, pp 93-95.

Barash, H., Neumark, H., Hepher, E., Iosif, B. and Itzkovich, J., 1983. Improvement of technology for determining diet digestibility of tilapia. *Int. Symp. on Tilapia in Aquac.*, Nazareth, Israel, May 8-13, 1983.

Bardach, J.E., Ryther, J.M. and McLarney, W.O., 1972. *Aquaculture. The farming and husbandry of freshwater and marine organisms.* A. Wiley - Interscience Publication. J. Wiley and Sons (Eds.), New York.

Barnicoat, C.R., 1945. Estimation of apparent digestibility coefficients by means of an inert reference-substance. *New Zealand Journal of Science and Technology*, 27: 202-212.

Barrington, E.J.W., 1957. The alimentary canal and digestion. In: M.E. Brown, (Ed.), *The Physiology of Fishes*. Academic Press, New York, pp 109-161.

Bauermeister, A.E.M., Pirie, B.J.S., and Sargent, J.R., 1979. An electron microscopic study of lipid absorption in the pyloric caeca of rainbow trout (*Salmo gairdneri*) fed wax ester-rich zooplankton. *Cell and Tissue Research*, 200, pp 475-486.

Beamish, F.W.H., 1972. Ration size and digestion in large mouth bass, *Micropterus salmoides* Lacepede. Can. J. Zool., 50, pp 153-164.

Beamish, F.W.H. and Thomas, E., 1984. Effects of dietary protein and lipid on nitrogen losses in rainbow trout, *Salmo gairdneri*. *Aquaculture*, 41, pp 359-371.

Bergot, F., 1981. Etude de l'utilisation digestive d'une cellulose purifieé chez la truite arc-en-ciel (*Salmo gairdneri*) et la carpe commune (*Cyprinus carpio*). *Reprod. Nutr. Develop.*, 21 (1), pp 83-93.

Bergot, F. and Breque, J., 1983. Digestibility of starch by rainbow trout: effects of the physical state of starch and of the intake level. *Aquaculture*, 34, pp 203-212.

Bergot, P., 1976. Démonstration par le rouge to ruthénium d'invaginations profondes de la membrane plasmique apicale des entérocytes dans l'intestin postérieur chez la truite arc-en-ciel. *Ann. Biol. Anim. Bioch. Biophys.*, 16(1), pp 37-42.

Bergot, P., 1981. Absorption des lipides. In: Fontaine, M. (Ed.), 1981. *Nutrition des Poissons*. Actes. Coll. CNERNA, Paris, Mai 1979, CNRS, Paris.

Bergot, P. and Fléchon, J.E., 1970a. Forme et voie d'absorption intestinale des acides gras a chaine longue chez la truite arc-en-ciel (*Salmo gairdneri* Rich.). I- Lipides en particules. *Ann. Biol. Anim. Bioch. Biophys.*, 10(3), pp 459-472.

Bergot, P. and Fléchon, J.E., 1970b. Forme et voie d'absorption intestinale des acides gros a chaine longue chez la truite arc-en-ciel (*Salmo gairdneri* Rich.) II- Lipides "étalés". *Ann. Biol. Anim. Bioch. Biophys.*,10(3), pp 473-480.

Bergot, P., Solari, A. and Luquet, P., 1975. Comparaison des surfaces absorbantes des caeca pyloriques et de l'intestin chez la truite arc-en-ciel (*Salmo gairdneri* Rich.). *Ann. Hydrobiologie*, 6(I), pp 27-43.

Beveridge, M.C.M., 1984. Cage and pen fish farming. Carrying capacity models and environmental impact. *FAO Fisheries Technical Paper*, 255, 131p, FAO, Rome.

Beveridge, M.C.M., Phillips, M.J. and Clarke, R.M., 1991. A quantitative and qualitative assessment of wastes from aquatic animal production. In: Brune, D.E. and Tomasso, J.R. (Eds.) *Aquaculture and Water Quality*, Advances in World Aquaculture, Vol.3, World Aquaculture Society, Baton Rouge, Louisiana, pp 506-533.

Block, E., Kilmer, L.H. and Muller, L.D., 1981. Acid insoluble ash as a marker of digestibility for sheep fed corn plants or hay and for lactating dairy cattle fed hay *ad libitum*. *J. Animal Science*, 52(5).

Bogé, G. and Pérès, G., 1983. Acquisition récentes concernant l'absorption intestinal de protides chez les poissons. *Ichthyophysiol. Acta*, 7, pp 114-118.

Bogé. G., Rigal, A. and Pérès, G., 1979. A study of intestinal absorption *in vivo* and *in vitro* of different concentrations of glycine by the rainbow trout (*Salmo gairdneri*, Richardson). *Comp. Biochem. Physiol.*, 62A, pp 831-836.

Bogé, G., Rigal, A. and Pérès, G., 1981. Rates of *in vivo* intestinal absorption of glycine and glycylglycine by rainbow trout (*Salmo gairdneri*, R.). *Comp. Biochem. Physiol.*, 69A., pp 455-459.

Bowen, S.H., 1978a. Chromic oxide in assimilation studies - A caution. *Trans. Am. Fish. Soc.*, 107(5), pp 755-756.

Bowen, S.H., 1978b. Reply to comments on "chromic oxide in food assimilation studies", by J.W. Foltz. *Trans. Am. Fish. Soc.*, 108, pp 651-652.

Brett, J.R., 1979. Environmental factors and growth. In: *Fish Physiology*, Vol. VIII, Bioenergetics and growth, Chapter 10. Hoar, W.S., Randall, D.J. and Brett, J.R. (Eds.), 1979. Academic Press, Inc.

Brett, J.R. and Higgs, D.A., 1970. Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka*. *J. Fish. Res. Bd. Canada*, 27(10), pp 1767-1778.

Bromley, P.J. and Adkins, T.C., 1984. The influence of cellulose filler on feeding, growth and utilisation of protein and energy in rainbow trout, *Salmo gairdneri*, Richardson. *J. Fish Biol.* 24, pp 235-244.

Buclon, M., 1974. Biolectric potentials and the transfer of amino acids across the digestive epithelium of the tench (*Tinca tinca* L.) *J. Physiol.* 68, pp 157-180.

Buddington, R.K., 1980. Hydrolysis-resistant organic matter as a reference for measurement of fish digestive efficiency. *Trans. Am. Fish. Soc.*, 109, pp 653-656.

Buhler, D.R. and Halver, J.E., 1961. Nutrition of salmonid fishes, IX. Carbohydrate requirements of chinook salmon. *J. Nutr.*, 74, pp 307-318.

Castell, J.D., 1979. Review of lipid requirements of finfish. In: Halver, J.E. and Tiews, K. (Eds.), *Finfish Nutrition and Fish Feed Technology.* Vol.1, H. Heeneman, Berlin, pp 59-83.

Castell,J.D., Lee, D.J. and Sinnhuber, R.A., 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): lipid metabolism and fatty acid composition. *J. Nutrition*, 102, pp 93-100.

Castledine, A.J. and Buckley, J.T., 1980. Distribution and mobility of ω3 Fatty Acids in rainbow trout fed varying levels and types of dietary lipid. *J. Nutrition* 110(4), pp 675-685.

Cho, C.Y., 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. *Aquaculture*, 100, pp 107-123.

Cho, C.Y., Bayley, H.S. and Slinger, S.J., 1974. Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.*, 31(9), pp 1523-1528.

Cho, C.Y., Castledine, A.J. and Lall, S.P., 1989. The status of Canadian aquaculture with emphasis on formulation, quality and production of fish feeds. In: *The Current Status of Fish Nutrition in Aquaculture.* The Proc. Third Int. Symp. on Feeding and Nutrition in Fish. Toba Aug. 28 - Sept. 1, Japan, 1989, pp 67-82.

Cho, C.Y., Hynes, J.D., Wood, K.R. and Yoshida, H.K., 1991. Quantitation of fish culture wastes by biological (nutritional) and chemical (limnological) methods; the development of high nutrient dense (HND) diets. In: *Nutritional strategies and aquaculture waste.* Cowey, C.B. and Cho, C.Y. (Eds.). Proc. First Int. Symp. on Nutritional Strategies in Management of Aquaculture Waste (NSMAW), 5-9 June, 1990, Univ. Guelph, Ontario, Canada.

Cho, C.Y. and Kaushik, S.J., 1985. Effects of protein intake on metabolizable and net energy values of fish diets. In: *Nutrition and Feeding in Fish*, Cowey, C.B., Mackie, A.M. and Ball, J.G. (Eds.). Academic Press, London, N.Y., Tokyo.

Cho. C.Y. and Slinger, S.J., 1979a. Significance of digestibility measurements in formulation of feeds for rainbow trout. In: Halver, J.E. and Tiwes, K. (Eds.) *Finfish Nutrition and Fishfeed Technology*, 2, H. Heenemann, Berlin, pp 239-247.

Cho, C.Y. and Slinger, S.J., 1979b. Effect of water temperature on energy utilisation in rainbow trout (*Salmo gairdneri*). In: *Energy Metabolism. Proceedings of the Eighth Symp. on Energy Metabolism.* Churchill Coll., Cambridge, September 1979.

Cho, C.Y. and Slinger, S.J., 1979c. Apparent digestibility measurement in feedstuffs for rainbow trout. *Proc. World Symp. on Finfish Nutrition and Feedstuffs Technology*, Hamburg 20-23 June, 1978. Vol. II, Berlin 1979.

Cho, C.Y., Slinger, S.J. and Bayley, H.S., 1976. Influence of level and type of dietary protein, and of level of feeding on feed utilisation by rainbow trout. *J. Nutrition*,106(11), pp 1547-1556.

Cho, C.Y., Slinger, S.J. and Bayley, H.S., 1982. Bioenergetics of salmonid fishes; energy intake, expenditure and productivity. *Comp. Biochem. Physiol.*, 73B(1), pp 25-42.

Choubert, G., 1983. Estimation des rejects solides chez le poisson. *La Pisciculture Française*, no. 72, pp 32-40.

Choubert, G., Blanc, J.M. and Luquet, P., 1984. Influence de la modification de la fréquence de distribution des repas sur la digestibilité du régime alimentaire chez la truite arc-en-ciel. *Ann. Zootech.*, 33, pp 255-262.

Choubert, G., Fauconneau, B. and Luquet, P., 1982a. Influence d'une élévation de la température 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. Dévelop.*, 22(6), pp 941-949.

Choubert, G., Noüe, de la J. and Luquet, P., 1979. Continuous quantitative automatic collector for fish faeces. *Prog. Fish Culturist*, 41(2), pp 64-67.

Choubert, G., Noüe, de la J., and Luquet, P., 1982b. Digestibility in fish: improved device for the automatic collection of faeces. *Aquaculture*, 29, pp 185-189.

Church, D.C. and Pond, W.G., 1982. *Basic Animal Nutrition and Feeding*. Ch.5 - Measurement of feed and nutrient utilisation and requirements by animals., 2nd Ed., Wiley & Sons Inc. (Eds.), pp 37-49.

Cowey, C.B. and Sargent, J.R., 1972. *Fish Nutrition. Advances in Marine Biology*, 10, pp 383-492.

Dabrowski, K., 1983. Comparative aspects of protein digestion and amino acid absorption in fish and other animals. *Comp. Biochem. Physiol.*, 74A.(2), pp 417-425.

Dabrowski, K. and Dabrowska, H., 1981. Digestion of protein by rainbow trout (*Salmo gairdneri* Rich.) and absorption of amino acids within the alimentary tract. *Comp. Biochem. Physiol.*, 69A., pp 99-111.

Dabrowski, K., Leray, C., Nonnotte, G. and Colin, D.A., 1986. Protein digestion and iron concentrations in rainbow trout *Salmo gairdneri* digestive tract in sea and fresh water. *Comp. Biochem. Physiol. A. Comp. Physiol.*, 83(1), pp 27-40.

Dabrowski, K., Poczyczynski, P., Köck, G. and Berger, B., 1989. Effect of partially or totally replacing fish meal protein by soybean meal protein on growth, food utilisation and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*). New *in vivo* Test for Exocrine Pancreatic Secretion. *Aquaculture*, 77, pp 29-49.

Dade, B.A., Aguirre, P., Blanc, D. and Kaushik, S.J., 1990. Incorporation of rapeseed meal or full fat rapeseed (colza 00) in rainbow trout (*Oncorhynchus mykiss*) diets: growth performance and digestibility. *Bull. Fr. Pêche Piscic.*, 317, pp 50-57.

Dansky, L.M. and Hill, F.W., 1952. Application of the chromic oxide indicator method to balance studies with growing chickens. *J. Nutrition*, 47, pp 449-459.

Dekker, I.W., 1986. Evolution of low pollution diets. In: *Proc. Int. Symp. Environment and Nutrition*. Determining factors in intensive fish farming. Acquacoltura' 1986, Verona 11th Oct., 1986, Italy, pp 57-62.

Depiereux, 1982. Utilisation critique de l'analyse en composantes principales et des "cluster analysis" pour la description d'echantillons d'invertébrés benthiques en eau douce. Étude de la répartition des invertébrés benthiques sur l'Outhe et la Lesse moyennes et leur affluents en relation avec la qualité de l'eau. *Dissertation présentée pour l'obtention du grade de Docteur en Sciences*, Fac. Universitaires Notre Dame de la Paix, Namur, pp 218.

De Silva, S.S. and Owoyemi, A.A., 1983. Effect of dietary quality on the gastric evacuation and intestinal passage in *Sarotherodon mossambicus* (Peters) fry. *J. Fish Biology*, 23, pp 347-355.

Di Benedetto, F.E. and Farmanfarmaian, A.,1975. Intestinal absorption of naturally occurring sugars in rainbow trout, *Salmo gairdneri*, under free swimming conditions. *Comp. Biochem. Physiol.*, 47, pp 1023-1033.

Duncan, D.B., 1955. Multiple range and multiple F tests. *Biometrics*, 11, pp 1-42.

Duncan, A. and Klekowski, R.Z., 1975. Parameters of an energy budget. In: *IBP handbook no.24. Methods for ecological bioenergetics.* Grodzinski, W., Klekowski, R.Z. and Duncan, A. (Eds.). Blackwell Scientific Publs., Oxford, London.

Edin, H. Kihlén, G. and Nordfeldt, S., 1944. A summarised description of "Edin's indicator method" for determination of the digestibility of feeds and feed mixtures. *The Annals of the Agricultural College of Sweden*, 12, pp 166-171.

Elam, C.J. and Davies, R.E., 1961. Lignin excretion by cattle fed a mixed ration. *J. Animal Sci.*, 20, pp 484-486.

Elam, C.J., Putman, P.A. and Davis, R.E., 1959. Faecal excretion pattern of chromic oxide administered to Hereford heifers in a completely pelleted ration. *J. Animal Sci.*, 18, pp 718.

Elliott, J.M., 1972. Rates of gastric evacuation in brown trout, *Salmo trutta*. *J. Freshwater Biology*, 2, pp1-8.

Elliott, J.M., 1976. Body composition of brown trout (*Salmo trutta* L.) in relation to temperature and ration size. *J. Animal Ecology*, 45, pp 273-289.

Ely, R.E., Kane, E.A.; Jacobson, W.C. and Moore, L.A., 1953. Studies on the composition of lignin isolated from orchard grass hay cut at four stages of maturity and from the corresponding faeces. *J. Dairy Sci.*,36, pp346-355.

Escoubert, P., Boge, G. and Rigal, A., 1974. Comparison de l'absorption intestinal du glycocolle et du glucose chez la truite (*Salmo gairdneri*, R.) à differentes temperatures. *Ann. Inst. Michel Pacha*, 6, pp 37-44.

Ezeasor, D.N. and Stokoe, W.M., 1980. Scanning electron microscopic study on the gut mucosa of the rainbow trout (*Salmo gairdneri*). *J. Fish Biol.*, 17, pp 619-634.

Ezeasor, D.N. and Stokoe, W.M., 1981. Light and electron microscopic studies on the absorptive cells of the intestine, caeca and rectum of the adult rainbow trout, *Salmo gairdneri*, Rich. *J. Fish Biol.*, 18, pp 527-544.

Falge, R. and Spannhof, L., 1976. The activity of amylase, esterease and protease in the intestinal contents of rainbow trout after feeding. *Vopr. Ikhtiol.*, 16, pp 739-744.

Falge, R., Spannhof, L. and Jurss, K., 1978. Amylase, esterease and protease activity in the intestine content of rainbow trout, *Salmo gairdneri* Rich., after feeding with feed containing different amounts of starch and protein. *Vopr. Ikhtiol.*, 18, pp 314-319.

Fänge, R. and Grove, D., 1979. Digestion, Ch.4. In: Hoar, W.S., Randall, D.J. and Brett, J.R., (Eds.) *Fish Physiology, Vol. VIII - Bioenergetics and Growth.* Academic Press Inc.

FAO, 1988. *World Aquaculture Production.* ADCP/Aquaculture minutes UNDP/FAO,ADCP/1988/No.1.

FAO, 1990. *World Aquaculture Production.* ADCP/Aquaculture minutes UNDP/FAO,ADCP/1990/No.8

FAO, 1991. *Aquaculture Production (1986-1989).* FIDI/C815(Rev.3).

Ferraris, R.P., Catacutan, M.R. Mabelin, R.L. and Jazul, A.P., 1986. Digestibility in milkfish, *Chanos chanos* (Forsskal): Effects of protein source, fish size and salinity. *Aquaculture*, 59, pp 93-105.

Fish, G.R., 1960. The comparative activity of some digestive enzymes in the alimentary canal of tilapia and perch. *Hydrobiol.*, 15, pp 161-178.

Flowerdew, M.W. and Grove, D.J., 1979. Some observations of the effects of body weight, temperature, meal size and quality on gastric emptying time in the turbot, *Scophthalmus maximus* (L.) using radiography. Rep. from *J. Fish Biol.*, 14, pp 229-238.

Foltz, J.W., 1978. Comments - Chromic oxide in food assimilation studies. *Trans.Am.Fish Soc.*, 108, pp 650-651.

Fry, F.E.J.,1978. The effect of environmental factors on the physiology of fish. In: *Fish Physiology*, Vol. VI, Ch.1, Hoar, W.S. and Randall, D.J., pp 1-99. Academic Press.

Furuichi, Y. and Takahashi, T., 1981. Evaluation of acid insoluble ash as a marker in digestion studies. *Agr. Biol. Chem.*, 45(10), pp 2219-2224.

Furukawa, A., 1976. Diet in yellowtail culture. In: Price, Jr., K.S., Shaw, W.N. and Danberg, K.S. (Eds.). *First Int. Conf. Aquac. Nutrition*, Oct. 1975. Coll. Mar. Studies, Univ. Delaware, Newark, Del., pp 85-104.

Furukawa, A. and 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. Jap. Soc. Sci. Fish*, 32(6), pp 502-506.

Garber, K.J., 1983. Effect of fish size, meal size and dietary moisture on gastric evacuation of pelleted diets by yellow perch, *Perca flavescens*. *Aquaculture*, 34, pp 41-49.

Georgopoulou, U., Sire, M.F. and Vernier, J.M., 1985. Macromolecular absorption of proteins by epithelial cells of the posterior intestinal segment and their intracellular digestion in the rainbow trout. Ultrastructural and biochemical study. *Biol.Cell*, 53, pp 269-282.

Georgopoulou, U., Sire, M.F. and Vernier, J.N., 1986. Intestinal absorption of macromolecular proteins and their digestion in the rainbow trout. Ultrastructural and biochemical study with respect to the first feeding. *Can.J.Zool.*, 64(6), pp 1231-1240.

Gómez-Jarabo, G., Higuera, M. de la., Mataix, F.J., Varela, G. and Illera, M., 1979. La edad como factor determinante de la utilización nutritiva de la proteína por la trucha. *Revista Española de Fisiologia*, 35, pp 273-278.

Grabner, M. and Hofer, R., 1985. The digestibility of the proteins of broad bean (*Vicia faba*) and soya bean (*Glycine max*) under *in vitro* conditions simulating the alimentary tracts of rainbow trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*). *Aquaculture*, 48, pp 111-122.

Grayton, B.D. and Beamish, F.W.H., 1977. Effects of feeding frequency on food intake, growth and body composition of rainbow trout (*Salmo gairdneri*). *Aquaculture*, 11(2), pp 159-172.

Grove, D.J., Loizides, L.G. and Nott, J., 1978. Satiation amount, frequency of feeding and gastric emptying in *Salmo gairdneri*. *J.Fish. Biol.*, 12, pp 507-516.

Haerlein, G.F.W., Smith, R.C. and Yoor, Y.M., 1966. Determination of the faecal excretion rate of horses with chromic oxide. *J.Animal Sci.*, 25, pp 1091.

Hanley, F., 1987. The digestibility of foodstuffs and the effects of feeding selectivity on digestibility determinations in tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 66, pp 163-179.

Hastings, W.H., 1967. Feed Formulation, Physical Quality of pelleted feed, Digestibility. *Fish Wildl.Serv. Resour.*, Publ.39., pp 137-141.

Hastings, W.H., 1969. Nutritional score. In: Neuhaus, O.W. and Halver, J.C. (Eds.) *Fish in Research*. Academic Press, New York, pp 263-292.

Henderson, R.J., and Sargent, J.C., 1985. Fatty acid metabolism in fish. In: Cowey, C.B., Mackie, A.M. and Bell, J.G. (Eds.) *Nutrition and Feeding in Fish*. Academic Press, London, pp 349-364.

Hepher, B., 1988. *Nutrition of Pond Fishes.*, Ch.2 - Ingestion, digestion and absorption of food. Cambridge Univ. Press.

Hilton, J.W., Atkinson, J.L. and Slinger, S.J., 1983. Effect of increased dietary fibre on the growth of rainbow trout (*Salmo gairdneri*). Rep. from *Can. Journ. Fish. Aq. Sciences*, 40(1), pp 81-85.

Hilton, J.W., Cho, C.Y. and 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*). *Aquaculture*, 25, pp 185-194.

Hilton, J.W. and Slinger, S.J., 1983. Effect of wheat bran replacement of wheat middlings in extrusion processed (floating) diets on the growth of juvenile rainbow trout (*Salmo gairdneri*). *Aquaculture*, 35, pp 201-210.

Hilton, J.W. and Slinger, S.J., 1986. Digestibility and utilisation of canola meal in practical-type diets for rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.*, 43, pp 1149-1155.

Hirao, S., Yamada, J. and Kikuchi, R., 1960. On improving the efficiency of feed for fish culture. I. Transit and digestibility of diet in eel and rainbow trout observed by use of P^{32}. *Bull.Tokai Regional Fish. Res.Lab.*, 7, pp 67-72.

Hiyama, Y. and Singh, R.P., 1966. Quantitative relationship between marine and freshwater preys and predators as determined by ^{14}C labelled glucose. *Bull Jap.Soc.Sci.Fish.*, 32(9), pp 710-715.

Hofer, R. and Sturmbauer, C., 1985. Inhibition of Trout and Carp Amylase by Wheat. *Aquaculture*, 48, pp 277-283.

Hokazono, S., Tanaka, Y., Katayama, T., Chichester, C.O. and Simpson, K.L., 1979. Intestinal transport of L-lysine in rainbow trout, *Salmo gairdneri*. *Bull. Jap. Soc. Sci. Fish.*, 45, pp 845-848.

Hudon, B. and de la Noüe, J., 1984. Influence of meal frequency on apparent nutrient digestibility in rainbow trout, *Salmo gairdneri*. *Bull. Fr. Piscic.*, 293-4, pp 49-51.

Huet, M., 1979. *Textbook of Fish Culture. Breeding and Cultivation of Fish*. 3rd Ed. Fishing News Books Ltd., England.

Hydén, S., 1960. The use of reference substances and the measurements of flow in the alimentary tract. *Proceedings of Univ. Nottingham's 7th Easter School in Agric. Scs.*, Ivins, J.D.,(Ed.).

Inaba, D., Ogino, C., Takamatsu, C., Sugano, S. and Hata, H., 1962. Digestibility of dietary components in fishes. I. Digestibility of dietary proteins in rainbow trout. *Bull. Jap. Soc. Sci. Fish*, 28(3), pp 367-371.

Inaba, D., Ogino, C., Takamatsu, C., Ueda, T. and Kurokawa, K., 1963. Digestibility of dietary components. II. Digestibility of dietary proteins and starch in rainbow trout. *Bull. Jap. Soc. Sci. Fish*, 29(3), pp 242-244.

Ingham, L. and Arme, C., 1977. Intestinal absorption of amino acids by rainbow trout. *J. Comp. Physiol.*, 117, pp 323-334.

Irwin, M.I. and Crampton, E.W., 1951. The use of chromic oxide as an index material in digestion trials with human subjects. *J. Nutrition*, 43, pp 77.

Jauncey, K., 1982. The effects of varying dietary protein level on the growth, food conversion, protein utilisation and body composition of juvenile tilapias (*Sarotherodon mossambicus*). *Aquaculture*, 27, pp 43-54.

Jobling, M., 1980. Gastric evacuation in plaice, *Pleuronectes platessa* L.: effects of dietary energy level and food composition. *J. Fish Biol* 17, pp 187-196.

Jobling, M., 1983. A short review and critique of methodology used in fish growth and nutrition studies. *J. Fish Biol.*, 23, pp 685-703.

Jobling, M., 1986. Gastrointestinal overload - A problem with formulated feeds? *Aquaculture*, 51, pp 257-263.

Jobling, M., Gwyther, D. and Grove, D.J., 1977. Some effects of temperature, meal size and body weight on gastric evacuation time in the dab, *Limanda limanda* (L.). Rep. from *J. Fish Biol* 10, pp 291-298.

Kane, E.A., Ely, R.E., Jacobson, W.C. and Moore, L.A., 1953. A comparison of various digestion trial techniques with dairy cattle. *J. Dairy. Sci.*, 36, pp 325-333.

Kane, E.A., Jacobson, W.C., and Moore, L.A., 1952. Diurnal variations in the excretion of Cr_2O_3 and lignin. *J. Nutr.*, 47, pp 263.

Kapoor, B.G., Smit, H. and Verighina, I.A., 1975. The alimentary canal and digestion in teleosts. *Adv. Mar. Biol.*, 13, pp 109-239.

Kaushik, S.J., 1980. Influence of nutritional status on the daily patterns of nitrogen excretion in the carp (*Cyprinus carpio* L.) and the rainbow trout (*Salmo gairdneri* R.). *Reprod. Nutr. Dévelop.*, 20(6), PP 1751-1765.

Kaushik, S.J., 1989a. Nitrogen and energy balance studies in fish. *Roche Res. Prize for Animal Nutrition* 1989, Nov. 9th, pp 71-95.

Kaushik, S.J., 1989b. Status of European aquaculture and fish nutrition. In: The current status of fish nutrition in aquaculture. *The Proc. Third Int. Symp. on Feeding and Nutrition in Fish*, Toba. Aug. 28 - Sept. 1, Japan, 1989, pp 3-12.

Kaushik, S.J., 1990. Nutrition et alimentation des poissons et contrôle des déchets piscicoles. *La Pisciculture Française*, 101, pp 14-23.

Kaushik, S.J., and Oliva Teles, A., 1985. Effect of digestible energy on nitrogen and energy balance in rainbow trout. *Aquaculture, 50, pp 89-101.*

Kawai, S. and Ikeda, S., 1973a. Studies on digestive enzymes of fishes. III. Development of the digestive enzymes of rainbow trout after hatching and the effect of dietary change on the activities of digestive enzymes in the juvenile stage. *Bull. Jap. Soc. Sci. Fish*, 39, pp 819-823.

Kawai, S. and Ikeda, S., 1973b. Studies on digestive enzymes of fishes. IV. Development of the digestive enzymes of carp and black sea bream after hatching. *Bull. Jap. Soc. Sci. Fish*, 39, pp 877-881.

Kennelly, J.J., Aherne, F.X. and Apps, M.J., 1980. Dysprosium as an inert marker for swine digestibility studies. *Can. J. Anim. Sci.*, 60, pp 441-446.

Kim, J.D. and Kaushik, S., 1989. Contribution of digestible energy from carbohydrates for growth of rainbow trout (*Oncorhynchus mykiss*). *The Third Int. Symp. on Feeding and Nutrition in Fish*, Aug. 28th-Sept. 1st, Toba, Japan.

Kionka, B.C. and Windell, J.T., 1972. Differential movement of digestible and indigestible food fractions in rainbow trout, *Salmo gairdneri. Trans. Am. Fish. Soc.*, 1972, No.1.

Kitamikado, M., Morishita, T. and Tachino, S. 1964a. Digestibility of dietary protein in rainbow trout. I - Digestibility of several dietary proteins. *Bull. Jap. Soc. Sci. Fish.*, 30(1), pp 46-49.

Kitamikado, M., Morishita, T. and Tachino, S., 1964b. Digestibility of dietary protein in rainbow trout. II - Effect of starch and oil contents in diets, and size of fish. *Bull. Jap. Soc. Sci. Fish.*, 30(1), pp 50-54.

Kitamikado, M. and Tachino, S., 1960a. Studies on the digestive enzymes of rainbow trout. I - Carbohydrases. *Bull. Jap. Soc. Sci. Fish.*, 26, pp 679-684.

Kitamikado, M. and Tachino, S., 1960b. Studies on the digestive enzymes of rainbow trout. II - Proteases. *Bull. Jap. Soc. Sci. Fish.*, 26, pp 685-690.

Kitamikado, M. and Tachino, S. 1960c. Studies on the digestive enzymes of rainbow trout. III - Esterases. *Bull. Jap. Soc. Sci. Fish.*, 26. pp 691-694.

Knapka, J.J., Barth, K.M., Brown, D.G. and Cragle, R.G., 1967. Evaluation of polyethylene, chromic oxide and ^{144}Cerium as digestibility indicators in burros. *J. Nutr.*, 92, pp 79-85.

Korn, S. and Macedo, D., 1973. Determination of fat content in fish with a non-toxic, non-inflammable solvent. *J. Fish. Res. Board Can.*, 30, pp. 1880-1881.

Kotb, A.R. and Luckey, T.D., 1972. Markers in nutrition. *Nutrition Abstracts and Reviews*, 42(3), pp 813-845.

Lall, S.P., 1991. Digestibility, metabolism and excretion of dietary phosphorus in fish. In: *Nutritional Strategies and Aquaculture Waste*. Cowey, C.B. and Cho, C.Y. (Eds.). Proc. First Int. Symp. on Nutrition Strategies in Management of Aquaculture Waste (NSMAW), 5-9 June 1990, Univ. Guelph, Ontario, Canada.

Lall, S.P. and Bishop, F.J., 1976. Studies on the nutrient requirements of rainbow trout (*Salmo gairdneri*) grown in sea water and freshwater. *FAO Tech. Conf. on Aquac.*, Kyoto, Japan, 26 May - 2 June, 1976.

Leary, D.F. and Lovell, R.T., 1975. Value of fibre in production-type diets for channel catfish. Repr. from *Trans. Am. Fish. Soc.*, 104(2), pp 328-332.

Lee, D.J. and Putnam, G.B., 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. *J. Nutr.*, 103, pp 916-922.

Legendre, L. and Legendre, P., 1984. *Ecologie Numérique. 2. La structure des données écologiques*. Collection d'Ecologie, 13. Masson. Presses de l'Université de Quebec, 2nd Ed.

Leger, C., Luquet, P. and Boudon, M., 1976. Effects de la suppression des glucides dans l'alimentation de la truite arc-en-ciel à la température de 16°C. II. Evolution des compartiments corporels avec réference particulière aux acides grass des familles ω9 ω6 ω3 et influence d'un changement de température. *Ann. Hydrobiol.*, 7, pp 185-201.

Lied, E. and Julsham, K., 1989. Determination of digestibility with chromium oxide. *The Third Int. Symp. on Feeding and Nutrition in Fish*, Aug. 28 - Sept. 1, 1989, Toba, Japan.

Lied, E., Julshamm, K. and Braekkan, O.R., 1982. Determination of protein digestibility in Atlantic Cod (*Gadus morhua*) with internal and external indicators. Reprinted from *Can. Journal Fish.and Aquatic Sciences*, 39(6),pp 854-861.

Lindsay, G.J.H., 1984a. Distribution and function of digestive tract chitinolytic enzymes in fish. *J. Fish Biol.*24, pp 529-536.

Lindsay, G.J.H., 1984b. Adsorption of rainbow trout (*Salmo gairdneri*) gastric lysozymes and chitinase by cellulose and chitin. *Aquaculture*, 42, pp 241-246.

Lindsay, G.J.H., and Harris, J.E., 1980. Carboxymethylcellulose activity in the digestive tracts of fish. *J. Fish Biol.* 16, pp 219-233.

Luckey, T.D., Kotb, A., Vogt, J.R., and Hutcheson, D.P., 1975. Feasibility studies in rats fed heavy metals as multiple nutrient markers. *J. Nutr.*,105, pp 660-669.

Luquet, P. and Fauconneau, B., 1979. Influence de la température sur quelques aspects de l'alimentation des poissons. *Bull. Cent. Etud. Rech. Sci.*, Biarritz, 12, pp 475-492.

Luquet, P. and Kaushik, S.J., 1981. Besoins en protéines et en acides aminés. In: *Nutrition des poissons.* Éd. du Centre Nationale de la Recherche Scientifique, Paris, pp 171-184.

Marcotte, G. and de la Noüe, J., 1984. Absence de transfert intestinal de protéines chez la truite arc-en-ciel, *Salmo gairdneri. Reprod. Nutr. Develop.*, 24, pp 117-125.

Maynard, L.A. and Loosli, J.K., 1969. *Animal Nutrition.* 6th Ed. McGraw-Hill, New York, 613 pp.

Maynard, L.A., Loosli, J.K., Hintz, H.F. and Warner, R.G., 1979. *Animal Nutrition.*, Ch. 3 - Digestive Processes in different species. McGraw-Hill Book Company, 7th Ed., pp 21-46.

McCarthy, J.J., Aherne, F.X. and Okai, D.B., 1974. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. *Can. J. Anim. Sci.*, 54, pp 107-109.

McLean, E. and Ash, R., 1990. Modified uptake of the protein antigen horseradish peroxidase (HRP) following oral delivery to rainbow trout, *Oncorhynchus mykiss* - short communication. *Aquaculture*, 87, pp 373-379.

McLeese, J.M. and Stevens, E.D., 1982. The effect of acclimation temperature, assay temperature and ration on the specific activity of trypsin and chymotrypsin from rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.*, 73B(3), pp 631-634.

McLeese, J.M. and Stevens, E.D., 1986. Trypsin from two strains of rainbow trout, *Salmo gairdneri*, is influenced differently by assay and acclimation temperature. *Can. J. Fish. Aquat. Sci.*, Vol. 43, pp 1664-1667.

McLeod, M.G., 1977. Effects of salinity on food intake, absorption and conversion in the rainbow trout *Salmo gairdneri. Marine Biology*, 43(2), pp 93-102.

Medale, F., Parent, J.P. and Vellas, F., 1985. Effet d'une réduction de l'oxygénation de l'eau sur l'excrétion d'ammoniaque chez la truite arc-en-ciel (*Salmo gairdneri* Rich.). *Sciences de l'eau*, 4, pp 341-347.

Medale, F., Parent, J.P. and Vellas, F., 1987. Responses to prolonged hypoxia by rainbow trout (*Salmo gairdneri*) I - Free amino acids and proteins in plasma, liver and white muscle. *Fish Phys. and Biochem.* 3(4) 183-189.

Merican, Z.O. and Phillips, M.J., 1985. Solid waste production from rainbow trout, *Salmo gairdneri* Richardson, cage culture. *Aquaculture and Fisheries Management*, 1, pp 55-69.

Micha, J.C., Dandrifosse, G. and Jeuniaux, C., 1973. Distribution et localisation tissulaire de la synthèse des chitinases chez les vertébrés inférieurs. *Arch. Int. Physiol. Biochim.*, 81, pp 439-451.

Moore, J.H., 1957. Diurnal variations in the composition of the faeces of pigs on diets containing chromium oxide. *Brit. J. Nutr.*, 11, pp 273-288.

Morishita, T., Noda, H., Kitamikado, M., Takahashi, T. and Tachino, S., 1964. The activity of the digestive enzymes in fish. *J. Fac. Pref.*, Univ. of Mie (Japan), 6(2), pp 239-246.

Morishita, T., Noda, H. Kitamikado, M., Takahashi, T. and Tachino, S., 1967. Studies on the esterase and lipase of fish. II. Esterolytic activities of esterase and lipase in the digestive organs from various fish. *J. Fac. Pref., Univ. Mie.*, 7, pp 37-45.

Mugdal, V.D., Dixon, R.M., Kennedy, P.M. and Milligan, L.P., 1982. Effect of two intake levels on retention times of liquid, particle and microbial markers in the rumen of sheep. *J. Anim. Sci.*, 54(5).

Mundheim, H. and Opstvedt, J., 1989. Effect of dietary level of protein and fibre on apparent protein digestibility in the rainbow trout (*Salmo gairdneri*) and salmon (*Salmo salar*) and comparison of protein digestibility in mink (*Mustela vison*), rainbow trout and salmon. *The Third Int. Symp. on Feeding and Nutrition in Fish*, Aug. 28th- Sept. 1st, Toba, Japan.

Munro, H.M. and Fleck, A., 1969. The determination of organic nitrogen in biological material. A Review. *Clin. Chem.Act.*, 11, pp 2-12.

Nikinmaa, M. and Soivio, A., 1982. Blood oxygen transport of hypoxic *Salmo gairdneri*. *J. Exp. Zool.*, Vol. 219, 173-178.

Nomura, M., Nose, T., Uematsu, Z. and Inaba, D., 1972. Application of imported Peru fish meal in fish feed - 1. Feeding experiment with rainbow trout. *Bull. Freshwater Fish Res. Lab.*, Tokyo, 22(1), pp 85-92.

Nose, T., 1960. On the digestion of food protein by goldfish (*Carassius auratus* L.) and Rainbow Trout (*Salmo gairdneri* R.). *Bull. Fresh. Fish Res. Lab.*, Tokyo, 10, pp 11-22.

Nose, T., 1961. Determination of nutritive value of food protein on fish - I. On the determination of food protein utilization by carcass analysis. *Bull. Fresh. Fish Res. Lab.*, Tokyo, 11, pp 29-42.

Nose, T., 1967a. On the metabolic faecal nitrogen in young rainbow trout. *Bull. Fresh. Fish Res. Lab.*, Tokyo, 17(2), pp 97-105.

Nose, T., 1967b. Recent advances in the study of fish digestion in Japan. FAO/EIFAC Tech. Pap. No.3 (1967), *Proc. Symp. on Feeding in Trout and Salmon Culture*, May 1966, pp 83-94.

Nose, T., 1989. Protein and amino acid nutrition in fish. *Roche Res. Prize for Animal Nutrition* 1989, pp 13-47.

Nose, T. and Mamiya, H., 1963. Protein digestibility of flatfish meal in rainbow trout. *Bull. Freshwater Fish. Res. Lab.*, Tokyo, 12(2).

Nose, T. and Toyama, K., 1966. Protein digestibility of brown meal in rainbow trout. *Bull. Freshwater Fish. Res. Lab.*, Tokyo, 15(2), pp 213-225.

Noüe, de la J. and Choubert, G., 1986. Digestibility in rainbow trout: comparison of the direct and indirect methods of measurement. *The Progressive Fish Culturist*, 48, pp 190-195.

N.R.C. (National Research Council), 1982. *Nutrient Requirements of Domestic Animals, No. 16 - Nutrient Requirements of Coldwater Fish.* Nat. Ac. Press, Washington, D.C.

Ogino, C., 1980. Requirements of carp and rainbow trout for essential amino acids. *Bull. Jap. Soc. Sci. Fish.*, 46(2), pp 171-177.

Ohlde, G. and Becker, K., 1982. Suitability of cell-wall constitutents as predictors of organic matter digestibility in some tropical and sub-tropical by-products. *Animal Feed Science and Technology*, 7, pp 191-199.

Oliva-Teles, A., and Rodrigues, A.M.P., 1991. The effect of high temperature and diet protein level on metabolic utilisation of diets by rainbow trout. In: *Fish Nutrition in Practice, IV Int. Symp. on Fish Nutrition and Feeding*, Biarritz, France, June 24-27, 1991.

Onishi, T., Murayama, S. and Takeuchi, M., 1973a. Sequence of digestive enzyme levels in carp after feeding. I - Amylase and protease of intestinal content, hepatopancreas and gallbladder. *Bull. Tokai Reg. Fish. Res. Lab.*, 75, pp 23-32.

Onishi, T., Murayama, S. and Takeuchi, M., 1973b. Sequence of digestive enzyme levels in carp after feeding. II - Protease in activated and zymogen forms of intestine, hepatopancreas, gallbladder and spleen. *Bull. Tokai Reg. Fish. Res. Lab.*, 75, pp 33-38.

Penning, P.D., and Johnson, R.H., 1983a. The use of internal markers to estimate herbage digestibility and intake. 1 - Potentially indigestible cellulose and acid insoluble ash. *J. Agric. Sci.*, 100, pp 127-131.

Penning, P.D. and Johnson, R.H., 1983b. The use of internal markers to estimate herbage digestibility and intake. 2 - Indigestible acid detergent fibre. *J. Agric. Sci.*, 100, pp 133-138.

Persson, G., 1988. Relationship between feed, productivity and pollution in the farming of large rainbow trout (*Salmo gairdneri*). *National Swedish Environmental Protection Board*, Report 3534:10.

Petrusewicz, K. and MacFadyen, A., 1970. *Productivity of terresterial animals. Principles and Methods*. IBP Handbook No.13. Blackwell Scientific Publs., Oxford and Edinburgh.

Pfeffer, E., Beckmann-Toussaint, J., Henrichfreise, B. and Jansen, H.D., 1991. Effect of extrusion on efficiency of utilisation of maize starch by rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 96, pp 293-303.

Phillips, A.M., Tunison, A.V. and Brockway, D.R., 1948. The utilisation of carbohydrates by trout. *Fish Res. Bull.*, 11, pp 1-44.

Philipson, J., 1964. A miniature bomb calorimeter for small biological samples. *Oikos*, 15, pp 130-139.

Pieper, A. and Pfeffer, E., 1979. Carbohydrates as possible sources of dietary energy for rainbow trout (*Salmo gairdneri*, Richardson). In: *Proc. World Symp. on Finfish Nutrition and Fishfeed Technology*, Hamburg 20-23 June, 1978, Vol.1, Berlin, 1979, pp 209-220.

Pike, I.H., Andorsdóttir, G. and Mundheim, H., 1990. The role of fish meal in diets for salmonids. *Int. Ass. Fish Meal Manufacturers (IAFMM)*, No. 24, 36 pp.

Plantikow, A. and Plantikow, H., 1985. Alanine aminopeptidase (AAP) activity in the midgut of rainbow trout (*Salmo gairdneri* R.). The influence of feed quantity and quality, temperature and osmolarity. *Aquaculture*, 48, pp 261-276.

Possompes, B., 1973. Influence de la température sur les besoins en protéines, le transit alimentaire et la digestibilité chez la truite arc-en-ciel, *Salmo gairdneri* Rich. *Thèse 3^e Cycle*, Univ. Paris, 6, pp 58.

Possompes, B.P., Bergot, P. and Luquet, P., 1975. Mise au point d'une méthode d'étude du transit gastro-intestinal chez la truite arc-en-ciel *Salmo gairdneri* Richardson: Influence du nombre de repas, des quantités ingérées et de la température d'acclimatation. *Ann. Hydrobio.*, 6(2), pp 131-143.

Pouliot, T. and de la Noüe, J., 1988. Apparent digestibility in rainbow trout (*Salmo gairdneri*): Influence of hypoxia. *Can. J. Fish. Aquat. Sci.*, 45, pp 2003-2009.

Pouliot, T. and de la Noüe, J., 1989. Feed intake, digestibility and brain neurotransmitters of rainbow trout under hypoxia. *Aquaculture*, 79, pp 317-327.

Prosser, C.L., 1973. *Comparative Animal Physiology*, 3rd Ed., W.B. Saunders, Philadelphia, Penn., USA.

Rajamani, M. and Job, S.V., 1976. Food utilisation by *Tilapia mossambica* (Peters); function of size. *Hydrobiologia*, 50, pp 71-74.

Reinitz, G.L. and Yu, T.C., 1981. Effects of dietary lipids on growth and fatty acid composition of rainbow trout (*Salmo gairdneri*). *Aquaculture*, 22, pp 359-366.

Ringo, E., 1991. Hatchery-reared landlocked Arctic charr, *Salvelinus alpinus* (L.), from Lake Takvatu reared in fresh and sea water. II. The effect of salinity on the digestibility of protein, lipid and individual fatty acids in a capelin roe diet and commercial feed. *Aquaculture*, 93, pp 135-142.

Roach, A.G., Sanderson, P. and Williams, D.R., 1967. Comparison of methods for the determination of available lysine values in animal and vegetable protein sources. *J. Sci. Fd. Agric.*,18, pp 274-278.

Rohlf, F.J., 1987. *NTSYS*, Ver. 1.21. Copyright (C), 1985 Borland Inc.

Rosenlund, G. and Njaa, L.R., 1982. Protein digestibility in rats determined with TiO_2 and Cr_2O_3 and comparisons with a rapid enzyme test. *Acta. Agri. Scand.*, 32, pp 115-121.

Ross, B. and Jauncey, K., 1981. A radiographic estimation of the effect of temperature on gastric emptying time in *Sarotherodon niloticus* (L.) x *S. aureus (Steindachner)* hybrids. *J. Fish Biology*, 19, pp 333-344.

Ross, L.G., McKinney, R.W. and Ross, B., 1988. Energy budgets for cultured tilapias. In: *Proc. Second Int. Symp. on Tilapia in Aquaculture*, 16-20 March, 1987, Bangkok, Thailand. Pullin, R.S.V., Bhukaswan, T., Tonguthai, K. and MacLean, J.L. (Eds.), pp 83-90.

Ross, L.G. and Ross, B., 1984. *Anaesthetic and Sedative Techniques for Fish.* Publ. by Inst. of Aquaculture, Univ. Stirling, Stirling, Scotland, 1984. B., Son and Ferguson Ltd. (Eds.), The Nautical Press, Glasgow.

Ross, L.G., Ward, K.M.H. and Ross, B., 1985. The effects of formalin, malachite green and suspended solids on the respiratory activity of rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture and Fisheries Management*, 16, pp 129-138.

Rumsey, G.L., Page, J.W. and Scott, M.L., 1983. Methionine and cystine requirements of rainbow trout. *Prog. Fish Cult.*, 45, pp 139-143.

Rychly, J., 1980. Nitrogen balance in trout. II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. *Aquaculture*, 20, pp 343-350.

Rychly, J. and Marina, B.A., 1977. The ammonia excretion of trout during a 24 hour period. *Aquaculture*, 11, pp 173-178.

Rychly, J. and Spannhof, L., 1979. Nitrogen balance in trout. I. Digestibility of diets containing varying levels of protein and carbohydrate. *Aquaculture*, 16, pp 39-46.

Sayer, M.D.J. and Davenport, J., 1987. The relative importance of the gills to ammonia and urea excretion in five seawater and one freshwater teleost species. *J. Fish Biology*, 31, pp 561-570.

Schürch, A.F., Lloyd, L.E. and Crampton, E.W., 1950. The use of chromic oxide as an index for determining the digestibility of a diet. *J. Nutrition*, 41, pp 629-636.

Shah, N., Atallah, M.T., Mahoney, R.R. and Pellett, P.L., 1982. Effect of dietary fibre components on faecal nitrogen excretion and protein utilisation in growing rats. *J. Nutrition*, 112, pp 658-666.

Shiau, S.Y., 1989. Role of fibre in fish feed. In: *Progress in Fish Nutrition, 1989. Proc. Fish Nutrition Symp.*, Keeling, Taiwan, 6-7 Sept., 1989. Shiau, S.Y. (Ed.) Marine Food Science Series, No.9, pp 208.

Shrivastava, V.S. and Talapatra, S.K., 1962. Pasture studies in Uttar Pradesh. II - Use of some natural indicators to determine the plane of nutrition of a grazing animal. *Indian Journal of Dairy Sci.*, 15, pp 154.

Singh, R.P. and Nose, T., 1967. Digestibility of carbohydrates in young rainbow trout. *Bull. Fresh. Fish. Res. Lab.*,17(1), pp 20-25.

Sire, M.F., Dorin, D. and Vernier, J.M., 1992. Intestinal absorption of macromolecular proteins in rainbow trout. *Aquaculture*, 100, pp 234.

Sire, M.F., Lutton, C. and Vernier, J.M., 1981. New view of intestinal absorption of lipids in teleostean fishes: an ultrastructural and biochemical study in the rainbow trout. *J. Lipid Res.*, 22, pp 81-94.

Smith, B.W., and Lovell, R.T., 1972. Digestibility of nutrients in semi-purified rations by channel catfish in stainless steel troughs. Rep. from *Proc. 25th Annual Conf. Southeastern Association of Game and Fish Commissioners*, 1971, pp 452-458.

Smith, B.W. and Lovell, R.T., 1973. Determination of apparent protein digestibility in feeds for channel catfish. Reprinted from *Trans. Am. Fish. Soc.*,102(4), pp 830-835.

Smith, L.S., 1982. *Introduction to Fish Physiology*. T.F.M. Publications Inc.

Smith, L.S., 1989. Digestive functions in teleost fishes. In: Halver, J.E., 1989. *Fish Nutrition*, 2nd Edition, Ch.7, pp 331-421. Academic Press Inc.

Smith, R.R., 1971. A method for measuring digestibility and metabolizable energy of fish feeds. *The Prog. Fish Cult.*, 33(3), pp 132-134.

Smith, R.R., 1979. Methods for determination of digestibility and metabolisable energy of feedstuffs for finfish. *Proc. World Symp. on Finfish Nutrition and Fish Feed Technology*, Hamburg 20-23 June, Vol. II, Berlin 1979.

Smith, R.R., 1980. Nutritional bioenergetics in fish. In: *Fish Feed Technology*, Rome: UNDP/FAO.

Smith, R.R., Peterson, M.C. and Allred, A.C., 1980. Effect of leaching on apparent digestion coefficients of feedstuffs for salmonids. *The Prog. Fish Culturist*, 42(4), pp 195-199.

Sneath, P.N., and Sokal, R.R., 1973. *Numerical Taxonomy. The Principles and Practice of Numerical Classification.* W.H. Freeman and Company, S. Francisco, 573 pp.

Spannhof, L. and Plantikow, H., 1983. Studies on carbohydrate digestion in rainbow trout. *Aquaculture*, 30 pp 95-108.

Spyridakis, P., Metailler, R., Gabaudan, J. and Riaza, A., 1989. Studies on nutrient digestibility in European sea bass (*Dicentrarchus Labrax*). 1 - Methodological aspects concerning faeces collection. *Aquaculture*, 77, pp 61-70.

Stickney, R.R., 1976. Cellulase activity in the stomachs of freshwater fishes from Texas. *Proc. S.E. Game Fish Comm.*, 29, pp 282-286.

Stickney, R.R. and Shumway, S.E., 1974. Occurrence of cellulase activity in the stomachs of fishes. *J. Fish Biol.*, 6, pp 779-790.

Stokes, R.M. and Fromm, P.O., 1964. Glucose absorption and metabolism by the gut of rainbow trout. *Comp. Biochem. Physiol.*, 13, pp 53-69.

Storebakken, T. and Austreng, E., 1987. Ration level for salmonids. II - Growth, feed intake, protein digestibility, body composition, and feed conversion in rainbow trout weighing 0.5-1.0kg. *Aquaculture*, 60, pp 207-221.

Storebakken, T., Austreng, E. and Steenberg, K., 1981. A method for determination of feed intake in salmonids using radioactive isotopes. *Aquaculture*, 24, pp 133-142.

Storebakken, T., Hung, S.S.O., Calvert, C.C. and Plisetskaya, E.M., 1991. Nutrient partitioning in rainbow trout at different feeding rates. *Aquaculture*, 96, pp 191-203.

Sumpter, J.P., 1992. Control of growth of rainbow trout *(Oncorhynchus mykiss)*. *Aquaculture*, 100, pp 299-320.

Sutton, E.I., Bowland, J.P. and McCarthy, J. F., 1977. Studies with horses comparing 4N-HCl insoluble ash as an index material with total faecal collection in the determination of apparent digestibilities. *Can. J. Anim. Sci.*, 57, pp 543-549.

Tacon, A.G.J. and Beveridge, M.M., 1982. Effects of dietary trivalent chromium on rainbow trout. *Nutrition Reports International*, 25(1), pp 49-56.

Tacon, A.G.J. and Ferns, P.N., 1976. The use of activated sludge from domestic sewage in trout diets. *Nutr. Rep. Int.*, 13, pp 549-562.

Tacon, A.G.J. and Jackson, A.J., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. In: Cowey, C.B., Mackie, A.M. and Bell. J.G. (Eds.) *Nutrition and Feeding in Fish*, Academic Press, London, pp 119-145.

Tacon, A.G.J. and 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(4), pp 391-400.

Takeuchi, T. and Watanabe, T., 1976. Nutrition value of ω3 highly unsaturated fatty acids in pollack liver oil for rainbow trout. *Bull. Jap. Soc. Sci. Fish.*, 42, pp 907-919.

Takeuchi, T., Watanabe, T. and Ogino, C., 1978a. Optimum ratio of protein to lipid in diets of rainbow trout. *Bull. Jap. Soc. Sci. Fish.*, 44(6), pp 683-688.

Takeuchi, T., Watanabe, T. and Ogino, C., 1978b. Supplementary effect of lipids in a high protein diet of rainbow trout. *Bull. Jap. Soc. Sci. Fish.*, 44(6), pp 677-681.

Talbot, C. and Higgins, P.J., 1982. Observations on the gallbladder of juvenile atlantic salmon (*Salmo salar* L.) in relation to feeding. *J. Fish Biol.* 21, pp 663-669.

Talbot, C. and Higgins, P.J., 1983. A radiographic method for feeding studies on fish using metallic iron powder as a marker. *J.Fish Biol.*, 23(2), pp 211-220.

Thonney, M.L., Duhaime, D.J., Moe, P.W., and Reid, J.T., 1979. Acid insoluble ash and permanganate lignin as indicators to determine digestibility of cattle rations. *J. Animal Sci.* , 49, pp 1112.

Ulla, O. and Gjedrem, T., 1985. Number and length of pyloric caeca and their relationship to fat and protein digestibility in rainbow trout. *Aquaculture*, 47, pp 105-111.

Van Dyke, J.M. and Sutton, D.L., 1977. Digestion of duckweed (*Lemna* spp.) by the grass carp (*Ctenopharyngodon idella*). *J. Fish. Biol.* 11, pp 273-278.

Van Keulen, J. and Young, B.A., 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Animal Sci.*, 44, pp 282.

Van Soest, P.J., 1963. Use of detergents in the analysis of fibrous feeds. II - A rapid method for the determination of fibre and lignin. *J. Ass. Offi. Agric. Chemists*, 46, pp 829-835.

Van Soest, P.J., 1982. Refractory and inhibitory substances. In: *Nutritional Ecology of the Ruminant.* O and B Books, Inc., Corvallis, OR., pp 118.

Vens-Cappell, B., 1985. Methodological studies on digestion in trout. I - Reliability of digestion coefficients in relation to methods for faeces collection. *Aquacultural Engineering*, 4, pp 33-39.

Vogtmann, H., Pfirter, H.P., and Prabucki, A.L., 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. *British Poultry Soc.*, 16, pp 531.

Waller, J., Merchen, N., Hanson, T. and Klopfenstein, T., 1980. Effect of sampling intervals and digesta markers on abossomal flow determinations. *J. Anim. Sci.*, 50(6).

Ward, AT. and Reichert, R.D., 1986. Comparison of the effect of cell wall and hull fibre from canola and soybean on the availability for rats of minerals, protein and lipid. *J. Nutr.*, 116, pp 233-241.

Watanabe, Y., 1981. Ingestion of horseradish peroxidase by the intestinal cells in larvae and juveniles of some teleosts. *Bull. Jap. Soc. Sci. Fish.*, 47, pp 1299-1307.

Watanabe, T., Kobayashi, T., Utsue, O. and Ogino, C., 1974. Effect of dietary methyl limolenate on fatty acid composition of lipids in rainbow trout. *Bull. Jap. Soc. Sci. Fish.*, 40, pp 393-397.

Watanabe, T., Takeuchi, T. and Ogino, C., 1979. Studies on the sparing effect of lipids on dietary protein in rainbow trout (*Salmo gairdneri*). In: Halver, J.E. and Tiews, K. (Eds.) *Finfish Nutrition and Fishfeed Technology*, Vol.I. Berlin: Heinemann Verkgsgesel, pp 113-125.

Watanabe, T., Takeuchi, T. and Satoh, S., 1989. The digestible crude protein content of practical feed ingredients determined in freshwater fish. *The Third Int. Symp. on Feeding and Nutrition in Fish*, Aug.28 - Sept.1, 1989, Toba, Japan.

Western, J.R.H. and Jennings, J.B., 1970. Histochemical demonstration of hydrochloric acid in the gastric tubules of teleost using an *in vivo* Prussian blue technique. *Comp. Biochem. Physiol.*, 35, pp 879-884.

Windell, J.T., 1967. Rates of digestion in fishes. In: *The Biological Basis of Freshwater Fish Production*. Gerking, S.D., (Ed.). Blackwell, Oxford, pp 151-173.

Windell, J.T., 1978. Digestion and the daily ration of fishes. In: *Ecology of Freshwater Fish Production*, Chapter 7, ed. by Shelby D. Gerking. Blackwell Scientific Publ., 1978.

Windell, J.T., Foltz, J.W., and Sarokon, J.A., 1978a. Methods of faecal collection and nutrient leaching in digestibility studies. *Prog. Fish Cult.*, 40(2),pp 50-55.

Windell, J.T., Foltz, J.W., and Sarokon, J.A., 1978b. Effect of fish size, temperature, and amount fed on nutrient digestibility of a pelleted diet by rainbow trout, *Salmo gairdneri*. *Trans. Am. Fish. Soc.*, 107(4), pp 613-616.

Windell, J.T., Hubbard, J.D. and Horak, D.C., 1972. Rate of gastric digestion in rainbow trout, *Salmo gairdneri*, fed three pelleted diets. *Prog. Fish Cult.*, 34(3), pp 156-159.

Windell, J.T., and Norris, D.O., 1969a. Dynamics of gastric evacuation in rainbow trout, *Salmo gairdneri*. *Am. Zool.*, 9, pp 584.

Windell, J.T. and Norris, D.O., 1969b. Gastric digestion and evacuation in rainbow trout. *Prog. Fish Cult.*, 31, pp 20-26.

Windell, J.T., Norris, D.O., Kitchell, J.F. and Norris, J.S., 1969. Digestive response of rainbow trout, *Salmo gairdneri*, to pellet diets. *J. Fish. Res. Board Can.*, 26, pp 1801-1812.

Yamada, J., Kikuchi, R., Matsushima, M. and Ogami, H., 1962. On improving the efficiency of feed for fish culture. II - Digestibilities of feedingstuff for rainbow trout and some trials on the improvement. *Bull. Jap. Soc. Sci. Fish.*, 28(9).

Yamane, S., 1973a. Localisation of amylase activity in digestive organs of carp determined by a substrate film method. *Bull. Jap. Soc. Sci. Fish.*,39, pp 497-504.

Yamane, S., 1973b. Localisation of amylase activity in the digestive organs of the Mozambique mouthbrooder, *Tilapia mossambica* and bluegill, *Lepomis macrochirus*, determined by starch substrate film method. *Bull. Jap. Soc. Sci. Fish.*, 39, pp 595-603.

Yen, J.T., Tess, M.W., Pond, W.G. and Dickerson, G.E., 1983. Digestibility and metabolism of dietary nitrogen and energy in contemporary, genetically lean and obese pigs as estimated by total faecal collection and acid insoluble ash. *Journal of Animal Science*, 56(2), pp 426-430.

Yu, T.C. and Sinnhuber, R.O., 1976. Growth response of rainbow trout (*Salmo gairdneri*), to dietary ω3 and ω6 fatty acids. *Aquaculture*, 8, pp 309-317.

Zar, J.H., 1984. *Biostatistical Analysis*, 2nd Ed., Prentice-Hall, Inc., Englewood Cliffs, N.J., 718 pp.